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Mitochondrial dysfunction in skeletal muscle during experimental Chagas disease

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ABSTRACT

Trypanosoma cruzi invasion and replication in cardiomyocytes and other tissues induce cellular injuries and cytotoxic reactions, with the production of inflammatory cytokines and nitric oxide, both sources of reactive oxygen species. The myocyte response to oxidative stress involves the progression of cellular changes primarily targeting mitochondria. Similar alterations could be taking place in mitochondria from the skeletal muscle; if that is the case, a simple skeletal muscle biopsy would give information about the cardiac energetic production that could be used as a predictor of the chagasic cardiopathy evolution. Therefore, in the present paper we studied skeletal muscle mitochondrial structure and the enzymatic activity of citrate synthase and respiratory chain complexes I to IV (CI–CIV), in Albino Swiss mice infected with *T. cruzi*, Tulahuen strain and SGO Z12 and Lucky isolates, along the infection. Changes in the mitochondrial structure were detected in 100% of the mitochondria analyzed from the infected groups: they all presented at least 1 significant abnormality such as increase in their matrix or disorganization of their cristae, which are probably related to the enzymatic dysfunction.

When we studied the Krebs cycle functionality through the measurement of the specific citrate synthase activity, we found it to be significantly diminished during the acute phase of the infection in Tulahuen and SGO Z12 infected groups with respect to the control one; citrate synthase activity from the Lucky group was significantly increased ($p < 0.05$). The activity of this enzyme was reduced in all the infected groups during the chronic asymptomatic phase ($p < 0.001$) and return to normal values (Tulahuen and SGO Z12) or increased its activity (Lucky) by day 365 post-infection (p.i.). When the mitochondrial respiratory chain was analyzed from the acute to the chronic phase of the infection through the measurement of the activity of complexes I to IV, the activity of CI remained similar to control in Tulahuen and Lucky groups, but was significantly augmented in the SGO Z12 one in the acute and chronic phases ($p < 0.05$). CII increased its activity in Tulahuen and Lucky groups by day 75 p.i. and in SGO Z12 by day 365 p.i. ($p < 0.05$). CIII showed a similar behavior in the 3 infected groups, remaining similar to control values in the first two stages of the infection and significantly increasing later on ($p < 0.0001$). CIV showed an increase in its activity in Lucky throughout all stages of infection ($p < 0.0001$) and an increase in Tulahuen by day 365 days p.i. ($p < 0.0001$); SGO Z12 on the other hand, showed a decreased CIV activity at the same time.

The structural changes in skeletal muscle mitochondria and their altered enzyme activity began in the acute phase of infection, probably modifying the ability of mitochondria to generate energy; these changes were not compensated in the rest of the phases of the infection. Chagas is a systemic disease, which produces not only heart damage but also permanent skeletal muscle alterations.

1. Introduction

The causative agent of Chagas disease is the intracellular protozoan parasite *Trypanosoma cruzi*; it affects approximately 20 million people (Moncayo and Silveira, 2009; WHO, 2007) and represents a serious health problem in Central and South America (Biolo et al., 2010). Chagas disease also denotes an increasing challenge for clinicians in the United States (Bern et al., 2007) and some European countries (Reesink, 2005) due to the continuous immigration of people from disease-endemic regions (Polo-Romero et al., 2011).

There are 3 stages in Chagas disease: the acute phase, with a local inflammatory lesions that appears at the site where the metacyclic trypomastigotes enter and the parasite spreads throughout the host organism (Prata, 2001; Umezawa et al., 2002); the cardiac chronic phase in which the diversity and severity of the symptoms range from a mild electrocardiographic alteration to sudden death due to cardiac

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dysrhythmias, varying in different patients and in different regions (Storino and Milei, 1994). In this stage of the disease, the heart is the organ most commonly involved and the dysrhythmias, branch blocks and cardiac heart failure are common symptoms of the 30% of patients that develop chagasic cardiomyopathy (Andrade, 1999; Storino and Milei, 1994). Patients however may also evolve into the digestive form or both cardiac and digestive forms together. Between the acute and the cardiac chronic phases exists a period called the chronic indeterminate stage, which is generally symptomless and may last for 10 to 20 years (Macêdo, 1999; Paglini-Oliva et al., 2012; Ribeiro and Rocha, 1998).

Trypanosoma cruzi entrance and replication in the cardiomyocytes cause cellular damage and a cytotoxic reactions, with inflammatory cytokines and nitric oxide production, both of them being source of reactive oxygen species (ROS) in the acute (Cardoni et al., 1997) and cardiac chronic phases of the infection (Talvani et al., 2004). A similar inflammatory response has been described for the chronic indeterminate phase. These responses appear to control parasite reproduction, but it may also have toxic effects upon host cellular components (Ueda et al., 2002).

Previous works from our laboratory (Báez et al., 2008, 2011, 2013) have demonstrated that the myocyte response to oxidative stress involves cellular changes, primarily targeting mitochondria (Long et al., 2004) and modifying therefore the energy supply; this bioenergetic dysfunction could be involved in the genesis and progression of heart failure (Guzmán Mentesana et al., 2010; Marin-García and Goldenthal, 2008; Tsutsui, 2006). We and other authors (Báez et al., 2008, 2011, 2013; Garg et al., 2003; Vyatkina et al., 2004), have demonstrated different structural and functional alterations in cardiac mitochondria isolated from mice infected with different *T. cruzi* strains throughout all stages of the experimental infection (Báez et al., 2013). We also reported the presence of the parasite in the skeletal muscle of these mice (Báez et al., 2013).

