Experimental Parasitology 171 (2016) 17–22

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Nitazoxanide induces *in vitro* metabolic acidosis in *Taenia crassiceps* cysticerci

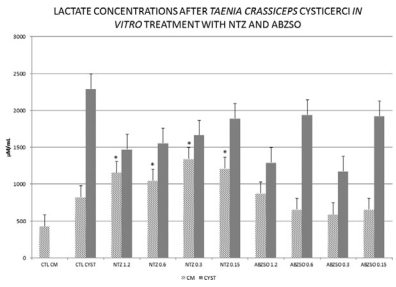
Eliana Isac, Guaraciara de A. Picanço, Tatiane L. da Costa, Nayana F. de Lima, Daniella de S. M. M. Alves, Carolina M. Fraga, Ruy de S. Lino Junior, Marina C. Vinaud*

Laboratory of Studies of the Host-Parasite Relationship, Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235, s/n, Setor Universitário, Goiania, Goias, CEP:74605-050, Brazil

HIGHLIGHTS

- NTZ induces impair in glucose uptake in *in vitro* cysticerci.
- NTZ did not influence the pyruvate concentrations in *in vitro* cysticerci.
- NTZ induces metabolic acidosis in *in vitro* cysticerci.
- The metabolic acidosis *in vitro* induced by NTZ is greater than ABZ.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form
10 October 2016
Accepted 13 October 2016
Available online 14 October 2016

Keywords:
Nitazoxanide
Albendazole sulfoxide
Anaerobic metabolism
Lactic acid
Taenia crassiceps

ABSTRACT

Nitazoxanide (NTZ) is a broad-spectrum anti-parasitic drug used against a wide variety of protozoans and helminthes. Albendazole, its active metabolite albendazole sulfoxide (ABZSO), is one of the drugs of choice to treat both intestinal and tissue helminth and protozoan infections. However little is known regarding their impact on the metabolism of parasites. The aim of this study was to compare the *in vitro* effect of NTZ and ABZSO in the glycolysis of *Taenia crassiceps* cysticerci. The cysticerci were treated with 1.2; 0.6; 0.3 or 0.15 μg/mL of NTZ or ABZSO. Chromatographic and spectrophotometric analyses were performed in the culture medium and in the cysticerci extract. Regarding the glucose concentrations was possible to observe two responses: impair of the uptake and gluconeogenesis. The pyruvate concentrations were increased in the ABZSO treated group. Lactate concentrations were increased in the culture medium of NTZ treated groups. Therefore it was possible to infer that the metabolic acidosis was greater in the group treated with NTZ than in the ABZSO treated group indicating that this is one of the modes of action used by this drug to induce the parasite death.

1. Introduction

Nitazoxanide (NTZ) is a broad-spectrum anti-parasitic drug used against a wide variety of protozoans and helminths. It has been established that NTZ as well as its reduced form, tizoxanide, are effective against most target pathogens. It is the only drug effective against *Cryptosporidium* infections and one of the most effective against intestinal giardiasis (Miyamoto and Eckmann, 2015). It has been described as an effective treatment of cutaneous leishmaniasis by *Leishmania donovani* (Dhawan et al., 2015). Also this drug has been indicated against intestinal and tissue helminth infection

* Corresponding author.
E-mail address: marinavinaud@gmail.com (M.C. Vinaud).

http://dx.doi.org/10.1016/j.exppara.2016.10.012
0014-4894/

such as cystic echinococcosis (Laura et al., 2015), soil-transmitted helminths (Somvanshi et al., 2014); *Ancylostoma ceylanicum*, *Ascaris suum*, *Trichuris muris and Caenorhabditis elegans* (Hu et al., 2013), *Taenia saginata* and *Hymenolepis nana* (Rossignol and Maisonneuve, 1984). Interestingly this drug has been successfully used against nosocomial pathogens (Gau et al., 2016), *Clostridium difficile* (Soriano and Johnson, 2015) and hepatitis C virus (Kohla et al., 2016).

This drug has been indicated as an alternative treatment when the resistance to traditional anti-parasitic drugs is detected. For example, NTZ has been described as effective against *T. saginata* niclosamide- and praziquantel-resistant (Lateef et al., 2008).

In protozoans both NTZ and tizoxanide inhibit central physiological enzymes such as pyruvate ferredoxin oxidoreductase, nitroreductase 1, quinone reductase and induces lesions in the cell membrane and vacuolization (Leitsch, 2015). While in *Neospora caninum*, NTZ inhibited the protein disulfide isomerase (Muller et al., 2008). In bacteria this drug inhibits the pilus biogenesis by the chaperone/usher pathway (Chahales et al., 2016). However, there are few studies determining the effect of this drug on helminths. Somvanshi et al. (2014) using the *C. elegans* experimental model for soil-transmitted nematodes determined that NTZ acts on an alpha-type subunit of a glutamate-gated chloride ion channel which is also responsible for ivermectin susceptibility in this organism.

Albendazole (ABZ) is one of the drugs of choice to treat both intestinal and tissue helminth and protozoan infections and its active metabolite is albendazole sulfoxide (ABZSO) (Van den Enden, 2009). However, regarding the *G. intestinalis* treatment it has been determined that other drugs such as tinidazole and NTZ are more effective than ABZ (Leitsch, 2015; Escobedo et al., 2016). Regarding the treatment of helminth's tissue infections there are few drugs used such as ABZ, ivermectin and praziquantel, easy to use and effective against most parasites such as *Echinococcus granulosus* (Hemphill et al., 2014), *Strongyloides stercoralis* (Luvira et al., 2014), ocular toxocariasis (Ahn et al., 2014) and cysticercosis (Del Brutto, 2014). The reliance on such drugs creates the danger of resistance which has already been reported (Geary, 2012) leading to the imperative need for the development of new drugs.

