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Research brief

DNA damage and nitric oxide synthesis in experimentally infected balb/c mice with *Trypanosoma cruzi*

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Abstract

This study aimed to evaluate whether experimental Chagas disease in acute phase under benznidazole therapy can cause DNA damage in peripheral blood, liver, heart, and spleen cells or induce nitric oxide synthesis in spleen cells. Twenty Balb/c mice were distributed into four groups: control (non-infected animals); *Trypanosoma cruzi* infected; *T. cruzi* infected and submitted to benznidazole therapy; and only treated with benznidazole. The results obtained with the single cell gel (comet) assay showed that *T. cruzi* was able induce DNA damage in heart cells of both benznidazole treated or untreated infected mice. Similarly, *T. cruzi* infected animals showed an increase of DNA lesions in spleen cells. Regarding nitric oxide synthesis, statistically significant differences ($p < 0.05$) were observed in all experimental groups compared to negative control, the strongest effect observed in the *T. cruzi* infected group. Taken together, these results indicate that *T. cruzi* may increase the level of DNA damage in mice heart and spleen cells. Probably, nitric oxide plays an important role in DNA damaging whereas benznidazole was able to minimize induced *T. cruzi* genotoxic effects in spleen cells.

Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Chagas disease; Single cell gel (comet) assay; Nitric oxide

1. Introduction

Human infection with the protozoan parasite *Trypanosoma cruzi (T. cruzi)* leads to Chagas disease, which affects about 17 million people in Latin America (Moncayo, 2003). Transmission of *T. cruzi* is predominantly via insect vectors of the family Reduviidae, sub-family Triatominae, genus Triatoma (Zeledon and Ponce, 1972), referred as "kissing bugs" (Zacks et al., 2005). Many countries have well-established vector-control programs. Blood transfusion and organ transplantation represent secondary transmission routes for *T. cruzi* (Zacks et al., 2005). The World Health Organization (WHO) estimates that approximately 300,000 new cases of Chagas disease occur every year (World Health Organization, 2002). To date, two drugs are currently in therapeutic use for Chagas disease: 4-[(5-nitrofurfurylidene) amino-3-methylthiomorpholine-1,1-di oxide], nifurtimox, and *N*-benzil-2-nitro-1-imidazole acetamide, benznidazole, which act by inducing oxidative or reductive damage to the parasite (Poli et al., 2002). Benznidazole is mainly recommended for acute phase treatment (Morilla et al., 2005).

The most proeminent manifestion of Chagas disease is related to cardiovascular system involvement, initially characterized by acute myocarditis (Zhang et al., 1999). In this phase, trypomastigotes invade cardiac muscle cells, become amastigotes and, then, multiply and differentiate into

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trypomastigotes. This picture leads to rupture with distention of the invaded myocytes, releasing the parasites into the interstitium (Andrade et al., 1994). There is also mononuclear cell infiltration, fibrosis, hypertrophy, and hyperplasia of the myocardium (Arnaiz et al., 2002).

The international agency for cancer research (IARC) has found sufficient evidence to recognize some pathogens as carcinogenic to humans; these include Human papillomaviruses, Hepatitis C viruses, *Helicobacter pylori*, and *Schistosoma haemotobium* (IARC, 1994). Regarding Chagas disease, data on the involvement of *T. cruzi* during the carcinogenic process is still matter for debate (Oliveira et al., 1997). Some authors have reported a greater frequency of malignant neoplasias in Chagas disease than non-Chagas disease patients (Murta et al., 2002). However, no association between Chagas disease and some specific carcinomas have been reported (Rocha et al., 2003). There are reports that *T. cruzi* infection may enhance resistance against some experimental tumors (Oliveira et al., 2001) and promote synergistic effects with nonsteroidal anti-inflammatory drugs (Escalante et al., 2006).

