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A benzimidazole derivative (RCB20) *in vitro* induces an activation of energetic pathways on *Taenia crassiceps* (ORF strain) cysticerci

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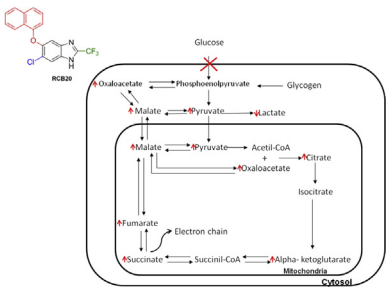
HIGHLIGHTS

- Cysticerci exposed to RCB20 presented a greater induction of glycolylis.
- Cysticerci exposed to RCB20 presented a greater induction of the TCA cycle.
- RCB20 induced the energetic pathways.
- RCB20 presents a greater effect on the metabolism than ABZSO.
- RCB20 is a promising substitute for ABZSO.

GRAPHICAL ABSTRACT



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ABSTRACT

Human cysticercosis caused by *Taenia crassiceps* is unusual; however, it is an useful experimental model for cysticercosis studies. Benzimidazole derivatives are important antihelminthic drugs widely used against helminths. A novel compound 6-chloro-5-(1-naphthyloxy) -2-(trifluoromethyl)-1*H*-benzimidazole (RCB20) is a benzimidazole derivative less polar and more lipophilic. The aim of this study was to detect the effect of the RCB20 on the *in vitro* energetic metabolism of *T. crassiceps* cysticerci. For this, products of the metabolism both produced and secreted/excreted (S/E) by the parasite were detected through spectrophotometry and high performance liquid chromatography after exposure to 6.5 and 13 μM of RCB20 and albendazole sulfoxide (ABZSO). There was a gradual increase in the concentrations of glucose not uptaken by parasites exposed to both concentrations RCB20 and ABZSO. There was a higher concentration of all the organic acids related to the tricarboxilic acid cycle int the parasites exposed to RCB20. The structural differences between RCB20 and ABZSO result in different targets within the parasite and in a greater induction of the energetic pathways, such as the glycolysis and the TCA cycle. RCB20 is a good candidate as a substitute for anthelminthic benzimidazoles due to a differentiated site of action with similar outcome.

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1. Introduction

Taenia crassiceps is a cestode which larval form also known as *Cysticercus longicollis* Rudolphi, 1810 is found in wild rodents such as voles (*Microtus arvalis*), groundhog (*Marmota monax*) and chinchillas (*Chinchilla lanigera*) (Freeman, 1962; Willms and Zurabian, 2010; Basso et al., 2014). One important characteristic of this species is the budding replication of the larval form which enables the increase in parasite burden within the intermediate host and in laboratory conditions. Also this parasite present antigenic and metabolic similarities with other *Taenia* species which makes it useful as an experimental model for cysticercosis studies (Vaz et al., 1997; Corbin et al., 1998; Vinaud et al., 2007).

Albendazole is a benzimidazole derivative developed initially against parasites of veterinary importance (Horton, 2000). Benzimidazoles such as albendazole, mebendazole and thiabendazole are widely used as antihelminthic; however, there are several reports on benzimidazole resistance in helminths of both animals and humans (Hoque et al., 2003; James et al., 2009; Soukhathammavong et al., 2012; Chaudhry et al., 2015; Muñiz-Lagunes et al., 2015). The benzimidazole resistance mechanisms is likely to be similar both in animal and in humans nematodes due to the extensive use of antihelminthic drugs for the control of gastrointestinal nematode parasites, mass drug administration to helminth infection control, misinformation regarding the antihelminthic mode of action, continued use of the same class of drug for a long period of time, overuse or inappropriate use of drugs without need (Prichard, 2007; Srivastava and Misra-Bhattacharya, 2015). While other authors stress that the increase in helminth resistance to currently available drugs imposes problems in controlling and eliminating such infections and, therefore, one of the strategies to reduce morbidity due to helminth infections is the development of new efficacious compounds associated to other approaches such as vaccines, antivectorial agents and new diagnostic tools (Srivastava and Misra-Bhattacharya, 2015).

