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Taenia crassiceps: Fatty acids oxidation and alternative energy source in *in vitro* cysticerci exposed to anthelminthic drugs

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ABSTRACT

Cysticerci metabolic studies demonstrate alternative pathways responsible for its survival, such as energy sources, fatty acids oxidation and excretion of β-hydroxybutirate, which indicates the capability of energy production from proteins. The aim of this study was to detect alternative metabolic pathways for energy production and its end products in *Taenia crassiceps* cysticerci *in vitro* exposed to praziquantel and albendazole, in sub lethal doses. Spectrophotometer and chromatographic analysis were performed to detect: propionate, acetate, β-hydroxybutirate, total proteins, urea and creatinine, SE by cysticerci *in vitro* exposed to praziquantel and albendazole. The drugs influenced the metabolism by inducing the creatinine phosphate phosphorylation as an alternative energy source, inhibiting the use of proteins and amino acids in the acid nucleic synthesis; and preventing the budding and replication of the cysticerci. This study also highlights the description of urea excretion, which is an important metabolic pathway to excrete toxic products such as ammonia, and the fatty acid oxidation as an alternative energy source in cysticerci exposed to anthelmintic drugs.

1. Introduction

Taenia crassiceps cysticerci are used as experimental model for cysticercosis caused by the *Taenia solium* metacestode larvae, presenting antigenic and metabolic similarities (Del Arenal et al., 2005; Espindola et al., 2002). Besides, there are reports of human beings, mostly immune-compromised, acting as hosts to *T. crassiceps* in cases of ocular and subcutaneous cysticercosis (Chermette et al., 1995; Maillard et al., 1998).

Cysticerci metabolic studies have shown biochemical differences and similarities between this parasite and its mammal host, highlighting the detection of alternative metabolic pathways which are responsible for the helminth survival in several habitats, such as the host tissues or its intestinal lumen (Corbin et al., 1998; Vinaud et al., 2007).

Taenia crassiceps cysticerci *in vivo* present as main energy source glucose molecules, removed from the host, or glycogen stored in the tegument (Willms et al., 2005). However, in the case of low glucose concentrations or metabolic alterations in the habitat, the parasite presents alternative energy sources such as fatty acids oxidation and its consequent secretion of β-hydroxybutirate, which indicates the ability of producing energy from lipids stored in its structure or removed from the host, allowing its survival (Vinaud et al., 2007). The detection of this alternative pathways *in vitro* or its final products, urea and creatinine, have not yet been described in the literature.

Praziquantel and albendazole, anthelminthic drugs widely used in cysticercosis treatment, interfere in the glucose uptake of the parasite leading to an alteration in the energy metabolism and the consequent decrease of glycogen stores, resulting in immobility and death of the parasites (Cioli et al., 1995; Nogales-Gaete et al. 2006; Köhler, 2001). However, the action of these drugs in inducing survival through alternative energy metabolism pathways, in other words, inducing the oxidation of fatty acid is yet to be described.

The aim of this study was to detect, through chromatography and spectrophotometry techniques, the alternative metabolic pathways and its final products SE by *T. crassiceps* cysticerci *in vitro* exposed to sublethal doses of anthelminthic drugs.

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2. Material and methods

2.1. Source of cysticerci

The cysticerci of *T. crassiceps* (ORF strain) were maintained in the animal facilities of the Tropical Pathology and Public Health Institute of UFG. The study was approved by the Ethics Committee of UFG. The infection of Balb/c mice was performed as described previously by Vaz et al. (1997) and Vinaud et al. (2007, 2008). The classification of cysticerci into three stages: initial, larval and final occurred according to characteristics described by Vinaud et al. (2007, 2008).

2.2. Exposure of cysticerci to anthelminthic drugs

Groups of 25 cysticerci at each stage were maintained in 10 mL of supplemented RPMI (D-glucose, non-essential amino acids, sodium pyruvate, L-glutamine) (Invitrogen, Gibco) culture media at 37 °C and exposed for 24 h to 0.03 μg mL^{-1} and 0.06 μg mL^{-1} of praziquantel and 0.05 μg mL^{-1} and 0.075 μg mL^{-1} of albendazole, corresponding to their DL_{50} and both were dissolved in ethanol accordingly to Palomares et al. (2004). As controls, 25 cysticerci of each stage were maintained separately in 10 mL of supplemented RPMI (Gibco) culture media at 37 °C and exposed to ethanol in the same concentrations as in the experimental groups.

After 24 h of exposure to the drugs, the samples were frozen in liquid nitrogen to stop metabolic reactions. The SE products from the cysticerci were analyzed as follows.