It is known that antihelminthic drugs are able to eliminate parasitic infections, however the mode of action of such drugs and their effect on the parasite metabolism has been little explored. It has been previously determined that ABZ in *in vitro* analysis induces a partial blockage of glucose uptake, decrease in lactate concentrations in the culture medium and a preference for the aerobic metabolism in *Taenia crassiceps* cysticerci (Vinaud et al., 2008). It has been described that one of the mode of actions of NTZ is to block the pyruvate ferredoxin oxidoreductase enzyme which is part a very energetic rentable pathway – the carbohydrate catabolism (Hoffman et al., 2007). This enzyme performs the decarboxylation of pyruvate (Hoffman et al., 2007) therefore the analysis of the metabolic pathway which produces pyruvate – anaerobic glycolysis - may indicate the effect of this drug on the energy production by the parasite. On the other hand it is important to present a comparison of the metabolic impact of NTZ in to a well-known and more studied drug, which is ABZSO.

T. crassiceps is a cestode widely used as experimental model of cysticercosis caused by *T. solium* due to their antigenic similarity (Vaz et al., 1997). It has been previously used to determine the impact of anti-helminthics such as ABZ, praziquantel and a benzimidazole derivative in their energetic metabolism (Vinaud et al., 2007, 2008; Fraga et al., 2016). Therefore the aim of this study is to compare the *in vitro* effect of NTZ and ABZSO in the glycolysis of *T. crassiceps* cysticerci.

2. Material and methods

2.1. Maintenance of T. crassiceps cysticerci

The maintenance of *T. crassiceps* cysticerci was performed according to the description by Vaz et al. (1997). Briefly, BALB/c female mice with 8–12 weeks old are intraperitoneally infected with 10 initial stage (no buds, translucent membrane and vesicular liquid) *T. crassiceps* cysticerci, maintained in cages with five animals maximum, received standard ration and water ad libitum. After 90 days of infection, the animals are euthanized, the cysticerci removed from the peritoneal cavity and inoculated into another mouse. The ethical principles of animal experimentation stipulated by the Brazilian Society of Laboratory Animals Science (SBCAL) were obeyed. This study was approved by the Ethics Committee in Animal Use from the Federal University of Goias (CEUA/UFG) (protocol number 050/2013).

2.2. Cysticerci culture and drugs exposure

A mouse with 30 days of intraperitoneal infection was euthanized within a laminar flow chamber and the cysticerci were removed and washed with sterile saline solution as to remove cells and any other contaminants (Márquez-Navarro et al., 2013). Afterwards the larval stage cysticerci (with buds, translucent vesicular membrane and fluid) (Vinaud et al., 2007) were collected and cultured as described by Fraga et al. (2016). Briefly, 30 larval stage cysticerci were added into 5 mL of supplemented RPMI culture medium. The cysticerci were treated with 1.2; 0.6; 0.3 or 0.15 μg/mL of NTZ or ABZSO. The drugs were diluted with dimethyl sulfoxide (DMSO) (0.60%). The control groups were constituted by a group of cysticerci without drugs and another one that received the concentration of DMSO used to dilute the drugs.

After 24 h of culture the cysticerci were separated from their culture medium and frozen into liquid nitrogen as to stop the metabolic reactions (Vinaud et al., 2008).

The molarity concentrations of the drugs are exposed in Table 1.

2.3. Biochemical analysis

The organic acids secreted/excreted into the culture medium were extracted through an ionic exchange solid phase extraction column (Bond Elut® Agilent®), as described by Vinaud et al. (2007).

The cysticerci extract was obtained after the liquid nitrogen metabolic stasis. The cysticerci were defrost and homogenized in 500 μL of tris-HCl 0.1 M buffer supplemented with a protease inhibitor (SIGMAFAST, protease inhibitor cocktail tablets, EDTA free, Sigma), pH 7.6. The extract obtained was centrifuged at 15,652*g* (10,000 rpm) (Beckman centrifuge, TA-10-250, 13.7 cm radius) per 10 min at 4 °C and then the organic acids were extracted through an ionic exchange solid phase extraction column (Bond Elut® Agilent®). The resulting samples were frozen at −20 °C for posterior analysis in high performance liquid chromatography (Vinaud et al., 2007).

Table 1
Molarity concentrations of nitazoxanide and albendazole sulfoxide used in the *in vitro* assay.

Concentration μg/mL	NTZ (MW = 307.28)	ABZSO (MW = 281.33)
1.2	3.90 μM	4.26 μM
0.6	1.95 μM	2.13 μM
0.3	0.97 μM	1.06 μM
0.15	0.48 μM	0.53 μM

NTZ – nitazoxanide; ABZSO – albendazole sulfoxide; MW – molecular weight.

For the chromatographic analysis an exclusion BIORAD-Aminex HPX-87H column was used. The eluent was sulfuric acid 5 mM, 0.6 mL/min, with spectrophotometric reading of absorbance at 210 nm. The results were analyzed through the Star Chromatography Workstation software (Agilent®), previously calibrated for the pyruvate and lactate identification which are indicative of anaerobic glycolisis (Vinaud et al., 2007). The glucose quantification was performed through an Architec C8000 Plus device, using a commercial kit protocol with an enzymatic method (Fraga et al., 2012).

