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Quantitative PCR and unconventional serological methods to evaluate clomipramine treatment effectiveness in experimental *Trypanosoma cruzi* infection

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ABSTRACT

Clomipramine (CLO), a tricyclic antidepressant drug, has been used for the treatment of mice infected with *Trypanosoma cruzi*. In this work we evaluated the effectiveness of CLO treatment upon *T. cruzi*-infected mice in the chronic phase of the experimental infection using Quantitative polymerase chain reaction (qPCR) and recombinant ELISA. Sixty Swiss albino mice were inoculated with 50 trypomastigote forms of *T. cruzi* (Tulahuen strain). CLO treatment consisted of 5 mg/kg/day during 60 days by intraperitoneal injection, beginning on day 90 post infection (p.i) when the mice presented electrocardiographic (ECG) alterations compatible with the chronic phase of the disease. The evolution of experimental infection and the treatment efficacy were studied through survival, electrocardiography, serology using a mixture and individual (1, 2, 13, 30, 36 and SAPA) recombinant proteins from epimastigotes and trypomastigotes of *T. cruzi;* and qPCR on days 180 and 270 p.i. CLO treatment in the chronic phase decreased the parasite load, reduced the levels of antibodies against antigen 13 throughout 270 days p.i and reversed the ECG abnormalities in the treated animals, from 100% of the mice with alterations at the beginning of the treatment to only 20% of the mice with alterations by day 270 p.i. This study shows that qPCR and the use of recombinant antigens are more sensitive to evaluate the effectiveness of the treatment and proves that clomipramine may be considered as a new chemotherapy for the chronic phase of the disease.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a zoonosis caused by the flagellate protozoan parasite, *Trypanosoma cruzi*. Between 6 and 7 million people are estimated to be infected worldwide, mostly in Latin America. Initially, Chagas disease was confined to the region of Central and South America, but now, due to migration, it has spread to other continents (WHO, 2015).

The disease is characterized by two phases: an acute phase which appears just after infection, and a chronic phase which may have no evident pathology (stage previously known as the indeterminate phase) or evolve into irreversible cardiac and/or digestive lesions (Mitelman et al., 2011).

Chagasic cardiopathy appears to carry the worst prognosis and has become the most frequent cause of heart failure and sudden death, as well as the most common cause of cardio-embolic stroke in Latin America (Bern et al., 2007).

Chagas disease specific treatment has recently been recommended for people in either the acute or the chronic phase. To date, only two drugs have been effectively used in Chagas disease chemotherapy: Nifurtimox and Benznidazole, which present several limitations mainly due to adverse secondary effects (Apt, 2010).

Experimental studies have identified several novel targets for chemotherapy, one of them being the parasite's enzyme trypanothione reductase (TR). TR has been widely identified as a drug target for Chagas disease treatment (Meiering et al., 2005). The essential role of TR in the parasite thiol metabolism and its absence from the mammalian host render the enzyme a highly attractive target for structure based drug development against trypanosomatids (Krauth-Siegel and Inhoff, 2003).

Abbreviations: bp, base pairs; CLO, clomipramine; EA, ECG alterations; ECG, electrocardiographic; NEA, no ECG alterations; NI, non-infected; NIT, non-infected treated with CLO; OD, optical density; p.i, post infection; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; T, treated with CLO; TR, trypanothione reductase; UT, untreated.

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The structure of tricyclic neuroleptic compounds has shown them to be a promising class of TR inhibitors (Gutierrez-Correa et al., 2001). Clomipramine (CLO), a tricyclic antidepressant drug with anti-TR and anti-calmodulin effects, has been used for the treatment of mice infected with *T. cruzi* (Rivarola et al., 2005). Previous works from our laboratory demonstrated that this drug was effective in preventing cardiac damage when used either in the acute or the chronic phase with no obvious pathology (Bazán et al., 2008; Rivarola et al., 2001;) not only upon infection with the Tulahuen strain, but also upon infection with an isolate obtained from an Argentinean endemic area (Rivarola et al., 2005).

In general, control programs have focus their budgets and strategies towards the elimination of the vector insects and the treatment of infected patients in the acute stage; patients with chronic Chagas disease however, also need anti-parasitic treatment, taking into consideration that the parasite has been frequently detected during this phase (Dias et al., 2002).

The goal of *T. cruzi* specific treatment is to eliminate the parasite from the infected individual, to decrease the probability of developing the characteristics of the chronic disease (cardiac or digestive) and to break the chain of infection (Sosa-Estani et al., 2009).

