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The immunomodulatory effects of the Enalapril in combination with Benznidazole during acute and chronic phases of the experimental infection with *Trypanosoma cruzi*

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ABSTRACT

Trypanosoma cruzi infection triggers a chronic inflammatory process responsible for the alterations in the extracellular matrix and functionality of the heart. The angiotensin converting enzyme (ACE) inhibitors affects *T. cruzi in vitro* surveillance and modulates *in vivo* some inflammatory mediators. In this study, we investigated the treatment with an ACE inhibitor (Enalapril) and the Benznidazole (Bz) in a single and combination therapies (CT) in C57BL/6 mice infected with VL-10 strain of the *T. cruzi*. Animals were treated during 20 days with different doses of Bz (100, 80, 60 mg/kg), Enalapril (25, 20, 15 mg/kg) and their CT (100 + 25; 80 + 20; 60 + 15 mg/kg) and euthanized at 30° (acute) and at 120° (chronic) days post infection. The plasma and heart were processed for immunopathological investigations. Our data shown that Bz and Enalapril controlled, in part, the parasite replication and reduced plasma levels of TNF, CCL2 and CCL5 in the acute and in chronic phase of infection. However, the CT doses reduced in around 20% the inflammatory parameters obtained with the Bz therapy. The CT doses of 100 + 25 and 80 + 20 mg/kg increased the IL-10 levels and reduced the cardiac inflammation while Bz inhibited the collagen neogenesis in the infection. In conclusion, we assume that the CT administrated in the initial stage of infection, presents a minor immunomodulatory effect when the VL-10 strain of *T. cruzi* is used. In contrast, Bz and Enalapril in monotherapies persist suggesting a potential protection against cardiac damages during experimental *T. cruzi* infection.

1. Introduction

Trypanosoma cruzi is a hemoflagellated protozoan that causes Chagas disease and is responsible for a chronic and persistent inflammatory response in different organs, especially in the heart (Zhang and Tarleton, 1999; Marin-Neto et al., 2007)

Pharmacological strategies have been proposed to eliminate the parasite, the main trigger-point of the cardiac inflammatory process, or to modulate the immune cells and their molecular components, thus preventing a faster development of *T. cruzi*-induced cardiomyopathy in infected hosts. For the first strategy, benznidazole (Bz) is continued to be used as the first-line treatment for parasite elimination in the acute phase of infection despite its high toxicity. This drug has also been shown to be effective in improving the clinical course of heart disease in humans and animal infection models (Marin-Neto et al., 2009; Lana et al., 2009; Caldas et al., 2013) as well as in interfering with the immune pathways. Bz was previously described decrease the expression of nitric oxide synthase gene and TNF by inhibition of the nuclear factor-kappaB (NF-kB) and MAPK in different models of inflammation (Piaggio et al., 2001; Ronco et al., 2011). In consequence, Bz is recognized for its ability to inhibit L-arginine-converting nitric oxide and reduce inflammatory cytokines and cellular recruitment into the affected organs. However, further studies toward the immunomodulatory effects of Bz is still necessary to recognize its systemic and local modulatory action during *T. cruzi* infection. Finally, the second pharmacological strategy here propose is based on drugs that improve heart functionality with an additional immune modulatory function.

In humans and experimental *T. cruzi* infection, angiotensin-

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converting enzyme (ACE) inhibitors have been shown to interfere with the cellular immune response and are also involved in the elimination of *in vitro* parasites (Leon et al., 2003; Botoni et al., 2007; Paula-Costa et al., 2010; Penitente et al., 2015). The binding of the peptide Angiotensin II to its AT1 receptor increases vascular permeability, induces rearrangement of the cytoskeleton, contributes to leukocyte recruitment, and regulates adhesion molecules and chemokines by resident cells (Singh and Karnik, 2016). Angiotensin II also participates in transcriptional regulation of intracellular signaling proteins such as NF-κB and the small G protein family (RhO), and contributes to free radical generation and mitochondrial dysfunction that result in tissue damage, repair, and remodeling (Yaghooti et al., 2011). Cardiomyopathy induced by *T. cruzi* requires distinct pharmacological treatment according to the severity and clinical characteristics of the patient, but ACE inhibitors are an important routine medicine prescribed to treat chronic chagasic patients.

A major research focus is the association between drugs with a direct action on the parasite and drugs with an immunomodulatory action to improve clinical benefit and reduce toxicity. Based on this, the aim of the present study was to characterize the parasite response and cellular immune response in mice treated with a combination of enalapril and Benznidazole during both the acute and chronic phases of infection with the VL-10 strain of *T. cruzi*.

2. Materials and methods

2.1. Ethics statement

All procedures described in the current study are in accordance with guidelines issued by the National Council for Control of Animal Experimentation (CONCEA), and this research was previously approved by the Ethics Committee on Animal Research of UFOP- CEUA (Protocol CEUA- N° 056/2010).