2.4. Statistical analysis

All experiments were repeated five times independently. The statistical analysis was performed through the Sigma Stat 4.0 software. The descriptive analysis was performed as to determine the normal distribution and homogenous variation as well as mean and standard deviation. As the values presented normal distribution, the analysis of variation test (ANOVA) was performed followed by the Bonferrone post-test. The differences were considered significant when $p < 0.05$.

3. Results

This study compared the *in vitro* effect of two widely used antihelminthic drugs in the glycolysis of *T. crassiceps* cysticerci. Therefore the concentrations of glucose and the main final products of glycolysis, pyruvate and lactate, were quantified both in the culture medium and in the cysticerci extract from *T. crassiceps* cysticerci after the exposure to different concentrations of NTZ and ABZSO. The analysis of the culture medium aimed to detect the excretion/secretion of glucose, pyruvate and lactate; while the analysis of the cysticerci extract aimed to determine the intracellular concentrations of these substances. The cysticerci were *in vitro* exposed to different concentrations of the drugs which were diluted with DMSO. The diluent did not influence in the metabolic analysis.

There was a significant difference between the glucose concentrations in the culture medium of the control group and the group treated with the highest concentrations of NTZ ($p < 0.05$). While the NTZ treatment induced an impairment of the glucose uptake the ABZSO treatment induced a greater uptake of glucose ($p < 0.05$) (Fig. 1).

In the cysticerci extract the glucose levels were significantly higher in the NTZ treated groups than in the control group ($p < 0.05$). While in the ABZSO treated group there was no significant difference when compared to the control group.

The pyruvate concentrations in the culture medium of the groups treated with ABZSO were significantly higher than the concentration detected in the control group ($p < 0.05$). The NTZ

