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Taenia crassiceps: Host treatment alters glycolisis and tricarboxilic acid cycle in cysticerci

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ABSTRACT

Human cysticercosis by *Taenia crassiceps* is rare although it is considered of zoonotic risk, especially to immunocompromised individuals. Albendazole and praziquantel are widely used and effective in its treatment. Their active forms inhibit the glucose uptake by the parasite and induce muscle contractions that alter its glycogen levels interfering in the energetic metabolism of the parasite and leading to its death. The aim of this study was to evaluate alterations in glycolysis, the tricarboxylic acid cycle and glucose concentrations caused by low dosage treatments of the hosts with albendazole and praziquantel. Therefore, *T. crassiceps* intraperitoneally infected mice were treated by gavage feeding with 5.75 or 11.5 mg/kg of albendazole and 3.83 or 7.67 mg/kg of praziquantel. The treated mice were euthanized after 24 h and the cysticerci collected were morphologically classified into initial, larval or final phases. Concentrations of the organic acid produced and glucose were evaluated to detect alterations into the glycolysis and the tricarboxylic acid cycle pathways through chromatography and spectrophotometry. The low dosage treatment caused a partial blockage of the glucose uptake by the cysticerci in spite of the non significant difference between its concentrations. An activation of the tricarboxylic acid cycle was noted in the cysticerci that received the treatment due to an increase in the production of citrate, malate and α-ketoglutarate and the consumption of oxaloacetate, succinate and fumarate. The detection of α-ketoglutarate indicates that the cysticerci which were exposed to the drugs after host treatment present different metabolic pathways than the ones previously described after *in vitro* treatment.

1. Introduction

Taenia crassiceps cysticercosis is a rare disease which occurs in domestic animals mainly in USA and Western Europe (Hoberg et al., 1999; Wünschmann et al., 2003; Ballweber, 2009). However it represents a zoonotic risk especially to immunocompromised individuals (Wünschmann et al., 2003). There are reports of cases from France, Germany and England where cysticerci were found in skeleton muscles, central nervous system and intra-ocular and subcutaneous regions. The albendazole and praziquantel treatments were effective in those cases (Klinker et al., 1992; Chermette et al., 1995; François et al., 1998; Maillard et al., 1998; Heldwein et al., 2006). However, there is little information on the mode of action of these antihelminthic drugs on the main biochemical pathways used by the parasite.

Albendazole is a drug from the group of the benzimidazoles which presents a rapid intestinal absorption after ingestion and is rapidly converted into its active forms: the sulfoxide albendazole and the sulfone albendazole, which are effective *in vivo* and *in vitro* against the main species of intestinal nematodes and cestodes that infect humans (Horton, 2000). In vertebrates, it induces the cytochrome P450 complex activity, responsible for its metabolism (Venkatesan, 1998). The average plasmatic half-life of its active form is of approximately eight and a half hours, being eliminated basically through urine (Venkatesan, 1998). Its active form inhibits β-tubulin polymerization in the parasites interrupting the formation of microtubules and inhibiting the glucose capture, thereby making the level of intracellular energy inadequate for helminth survival through immobilization and then death due to insufficient energy production (Horton, 2000; Palomares et al., 2006).

On the other hand praziquantel is a compound well tolerated by the human organism and due to its excellent pharmacological properties and is often used in mass chemotherapy treatment (Cioli et al., 1995; Cioli and Pica-Mottoccia, 2003; Doenhoff et al., 2009). After oral administration its metabolism is fast and its plasma half-life is of about an hour. Generally, its metabolites have a longer plasma half-life of about four hours and are represented mainly by monohydroxylated praziquantel, with 4-hydroxycyclohexylcarbonyl as the principal metabolite. These drug substances are eliminated mostly through urine but also through bile and feces and, to a lesser

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degree, through human milk (Cioli et al., 1995; Cioli and Pica-Mottoccia, 2003). Although often utilized, its action on cestodes has yet to be fully elucidated. Cioli et al. (1995) described its action on helminths of the *Schistosoma* genus. In these parasites, praziquantel causes muscle contraction, tegumental damage and metabolic alterations directly or indirectly related to the redistribution of the Ca^{2+} ions in parasite tissue.

Studies found in the literature describe ultra-structural alterations (Palomares et al., 2004, 2006; Palomares-Alonso et al., 2007) and structural alterations (Venkatesan, 1998; Cioli et al., 1995; Cioli and Pica-Mottoccia, 2003; Horton, 2000) related to the action of the active forms of these drugs. *In vitro* studies on the energetic and respiratory metabolism of cysticerci described glucose degradation pathways and the citric acid cycle (Corbin et al., 1998) with an active electron transport chain (Del Arenal et al., 2001). *In vitro* studies of the cysticerci exposed to praziquantel and albendazole evaluated metabolic alterations in the excretion/secretion of organic acids present in the glucose degradation and in the citric acid cycle (Vinaud et al., 2007, 2008). Yet no descriptions were found in the literature of the effects of drugs in the main energy producing metabolic pathways in resident parasites. Therefore, the purpose of this study was to evaluate the alterations in glycolysis, in the citric acid cycle and in the detection of glucose in *T. crassiceps* cysticerci after the host treatment with low doses of albendazole and praziquantel.

