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ORIGINAL PAPER

Effects of homeopathy in mice experimentally infected with *Trypanosoma cruzi*

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Aim: **The aim of this study was to evaluate the action of homeopathic treatment on mice experimentally infected with *Trypanosoma cruzi*.**
Methods: **Eighty adult male C57BL/6 inbred mice were randomly allocated to five groups treated with biotherapy (nosode) of *T. cruzi* 12dH (12×) pre- and post-infection; *Phosphorus* 12dH post-infection; infected control treated with control solution and uninfected control. The biotherapy was prepared by the Costa method from the blood of mice experimentally infected with the Y strain of *T. cruzi*. *Phosphorus* was used because of its clinical and reportorial similarity to Chagas disease. *T. cruzi* (10^4) sanguineous forms were inoculated intraperitoneally per animal. Parasitaemia was monitored, leukocyte and serological responses were evaluated at 0, 7, 14 and 42 days after infection. The prepatent and patent periods of parasitaemia, maximum of parasitaemia, day of maximum parasitaemia and mortality rates were compared between groups.**
Results: **A significantly shorter period of patent parasitaemia was observed in the group treated with the biotherapy before infection ($p < 0.05$) than in the other groups. This group also had the lowest parasitaemias values at 9, 13, 15 ($p < 0.05$), 17 ($p < 0.05$), 22, 24 and 28 days, a lower rate of mortality and a significant increase of lymphocytes compared to the infected control group. The *Phosphorus* group had the longest period of patent parasitaemia, higher maximum parasitaemia, and a significant reduction of lymphocyte numbers, but no mortality. The infected control group had the highest mortality rate (not statistically significant), and the highest IgG titres at 42 days post-infection ($p < 0.05$).**
Conclusions: **The results suggest that pre-treatment with biotherapy modulates host immune response to *T. cruzi*, mainly during the acute phase of the infection. *Phosphorus* shows an action on the pathogenicity by *T. cruzi* infection. Homeopathic treatment of *T. cruzi* infection should be further investigated.** *Homeopathy* (2008) **97**, 65–69.

Keywords: Chagas disease; *Trypanosoma cruzi*; Mice; Homeopathy; Biotherapy; Nosode; *Phosphorus*

Introduction

Chagas disease (American trypanosomiasis), a parasitic disease caused by kinetoplastid protozoan *Trypanosoma* (*Schizotrypanum*) *cruzi*, remains an important public health problem in South America even with advances in vectorial intradomicilliary transmission control. There is no safe chemotherapy treatment available, traditional antiparasitics are effective only in the acute phase (AP) of infection. Most infected persons have the established chronic form of the disease, 30–40% of such cases have irreversible heart or gastrointestinal tract lesions as a consequence of a sustained

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inflammatory process, probably associated with persistence of the parasites.[1,2] The pathogenesis of Chagas disease is a consequence of the complex relation between persistence of parasites and impaired host immunomediated mechanisms, probably established during the AP of the infection.

One of the main characteristics of homeopathic medicines is the low concentration of substances they contain. With potencies above approximately 12cH, there are no molecules of the original substance present to provide a specific molecular stimulus.[3] However, there is much evidence that these 'ultramolecular' dilutions exert biological effects in living systems[4] that cannot be explained with our current knowledge. There are numerous speculative hypotheses as to how such information might be captured and stored at ultradiluted preparations, if this indeed occurs.[5]

Biotherapies, also called nosodes, are homeopathic medicines, in which the aetiologic agent itself is diluted, in accordance with the homeopathic pharmacotechnic method. Biotherapies are widely used in homeopathy, and their use in treatment and prophylaxis of infectious and parasitic diseases have been investigated and questioned by many authors. They have been used as a substitution for vaccines, in the absence of scientific evidence.[6] But there is little information available based on adequate methodology, on their action.