Nitric oxide production is heavily involved during infection with *T. cruzi* (Bergeron and Olivier, 2006). Synthesis occurs through the conversion of L-arginine to citrulline by different isoforms of nitric oxide synthases. NO synthases are expressed in response to an inflammatory stimulus and result in a large amount of nitric oxide; this appears to be closely involved in the pathogenesis of Chagas disease (Silva et al., 2005). Considering the strong evidence linking nitric oxide synthesis, DNA damage, and carcinogenesis (Nakamura et al., 2006) this study aimed to evaluate whether experimental Chagas disease in acute phase and with benznidazole therapy can cause DNA damage in mouse peripheral blood, liver, heart, and spleen cells, and induce nitric oxide synthesis in spleen cells. DNA damage, in this experimental model, was evaluated by single cell gel (comet) assay, a new, fast, simple and reliable biochemical technique based on the DNA fragment migration in electrophorized matrix (Olive et al., 1990; Tice et al., 2000).

2. Materials and methods

2.1. Experimental groups

A total of 20 twelve-week-old isogenic Balb/c male mice, weighing approximately 29 g, were obtained from the Department of Tropical Diseases, Botucatu Medical School, UNESP. All animals were housed in plastic cages (five mice per cage) with white wood chips for bedding, free access to commercial food (Labina, Sao Paulo, SP, Brazil) and drinking water, and under controlled light (12 h light/12 h dark cycle) and temperature (22 ± 2 °C) conditions. Animals were randomly distributed into four groups: control (G1)—non-infected animals; *T. cruzi* infected animals (G2); *T. cruzi* infected animals and submitted to benznidazole therapy (G3); and treated with benznidazole only (G4).

2.2. Animal infection

T. cruzi Y strain (Malvezi et al., 2004) derived from mice-infected peripheral blood (blood tripomastigotes) was obtained from Department of Tropical Diseases, Medical School—UNESP. After 14 days of climatization, mice were i.p. infected with the protozoan parasite at concentration of 1×10^3/mL in a final volume of 0.1 mL per animal, in the morning (9:00 a.m.). A quantity of 0.2 mL benznidazole (Roche, Brazil; 100 mg/kg b. wt. per day) was i.p. administered on the first and in the successive four days, i.e., second, third, fourth, and fifth days after infection. Negative controls and infected only mice were i.p. inoculated with saline solution (NaCl at 0.9% concentration).

Twelve hours before completing the experimental period (six days), mice were submitted to fasting, and then killed by intraperitoneal administration of 0.4% sodium pentobarbital (1 mL/kg). The peritoneal and thoracic cavities were exposed, and the left ventricle punctured with a fine needle for collecting peripheral blood (1 ml). Liver, heart, and spleen samples were also collected and maintained in 0.9% cold NaCl solution. The Ethical Committee for Animal Research, Botucatu Medical School (UNESP), approved the experimental protocols.

2.3. Parasitaemia evaluation

Blood examination was performed at the end of experiment period. Briefly, 5 µl of blood was transferred to a microscope slide, covered with a coverslip and examined for living flagellated by direct optical microscope.

2.4. DNA damage

The protocol used for peripheral blood, liver, heart, and spleen cells followed the guidelines purposed by Sasaki et al. (2002) with some modifications. Namely, 5 µl of peripheral blood was added to 120 µl 0.5% low-melting-point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip. A central fragment of liver, heart, and spleen was collected and minced in Hank's solution. The supernatant (cellular suspension; 10 µl) was added to 120 µl 0.5% low-melting-point agarose at 37 °C, layered onto a pre-coated slide with 1.5% regular agarose, and covered with a coverslip. After brief agarose solidification in refrigerator, the coverslip was removed and slides immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl buffer, pH 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO) for about 1 h. Prior to electrophoresis, the slides were left in alkaline buffer (pH > 13) for 20 min and electrophoresed for another 20 min, at 0.7 V/cm, 300 mA. After electrophoresis, slides were neutralized with 0.4 M Tris–HCl (pH 7.5), fixed in absolute ethanol and stored until analysis. Independent positive controls (blood, liver, heart, and spleen cells) were treated with 100 µM H_2O_2 for 5 min, in triplicate, to ensure assay reproducibility and sensitivity. All steps were