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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 actiologic 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 ± 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

to repertory rubrics, reflecting the clinical and repertorial images of the infection (Table 1). The mathematical repertorization of these rubrics is shown in Table 2, 11 medicines were selected, ordered by symptoms represented and weighting. The homeopathic medicine *Phosphorus* mostly fully reflected the clinical and repertorial image of the *T. cruzi* infection, covering the greatest number of symptoms, with the heaviest weighting.

The pathogenetic image of *Phosphorus* is associated with weakness and sudden prostration. It also has been related to heart and liver disorders, inflammation of the intestines and other inflammatory alterations[12] as well as haemorrhage caused by irritative, inflammatory and degenerative actions resulting in blood vessel damage.[13] These characterize *Phosphorus* as a medicine of syphilitic diathesis.[14] The study of Chagas disease diathesic origin shows a statistically significant relation between syphilitic diathesis and Chagas disease.[9] These findings possibility leads to the adoption of a new prophylactic and therapeutic approaches.

Experimental evaluations

At D5 *T. cruzi* tripomastigotes forms were detected in the blood of infected mice. The results of parasitological parameters (Table 3) did not show any variation of the prepatent period between infected groups, nor did the day of maximum parasitaemia (mean D7 for all groups). But variation of parasitaemia values was found (Figure 1). A shorter patence period ($p < 0.05$) and lower parasitaemia values were observed in the *T. cruzi* biotherapic pre-treated group (Bbi) than in the placebo group at D09, 13, 15 ($p < 0.05$), 17 ($p < 0.05$), 22, 24 and 28. *Phosphorus* treated mice showed a longer patent period than the remaining groups and higher parasitaemias at D7 ($p = 0.05$) and 20 ($p < 0.01$) than the infected control group and at D07 (<0.05), 17(<0.05), 20(<0.05), and 24 (<0.05), compared to Bbi. Nevertheless, no death was observed in the *Phosphorus* group during the experimental period.

The mortality in *T. cruzi* experimentally infected mice is a result of both the parasite and of the host's own immune response. Excessive parasite multiplication in the host can lead to death in susceptible animals.[15] The 100% survival rate observed in the *Phosphorus* treated group, although not statistically significant (which may be due to relatively small numbers), suggests an action on the *T. cruzi* induced morbidity, since this group showed higher maximum of parasitaemia ($p < 0.05$) than Bbi and infected control groups, and more resistance, since all the animals attained the CP of infection. High parasitaemia with low mortality in *T. cruzi* infected mice suggests a regulatory and anti-inflammatory action,[16] or an action on the balance between pro- and anti-inflammatory mediators, that should be studied when homeopathy is employed. The results suggest a difference in effect between the biotherapy pre-treated group (Bbi), the *Phosphorus* treated group and the infected control group, the latter exhibited the highest mortality rate.

All infected groups showed a decrease in number of lymphocytes from 0 to 7 days post-infection, characteristic of the AP of infection, and already well described. Nevertheless, after D7, only the group treated before infection (Bbi) showed a significant increase of lymphocytes ($p < 0.05$) during the course of infection (Figure 2), and a significant increase in neutrophils count ($p < 0.05$) (Figure 3), showing an action of innate and acquired immunity on parasitaemia control during the AP in this group.

Table 1 Repertorial rubrics of *T. cruzi* infection symptoms (Sihore Max Software). *Italic* = grade 2, **bold** = grade 3

Section	*Rubric*	*Sub-rubric*
Chest	*Heart congestion*	
Chest	**Heart inflammation**	
Chest	*Heart inflammation*	*Myocardium*
Chest	**Heart affections**	
Chest	*Heart affections*	*Chronic*
Chest	**Heart dilatation**	
Abdomen	*Enlarged spleen*	
Abdomen	**Swollen spleen**	
Throat	*Oesophagus inflammation*	
Head	**Brain inflammation**	
Generalities	*Ganglion inflammation*	
Generalities	**Muscle inflammation**	

Table 2 Repertorization of *T. cruzi* infection

Medicines	*Number of symptoms covered*	*Symptoms × weighting*
Phos	10	20
Acon	7	17
Ars	7	14
Iod	7	13
Puls	7	13
Sulph	7	8
Lach	6	12
Bry	6	11
Nat mur	6	11
Nux vom	6	11
Kali iod	6	7

Medicines ordered by number of symptoms covered.

Discussion

More experiments are needed to elucidate the specific mechanism of the biotherapy immunomodulatory action in *T. cruzi* infection. It is important to develop strategies aiming to reduce the inflammation that leads to severe heart dysfunction, without compromising the parasitism control.