2.3. Quantification of urea, creatinine, total proteins and organic acids SE

2.3.1. Analysis of urea, creatinine and total proteins

The concentrations of urea, creatinine and total proteins SE by the cysticerci were measured by spectrophotometric analysis of the culture media. An Ultrospec 2100pro UV/visible spectrophometer was used accompanied by the following analysis kits: urea 500, Doles®, with an absorbance reading at 600 nm; creatinine dosage, using an enzymatic method, Doles®, with an absorbance reading at 520 nm; and total proteins dosage, using an enzymatic method, Doles®, with an absorbance reading at 550 nm.

2.3.2. Organic acids analysis

The organic acids SE in the culture media were extracted and analyzed as described previously by Vinaud et al. (2007, 2008). The organic acids related to fatty acid metabolism are: acetate, β-hydroxybutirate and propionate.

2.4. Statistical analysis

Every analysis was performed in triplicate. Statistical analysis was performed using the Sigma Stat 3.2 programme. For the statistical analysis of the organic acid concentrations the ANOVA test followed by Tukey post-test were used and statistically significant differences were considered when $p < 0.05$.

3. Results and discussion

This study evaluated the presence of metabolic pathways related to the fatty acids oxidation and to the degradation of proteins used as alternative sources in energy production in *T. crassiceps* cysticerci. The fatty acids oxidation was previously described in *in vivo* cysticerci by our research group (Vinaud et al., 2007), however, reports of the action of anthelminthic drugs in the performance of these metabolic pathways have not yet been described in the literature.

3.1. Fatty acid oxidation

The β-hydroxybutirate SE by final stage cysticerci was significantly higher when they were exposed to praziquantel at 0.03 μg mL^{-1} when compared to the control group and the other tests groups, which indicates higher consumption of acetyl-CoA and acetate resulting in β-hydroxybutirate production when the cysticerci is exposed to sublethal doses of the drug (Table 1). These data explain the non detection of acetoacetate, which was probably consumed by the α-β-hydroxybutirate hydrogenase and consequently producing β-hydroxybutirate which was SE (Lehninger et al., 2005).

There was no significant difference between the concentrations of acetate SE by the cysticerci exposed or not to the drugs. According to the literature, in some helminthes, such as *Echinococcus granulosus* and *Hymenolepis diminuta*, cestodes such as *T. crassiceps*, the acetate secretion is detected when there is excess of intramitochondrial pyruvate which can not be converted into acetyl-CoA used in the citric acid cycle (Fig. 1) (Melhorn et al., 1988).

There was no significant difference between the concentrations of propionate SE by the control cysticerci and the ones exposed to the drugs which indicates that the sublethal doses used did not affect the secretion of this organic acid, therefore not affecting the citric acid cycle and confirming aerobiosis as the preferential energy production pathway (Table 1). This data is in accordance to the study by Vinaud et al. (2008) which showed the capability of the cysticerci to maintain aerobiosis when exposed to a hostile environment.

3.2. Total proteins, urea and creatinine quantification

The concentration of total proteins SE by final stage cysticerci exposed to albendazole at 0.075 μg mL^{-1} was significantly higher than its concentration from cysticerci at the same stage exposed to albendazole at 0.05 μg mL^{-1}. The concentration of total proteins SE by initial stage cysticerci exposed to praziquantel at 0.06 μg mL^{-1} was significantly higher than the concentration from cysticerci at the same stage exposed to praziquantel at 0.03 μg mL^{-1}. This is explained by the probable blockage of protein break down and/or availability and its consequent use as energy source. This data shows that the presence of the drug interferes in the glucose uptake and induces the use of glycogen and glucose stores as energy sources such as described previously by Vinaud et al. (2008). Other reports on helminth proteic catabolism were not found in the literature.

The concentrations of urea SE by larval stage cysticerci in control group were significantly higher than the urea concentrations SE by cysticerci exposed to the drugs. This data indicates the probable use of the carbonic chain from the amino acids for nuclei acids synthesis during the cellular replication (Lehninger et al., 2005) which is intense in larval stage cysticerci which produces large amounts of buds. A possible explanation for this statistically significant difference in urea concentrations detected in control cysticerci when compared to those exposed, is that one mode of action of these drugs is to prevent the use of the amine chain in nuclei acids synthesis, which is characteristic of stages with intense cellular replication, preventing budding and leading to the decrease of parasite replication, and consequently, its parasitary burden.

Helminths capable of secreting ammonium ions such as uric acid or urea are called uricotelic. Urea may be produced from one of the following four sources: (1) arginine uptake from the environment, which is catabolized by arginasis; (2) urea uptake