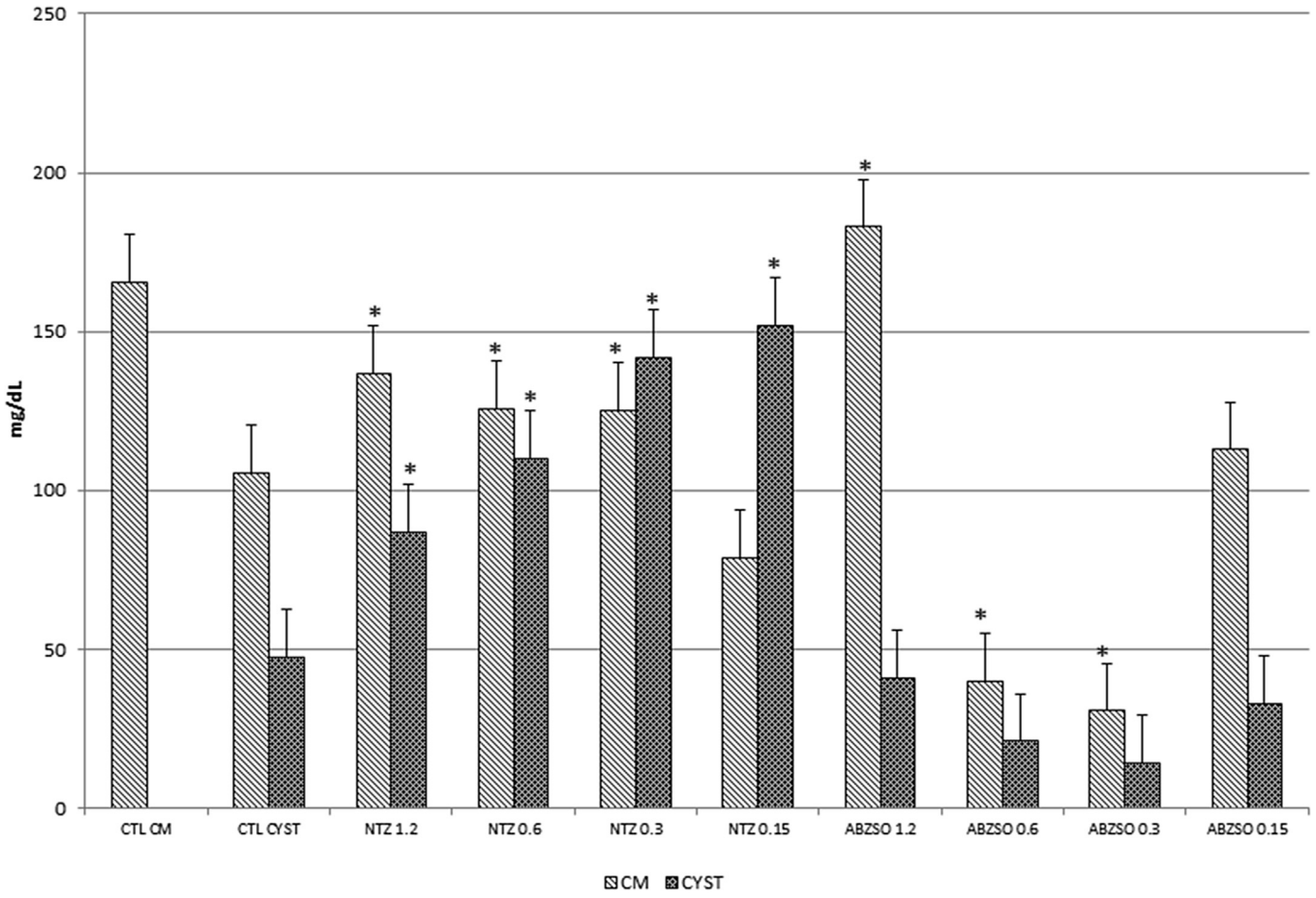


Fig. 1. Glucose concentrations in the culture medium and in the cysticerci extract from groups *in vitro* treated with different concentrations of nitazoxanide and albendazole. CM: concentrations detected in the culture medium analysis; CYST: concentrations detected in the cysticerci extract; CTL CM: RPMI culture medium without cysticerci; CTL CYST: cysticerci without drugs exposure; NTZ 1.2: cysticerci exposed to 1.2 μg/mL of nitazoxanide; NTZ 0.6: cysticerci exposed to 0.6 μg/mL of nitazoxanide; NTZ 0.3 cysticerci exposed to 0.3 μg/mL of nitazoxanide; NTZ 0.15: cysticerci exposed to 0.15 μg/mL of nitazoxanide; ABZSO 1.2: cysticerci exposed to 1.2 μg/mL of albendazole sulfoxide; ABZSO 0.6: cysticerci exposed to 0.6 μg/mL of albendazole sulfoxide; ABZSO 0.3: cysticerci exposed to 0.3 μg/mL of albendazole sulfoxide; ABZSO 0.15: cysticerci exposed to 0.15 μg/mL of albendazole sulfoxide. * statistical difference when compared to the control group.

treatment did not influence the pyruvate concentrations in the culture medium. The pyruvate concentrations in the cysticerci extract were not influenced by the exposure to ABZSO nor to NTZ (Fig. 2).

There was a significant increase in the lactate concentrations in the culture medium of the group exposed to all concentrations of NTZ ($p < 0.05$). While the exposure to different concentrations of ABZSO did not influence the concentration of this organic acid in the culture medium. The lactate concentrations in the cysticerci extract were not influenced by the *in vitro* exposure nor to ABZSO neither to NTZ (Fig. 3).

4. Discussion

Glucose is the main energy source for cestodes such as *T. crassiceps* (Smyth and McManus, 1989). Due to the importance of such molecule in the parasite metabolism this study compared the effect of two widely used antihelminthic drugs in the *in vitro* glycolysis of *T. crassiceps* cysticerci.

The anaerobic energy metabolism in helminthes is enhanced when there are stressful factors affecting the cell such as inadequate oxygen supply or exposure to drugs. This may lead to metabolic acidosis and cell death (Smyth and McManus, 1989). In our study was possible to observe the metabolic acidosis due to high levels of lactate in the culture medium of the cysticerci treated with all concentrations of NTZ. This fact may be understood as the response of the parasitic cell to the stressful factor present in the culture medium, i.e., the different concentrations of the drugs. Also it indicates that the parasitic cell is evolving to death, even under little time of exposure (24 h), single dose of treatment and non-lethal doses (0.15–1.2 μg/mL).

The impair of glucose uptake is one of the mode of action observed in benzimidazole drugs alongside with the inhibition of tubulin formation (Kohler, 1985; McCracken and Stillwell, 1991; Martin, 1997) and was observed in the group treated with 1.2 μg/mL of ABZSO in our study. Also as there was an increase in the glucose levels in the culture medium it is possible to infer that gluconeogenesis also happened in this group.

Xiao et al. (1993) described that mebendazole also inhibited the glucose uptake in *Echinococus granulosus* cyst wall. This effect was observed in the ABZSO (1.2 μg/mL) and in the NTZ (1.2, 0.6 and 0.3 μg/mL) treated groups in our study. It seems that one of the

