Use of Noninvasive Parameters to Evaluate Swiss Webster Mice During Trypanosoma cruzi Experimental Acute Infection

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Abstract: Until now, there has been neither an agreed-upon experimental model nor descriptors of the clinical symptoms that occur over the course of acute murine infection. The aim of this work is to use noninvasive methods to evaluate clinical signs in Swiss Webster mice that were experimentally infected with the Y strain of Trypanosoma cruzi during acute phase (Inf group). Infected mice showed evident clinical changes beginning in the second week of infection (wpi) when compared to the noninfected group (NI): (1) animals in hunched postures, closed eyes, lowered ears, peeling skin, increased piloerection, prostration, and social isolation; (2) significant decrease in body weight (Inf: 26.2 ± 2.6 g vs. NI: 34.2 ± 2.5 g) and in chow (1.5 ± 0.3 vs. 6.3 ± 0.5 mg) and water (2.4 ± 0.5 vs. 5.8 ± 0.7 ml) intake; (3) significant decrease of spontaneous activity as locomotor parameters: distance (0.64 ± 0.06 vs. 1.8 ± 0.13 m), velocity (1.9 ± 0.3 vs. 6.7 ± 1.5 cm/sec), and exploratory behavior by frequency (1.0 ± 0.5 vs. 5.7 ± 1.6 events) and duration (1.4 ± 0.3 vs. 5.1 ± 0.5 sec in central arena region); (4) significant increase in the PR (41.7 ± 8.7 vs. 27.6 ± 1.9 msec) and QT intervals (39.7 ± 2.0 vs. 27.5 ± 4.0 msec), and a decreased cardiac frequency (505 ± 52.8 vs. 774 ± 17.8 msec), showing a marked sinus bradycardia and an atrioventricular block. At 3 and 4 wpi, the surviving animals showed a tendency of recovery in body weight, food intake, locomotor activity, and exploratory interest. Through the use of noninvasive parameters, we were able to monitor the severity of the infection in individuals prior to death. Our perspective is the application of noninvasive methods to describe clinical signs over the course of acute infection complementing the preclinical evaluation of new agents, alone or in combination with benznidazole.

The aim of the present study was to extend to Swiss Webster mice infected with the Y strain of Trypanosoma cruzi the use of noninvasive methods to establish clinical criteria to monitor the development of the acute phase, as previously reported in Balb/C mice (Silva et al., 2012). The pair outbred mouse/parasite strain is the classical model for evaluation of acute phase, as previously reported in Balb/C mice (Silva et al., 2012). The parasite strain led to high parasitemia levels in the second week of infection and trypomastigote forms were isolated at the peak of parasitemia. Male Swiss Webster mice (11–13 g) were obtained from the FIOCRUZ outbred mouse is extremely susceptible to different strains of T. cruzi (Araújo-Jorge, 2000). However, there are important differences between the natural disease and experimental models. Disease models often hinge on the description of specific clinical signs during the course of experimental infection (Chatelain and Konar, 2015). Cardiological monitoring is very important for the clinical evaluation of Chagas disease. Initial electrocardiographic studies conducted in Swiss Webster mice revealed significant differences in the tracings between infected and control groups (Packchanian and Robinson, 1958; Andrade and Sadigursky, 1987). In a previous study, we demonstrated that the treatment of infected mice with GW788388, an inhibitor of transforming growth factor beta (TGF-β), besides decreasing the parasitemia and mortality levels, improved the electrocardiogram profile (Oliveira et al., 2012).
The parasitemia was determined daily from 6 to 30 days postinfection (dpi) by using the Pizzi-Brener method (Brener, 1962) quantifying the parasites in 5 μl blood drawn from the tail of mice and diluted, when necessary, in PBS. Mortalities were noted daily and the index of cumulative mortality was calculated at 30 dpi. Body weights were evaluated weekly (at 0, 7, 14, 21, and 30 dpi).

Besides the daily physical inspection of the animals, the following parameters were evaluated: body posture, skin integrity (injury and/or peeling), fur appearance (piloerection, dull fur, focal or diffuse alopecia), and the presence of clinical signs related to secondary bacterial infections, such as dermatitis and conjunctivitis. Mouse food and water consumption were measured every 3 days by calculating the differences between the weight/volume offered (250 g/250 ml).

To characterize the spontaneous activity of the animals, we used the video-tracking tool Noldus EthoVision XT6 (Noldus Information Technology, Leesburg, Netherlands). The arena was defined as 12 rectangles, which were divided into lateral and central areas. In the total arena, the rectangles were calibrated with equal areas to ensure the consistency of the parameters with which the apparatus detected transitional mouse movements. This analysis was used to measure, at different times, the following parameters: (1) locomotor activity, i.e., covered distance (m) and average velocity (cm/sec); and (2) exploratory activity, the frequency of travel to the central region (number of events) every 5 min and the time spent in this region (sec) (Silva et al., 2012).

