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Weekly electrocardiographic pattern in mice infected with two different *Trypanosoma cruzi* strains

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Abstract

Background: Chagas' disease, which is caused by *Trypanosoma cruzi*, affects 20 million people. The electrocardiographic alterations are usually the first evidence of disease progression. In this work, we evaluated if two different *T. cruzi* strains presented electrocardiographic and heart histopathological alterations that could be characteristic and only achieved to the parasite strain. The moment when the electric alterations began was also studied.
Methods: Albino mice (n=100) were inoculated with 50 (n=50) and 500 (n=50) trypomastigotes of *T. cruzi*, for Tulahuen strain and SGO-Z12 isolate, respectively. Electrocardiograms were obtained before infection and once a week from 7 to 147 days post infection (d.p.i). Dipolar and unipolar leads were analyzed. Hearts were removed by necropsy on 14, 90 and 147 d.p.i. Each heart was cut horizontally into 5-μm sections and they were stained with Hematoxilin–Eosine.
Results: At 147 d.p.i., 30% of Tul-infected mice were found alive, while in the SGO-Z12 infected group, 75% were alive at the same moment. The Tul-infected group showed more intraventricular blockage alterations than the other groups from 49 to 70 d.p.i, (p<0.01). No structural cardiac alterations were detected in SGO-Z12-infected mice at 7 d.p.i., while the Tul-infected group showed mononuclear cell infiltrates. At 147 d.p.i., fiber disorganization and cell infiltration were observed in the SGO-Z12 and Tul-infected groups.
Conclusions: We demonstrated that *T. cruzi* Tulahuen strain and SGO-Z12 isolate determined different electrocardiographic alterations which were characteristic for each stage of the experimental Chagas' disease. These results highlight the importance of the *T. cruzi* strain in the severity of the cardiopathy.

Keywords: *Trypanosoma cruzi*; Electrocardiography; *T. cruzi* strains

1. Introduction

Chagas' disease is a major endemic disease caused by the protozoan *Trypanosoma cruzi*. This parasitic disease is widely distributed throughout Latin America, affecting 20 million people [1]. Upon infection, the initial acute phase of the disease ensues and is characterized by a transient parasitemia. Years or even decades after the initial infection, 20–30% of all infected individuals develop a chronic inflammatory disease mainly affecting the heart [2–5].

The electrocardiographic alterations are usually the first clinical evidence of Chagas' disease progression, but the onset of these abnormalities are rarely detected because they tend to be asymptomatic at the beginning and the heart involvement is usually clinically detected in the chronic phase [6,7]. The electrocardiographic study gives important information about aspects of cardiac function and is a useful tool to measure the severity of the cardiopathy as well as the possible prognosis of the disease [8]. The mice model for Chagas' disease was previously described by Laguens et al. [9] from a pathological, immunological and electrocardiographic point of view. Moreover, electrophysi-

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ology of normal mice was also studied and reviewed [10,11].

For many years, it has been noticed that there is a significant geographical variation in both the severity and prevalence of diverse clinical forms of Chagas' disease. These observations suggest that parasite strains are one of the factors that may be important in the physiopathology of the malady [12–16].

Concerning this, Espinoza et al. [17] found two clearly biological behaviors among five Mexican stocks of *T. cruzi* isolated from human patients. This two patterns correlate with the capacity of the isolates to kill infected mice. This high degree of heterogeneity was also detected by other authors [12,14,18,19].

In the present paper, we studied whether two different *T. cruzi* strains produce electrocardiographic and heart histopathological alterations that could be characteristic and only achieved to the parasite strain. Besides, the beginning of the electrical alterations of hearts and survival of infected mice were analyzed and qualified in order to make a possible correlation between these parameters and their evolution to a chagasic cardiac disease.

2. Material and methods

2.1. Parasites

Bloodstream trypomastigotes of *T. cruzi* Tulahuen strain, designated as TINF/CL/1945/Tulahuen (*T. cruzi* I), and SGO-Z12 isolate belonging to zimodeme 12 from Argentina [15,20,21], designated as MHOM/ARG/1999/SGO-Z12 (*T. cruzi*) in accordance with the World Health Organization classification and with the recommendations from the Satellite Meeting at the International Symposium to commemorate the 90th anniversary of the discovery of Chagas' disease, were obtained from infected mice. Infected heparinized blood (without parasite purification) was used for the assays described below.

2.2. Infection

Albino Swiss female mice weighing 30±1 g (n=100) were inoculated with 50 (n=50) as we previously described [22–24] and 500 (n=50) trypomastigote forms of *T. cruzi*, by intraperitoneal injection for Tulahuen strain and SGO-Z12 isolate, respectively.

The amount of SGO-Z12 parasites used for infection was the appropriate number to reproduce the acute (from 7 to 42 days postinfection; d.p.i.) indeterminate (from 42 to 105 d.p.i.) and chronic (from 105 to 147 d.p.i) phases of the experimental disease as we previously described [20,21,25] because less than 50 parasites did not provoke the characteristic phases. The number of parasites/ml of blood was determined in each group using a Neubauer haemocytometer. The blood (0.1 ml) was diluted in a physiological solution and 30% of BSA (V fraction) in order to obtain the appropriate parasite concentration.

The animals were divided into the following groups: the Tul-infected group (mice infected with 50 trypomastigotes of *T. cruzi* Tulahuen strain n=50), the SGO-Z12-infected group (mice infected with 500 trypomastigotes of *T. cruzi*, SGO-Z12 isolate n=50) and the noninfected group; n=50. Survival was monitored every day at 1 p.m.

The investigation was carried out according to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health, NIH publication (No. 85-23, revised 1996).

2.3. Electrocardiographic study

Electrocardiograms were obtained with an electrocardiographic unit (Model FD-16 Fukuda Denshi) under Ketamine CLH (Ketalar®, Parke Davis) anesthesia, 10 mg/kg before infection and once a week from 7 to 147 d.p.i for the animals infected with either *T. cruzi* Tulahuen strain or SGO-Z12. The electrocardiographic tracings were obtained with six standard leads (dipolar leads DI, DII, DIII and unipolar leads aVR, aVL, aVF), recording at 50 mm/s with amplitude set to give at 1 mV/10 mm. The following criteria were used to characterize an intraventricular conduction abnormality: (a) absence of the Q wave in DI and AVL with QRS complexes that may be thick and wide; single R wave in DI and AVL; slowly rising, broad R and S–T segments and T wave in opposition to QRS complex; (b) broad S way mainly in DI and AVL: QRS complex with the M form at lead A; QRS duration of more than 0.03 s. The criteria established for atrioventricular blockages were the same as those classically described for humans but taking into account that the maximum duration of the P–R interval in the mouse is of 0.04 s.

2.4. Histopathological studies

The hearts were removed from mice by necropsy on 14, 90 and 147 d.p.i. for the animals infected with *T. cruzi* Tulahuen or SGO-Z12 isolate, fixed in buffered (pH: 7.0) 10% of formaldehyde and embedded in paraffin. Each heart was cut horizontally into 5-μm sections from the apex to the auricles. The sections were stained with Hematoxilin–Eosine. A total of 50 slices from each group was analyzed and at least 30 areas from each slice were examined with a 40× objective.

2.5. Statistical analysis

The obtained data were compared by variance analysis of two-way (difference between time and groups) for the electrocardiographic studies. Chi square was used to analyze histopathology and electrocardiographic abnormalities, and survival was analyzed by Kaplan–Meier statistics. The significance level was set at $p<0.05$.