2. Materials and methods

2.1. Maintenance of the T. crassiceps biological cycle

The biological cycle of *T. crassiceps* (ORF strain) has been maintained in the vivarium of the Instituto de Patologia Tropical e Saúde Pública da Universidade Federal de Goiás (IPTSP/UFG) since 2002. Ten initial phase cysticerci were inoculated in the intraperitoneal cavity of 8–12 week old female BALB/c mice where they multiplied by budding. Approximately 90 days after inoculation the animals were euthanized and necropsied. The cysticerci were removed from the intraperitoneal cavity, 10 initial phase specimens were selected (Vinaud et al., 2008) and inoculated in other non-infected mice for cycle continuation (Espindola et al., 2002; Vaz et al., 1997).

The ethical principles for animal experimentation professed by the Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL) were followed and this study was authorized by the Committee for Ethical Research of the Federal University of Goias (CoEp/UFG) (registration number 008/09).

The mice received daily care, acidified water and standard rations.

2.2. Mice infection and treatment

The BALB/c female mice were intraperitoneally inoculated with 10 initial phase *T. crassiceps* cysticerci (Vinaud et al., 2008), using 1 mL syringes. Thirty days after infection they were gavage treated with low single doses of praziquantel (Merck®) and albendazole (Vitapan®), 24 h after treatment they were euthanized for better visual observation of the biochemical effects of the drugs on the cysticerci.

The infected mice were divided into four groups, namely, group A: consisting of 5 infected mice treated with a single dose of 5.75 mg/kg of albendazole; group B: consisting of 5 infected mice treated with a single dose of 11.5 mg/kg of albendazole; group C: consisting of 5 infected mice treated with a single dose of 3.83 mg/kg of praziquantel; group D: consisting of 5 infected mice treated with a single dose of 7.67 mg/kg of praziquantel. A control group was formed consisting of 5 infected mice that did not receive treatment.

All the experiment was performed in quintuplicate. The doses applied were determined according to the manufacturer's recommendation and then reduced to a level lower than necessary to eliminate the parasite, as the purpose of the study was to access the adaptability of the parasite to the drugs.

2.3. Cysticerci biochemical analysis

The cysticerci removed from the mice were classified macroscopically according to their evolutionary phase as initial, larval and final (Vinaud et al., 2007). The specimens were fixed in liquid nitrogen, homogenized with 12% perchloric acid as described by Vinaud et al. (2007), and then the organic acids were extracted for chromatographic analysis as described by Bezerra et al. (1999) and Vinaud et al. (2007, 2008).

The organic acids were identified through high performance liquid chromatography (HPLC) according to the previously determined retention time and calibration. Analyses were performed on the acids present in the glycolytic pathway (pyruvate and lactate) and in the citric acid cycle (oxaloacetate, citrate, α-ketoglutarate, succinate, fumarate and malate) (Bezerra et al., 1999; Vinaud et al., 2007, 2008).

2.4. Spectrophotometric analysis

The glucose concentrations were also analyzed from the cysticerci after the perchloric acid procedure by dose response spectrophotometry using a KoneLab 60i apparatus, according to the commercial kit protocol, enzymatic method, Wiener lab®.

2.5. Statistical analysis

The statistical analysis was performed using the Sigma Stat 2.3 program. Descriptive statistics were applied to determine the mean and standard deviation and to evaluate the differences between the groups analyzed. The variables were tested for normal distribution and homogeneous variance. As they presented normal distribution, variance analysis was used. The differences noted were considered significant when $p < 0.05$.

3. Results and discussion

This study evaluated the effect of the host treatment with low doses of antihelminthic drugs on the energetic metabolism and the glucose uptake of resident *T. crassciceps* cysticerci. Although *T. crassiceps* cysticerci may be used as an experimental model for *Taenia solium* cysticercosis studies due to their antigenical similarity there are no studies reporting their biochemical similarity. There are some studies reporting the effect of antihelminthic drugs on *T. solium* cysticerci such as the report from Mahanty et al. (2011) who demonstrated the *in vitro* effect of praziquantel and albendazole on the excretion of alkaline phosphatase by *T. solium* cysticerci showing that these drugs may affect more than one biochemical pathway in order to kill this parasite. Also other effects on morphological characteristics of the parasite have been described such as the effect of praziquantel on the evagination of *T. solium* cysticerci *in vitro* in contrast to ivermectin and oxfendazole (Cederberg et al. 2011).

In the parasites from non treated hosts a greater concentration of glucose was noted in larval phase cysticerci ($p < 0.05$). No statistical differences were noted when comparing the groups treated with both the drugs and the control group. In spite of exposure to the drug, the dose applied was not sufficient to totally block