Electrocardiographic parameters were evaluated in nonsedated mice by using transducers that were carefully placed under the skin in accordance with a chosen preferential derivation (DII), based on the electrocardiographic triangle (Benett et al., 2005). Traces were recorded with a digital system (Power Lab 2/20) connected to a bio-amplifier at 2 mV for 1 sec (PanLab Instruments, LSI, Barcelona, Spain). The filters were standardized between 0.1 and 100 Hz, and the traces were analyzed by using Scope software for Windows V3.6.10 (PanLab Instruments).
measured the cardiac frequency (beats per minute [bpm]) and the duration in milliseconds (msec) of the intervals PR (period between the start of the P wave and the beginning of the QRS complex; indicative of atrioventricular conduction), QRS (combination of the Q, R, and S waves; indicative of sino-atrial, atrio-ventricular and intraventricular conduction), and QT (period between the start of the Q wave and the end of the T wave; indicative of ventricular activity including both depolarization and repolarization) at 0, 8, 15, 22, and 29 dpi. We individually assessed the relations between the QT and RR (the interval between successive Rs waves, corresponding to the peak of the QRS) intervals to obtain physiologically relevant values for the heart-rate–corrected QT interval (QTc) as described by Mitchell et al. (1998).

Statistical significance \( (P < 0.05) \) was evaluated by the Mann–Whitney nonparametric test to compare the 2 experimental groups (Software SPSS version 8.0).

The clinical condition is an important parameter that is used in the evaluation of experimental animals. This subjective method employs a physical appearance and behavioral analysis for each animal during the trial. Healthy lab mice showed characteristics such as lively and bright eyes, lined and brilliant fur, good skin integrity, and no signs of peeling or alopecia. On the first week of infection, both the Inf and NI groups appeared to be physically healthy, with shiny and well-groomed fur and alert and active behavior. However, at the beginning of the second week of infection (2 wpi), progressive clinical alterations were observed, which were characterized by the presence of piloerection and a soiled coat, a hunched guarding posture, reduced mobility, severely despondent behavior, and lethargic reflexes. Individuals with more severe disease signs showed hunched postures (primarily in the thoracic region) on 13 dpi, along with closed eyes, lowered ears, peeling skin, increased piloerection, prostration, and social isolation (data not shown).

Figure 1 depicts a typical experiment with the curve of parasitemia with a peak of \( 3.1 \times 10^6 \) parasites/ml at 8 dpi and the cumulative mortality of 67% at 30 dpi (Fig. 1A). A significant decrease in body weight (Fig. 1B) was observed in the Inf group when compared with NI, at 14 dpi (26.2 \( \pm \) 2.6 vs. 34.2 \( \pm \) 2.5 g), 21 dpi (29.4 \( \pm \) 5.6 vs. 37.8 \( \pm \) 2.8 g), and at 30 dpi (30.9 \( \pm \) 5.1 vs. 41.2 \( \pm \) 3.9 g).

With regards to food consumption (Fig. 2), the chow intake levels (Fig. 2A) were similar for both groups until 1 wpi, and then there was a statistically significant decrease for the Inf group when compared with NI at 2 wpi (1.5 \( \pm \) 0.3 vs. 6.3 \( \pm \) 0.5 mg), with a partial recovery at 3 wpi (4.2 \( \pm \) 0.3 vs. 6.0 \( \pm \) 0.5 mg) and 4 wpi (4.8 \( \pm \) 0.8 vs. 5.8 \( \pm \) 0.5 mg). For water intake (Fig. 2B), there was a significant decrease for the Inf group at 2 wpi (2.4 \( \pm \) 0.5 vs. 5.8 \( \pm \) 0.7 ml) and 3 wpi (4.2 \( \pm \) 0.3 vs. 6.0 \( \pm \) 0.5 ml), and both groups presented similar levels at 4 wpi (7.0 \( \pm \) 0.5 vs. 6.2 \( \pm \) 0.5 ml). The NI group showed no significant alterations during the 30 days of observation.

We measured the spontaneous activity and exploratory behavior of the 2 experimental groups (Fig. 3). The motor activity was measured as the
distance covered (Fig. 3A) and the average velocity (Fig. 3B) by each animal in a delimited arena within 5 min. The exploratory activity was measured as the frequency (Fig. 3C) and duration of time (Fig. 3D) during which each mouse inhabits the central quadrant. At 6 dpi, the distances covered, frequency and duration of the events, and average velocity were lower for Inf than NI, but statistically significant differences were observed only at 13 dpi, namely, the distance (0.64 ± 0.06 vs. 1.80 ± 0.13 m), the velocity (1.9 ± 0.3 vs. 6.7 ± 1.5 cm/sec), the frequency (1.0 ± 0.5 vs. 5.7 ± 1.0 events), and the duration of events (1.4 ± 0.3 vs. 5.1 ± 0.5 sec) with a partial recovery at 20 and 27 dpi.