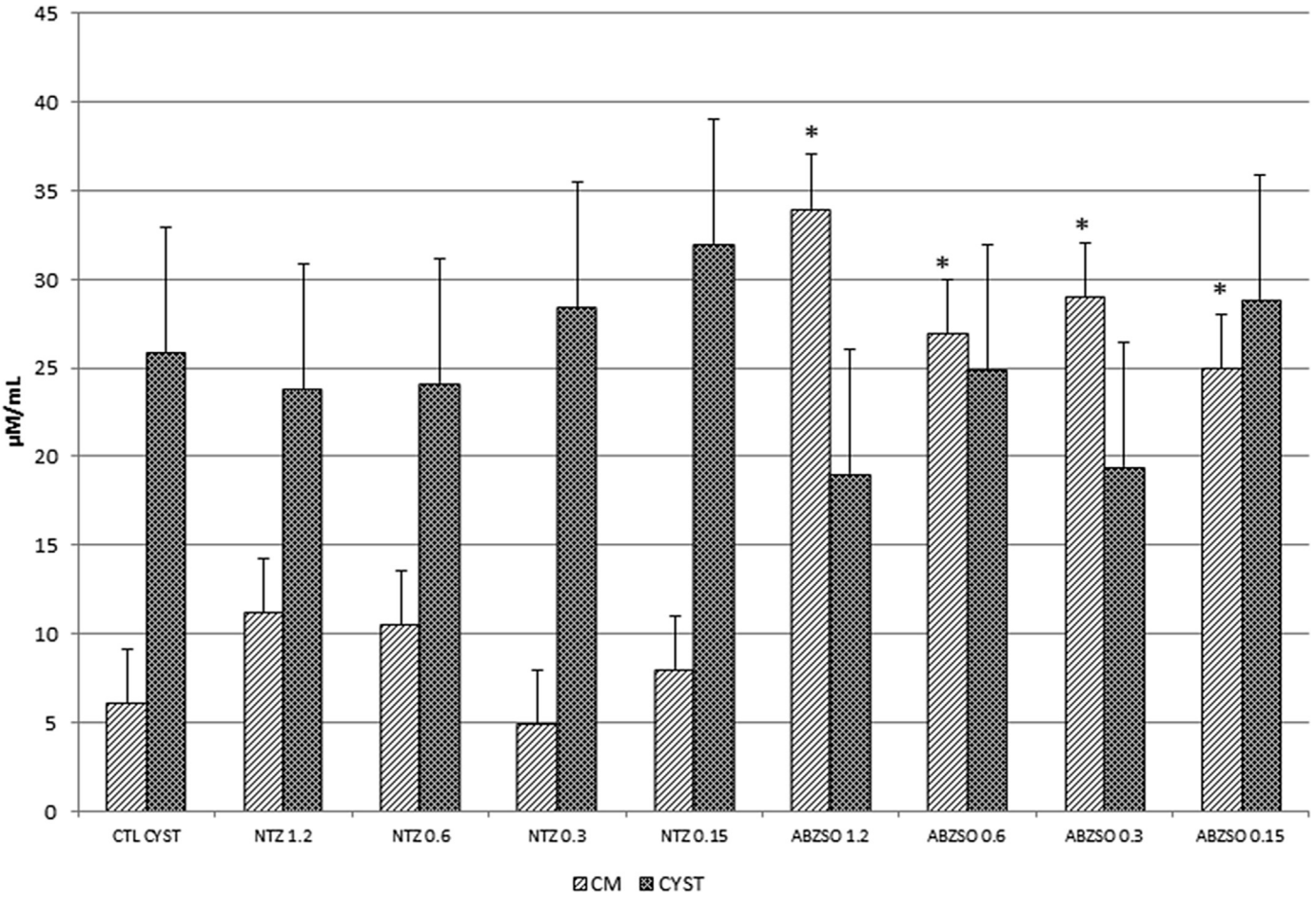


Fig. 2. Pyruvate concentrations in the culture medium and in the cysticerci extract from groups *in vitro* treated with different concentrations of nitazoxanide and albendazole. CM: concentrations detected in the culture medium analysis; CYST: concentrations detected in the cysticerci extract; CTL CYST: cysticerci without drugs exposure; NTZ 1.2: cysticerci exposed to 1.2 μg/mL of nitazoxanide; NTZ 0.6: cysticerci exposed to 0.6 μg/mL of nitazoxanide; NTZ 0.3 cysticerci exposed to 0.3 μg/mL of nitazoxanide; NTZ 0.15: cysticerci exposed to 0.15 μg/mL of nitazoxanide; ABZSO 1.2: cysticerci exposed to 1.2 μg/mL of albendazole sulfoxide; ABZSO 0.6: cysticerci exposed to 0.6 μg/mL of albendazole sulfoxide; ABZSO 0.3: cysticerci exposed to 0.3 μg/mL of albendazole sulfoxide; ABZSO 0.15: cysticerci exposed to 0.15 μg/mL of albendazole sulfoxide. * statistical difference when compared to the control group.

