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Physical exercise protects myenteric neurons and reduces parasitemia in *Trypanosoma cruzi* infection

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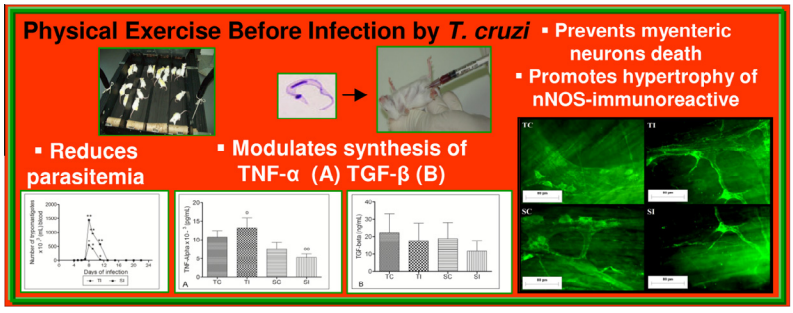
HIGHLIGHTS

- Moderated physical exercise in mice pre-infected by *T. cruzi* decreases parasitemia.
- Protects of the number of nNOS-immunoreactive neurons.
- Induces hypertrophy of nNOS-immunoreactive neurons.
- Modulates the synthesis of TNF-α and TGF-β.

GRAPHICAL ABSTRACT



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ABSTRACT

To evaluate the parasitemia, nitrergic neurons, and cytokines in *Trypanosoma cruzi*-infected mice subjected to moderate physical exercise, forty male Swiss mice, 30 days of age, were divided: Trained Control (TC), Trained Infected (TI), Sedentary Control (SC), and Sedentary Infected (SI). The moderate physical exercise program on a treadmill lasted 8 weeks. Three days after completing the moderate physical exercise program, the TI and SI groups were inoculated with 1300 blood trypomastigotes of the Y strain of *T. cruzi*, and parasitemia was evaluated from day 4 to day 22 after inoculation. After 75 days of infection, cytokines were measured and colonic neurons were quantified using immunofluorescence to identify neuronal nitric oxide synthase (nNOS). The results were analyzed using analysis of variance – Tukey and Kruskal–Wallis tests, to 5% significance. Moderate physical exercise reduced the parasite peak on day 8 of infection and total parasitemia ($p < 0.05$), contributed to survival of number of nNOS-immunoreactive neurons ($p < 0.01$) and promoted neuronal hypertrophy of the neurons ($p < 0.05$), increased the synthesis of tumor necrosis factor-α ($p < 0.01$) and transforming growth factor-β ($p > 0.05$), providing beneficial effects to the host by acting on the immune system to preserve nitrergic neurons.

1. Introduction

Chagas' disease is a parasitic disease caused by *Trypanosoma cruzi* that affects various regions of the Americas, particularly Latin

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America where it is endemic (World Health Organization, 2013). Approximately 10 million people in this region are infected by this parasite, and 25 million people are at risk of infection in countries where the disease is prevalent (World Health Organization, 2013). In Brazil, approximately 3 million people are currently infected (Portal da Saúde, 2013).

With the evolution of the disease, the patient may have symptoms related to the cardiovascular and digestive systems caused by changes in the anatomical physiognomy of the myocardium and digestive tract, mostly the esophagus and colon (De Lana and Tafuri, 2011). In the digestive tract, gastrointestinal transit impairment occurs as a result of the absence of peristalsis (De Lana and Tafuri, 2011). Peristalsis (i.e., contraction and dilation) is promoted by excitatory and inhibitory neurons in the enteric nervous system (Furness, 2000). Inhibitory neurons represent 18% of the neurons of the myenteric plexus where nitrergic neurons are expressed, which are responsible for reflex dilation of the intestinal smooth muscle (Furness, 2000). In the absence of intestinal peristalsis, the individual begins to show an accumulation of feces and consequent megacolon development with permanent dilation and compromised visceral diffusion (De Lana and Tafuri, 2011). Studies of the involvement of nitrergic neurons in alternative treatments for Chagas' disease are necessary because *T. cruzi* promotes the destruction of enteric nervous system neurons.

The medications for the treatment of Chagas' disease include benznidazole and nifurtimox. When administered during the acute phase of infection, these medications can cure up to 70% of patients. However, they have limited efficacy in the treatment of the chronic phase (Cançado, 2002; Coura and Castro, 2002). Thus, patients who undergo etiologic treatment may present symptoms of constipation and severe intestinal complications (Santos Júnior, 2002), constituting a substantial challenge in the implementation of alternative strategies to improve the quality of life of these patients.