A new concept of immunomodulation was proposed based on a new response ability of the organism, rather

Table 3 Prepatent and patent periods, mortality rate (means ± sd) and time to death of mice experimentally infected with *T. cruzi*

Experimental groups	*Prepatent (days)*	*Patent (days)*	*Mortality at 42 days PI*		
			(%)	*Number*	*Days PI*
BBI	5.0 ± 0.0	11.0 ± 3.5[1]	12.5	1/8	13 ± 0
BPI	5.2 ± 0.8	16.4 ± 4.8	37.5	3/8	14 ± 3
Phosphorus	5.3 ± 0.8	19.8 ± 2.9	0.0	0/8	–
Infected control	5.5 ± 2.1	17.5 ± 7.5	42.8	3/7	21 ± 7

BBI: treated with biotherapic pre-infection; BPI: treated with biotherapic post-infection; *Phosphorus*: treated with *Phosphorus* post-infection; infected control: treated with vehicle only pre- and post-infection.
[1] Statistical significance (ANOVA, 5% sig.).

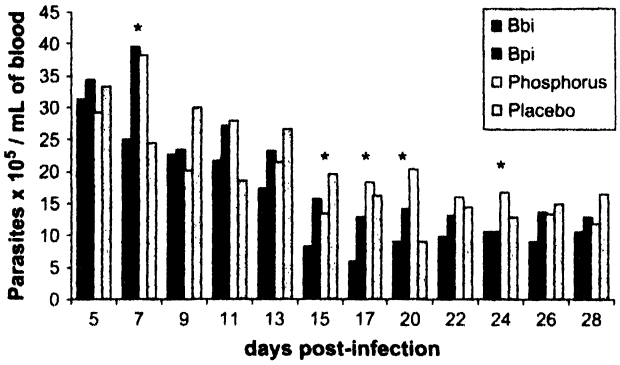

Figure 1 Parasitaemia values. $p < 0.05$, Kruskal–Wallis test.

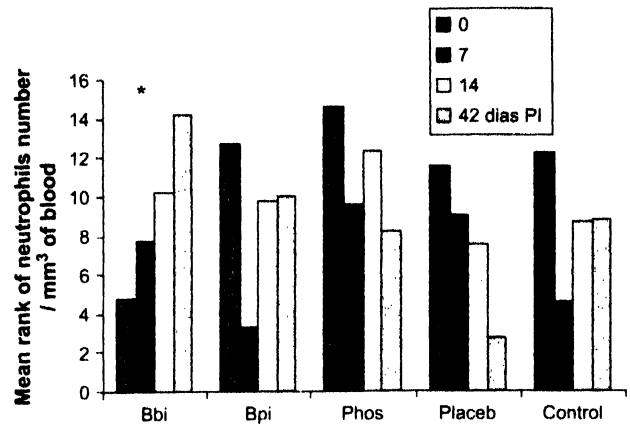

Figure 3 Mean rank of neutrophils number in experimental groups treated with homeopathy, placebo and control group not infected, at 0, 7, 14 and 42 days post-*T. cruzi* infection. *Variation statistically significant. Kruskal–Wallis test.

than on new molecules. Since the immune system is subjected to a complex network of cellular and molecular interactions, it seems that the "qualitative" aspect rather than the quantitative can be important and a single 'information' of an immunomediator can be understood by the organism.[3] Recent studies report that ultradiluted antigens can transfer signals to the immune system and modulate its response when the organism is in challenge with the related antigen,[17,5,3,18] and even before the challenge.[5] The autoreactivity of T-cells is managed by the immune system, dependent upon the concentration of the antigen they encounter: if they "see" high concentrations of a self-antigen they are deleted (killed), but when exposed to low doses they undergo a special kind of active inhibition (called 'bystander suppression').[19]

Therefore, the ability of ultradiluted antigen to manipulate the nature of a specific immune response to *T. cruzi* antigens, stimulating regulatory T-cells, avoiding immunomediated tissue lesions observed at *T. cruzi* infection should be studied.

The *Phosphorus* treated group showed a significant ($p < 0.05$) decrease of lymphocytes number at D42 (Figure 2). This could be associated with increased monocytes count ($p < 0.05$) (Figure 4) observed in this group, which could be due to the greater parasitaemia in this group during the AP of infection. The excessive nitric oxide (NO) production by macrophages during *T. cruzi* infection, among others factors, is suppressive to T cell.[20] Nevertheless, the decrease of lymphocyte number in the CP of infection could be important to avoid immunomediated lesions. These findings should be better explained by further histopathological evaluation.