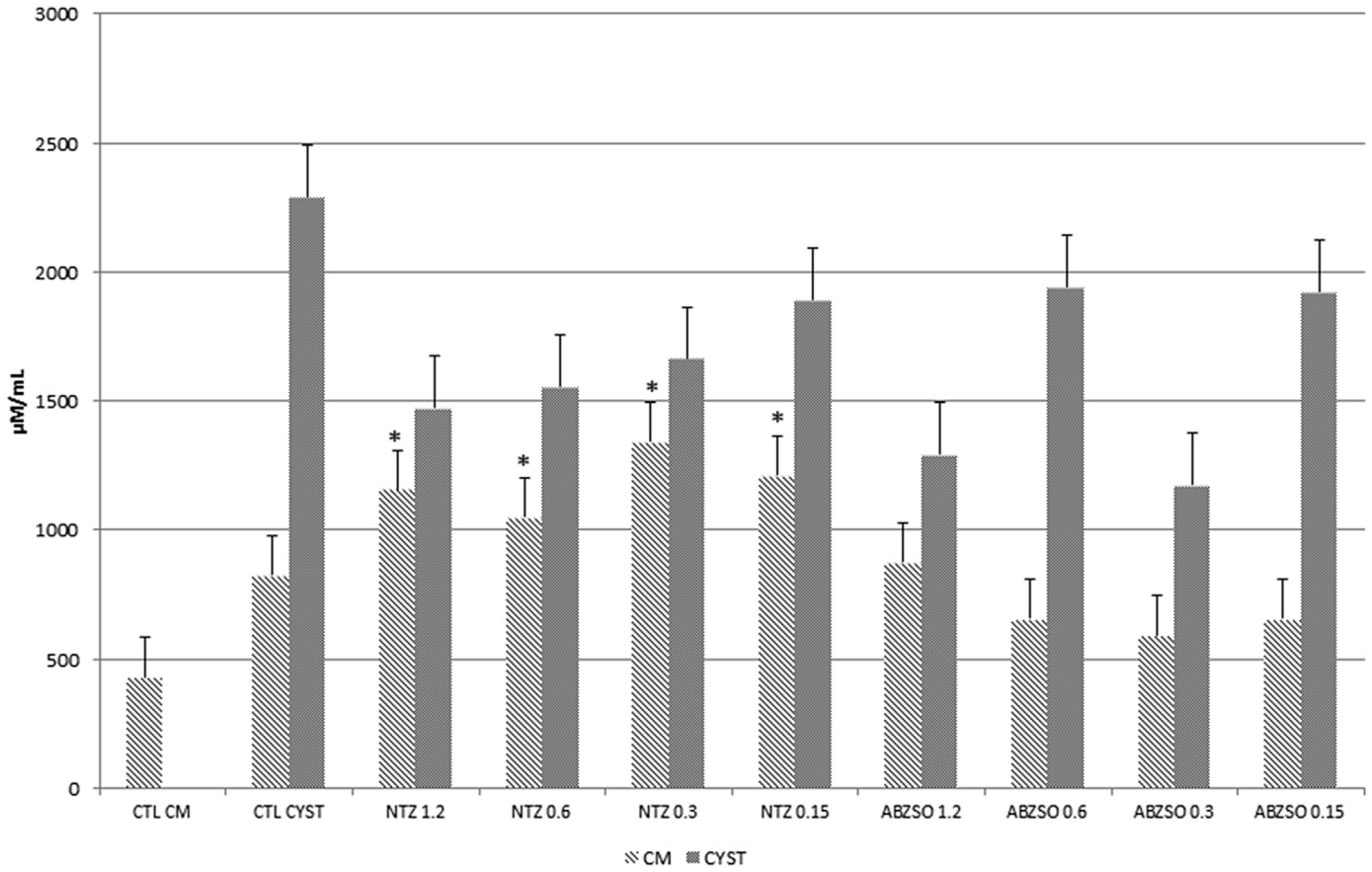


Fig. 3. Lactate concentrations in the culture medium and in the cysticerci extract of cysticerci exposed to different concentrations of albendazole and nitazoxanide. CM: concentrations detected in the culture medium analysis; CYST: concentrations detected in the cysticerci extract; CTL CM: RPMI culture medium without cysticerci; CTL CYST: cysticerci without drugs exposure; NTZ 1.2: cysticerci exposed to 1.2 µg/mL of nitazoxanide; NTZ 0.6: cysticerci exposed to 0.6 µg/mL of nitazoxanide; NTZ 0.3 cysticerci exposed to 0.3 µg/mL of nitazoxanide; NTZ 0.15: cysticerci exposed to 0.15 µg/mL of nitazoxanide; ABZSO 1.2: cysticerci exposed to 1.2 µg/mL of albendazole sulfoxide; ABZSO 0.6: cysticerci exposed to 0.6 µg/mL of albendazole sulfoxide; ABZSO 0.3: cysticerci exposed to 0.3 µg/mL of albendazole sulfoxide; ABZSO 0.15: cysticerci exposed to 0.15 µg/mL of albendazole sulfoxide. * statistical difference when compared to the control group.

modes of action of benzimidazole antihelminthics, such as mebendazole and ABZ, is to impair the transmembrane proton gradient which results in diminished levels of cellular ATP (McCracken and Stillwell, 1991) and in the use of alternative energy sources such as protein catabolism and fatty acids oxidation (Vinaud et al., 2009; Fraga et al., 2012).

One of the metabolic effects of NTZ is to interfere in the pyruvate oxidoreductase activity (Sisson et al., 2002). This enzyme catalyzes the interconversion of pyruvate into acetyl-CoA. The blockage of this enzyme leads to an excess of pyruvate which may be used in two different pathways: 1. Gluconeogenesis from the activity of phosphoenolpyruvate synthase and phosphoenolpyruvate carboxykinase; 2. Anaerobic metabolism through lactate dehydrogenase activity and lactate production (Smyth and McManus, 1989). Both pathways were observed in the NTZ treated groups due to the high concentrations of glucose in the culture medium analysis and due to the high concentrations of lactate both in the culture medium and in the cysticerci extract analysis. Also gluconeogenesis was observed in the ABZSO 1.2 µg/mL treated group due to the increase in the glucose levels in the culture medium analysis.

Interestingly, the pyruvate concentrations were not altered in the NTZ treated groups. This indicates that this organic acid is being used as substrate for lactate dehydrogenase as to produce lactate and also as substrate for malic enzyme as to produce malate, both in the cytoplasm and in the mitochondria (Smyth and McManus, 1989). The ABZSO treated groups presented an increase in the pyruvate concentrations in the culture medium analysis. The pyruvate may have been produced by the activity of lactate dehydrogenase and malic enzyme which are not influenced by the presence of the drug (Escobedo et al., 2016).

Palomares-Alonso et al. (2007) investigated the effect of nitazoxanide, tizoxanide and albendazole sulphoxide in the morphology and survival of *T. crassiceps* cysticerci. These authors describe that after 11 days of culture the parasites exposed to nitazoxanide and tizoxanide did not present alterations in the ruggedness and distribution of microtriches when analyzed under light microscopy. In our study we also did not observe alterations in the morphology of the parasites after 24 h of exposure to the drugs. However under electron microscopy it is possible to observe vacuolar bodies in the laminated layer of *Echinococus multilocularis in vitro* exposed to NTZ (Stettler et al., 2003). There was high accumulation of lipid droplets within the germinal layer of *T. crassiceps in vitro* exposed to NTZ while there microtriches and muscular cells were not influenced by this drug (Palomares-Alonso et al., 2007).

Therefore, it is possible to conclude that the NTZ concentrations tested induced metabolic acidosis in *in vitro T. crassiceps* cysticerci which is a signal of cell death. Also the drug impacted the glucose uptake by the parasite which may have contributed to the lactate production. It was possible to infer that the metabolic acidosis was greater in the group treated with NTZ than in the ABZSO treated group indicating that this is one of the modes of action used by this

drug to induce the parasite death.