The skeletal muscle is a highly oxidative tissue that depends on mitochondria to provide the energy to perform its metabolic activities. Mitochondria represent approximately 39 to 47% of the total volume of the muscle fibers and provide 90% of the energy that skeletal muscle needs for its activity.

Some abnormalities in the function and structure of skeletal muscle fibers had been found with increasing frequency associated with Chagas disease and other pathologies (Iqbal and Hood, 2015).

Chagasic patients present increased glycolytic and reduced oxidative activity; some studies also demonstrated a decrease in type I and an increase in type II fibers (Montes de Oca et al., 2004) or vice versa (Ramirez-Archila et al., 2011).

The entrance of *T. cruzi* into myocardial cells generates an intense inflammatory process with cytokine and free radical production directly affecting mitochondria. In addition, the parasite remains in the host throughout the infection, inducing a chronic inflammatory process of different magnitude in relation to the parasite strain.

Inflammatory infiltrates are frequently observed when the parasite invades the skeletal muscle (Molina et al., 1987; Monteón et al., 1996; Ramirez-Archila et al., 2011) generating myositis, atrophy and necrosis of myofibrils according to the phase of the infection (Acquatella, 2008; Molina et al., 1987; Monteón et al., 1996).

It has been proposed that the functional and structural alterations of cardiac mitochondria during the evolution of *T. cruzi* infection would be involved in the pathophysiologic mechanism of chronic chagasic myocardiopathy (Báez et al., 2008, 2011, 2013) and that similar damage in skeletal muscle mitochondria could be found (Báez et al., 2011; Guzmán Mentesana et al., 2014; Marin-Garcia et al., 1999). If that would be so, a simple skeletal muscle biopsy would give information about the cardiac energetic production that could be used as a predictor of the evolution of the cardiopathy.

Taking this into account, our current study investigates the structure and function of skeletal muscle mitochondria in mice infected with different *T. cruzi* strains in the different stages of the experimental *T. cruzi* infection.

2. Materials and methods

2.1. Infection

Three month old female and male Swiss Albino mice weighing 30 ± 1 g (n = 120) were used as follows: 30 mice were inoculated, by intraperitoneal injection, with 50 trypomastigote forms of *T. cruzi*, Tulahuen strain, 30 mice with 50 trypomastigote forms of the Lucky isolate and 30 mice with 50 trypomastigote forms of the SGO Z12 isolate. The number of parasites/ml of blood was determined in each group using a Neubauer hemocytometer. A non-infected group (n = 30) was also studied. Parasitemias in all groups were determined in a Neubauer hemocytometer using blood samples obtained from the tail of the mice, twice a week, beginning seven days after the infection. The investigation was carried out according to the Guide for the Care and Use of Laboratory Animals (Tolosa de Talamoni et al., 2010).

2.2. Mitochondria isolation

Sections of skeletal muscles from hind legs (rectus femoris, sartorius and vastus medialis and lateralis muscles) were removed on days 35, 75 and 365 post-infection (p.i.), which correspond to the acute, chronic asymptomatic and chronic symptomatic stages of the experimental infection. They were washed and suspended in ice-cold isolation buffer (5 mM HEPES, pH 7.2 containing 210 mM mannitol, 70 mM sucrose, 1 mM EGTA, and 0.5% BSA (fatty acid-free), tissue/buffer ratio, 1:10 w/v) and immediately homogenized. Homogenates were centrifuged at 1500 *g*, 4 °C for 20 min and the supernatant transferred to a new tube. The pellet was resuspended in isolation buffer, homogenized, and centrifuged again at 10,000 *g*, 4 °C for 5 min. The supernatant was discarded and the pellet was resuspended in buffer and centrifuged at 10,000 *g*, 4 °C for 10 min (twice = purification). The mitochondrial pellet was resuspended in isolation buffer (tissue/buffer, 1:1 ratio, w/v), and the aliquots stored at −80 °C.

2.3. Respiratory complex and citrate synthase activity

The enzymatic activity of the respiratory complexes I to IV (CI–CIV) and the citrate synthase was monitored by spectrophotometric methods as previously described (Báez et al., 2008, 2011, 2013; Jarreta et al., 2000; Trounce et al., 1996; Vyatkina et al., 2004) with slight modifications. Protein concentrations were calculated by Bradford assay (Bradford, 1976). All assays were performed in 1 ml final volume with 30–40 mg (for complexes I and II), 20–30 mg (for complex III) and 15 mg (for complex IV) of mitochondrial protein, and the linear change in absorbance was measured for 3 min.

2.3.1. CI (NADH-ubiquinone oxidoreductase)

The reaction mixture consisted of 10 mM Tris–HCl buffer, pH 8.0, 80 μM 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DB), 1 mg/ml BSA, 0.25 mM KCN. After incubating the mitochondria in the reaction mixture at 30 °C for 10 min., oxidation of NADH (200 μM) was monitored at 340 mM (e 8 mM^{-1} cm^{-1}).

2.3.2. CII (succinate-ubiquinone oxidoreductase)

Mitochondria were incubated in 1 M potassium phosphate buffer, pH 7.0, containing 0.1 ml succinate phosphate 0.1 M. After addition of assay mixture consisting of 50 μM 2,6-dichlorophenolindophenol (DCPIP), 5 μl EDTA 1 mM, 10 μl of Triton X-100 1%; 50 μl of DB. All the components were mixed. The reduction of DCPIP in association with CII catalyzed DB reduction was measured at 600 nm (e 20.5 × 10^6 M^{-1} cm^{-1}).