2.2. Animals, parasites, and infection

Eight-week-old female C57BL/6 mice were bred and maintained at the Center of Animal Science from the Universidade Federal de Ouro Preto (UFOP), Brazil. Animals were inoculated intraperitoneally with 5000 blood trypomastigote forms of the VL-10 strain of *T. cruzi*. The VL-10 strain belongs to the TcII *Discrete Typing Units* (DTU) of *T. cruzi*, and is described as resistant to benznidazole therapy, with cardiac tropism, but it causes a slight fibrosing inflammation in C57BL/6 mice (Filardi and Brener, 1987; Penitente et al., 2015). Parasitemia was determined daily, according to the method described by Brener (1962) and animals were euthanized on the 30th and 120th days post-infection (Fig. 1), when blood and heart tissues were collected for immune and histopathological assays.

2.3. Pharmacological treatment

Enalapril (Pharlab, Brazil) and Benznidazole (Bz), an *N*-Benzyl-2-(2-nitro-1H-imidazol-1-yl) acetamide (LAFEPE, Brazil) were commercially acquired. They were diluted in phosphate buffer suspended in 0.05% (w/v) methyl cellulose at a final concentration of enalapril (15, 20 and 25 mg/kg/day), and Bz (60, 80 and 100 mg/kg/day), and the respective combinations (15/60, 20/80, and 25/100 mg/kg/day). Drugs were administered orally by gavage on 20 consecutive days, initiated after the establishment of the *T. cruzi* infection.

Animals were grouped (n = 10) into five groups; (i) uninfected and

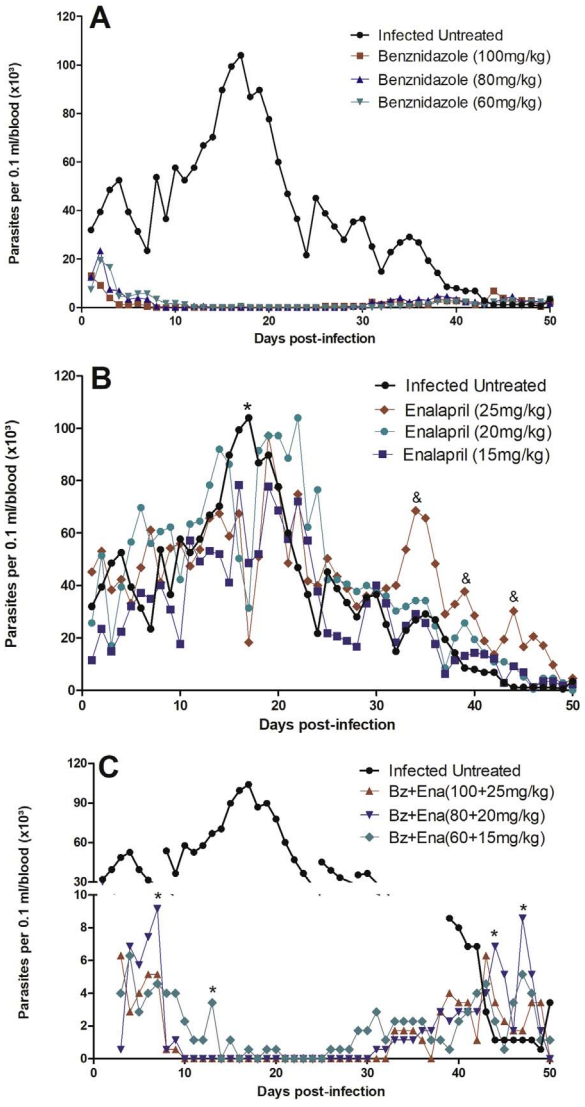

Fig. 2. Parasitemia curve of the VL-10 strain of *Trypanosoma cruzi*. C57BL/6 mice infected with 5000 trypomastigote forms of the VL-10 strain of *T. cruzi* were grouped (n = 10) and submitted to treatment with either Benznidazole, Bz (A), enalapril, Ena (B) or a combination of both drugs (C). The data at each point of the curve are representative of the mean of the parasitemia/day for each group. * P < 0.05 between the infected treated (with different drugs) and untreated group.

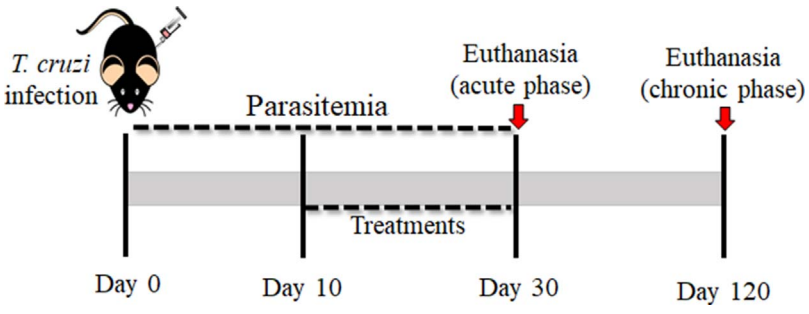

Fig. 1. Time line of infection and treatment. C57BL/6 mice were infected with the VL-10 strain of *T. cruzi* and treated with Enalapril, Benznidazole or with both drugs (combination) for 20 days and, on the 30th and 120th, animals were euthanized and biological samples collected.