Infected animals displayed ECG disturbances characterized by the presence of an AV block, an increased QT interval, and sinus bradycardia (Fig. 4). A significant increase in the PR interval time was observed when Inf was compared to NI at 15 dpi (41.7 ± 8.7 vs. 27.6 ± 1.9 msec), 22 dpi (49.2 ± 9.0 vs. 31.5 ± 4.4 msec), and 29 dpi (38.3 ± 7.6 vs. 28.7 ± 2.1 msec) (Fig. 4A). The cardiomyocyte electric depolarization/repolarization mechanism and the electrolyte level balance described by the QT interval showed significant increase at the 15 dpi in the Inf group (39.7 ± 2.0 vs. 27.5 ± 4.0 msec) (Fig. 4B). There were no significant differences in the QRS interval values between the 2 groups (Fig. 4C). At 15 dpi, there was also a decreased cardiac frequency in the Inf group (500.0 ± 52.8 vs. 774.0 ± 17.8 msec), showing a marked sinus bradycardia.

The following aspects of T. cruzi infection have been studied by using mouse models: infectivity in different tissues (Lenzi et al., 1996), the presence of cardiac inflammatory lesions (Andrade et al., 2006; Pavanelli et al., 2010), the disturbance of the cardiac electrical conduction system (Eickhoff et al., 2010), acute kidney injury (AKI) (Oliveira et al., 2009a, 2009b) and the efficacy of drug treatments (Soeiro et al., 2009). However, the clinical/behavioral comparison between treated and untreated animals by noninvasive parameters is an important tool to complement the evaluation of a potential trypanocidal agent.

The course of the infection in Swiss Webster mice inoculated with the Y strain of T. cruzi is consistent with previous results from our group and the data in the literature (Salomão et al., 2010; Timm et al., 2014). Through the noninvasive parameters of food consumption, locomotor (distance covered and average velocity), and exploratory behavior (frequency and duration in the central point of the arena), we were able to monitor the severity of the infection in individuals prior to death. In previous studies with male Balb/c mice also infected by the Y strain of T. cruzi, severe compromise in the physical and physiological characteristics of the animals was observed only at 3 wpi (Silva et al., 2012). In the present model, at 6 dpi, the Inf group developed a tendency towards immobility and a loss of exploratory interest in the environment, and at 2 wpi the measured parameters were statistically lower than in the control animals. The infected mice showed clinical signs of the disease, such as prostration and loss of curiosity, and these signs were aggravated by decreased food and water intake and by weight loss. In total, 67% of infected mice died between 15 and 21 dpi. The surviving animals (33%) showed a tendency of recovery in food intake, body weight, locomotor activity, and exploratory interest at 21 dpi. At 30 dpi, no parasites were detected in the bloodstream and the values of all parameters were similar to those of the NI group, which marked the beginning of the chronic asymptomatic phase in the surviving individuals.
We can correlate the results of the clinical signs with an evaluation of the cardiac electrical system. At 2 wpi, the effects on appearance, food consumption, locomotor activity and exploratory interest correlate with heart failure. This disturbance (AV block and arrhythmias) occurred to varying degrees of severity depending on the individuals, but in the surviving animals, the recovery of clinical frequency cardiac and despolarization/repolarization ventricular mechanism was observed. But increased PR interval (msec) was persistent during the last infection weeks.

The acute experimental infection with T. cruzi seems not to be restricted to acute myocarditis. Early work by Seneca (1969) described the development of toxema in T. cruzi-infected mice, which was characterized by the physical weakening to a state of immobility stemming from appetite loss, asthenia, and anemia (Bossola et al., 2007; Lainscak et al., 2007). Hölscner et al. (2000) correlated overproduction of TNF-α with a toxic-shock-like syndrome in IL-10-deficient infected mice. In addition, the relation between toxema and heart failure is characterized by clinical conditions and ECG changes in most of the susceptible mice during experimental infection, with a severe cachexia and toxema observed in moribund animals (progressing to death) (Hölscner et al., 2000; Oliveira et al., 2012). The systemic clinical disturbance, promoted by the parasite presence and toxema (sepsis), can be followed through a detailed evaluation of experimental acute phase of the Chagas disease by the use of noninvasive parameters.

We have previously shown that the treatment of T. cruzi acutely infected Balb/c mice with spironolactone decreased the mortality rate (Chumbinho et al., 2012). Moreover, the combination of benzimidazole and spironolactone significantly improved clinical/behavioral parameters, as, for example, the maintenance in body weight, elevated motor and exploratory activities in same mouse model (Alves et al., 2012). Our perspectives are to evaluate the efficiency of new agents, alone or in combination with benzimidazole, in the model Swiss Webster infected with the Y strain of T. cruzi, including, besides parasitological parameters, the application of the noninvasive methods of body weight, food consumption, physical activity, and electric cardiac system conduction. Independent of the murine lineage (Balb/c or Swiss Webster), these methods comprise an effective way to evaluate and describe clinical signs over the course of the experimental infection.

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LITERATURE CITED


**Chagas, C. 1909.** Nova tripanosomiasi humana: Estudos sobre a morfologia e o ciclo evolutivo do Schizotrypanum cruzi, gen. n. sp., agente etiologico de nova entidade morbida do homem. Memórias do Instituto Oswaldo Cruz 1: 159–218.