A major difficulty and controversy in accurate evaluation of therapeutic efficacy depends on reliable cure criteria when blood samples are assessed by serological and parasitological techniques after drug treatment. Changes in serology, parasite load and clinical evaluation have been used as criterion of cure in clinical trials of Chagas disease treatment (Guedes et al., 2011). After etiological treatment, cure criteria relies on serological; in patients initiating therapy at the chronic phase without evident pathology however, seroconversion usually occurs several years after treatment, requiring long-term follow-up to determine effectiveness (Viotti et al., 1994).

In Latin America, a recombinant ELISA was developed using a mixture of six recombinant proteins from epimastigotes and trypomastigotes of *T. cruzi*. These antigens were: SAPA (which is reactive during the acute stage of infection), 1, 2 and 30 (which detect antibodies primarily in chronic phase) and 13 and 36 (which are reactive for both acute and chronic stages) (Rassi and Luquetti, 2003; Vergara et al., 1991). Other authors carried out ELISAs with each of the individual recombinant antigens (1, 2, 13, 30, 36 and SAPA) separately using pre and post-treatment sera, monitoring antibody levels in 18 chronic chagasic patients for three years; recombinant antigen 13 prove to be the most sensitive to treatment, since 66.6% of the patients had an early decline in this antigen and seroconversion (Sánchez Negrette et al., 2008).

Quantitative polymerase chain reaction (qPCR) has the potential to become a novel parasitological tool for prompt evaluation of trypanocidal treatment. Conventional PCR is useful to verify infection when contradictory serological results appear, and to confirm treatment failure when seropositive results persist following treatment. Quantitative PCR is considered more sensitive for parasite detection than conventional methods and therefore may be a better tool to assess treatment effectiveness (Duffy et al., 2009).

In this work we evaluated the effectiveness of CLO treatment upon *T. cruzi*-infected mice (Tulahuen strain) in the chronic phase of the experimental infection using qPCR and recombinant ELISA.

2. Materials and methods

2.1. Animals and experimental design

Sixty female and male Swiss albino mice weighing 30 ± 1 g were intraperitoneally inoculated with 50 trypomastigote forms of *T. cruzi* (Tulahuen strain). The number of parasites/mL of blood was determined in each group using a Neubauer hemocytometer. Mice were divided into the groups described in Fig. 1. Electrocardiographic (ECG) studies were performed on day 90 post infection (p.i) to determine the chronic phase of the disease.

2.2. Treatment

CLO (Sigma Chemical, St. Louis, MI, USA) treatment consisted of 5 mg/kg/day during 60 days by intraperitoneal injection, beginning on

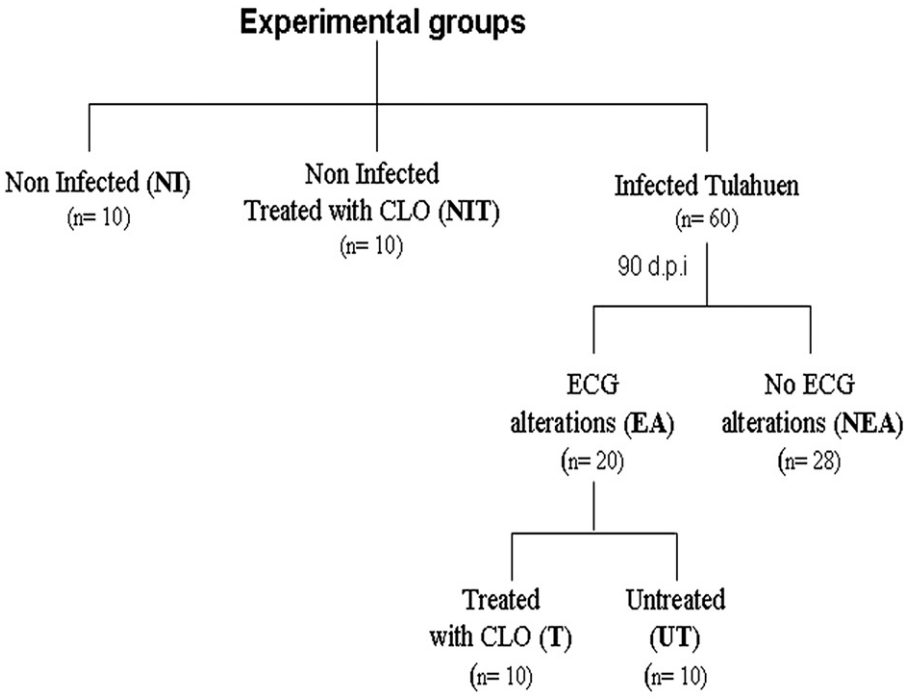

Fig. 1. Schematic representation of the experimental groups studied in the present work: non-infected mice, mice infected with *T. cruzi* (Tulahuen strain) left untreated or treated with CLO (5 mg/kg/day).