The *in vitro* studies of the metabolic effect of albendazole on *T. crassiceps* cysticerci show that this drug induces a preference for the aerobic pathways of energy production due to a decrease in lactate production (Vinaud et al., 2008, 2009). However, *in vivo* studies with this drug demonstrate the induction of the fatty acids oxidation and alternative energy production pathways. These differences were due to the differential effect of albendazole and its active molecule, albendazole sulphoxide (ABZSO) (Fraga et al., 2012a, 2012b). The presence of the benzimidazole nucleus is an important feature of therapeutic agents, and has been valued in active molecules as antimicrobials, antifungic, antivirals and antiparasites (Bansai and Silakari, 2012). Therefore to identify the potential of the 2-(trifluoromethyl)-1*H*-benzimidazole system as antiparasitic agent the o 6-chloro-5-(1-naphthyloxy)-2-(trifluoromethyl)-1*H*-benzimidazole (RCB20) was developed (Márquez-Navarro et al., 2013). According to Márquez-Navarro et al. (2013) RCB20 apparently has a better activity against *T. crassiceps* cysticerci than ABZSO regarding morphological alterations and α-tubulin expression. However the effects of this compound on the metabolism of these parasites are unknown. Thus, the aim of this study was to evaluate the *in vitro* energetic metabolism of *T. crassiceps* cysticerci exposed to different concentrations of RCB20.

2. Methodology

2.1. Maintenance of Taenia crassiceps

The maintenance of *T. crassiceps* was performed according to the description by Vaz et al. (1997). 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 in Research Committee from the Federal University of Goias (CoEp/UFG) (protocol number 011/11).

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 physiological solution as to remove cells and any other contaminants (Márquez-Navarro et al., 2013). Afterwards the cysticerci were macroscopically classified into the following evoluative stages: initial (absence of buds, vesicular fluid and membrane translucent), larval (presence of buds, vesicular fluid and membrane translucent) and final (absence of buds, vesicular fluid and membrane opaque) (Vinaud et al., 2007).

The cysticerci culture was performed as described by Vinaud et al. (2008) and Márquez-Navarro et al. (2013). 30 larval stage cysticerci were added into 5 mL of supplemented RPMI culture medium. The treatments were as follows: albendazole sulfoxide (ABZSO) (Sigma-Aldrich®) (6.5 μM and 13 μM), RCB20 (6.5 μM and 13 μM). The control groups did not receive the drugs. There was another control group that received DMSO (0.6%) at the same concentration 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).

2.3. Biochemical analysis

The organic acids present in the culture medium were extracted through an ionic exchange solid phase extraction column (Bond Elut® Agilent®), as described previously (Vinaud et al., 2007).

After the liquid nitrogen metabolic stasis, the cysticerci were defrost and homogenized in 500 μL of tris-HCl 0.1M buffer supplemented with a protease inhibitor (SigmaFast protease inhibitor cocktail tablets, EDTA-free, Sigma®), pH 7.6 (Rendón et al., 2004; 2008). The extract obtained was centrifuged at 15,652*g* (10,000 rpm) per 10 min at 4 °C and then the organic acids present in the vesicular fluid were extracted through an ionic exchange solid phase extraction column (Bond Elut® Agilent®) (Vinaud et al., 2007).

The resulting samples were frozen at −20 °C for posterior analysis in high performance liquid chromatography.

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 following organic acids identification: pyruvate and lactate (glycolytic pathway), oxaloacetate, citrate, alpha-ketoglutarate, succinate, fumarate and malate (tricarboxylic acid cycle) (Vinaud et al., 2007).

The glucose dosages were performed in the culture medium through an Architec C8000 Plus device, using a commercial kit protocol and enzymatic method. The samples from the cysticerci extract resulted in a very low final volume and did not allow the glucose dosage.

2.4. Statistical analysis

All experiments were repeated five times independently. The