In Chagas disease it is important to develop strategies to reduce the inflammation that leads to severe organic dysfunction without compromising the control of parasitism.[7] Hahnemann developed the concept that chronic disease is based on an acquired and/or hereditary principle, which he called ''miasms'', now sometimes called diatheses. The miasm or diathesis is a state of, or a disposition of the body to be susceptible to certain types of diseases, and the impact of an aetiological agent on an individual's predisposition.[8]

In the present study, we analyzed whether an ultradiluted and dynamized biotherapy preparation from blood of *T. cruzi* infected mice and the homeopathic medicine *Phosphorus* induced protective or therapeutic effects in mice experimentally infected with *T. cruzi*. We used a murine model of Chagasic infection by *T. cruzi* Y strain since it is well characterized as having defined AP and chronic phase (CP).

Material and methods

Animals

Eighty adult C57BL/6 male mice bred at CECAL-Fiocruz, RJ, living in conventional cages (maximum of 8 mice/cage), with a controlled temperature ($22 \pm 3°C$) and light cycle (12 h dark/day, lights on at 06:30), and receiving food and water *ad libitum*. The methodology was authorized by the Ethics Committee for Animal Experimentation (CEUA-Fiocruz, license number: P0209-04).

Parasite. *T. cruzi* Y strain from human infected patient maintained at a laboratory by serial passages in mice.

Homeopathic medicines. The biotherapy was made in a specialized homeopathic pharmacy from blood of experimentally infected mice with the *T. cruzi* Y strain (1×10^4 blood forms of *T. cruzi*/mL), according to Living Nosodes technique,[10] the blood containing living microorganisms initially diluted in a saline solution to the 11dH potency then in hydroalcoholic solution. *Phosphorus* was selected by correlation of clinical and repertorial images of *T. cruzi* infection, homeopathic computer repertorization (Sihore Max Software) and considering the diathesis of the Chagas disease[9] and was prepared according to Brazilian Homeopathic Pharmacopoiea.

Both were used in 12dH potency liquid form (15% ethanol in water). Control solution was 15% ethanol in water.

Treatment. Mice were randomly allocated to five experimental groups ($n = 16$):

Treated with biotherapy before experimental infection for a total of 20 days, with an interval of 10 days in the middle of this period (Bbi).
Treated with biotherapy post-infection for 20 days following infection (Bpi).
Treated with *Phosphorus* post-infection for 20 days (*Phosphorus*).
Treated with control solution before and after infection (infected control).
Control group not infected (uninfected control).

Dose was 3 drops (0.6 mL) daily, orally by dropper.

Experimental infection of mice. Animals of experimental groups were inoculated intraperitoneally with 1×10^4 blood forms of *T. cruzi* Y strain. After detection of parasitaemic peak mice were euthanized, blood was obtained by cardiac punction and the inoculum was adjusted in a Neubauer chamber.

Parameters evaluated. From the fifth day after infection, parasitaemia (parasites/mL of blood) was monitored at 2 days intervals in a Neubauer chamber. Prepatent and patent periods, maximum of parasitaemia, day of maximum parasitaemia, mortality rate and time of death were determined. The period post-infection during which the parasitaemia was detectable at fresh blood examination was considered the AP, and the period post-infection when the mice presented parasitaemia sub-patent was considered the CP. At 0, 7, 14 and 42 days post-infection (D0, 7, 14, 42) blood was sampled to analyze the leukocyte response, by global (Neubauer chamber) and differential (smears stained with Panotico Kit) leukocytes count. At D7, 14, and 42 the humoral immune response was evaluated by Indirect Immunofluorescence[11] for anti-*T. cruzi* IgM and IgG.

Statistical analysis. Statistical analysis of the prepatent and patent periods was completed using the analysis of variance (ANOVA), the mortality rate was analyzed by Chi-square test. To analyze the parasitaemia, serological titres and leukocyte parameters were analyzed by the non-parametric Kruskal–Wallis test followed by the Mann–Whitney test when necessary.

Results and discussion

Clinical and repertorial images of *T. cruzi* infection

The symptoms of *T. cruzi* infection in the human and murine model, described in the literature, were transcribed