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ORIGINAL PAPER

Trypanosoma cruzi: Biotherapy made from trypomastigote modulates the inflammatory response

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This study evaluates the effect of *Trypanosoma cruzi* biotherapy 17dH (BIOT) on mice of different ages, infected with the protozoa concerned.
Method: Performing a blind, controlled, randomized by drawing experiment, 110 animals four or eight-week-old, Swiss, male mice were divided into infected control treated hydroalcoholic 7% (CI-4 = 34 or CI-8 = 21 animals) and infected control treated with biotherapy 17dH−0.2 mL/animal/20 consecutive days/oral regimen (BIOT-4 = 33 or BIOT-8 = 21 animals). Animals were inoculated intraperitoneally with 1400 trypomastigote, *T. cruzi* Y-strain. Parasitological, immunological and histopathologic parameters were evaluated statistically, using Statistica-8.0 and R 3.0.2 program to analysis of survival. The study was approved by the Ethics Committee for Animal Experimentation/UEM.
Results: Four-week-old mice showed no statistical difference in parasitemia ($P = 0.5718$) between the treated and control group. Eight-week-old mice from the treated group had a higher parasite peak ($P = 0.0424$) and higher parasitemia ($P < 0.005$) than the control. To both groups of 4 and 8 weeks of age, treated or untreated, survival of mice was higher in the treated group than in the control, although it was not statistically significant (p-value = 0.32, 0.55 respectively). Four-week-old mice displayed a spleen section with a number of amastigote nests significantly higher in BIOT-4 than CI-4 ($P = 0.01$). In eight-week-old mice the number of amastigote nests ($P < 0.001$) and inflammatory foci ($P < 0.06$−10% significance) in the liver section were smaller in BIOT-8 than CI-8. Spleen giant cells were significantly higher in CI-8 than in BIOT-8 ($P < 0.01$). Eight-week-old animals treated with biotherapy showed higher parasitemia and lower tissue parasitism. Opposite pattern was observed in four-week-old animals.
Conclusion: There is a difference of high diluted medication effect in four and eight-week-old mice. In the group of animals 8 weeks the immunomodulatory effect seems to have been higher. Hence, treatment with the medicine produced from *T. cruzi* modulates the inflammatory response with increased apoptosis and decreased serum levels of TGF-β. *Homeopathy* (2015) **104**, 48–56.

Keywords: *Trypanosoma cruzi*; Murine infection; Homeopathy; Biotherapy; Apoptosis; TGF-β

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Introduction

Infection by *Trypanosoma cruzi* causes a predominantly inflammatory disease in which the presence of the parasite is linked to the pathogenesis.[1] The disease has autoimmunity features in which the mechanisms produced against the parasite are also able to destroy the host cells.[2] The immune response against *T. cruzi* is an important factor in resistance, infection control and effectiveness of etiological treatment.[3,4] In establishing intracellular infection, the pathogen develops mechanisms of subversion of the cellular defenses of the host. Apoptosis may be a reaction to escape interaction between parasite and host, inhibiting the proliferation of the parasite, which requires intact cells for its multiplication.[5] The regulation of apoptosis is directly linked to the immune system that, in this interaction process, induces the production of cytokines, important in parasite control. One of these cytokines is TGF-β, an anti-inflammatory cytokine with late regulation related to apoptosis and infection with *T. cruzi*.[6,7]

A neglected disease, just one drug is available in Brazil for Chagas disease, research that attempts to find new treatment options is required.[8] Literature shows the potential of alternative medicines in assisting the treatment of infectious diseases, maintaining homeostasis of the infected organism.[9–11] About half of the world's population has used Complementary and Alternative Medicine (CAM).[12] In this therapeutic line, highly diluted medications arise as an alternative intervention for diseases where the immune system is related to morbidity. The probable mechanism of action in these treatments is immunomodulation through specific cells or their products.[13,14] These include those produced from the etiological agent of infections.[11,14–16]

Objectives

The aim of this study is to evaluate the influence of treatment with highly diluted medicine made from trypomastigotes against *T. cruzi* infection on infected mice of different ages, measuring parasitological and immunological parameters, in order to better explore the effects observed.

Material and methods

Ethics

The study design was approved by the State University of Maringa (UEM) Ethics Committee for Experiments in Animals – Registration number 030/2008.

Experimental groups

One hundred and ten (110) Swiss male mice were used, at four and eight weeks of age, from the Central Animal Facility of the Universidade Estadual de Maringá. The animals were intraperitoneally infected with 1400 blood trypomastigotes of *T. cruzi-Y* strain[17] and were divided into four groups: CI4 – control group with infected animals aged four weeks old (n = 34); CI8 – control group with infected animals aged eight weeks old (n = 21); BIOT4 – infected animals aged four weeks old, treated with biotherapy 17dH (n = 34); BIOT8 - infected animals aged eight weeks old, treated with biotherapy 17dH (n = 21). The experiment was performed twice as a blind, randomized, controlled trial. Animals were divided into separate cages so that the mean initial weights of mice in each group were statistically equal. The animals were kept in a climate-controlled vivarium, under controlled temperature (22.7 ± 1.2°C), 70% humidity, light/dark cycles of 12 h, with free access to food and water. The animals were a conventional lineage with intestinal and ecto parasites control bred in a central vivarium.

Medication

The medication was produced from the buffy coat of blood containing bloody trypomastigotes collected from the orbital plexus of mice on the 7th day of infection by *T. cruzi Y*-strain. Blood was centrifuged at 840 rpm for 10 min. The leukocyte layer (with higher concentration of trypomastigotes and others figurative and liquid blood components) was carefully collected for use. The trypomastigotes were not purified utilizing chemical ingredients in order to avoid incorporating foreign substances to the final product. The medication was prepared by adding 0.9 mL of this leukocyte layer (with 4.1×10^7 trypomastigotes/mL of *T. cruzi*) in 9.1 mL of distilled water in a glass bottle (30 mL). Subsequently, dilutions were made in a 70% ethanol-water solution until $1{:}10^{16}$ concentration. The medication offered to animals, corresponding to a dilution of $1{:}10^{17}$ or 17dH of the initial suspension and was prepared with 7% ethanol-water solution.[18] Amongst dilutions, successive and rhythmic agitations were made using mechanical stirrer (Denise 10-50, AUTIC, Brazil). Microbiological test and biological risk *in vivo* were negative, according to the regulations of the Brazilian Ministry of Health.[19] The drug was stored in an amber glass for better conservation. The chosen dilution ($1{:}10^{17}$) was based on the results obtained by Aleixo et al.[11]

Treatment schedule

The medication was administered by gavage, 0.2 mL daily, for 20 consecutive days. Control groups received 0.2 mL hydroalcoholic 7% by gavage, during 20 days as well. Treatment was initiated on the 5th day after inoculation, when infection was confirmed for both control and treated groups.

Parasitological parameters

Parasitemia was assessed using Brener's technique: 5 μL of blood collected from the caudal vein were examined on microscope slides.[20] Parasites count was performed daily from the 4th day after infection until the animal's death. The parasitemia curve was drawn by using the mean parasitemia values of the animals inoculated in each group. From this parasitemia curve the following information was obtained: parasitemia peak (highest mean parasitemia