Acknowledgments

This work was supported by CNPq - National Counsel of Technological and Scientific Development (grant number 471009/2013-0).

References

Ahn, S.J., Ryoo, N.K., Woo, S.J., 2014. Ocular toxocariasis: clinical features, diagnosis, treatment, and prevention. Asia Pac. Allergy 4, 134–141. http://dx.doi.org/10.5415/apallergy.2014.4.3.134.

Chahales, P., Hoffman, P.S., Thanassi, D.G., 2016. Nitazoxanide inhibits pilus biogenesis by interfering with folding of the usher protein in the outer membrane. Antimicrob. Agents Chemother. 60, 2028–2038. http://dx.doi.org/10.1128/AAC.02221-15.

Del Brutto, O.H., 2014. Neurocysticercosis. Handbook of Clinical Neurology, vol. 121, pp. 1445–1459. http://dx.doi.org/10.1016/B978-0-7020-4088-7.00097-3.

Dhawan, A.K., Bisherwal, K., Gandhi, V., Singal, A., Sharma, S., 2015. Successful treatment of cutaneous leishmaniasis with nitazoxanide. Indian J. Dermatol. Venereol. Leprol. 81, 644–646. http://dx.doi.org/10.4103/0378-6323.165541.

Escobedo, A.A., Ballesteros, J., González-Fraile, E., Almirall, P., 2016. A meta-analysis of the efficacy of albendazole compared with tinidazole as treatments for *Giardia* infections in children. Acta Trop. 153, 120–127. http://dx.doi.org/10.1016/j.actatropica.2015.09.023.

Fraga, C.M., Costa, T.L., Bezerra, J.C.B., Lino Junior, R.S., Vinaud, M.C., 2012. Fatty acids oxidation and alternative energy sources detected in *Taenia crassiceps* cysticerci after host treatment with antihelminthic drugs. Exp. Parasitol. 131, 111–115. http://dx.doi.org/10.1016/j.exppara.2012.03.017.

Fraga, C.M., Costa, T.L., Castro, A.M., Reynoso-Ducoing, O., Ambrosio, J., Hernández-Campos, A., Castillo, R., Vinaud, M.C., 2016. Alternative energy production pathways in *Taenia crassiceps* cysticerci *in vitro* exposed to a benzimidazole derivative (RCB20). Parasitology 143, 488–493. http://dx.doi.org/10.1017/S0031182015001729.

Gau, J.S., Lin, W.P., Kuo, L.C., Hu, M.K., 2016. Nitazoxanide analogues as antimicrobial agents against nosocomial pathogens. Med. Chem. http://dx.doi.org/10.2174/1573406412666160129105719 [Epub ahead of print].

Geary, T.G., 2012. Are new anthelmintics needed to eliminate human helminthiases? Curr. Opin. Infect. Dis. 25, 709–717. http://dx.doi.org/10.1097/QCO.0b013e328359f04a.

Hemphill, A., Stadelmann, B., Rufener, R., Spiliotis, M., Boubaker, G., Müller, J., Müller, N., Gorgas, D., Gottstein, B., 2014. Treatment of echinococcosis: albendazole and mebendazole—what else? Parasite 21, 70. http://dx.doi.org/10.1051/parasite/2014073.

Hoffman, P.S., Sisson, G., Croxen, M.A., Welch, K., Harman, W.D., Cremades, N., Morash, M.G., 2007. Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductase of *Helicobacter pylori*, selected anaerobic bacteria and parasites, and *Campylobacter jejuni*. Antimicrob. Ag. Chemother. 51, 868–876. http://dx.doi.org/10.1128/AAC.01159-06.

Hu, Y., Ellis, B.L., Yiu, Y.Y., Miller, M.M., Urban, J.F., Shi, L.Z., Aroian, R.V., 2013. An extensive comparison of the effect of anthelmintic classes on diverse nematodes. PLoS ONE 8, e70702. http://dx.doi.org/10.1371/journal.pone.0070702.

Kohla, M.A., El-Said, H., El-Fert, A., Ehsan, N., Ezzat, S., Taha, H., 2016. Impact of nitazoxanide on sustained virologic response in Egyptian patients with chronic hepatitis C genotype 4: a double-blind placebo-controlled trial. Eur. J. Gastroenterol. Hepatol. 28, 42–47. http://dx.doi.org/10.1097/MEG.0000000000000492.

Kohler, P., 1985. The strategies of energy conservation in helminths. Mol. Biochem. Parasitol. 17, 1–18.

Lateef, M., Zargar, S.A., Khan, A.R., Nazir, M., Shoukat, A., 2008. Successful treatment of niclosamide- and praziquantel-resistant beef tapeworm infection with nitazoxanide. Int. J. Infect. Dis. 12, 80–82. http://dx.doi.org/10.1016/j.ijid.2007.04.017.

Laura, C., Celina, E., Sergio, S.B., Guillermo, D., Carlos, L., Luis, A., 2015. Combined flubendazole-nitazoxanide treatment of cystic echinococcosis: pharmacokinetic and efficacy assessment in mice. Acta Trop. 148, 89–96. http://dx.doi.org/10.1016/j.actatropica.2015.04.019.

Leitsch, D., 2015. Drug Resistance in the microaerophilic parasite *Giardia lamblia*. Curr. Trop. Med. Rep. 2, 128–135. http://dx.doi.org/10.1007/s40475-015-0051-1.

Luvira, V., Watthanakulpanich, D., Pittisuttithum, P., 2014. Management of *Strongyloides stercoralis*: a puzzling parasite. Int. Health 6, 273–281. http://dx.doi.org/10.1093/inthealth/ihu058.