The humoral immune response was studied by analysis of IgM and IgG levels at D7, 14 and 42 (Table 4). Significant variations of IgM levels between groups were not observed, but Bbi, Bpi and *Phosphorus* group tended to show higher IgM titres than the infected control group. At D7 the mice of the biotherapy pre-treated group (Bbi) showed an IgG response not observed in the other groups. At D42 mice of infected control group showed the highest IgG titres (1:128) ($p < 0.05$). It may demonstrate a lower control of immune response in the CP of infection, this may be associated with host immunomediated lesions. Previous studies have indicated that parasite-specific antibody production is important in both resistance to and pathogenesis of disease.[20–22] B cells and specific immunoglobulins are related to the autoimmune mechanisms of Chagas disease during the CP and higher serum IgG levels are found in symptomatic patients.[22]

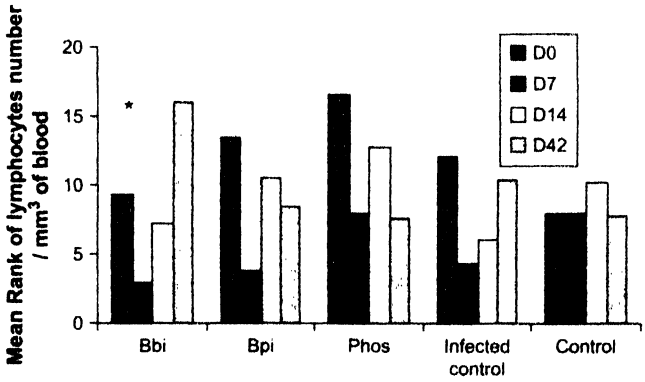

Figure 2 Mean rank of lymphocytes number in experimental groups treated with homeopathy, placebo and control group not infected, at 0, 7, 14 and 42 days post-*T. cruzi* infection. *Variation statistically significant. Kruskal–Wallis test.

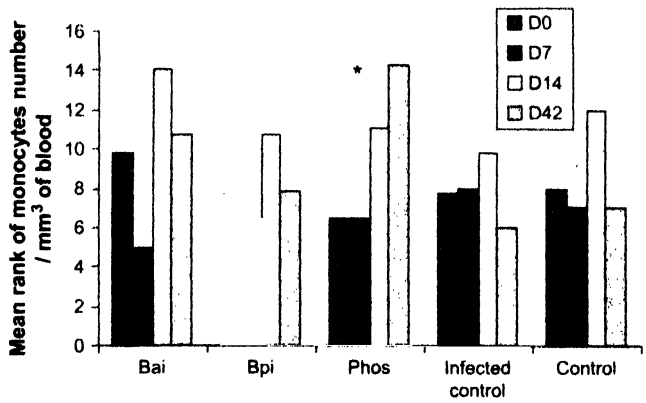

Figure 4 Mean rank of monocytes number at 0, 7, 14 and 42 days post-*T. cruzi* infection. *p, 0.05, Kruskal–Wallis test.

Table 4 Percentage of *T. cruzi*-specific IgG and IgM post-*T. cruzi* experimental infection in serum

Treatment group	*D7*		*D14*		*D42*	
	IgG	*IgM*	*IgG*	*IgM*	*IgG*	*IgM*
Bbi	100.0	25.0	100.0	50.0	100.0	56.0[2]
Bbi	25.0	50.0	75.0	75.0	[2]	
Phosphorus	75.0	25.0	100.0	50.0	100.0	75.0
Infected control	50.0	0.0	100.0	100.0	100.0[1]	100.0

Bbi: treated with biotherapic before *T. cruzi* experimental infection; Bpi: treated with biotherapic post-*T. cruzi* experimental infection; *Phosphorus*: treated with *Phosphorus* after *T. cruzi* experimental infection; infected control: treated with control solution before and after *T. cruzi* experimental infection.
[1] Statistically significant (Kruskal–Wallis test, 5% sig.).
[2] Compromised samples.

Conclusions

Our research suggests that oral pre-treatment with biotherapy modulates host immune response to *T. cruzi* infection, mainly in the AP of the infection. These results were not observed when biotherapic was given after an infectious challenge.

Based on the principle of similarity, the homeopathic medicine *Phosphorus* seems indicated in *T. cruzi* infection symptoms, as well as its diathesis. Our results suggest an action on the impact of *T. cruzi* infection, since no death occurred in this group. The action of the biotherapy and *Phosphorus* on the *T. cruzi* infection should be better studied.

There are still many questions and studies possible, mainly about the ultradilutions employed; the way and the moment of administration of the medicine and the preparation of the biotherapic: infected blood or isolated parasite?

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