The literature contains studies that have investigated alternative/complementary treatments for Chagas, disease, including natural products (Aleixo et al., 2008; Pupulin et al., 2010; Urbina, 1997). However, no conclusive results have yet been obtained.

Moderate physical exercise has been shown to significantly contribute to the survival of myenteric neurons (Silva, 2006) and improve appetite, functional capacity, and general well-being by positively changing mood in gastrointestinal disease (Lopes et al., 2011). Moderate physical exercise also represents a resistance factor for the development of protozoan infections in animals (Malm, 2006). It stimulates the immune response (Malm, 2004; Nagatomi, 2006; Rosa and Vaisberg, 2002) and positively modulates the neuroimmunological changes in patients with chronic heart failure (Rosa and Júnior, 2005). Animal models of physical exercise allow the control of the load of exercise and presence of infectious agents (Schebeleski-Soares et al., 2009). However, no studies of which we are aware have reported changes in nitrergic neurons in the colon in mice subjected to moderate physical exercise and subsequently infected with *T. cruzi*.

The present study evaluated the effects of moderate physical exercise on parasitemia, nitrergic neurons in the myenteric plexus, and the production of pro- and antiinflammatory cytokines in mice infected with *T. cruzi*.

2. Materials and methods

2.1. Ethical considerations

The present study was endorsed by the Ethics Committee on Animal Experiments (ECAE), State University of Maringa, Brazil (No. 046/2009).

2.2. Animals

The experiment was performed as a controlled, randomized, blind trial and repeated twice. In each replication, 40 mice male Swiss mice, 30 days of age, were used and divided into two groups: trained ($n = 20$) and sedentary ($n = 20$).

The animals were housed in polypropylene boxes ($414 \times 344 \times 168$ mm) with a galvanized grid closure and a central depression where food and water were provided. The boxes were lined with wood shavings and cleaned twice weekly. The boxes remained in the conditioning room (temperature, 21–23 °C) under a 12/12 h light/dark cycle. The animals were given *ad libitum* access to chlorinated drinking water and food (Nuvilab Cr-1, Nuvital).

2.3. Moderate physical exercise protocol

The 30-day-old animals were subjected to an aerobic physical exercise program on a treadmill (Inbrasport Classic CI model, Maringa, Brazil) for 8 weeks. The exercise consisted of a daily training session five times per week that included the following: 30–45 min sessions at a speed of 6–14 m/min in the first week, 45–60 min sessions at a speed of 8–16 m/min in the second week, and 60 min sessions at a speed of 10–20 m/min in the subsequent weeks. The mean speed was 13 m/min in the first 4 weeks and 17.5 m/min in the last 4 weeks. The sessions began at 6:00 PM during the dark/active phase of the light/dark cycle. The temperature was maintained at 20–22 °C during the training sessions. This physical exercise protocol is considered to require light or mild effort (Lerman et al., 2002; Schebeleski-Soares et al., 2009).

The treadmill had an adapter for small-animal training and a system that allowed both training-session planning and digital speed control with 2 m/min sensitivity. The training was conducted in the Exertion Physiology Laboratory, Department of Physiological Sciences, State University of Maringa.

Shock or similar mechanisms were not used to induce the animals to exercise, and a cardboard support was adapted to the top of each lane so that the animals could rest during training when they felt tired.

2.4. Infection

After completing the moderate physical exercise program, the animals were assigned to four groups: Trained Control (TC; subjected to physical exercise and uninfected; $n = 10$), Trained Infected (TI; subjected to physical exercise and subsequently infected; $n = 10$), Sedentary Control (SC; not subjected to physical exercise and uninfected; $n = 10$), and Sedentary Infected (SI; not subjected to physical exercise and subsequently infected; $n = 10$).

An intraperitoneal inoculum of 1300 bloodstream forms of the Y strain of *T. cruzi* was used. The TI and SI groups were inoculated 3 days after the moderate physical exercise program. Chronic infection was obtained with five doses of benznidazole (LAFEPE, PE, Brazil; three doses of 100 mg/kg/b.m. 11, 15, and 22 days after inoculation; two doses of 250 mg/kg/b.m. 18 and 41 days after injection) via oral gavage, otherwise the animals begin to die at around 11 days after injection (Moreira et al., 2013).

2.5. Evaluation of the course of infection

Parasitemia was evaluated in all of the animals infected using Brener's technique (Brener, 1962). The parasite count was performed daily from day 4 to day 9 after inoculation and on alternate days from day 9 to day 22. The curve was plotted using the average parasitemia in inoculated animals in each group. The following data were calculated: