

Lycopodiumclavatum* and *Conium Maculatum* effect on Hearts of Mice Infected with *Trypanosomacruzi

Gislaine Janaina Falkowski Temporini*, Camila Rafaela Cavagliar, Melissa Ayumi Tanaka**, Silvana Marques de Araújo*, Denise Lessa Aleixo*****

*Discente Unicesumar ***Docente Unicesumar, Centro de Ciências Biológicas e da Saúde, Departamento de Medicina, **Universidade Estadual de Maringá, Departamento de Ciências Básicas da Saúde/Parasiologia, Maringá, PR, Brazil.

Abstract

Chagas disease is a potentially fatal condition that presents high capacity to infect cardiac muscle cells and frequently evolves to dilated cardiomyopathy and congestive heart failure. The treatments available nowadays has low efficacy and several side effects, thus homeopathic medication is being studied as an alternative. Studies showed a decrease in the number of amastigotes in the heart of animals treated with *Lycopodiumclavatum*, a medication with anticholinesterasic, anti-inflammatory, antibacterial, antiviral and antifungal actions; and treated with *Conium maculatum*, a medication that acts in the nervous system by binding to nicotinic receptors. Therefore, the objective of this study was to describe the alterations verified in cardiac muscle cells of 12 Swiss male mice, 8 weeks old, infected with 1,400 trypanomastigotes forms of *T. cruzi* (Y strain) and treated with *Lycopodiumclavatum*, 13cH -1:10 [26] dilution (GLy) and *Conium maculatum* 13cH -1:10 [26] dilution (GCo). The results showed that the mean relative heart weight of the animals in group GCO (0.76g) was significantly higher than the mean relative heart weight of the animals in group GCI - control group (0,52g) (p=0,01). The animals in group GLy presented a mean relative weight of 0,62g, showing no statistic difference compared to group GCI.

Keywords: *Trypanosomacruzi*; Chagas Disease; Heart.

Introduction

Chagas disease, caused by the *Trypanosomacruzi* protozoan, is a potentially fatal condition, considered neglected by the World Health Organization, and affects approximately seven million people worldwide, most of them in the American continent [1,2,3]. According to recent articles published in the II Brazilian Consensus on Chagas Disease of 2015 [4], in Brazil, the number of individuals infected varies from 1,9 million to 4,6 million, most of whom are in the chronic phase [5]. The disease has two phases: acute and chronic, and presents high capacity to infect cardiac muscle cells and frequently evolves to dilated cardiomyopathy and congestive heart failure [6]. Around 30% of the patients in the chronic phase have

cardiac manifestation, representing the main responsible for the mortality [7].

The treatments available nowadays have low efficacy and several side effects, compromising patients' adherence to treatment [8]. Thus, the search for new therapy options has become the goal of many researchers, and homeopathic medication is being studied as an alternative [9].

Lycopodiumclavatum is a homeopathic medicine widely used for its proven anticholinesterasic, anti-inflammatory, antibacterial, antiviral and antifungal activities, in the most diverse types of diseases, including those that cause heart alterations [10]. *Conium maculatum* has amongst its effects, action on the nervous system by binding to nicotinic receptors [11,12]. Lopes *et al.* (2015) showed a decrease in the number of amastigotes in the hearts of animals treated with *Lycopodiumclavatum* and *Conium maculatum* [13]. Although the animals treated with *Conium* displayed a decrease in the number of parasites, they also showed premature death when compared to other groups [14].

Corresponding Author: Denise Lessa Aleixo, Av. Mario Clapier Urbinati, PR Brazil.
E-mail: denise.aleixo@unicesumar.edu.br

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In this context, the present study aims to describe the changes observed in cardiac muscle cells (opinião da banca - ser mais sincere e falar que foi só o tamanho do curacao???) size of hearts of mice infected by *T. cruzi* and treated with the homeopathic medicines *Lycopodiumclavatum* and *Conium maculatum*.

Methodology

Ethics

The study was approved by the Ethics Committee on the Use of Animals in Research (CEAE) from the State University of Maringá, under the protocol 054/2011, according to the Brazilian guideline for the use and care of animals (DBCA) elaborated by the National Council for Control of Animal Experimentation (CONCEA).

Animals

Twelve Swiss male mice, eight weeks old, were utilized in this study. The mice were obtained from the Central Bioterium, from the State University of Maringá. The animals were intraperitoneally infected with 1,400 trypomastigotes forms of *T. cruzi* (Y strain). The animals were maintained in controlled conditions regarding temperature (22,7 ± 1,2°C), and light/dark cycle (12 h/12 h) and received food and water *ad libitum*.

Experimental Groups

The animals were divided in experimental groups according to the treatment:

CI - Infected Control, treated with 7% hydroalcoholic solution, the vehicle preparation for the other medications.

Ly - Animals infected, treated with the homeopathic medicine *Lycopodiumclavatum* 13cH (1:10²⁶ dilution).

Co - Animals infected, treated with the homeopathic medicine *Conium maculatum* 13 cH (1:10²⁶ dilution).

The animals were divided into these different groups so that the mean weight in each group was not significantly different. The sample size (n=4) considered the experimental design in double trial and the 3Rs (reduction, refinement and replacement).

Medicine Selection and Treatment Schedule

For the analysis of the effect of the homeopathic medicine, a repertorization with the observation of

healthy animals was made by three homeopaths, one of them being a veterinarian. Soon after, the medicine was prepared in 13 cH potency in a 1:10 [26] dilution, indicated by the homeopathic veterinarian, because the objective was to treat an acute infection in small animals.

The treatment was given 48 hours before the infection, and 48, 96 and 144 hours after the parasite inoculation. The medicine offered was diluted in water (1ml/100ml) *ad libitum*, in amber drinking fountain for 16 hours. The control group received only the 7% hydroalcoholic solution, also diluted in 1ml/100ml of water.

The choice of the treatment schedule was based on preliminary studies that showed its benefits [15].

Medicine Preparation

The preparation of the medicine took into account the protocols established by the Brazilian Homeopathic Farmacopeia [16]. Microbiological and biological risk tests were made according to the regulations of the Brazilian Ministry of Health (RDCn67) [17].

The homeopathic medicine *Lycopodiumclavatum* and *Conium maculatum* were prepared according to the protocol established for decimal scale [16]. The preparation was made from 0.9 ml of mother tincture diluted in 9.1 ml of alcohol 70%, followed by 100 succussions to obtain the 1dH boost.

Intermediate dynamizations were prepared with 70% hydroalcoholic solution. The dynamizations used in the treatments *Lycopodiumclavatum* 13cH and *Conium maculatum* 13cH were prepared with 7% hydroalcoholic solution and stored in an amber bottle until the time of treatment.

Heart Evaluation

The relative weight of the heart was obtained by dividing the weight of the organ by the body weight of the animal and multiplied by 100.

To collect the heart, the animals were euthanized with 90 mg/kg of ketamine + 13 mg/kg of xilazinai.p. The organs were collected in the twelfth day of infection and weighted in semi analytical weight scale.

Statistical Analysis

The data was analyzed statistically by the R3.1.1.-test ANOVA/ LSD program. Significance level of 5%.

Results and Discussion

This is the first paper to show the effect of *Lycopodiumclavatum* and *Conium maculatum* on the relative heart weight of animals infected and treated. The results are shown in the table.

In the eighth week of infection by *T. cruzi*, the mean relative heart weight of the animals from group Gco (0,76g) was significantly higher than the mean relative heart weight of the animals in group GCI (0,56g) (p=0,01). The animals in group GLy showed a mean relative heart weight of 0,62g, presenting no statistical

difference with group GCI. The comparison between treatments showed statistical difference between groups Gco and GLy (p<0,05).

Studies showed that in Brazil, the number of people infected by *T. cruzi* vary from 1,9 million to 4,6 million people [4,5]. In humans, the cardiac presentation of Chagas disease is responsible for the elevated morbidity and mortality, being considered the most frequent presentation among all symptomatic forms of the disease [18,19,20]. The social compromise also deserves to be highlighted, justifying the need of basic and clinical researches to seek new alternatives of treatment for this disease.

Table 1: Mean, median, minimum value (VMin.), maximum value (Vmax.) and standard deviation value of the relative heart weight of Swiss mice, 8 weeks old, inoculated with 1400 trypomastigotes forms of *T. cruzi* (Y strain) submitted to different treatments with highly diluted medicine.

	Mean	Median	VMin.	Vmax.	Standard Deviation
GLy	0,62*	0,58*	0,57*	0,76*	0,09*
Gco	0,76	0,78	0,58	0,88	0,13
GCI	0,56*	0,59*	0,45*	0,62*	0,08*

*ANOVA test considering the significanci of 5%.(p =0.014452).

Although pathologic differences between rodents and humans have raised concerns over the extrapolation from these models, they are, nonetheless, considered the most appropriate and comprehended to the process that involves the search of new treatments [21].

Parasitemia, associated with the hypomodulation of the immune system of the host, lead to acute cardiac neural denervation, with dilatation of the smooth muscle of the myocardium. Along with the chronification of the disease, cardiac skeletal muscle cells began to undergo processes of fibrosis and necrosis, leading to cardiac enlargement due to dilatation of the heart and muscular hypertrophy [22-25].

Considering the morbidity of *T. cruzi* infection, the results shown in our study indicated no benefits in the use of *Lycopodiumclavatum* and *Conium maculatum* for cardiac alterations in mice infected by *T. cruzi*. In contrast, increased cardiac weight was observed in animals treated with *Conium maculatum*, reflecting a worsening of the condition, as an increased heart weight is related to the changes that leads to greater morbidity and mortality in the infection [26]. In addition, there are studies that have shown that *Conium maculatum* may present some degree of toxicity to the heart and may increase the chance of cellular apoptosis at the site [13,14,27,28].

Even though no difference was observed between the parameter evaluated in animals treated with *Lycopodiumclavatum*, previous studies showed that

treatment with this medicine decreased parasitemia, tissue parasitism and inflammation on hearts, as well as increase the survival of these animals [15].

Current treatments for cardiopathy in patients with Chagas disease are merely symptomatic and not very effective. In this sense, a treatment that accompanies an improvement in parasitological parameters, with a decrease in the number of parasites, a decrease in inflammation and a higher survival, even if it does not show benefits in reducing cardiac weight, should be considered as an alternative in this infection model.

Conclusion

Treatment for murine infection by *T. cruzi* using *Conium maculatum* 13dH should not be considered because of the cardiac harm observed by the increase in heart weight of treated animals. The treatment using *Lycopodiumclavatum*, although it did not show improvement in the parameter of heart weight, should be better evaluated, considering that preliminary studies demonstrated benefits of this treatment with a decrease in the number of parasites, a decrease in inflammation and a higher survival. Thus, this study shows that the use of homeopathic medicine is a valid alternative of treatment for several diseases, including Chagas.

Conflict of Interest

The authors declare no conflict of interests.

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