Márquez-Navarro, A., Pérez-Reyes, A., Zepeda-Rodrigues, A., Reynoso-Ducoing, O., Hernández-Campos, A., Hernández-Luis, F., Castillo, R., Yépez-Muli, A.L., Ambrosio, J.R., 2013. RCB20, an experimental benzimidazole derivative, affects tubulin expression and induces gross anatomical changes in *Taenia crassiceps* cysticerci. Parasitol. Res. 112, 2215–2226. http://dx.doi.org/10.1007/s00436-013-3379-2.

Martin, R.J., 1997. Modes of action of anthelmintic drugs. Vet. J. 154, 11–34.

McCracken, R.O., Stillwell, W.H., 1991. A possible biochemical mode of action for benzimidazole anthelmintics. Int. J. Parasitol. 21, 99–104. http://dx.doi.org/10.1016/0020-7519(91)90125-Q.

Miyamoto, Y., Eckmann, L., 2015. Drug development against the major diarrhea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia*. Front. Microbiol. 6, 1208. http://dx.doi.org/10.3389/fmicb.2015.01208.

Muller, J., Naguleswaran, A., Muller, N., Hemphill, A., 2008. *Neospora caninum*: functional inhibition of protein disulfide isomerase by the broad-spectrum antiparasitic drug nitazoxanide and other thiazolides. Exp. Parasitol. 118, 80–88. http://dx.doi.org/10.1016/j.exppara.2007.06.008.

Palomares-Alonso, F., Piliado, J.C., Palencia, G., Ortiz-Plata, A., Jung-Cook, H., 2007. Efficacy of nitazoxanide, tizoxanide and tizoxanide/albendazole sulphoxide combination against *Taenia crassiceps* cysts. J. Antimicrob. Chemother. 59, 212–218. http://dx.doi.org/10.1093/jac/dkl463.

Rossignol, J.F., Maisonneuve, H., 1984. Nitazoxanide in the treatment of *Taenia saginata* and *Hymenolepis nana* infections. Am. J. Trop. Med. Hyg. 33, 511–512.

Sisson, G., Goodwin, A., Raudonikiene, A., Hughes, N.J., Mukhopadhyay, A.K., Berg, D.E., Hoffman, P.S., 2002. Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans, and metronidazole in Helicobacter pylori. Antimicrob. Agents Chemother. 46, 2116–2123. http://dx.doi.org/10.1128/AAC.46.7.2116-2123.2002.

Smyth, J.D., McManus, D.P., 1989. The Physiology and Biochemistry of Cestodes. Cambridge University Press, Cambridge, UK.

Somvanshi, V.S., Ellis, B.L., Hu, Y., Aroian, R.V., 2014. Nitazoxanide: nematicidal mode of action and drug combination studies. Mol. Biochem. Parasitol. 193, 1–8. http://dx.doi.org/10.1016/j.molbiopara.2013.12.002.

Soriano, M.M., Johnson, S., 2015. Treatment of Clostridium difficile infections. Infect. Dis. Clin. North Am. 29, 93–108. http://dx.doi.org/10.1016/j.idc.2014.11.005.

Stettler, M., Fink, R., Walker, M., Gottstein, B., Geary, T.G., Rossignol, J.F., Hemphill, A., 2003. *In vitro* parasiticidal effect of nitazoxanide against *Echinococcus multilocularis* metacestodes. Antimicrob. Agents Chemother. 47, 467–474. http://dx.doi.org/10.1128/AAC.47.2.467-474.2003.

Van den Enden, E., 2009. Pharmacotherapy of helminth infection. Expert Opin. Pharmacother 10, 435–451. http://dx.doi.org/10.1517/14656560902722463.

Vaz, A.J., Nunes, C.M., Piazza, R.M., Livramento, J.A., Da Silva, M.V., Nakamura, P.M., Ferreira, A.W., 1997. Immunoblot with cerebrospinal fluid from patients with neurocysticercosis using antigen from cysticerci of *Taenia solium* and *Taenia crassiceps*. Am. J. Trop. Med. Hyg. 57, 354–357.

Vinaud, M.C., Ferreira, C.S., Lino Junior, R.S., Bezerra, J.C.B., 2008. *Taenia crassiceps*: energetic and respiratory metabolism from cysticerci exposed to praziquantel and albendazole in vitro. Exp. Parasitol. 120, 221–226. http://dx.doi.org/10.1016/j.exppara.2008.07.008.

Vinaud, M.C., Ferreira, C.S., Lino Junior, R.S., Bezerra, J.C.B., 2009. Taenia crassiceps: fatty acids oxidation and alternative energy source in in vitro cysticerci exposed to anthelminthic drugs. Exp. Parasitol. 122, 208–211. http://dx.doi.org/10.1016/j.exppara.2009.03.015.

Vinaud, M.C., Lino Junior, R.S., Bezerra, J.C.B., 2007. *Taenia crassiceps* organic acids detect in cysticerci. Exp. Parasitol. 116, 335–339. http://dx.doi.org/10.1016/j.exppara.2007.01.013.

Xiao, S.H., Feng, J.J., Guo, H.F., Jiao, J.Y., Yao, M.Y., Jiao, W., 1993. Effects of mebendazole, albendazole, and praziquantel on succinate dehydrogenase, fumarate reductase, and malate dehydrogenase in *Echinococcus granulosus* cysts harbored in mice. Acta Pharmacol. Sin. 14, 151–154.