Homeopathy (2016) 105, 186–193

http://dx.doi.org/10.1016/j.homp.2015.09.005, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Highly diluted medication reduces tissue parasitism and inflammation in mice infected by *Trypanosoma cruzi*

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Aim: **To evaluate the effects of *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* 13cH in mice infected by *Trypanosoma cruzi*.**
Materials and methods: **In a blind, controlled, randomized study, 102 male Swiss mice, 8 weeks old, were inoculated with 1400 trypomastigotes of the Y strain of *T. cruzi* and distributed into the following groups: Cl (treated with 7% hydroalcoholic solution), Ca (treated with *Kalium causticum* 13cH), Co (treated with *Conium maculatum* 13cH), and Ly (treated with *Lycopodium clavatum* 13cH). The treatments were performed 48 h before and 48, 96, and 144 h after infection. The medication was repertorized and prepared in 13cH, according to Brazilian Homeopathic Pharmacopoeia. The following parameters were evaluated: infectivity, prepatent period, parasitemia peak, total parasitemia, tissue tropism, inflammatory infiltrate, and survival. Statistical analysis was conduced considering 5% of significance.**
Results: **The prepatent period was greater in the Ly group than in the Cl group ($p = 0.02$). The number of trypomastigotes on the 8th day after infection was lower in the Ca group than in the Cl group ($p < 0.05$). Total parasitemia was significantly lower in the Ca, Co, and Ly groups than in the Cl group. On the 12th day after infection, the Ca, Co, and Ly groups had fewer nests and amastigotes/nest in the heart than the Cl group ($p < 0.05$). Decreases in the number of nests and amastigotes in the intestine were observed in the Ly group compared with the Cl group ($p < 0.05$). In the liver (day 12), Ly significantly prevented the formation of inflammatory foci compared with the other groups. In skeletal muscle, Co and Ly decreased the formation of inflammatory foci compared with Cl ($p < 0.05$). Ly afforded greater animal survival compared with Cl, Ca, and Co ($p < 0.05$). The animals in the Co group died prematurely compared with the Cl group ($p = 0.03$).**
Conclusions: **Ly with 13cH potency had significantly more benefits in the treatment of mice infected with *T. cruzi*, reducing the number of blood parasites, amastigote nests in tissue, and the number of amastigotes per nest and increasing animal survival.** *Homeopathy* (2016) **105**, 186–193.

Keywords: *Trypanosoma cruzi*; Homeopathy; Chagas disease

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Received 6 October 2014; revised 13 May 2015; accepted 21 September 2015

Introduction

Trypanosoma cruzi is the etiologic agent of Chagas disease, which is disseminated throughout the America.[1–3] An estimated 10 million people are infected, mainly in Latin America, where 25 million are at potential risk of acquiring the disease.[4] Chagas disease has reached countries like the United States and Canada and even extended to Europe.[4] In Brazil, three million people are in the chronic phase of the disease.[5] Tissue parasitism by *T. cruzi* in vertebrate hosts causes an inflammatory process, with the development of an immune response in an attempt to destroy the parasite.[1,6]

The available medications for the etiological treatment of Chagas disease include benznidazole and nifurtimox.[7] Both of these present low efficacy and significant side effects, often leading patients to cease treatment.[8–10] The search for new therapeutic approaches with fewer side effects and better treatment efficacy has been a major challenge.

Alternative treatment approaches have included moderate exercise[11–14] and the use of highly diluted medications.[15–18] Homeopathy is an interesting option. Medications that are "constitutional" have been prescribed according to the individual physical and behavioral characteristics of patients, considering their present and former status and future trends.[19] Although studies have been performed with highly diluted medications in murine models that are experimentally infected with *T. cruzi*,[15–17] the effects of homeopathic medications that are prescribed according to the physical and psychological characteristics of Swiss mice ("constitutional" medications) have not yet been studied. Thus, the present study evaluated the effects of *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* as "constitutional" medications in Swiss mice on blood and tissue parasitism and in the inflammatory *foci* formation.

Objectives

To evaluate the effect of *Kalium causticum*, *Conium maculatum, Lycopodium clavatum* in boosting 13cH (dilution $1{:}10^{26}$), inoculated with *T. cruzi* mice.

Material and methods

Ethics

The study was approved by the Ethics Committee on the Use of Animals in Research (CEAE), Universidade Estadual de Maringá (registration no. 054/2011).

Animals

Swiss male mice (n = 102), 8 weeks old, were obtained from the Central Bioterium, at Universidade Estadual de Maringá. The animals were intraperitoneally infected with 1400 blood trypomastigotes of the Y strain of *T. cruzi*. The study was conducted in two stages: (1) parasitological analysis and (2) histological analysis. For parasitological analysis, 42 animals were used. For histological analysis, 60 animals were used.

Experimental design

The experiment was conduced as a blind, controlled, randomized assay. The animals were maintained in a controlled environment with a 12 h/12 h light/dark cycle and received food and water *ad libitum*.

Experimental groups

The animals were allocated to the following groups: CI (infected control; treated with 7% hydroalcoholic solution; i.e., the vehicle preparation for the other medications; $n = 26$), Ca (treated with homeopathic *Kalium Causticum* 13cH; $1{:}10^{26}$ dilution; $n = 25$), Co (treated with homeopathic *Conium maculatum* 13cH; $1{:}10^{26}$ dilution; $n = 26$), and Ly (treated with homeopathic *Lycopodium clavatum* 13cH; $1{:}10^{26}$ dilution; $n = 25$). The animals were divided into these different groups so that the mean weight in each group was not significantly different.

For both the parasitological and histopathological analyses, fragments of heart, spleen, liver, large intestine (distal colon), and skeletal muscle (posterior thigh) were analyzed.

Medication selection

For medication selection, three homeopaths (one of whom was a veterinarian) made numerous observations of healthy animals. Using Lince Expert System Software (Albuquerque, NM, USA), they evaluated the behavioral, social, mental, and physiological characteristics of the mice, including need for company, fear, conscientious, shy, small amount of water, diminished vision, keen hearing, and heightened sense of smell. Using these characteristics were then selected the medicines *Kalium causticum*, *Conium maculatum*, and *Lycopodium clavatum* were then selected. The medications were used in 13cH potency at a $1{:}10^{26}$ dilution according to the recommendation of an homeopathic veterinarian because *T. cruzi* is an acute infection in small animals.

Medication manufacturing: The medications were produced from mother tinctures (all from Deutsche Homöopathie-Union, Karlsruhe, Germany) and prepared in 70% grain alcohol (Agro-Industrial Tarumã Ltda, São Pedro do Turvo, Brazil) until 12cH[20] dynamization was reached and then 7% alcohol until 13cH was reached. Mechanical dynamization (AUTIC Dinamizador, Denise Model, Campinas, Brazil) was used. The steps for the preparation of the medications followed the techniques recommended by Farmacopéia Homeopática Brasileira (2011).

Treatment schedule: The treatments were performed according to Falkowski.[21] The medications were administered 48 h before inoculation and 48, 96, and 144 h after infection, for a total of four administrations. The medications were diluted in water (1 mL/100 mL). The animals had *ad libitum* access to the water in an amber water dispenser for 16 h. The control group received only 7% hydroalcoholic solution (i.e., an inert ingredient used in medication preparation), which was also diluted 1 mL/100 mL water. The choice of this treatment regimen was based on our previous studies, in which we found that treatment before infection provided protection against the infective organism,[17] and continuous