Etanercept Induces Low QRS Voltage and Autonomic Dysfunction in Mice with Experimental Chagas Disease

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Abstract

Background: Chagas disease is a tropical parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. Chagasic cardiomyopathy is characterized by disorders of autonomic regulation and action potential conduction in the acute and chronic phases of infection. Although tumor necrosis factor alpha (TNF-α) has been linked to cardiomyopathy in experimental models and in patients with Chagas disease, other reports suggest that TNF-α may exert anti-parasitic actions during the acute phase of infection.

Objectives: This study aimed to determine the effects of a soluble TNF-α blocker, etanercept, on electrocardiographic parameters in the acute phase of experimental infection with *Trypanosoma cruzi*.

Methods: Electrocardiograms were obtained from untreated infected mice and infected mice who were treated with etanercept 7 days after infection. ECG wave and heart rate variability parameters were determined using Chart for Windows.

Results: Etanercept treatment resulted in a low QRS voltage and decreased heart rate variability compared with no treatment. However, the treated mice exhibited a delay in the fall of the survival curve during the acute phase.

Conclusion: The results of this study suggest that although etanercept treatment promotes survival in mice infected with a virulent *T. cruzi* strain, TNF-α blockade generates a low voltage complex and autonomic dysfunction during the acute phase of infection. These findings indicate that mortality during the acute phase can be attributed to a systemic inflammatory response rather than cardiac dysfunction. (Arq Bras Cardiol. 2013;101(3):205-210)

Keywords: Mice; Chagas Disease; Immunoglobulin G / adverse effects; Etanercept; Nervous System.

Abbreviations

TNF-α: Tumor necrosis factor alpha
CC: Chagasic cardiomyopathy
TNFR: Tumor necrosis factor receptor
HRV: Heart rate variability
ECG: Electrocardiography
SDNN: Standard deviation of R–R intervals
IFN-γ: Interferon gamma

Introduction

Chagasic cardiomyopathy (CC) is one of the most devastating consequences of Chagas disease, protozoan *Trypanosoma cruzi* infection, and it is closely related to mortality in the chronic phase of infection[1]. CC afflicts approximately 10%–30% of infected patients and is characterized by dilatation of both ventricles, apical aneurysm, and conduction disorders such as right bundle branch and atrioventricular blocks[2]. The role of inflammatory mediators in CC evolution remains controversial[3].

Tumor necrosis factor alpha (TNF-α) is a cytokine that is produced mainly by macrophages, B and T lymphocytes, and other cellular lineages such as endothelial cells, neurons, and cardiomyocytes. It is produced in response to inflammatory and infectious stimuli[4] and is initially synthesized as a transmembrane molecule, cleaved by a TNF-α converting metalloprotease (TACE or ADAM17), and secreted as a monomer grouped to form biologically active trimers[5]. TNF-α binds specific membrane receptors (TNF receptors; TNFRs), namely p55TNFR (TNFR1) and p75TNFR (TNFR2), by triggering nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways[6]. Etanercept (Enbrel®) is a dimeric fusion protein comprising the extracellular ligand-binding portion of the human 75 kDa (p75) TNFR linked to the Fc portion of human IgG1[4].

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Manuscript received November 10, 2012; revised manuscript December 20, 2012; accepted February 21, 2013.

DOI: 10.5935/abc.20130149

TNF-α has been associated with the evolution of CC. Lula and colleagues reported high serum levels of TNF-ligands in patients with cardiac dysfunction without associated arrhythmias[7]. Other reports have shown that TNF/TNFR1 signaling promotes myocarditis by stimulating CD8+ cell infiltration[8]. Importantly, it is known that TNF-α blockade decreased CC in experimental models[8,9]. However, worsening ventricular dysfunction in chronic experimental Chagas disease caused by TNF-α blockade with etanercept has been reported[10]; therefore, the role of TNF-α role in the pathophysiology of CC remains unclear. In addition, there is little information on the role of proinflammatory cytokines in the integrity of cardiac action potential conduction in Chagas disease. Therefore, the present study aimed to determine the effects of etanercept on electrocardiographic parameters during the acute phase of infection induced by a wild-type virulent strain of *T. cruzi* in experimental mice.

Methods

Animal care

Male NMRI (IVIC strain) mice weighing 30 g were housed, five per cage, in 40 × 25 × 15-cm transparent cages placed in a temperature- and humidity-controlled room maintained under 12-h light/dark cycles. All mice had free access to food and water and were habituated to these conditions for one week. All experimental procedures were in accordance with the Care and Use Handbook of Laboratory Animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1996).

After habituation, mice were administered 100-μL intraperitoneal injections, with 1000 Vero cell culture-derived trypomastigotes per gram. The inoculum was a Venezuelan wild isolate of *T. cruzi*. This strain is currently being characterized by molecular biology in our laboratory. The isolate was obtained from a specimen of *Panstrongylus geniculatus* captured from a rural community in the state of Miranda, and it was maintained by successive passages in Vero cells. Infected and uninfected mice were housed in separate cages within the controlled environment.

Etanercept treatment

The infected mice were divided into an untreated (n = 9) group and a treated group (n = 13). A single dose of etanercept was administered intraperitoneally (0.83 mg/kg) on day 7 after infection using a 27.5-gauge hypodermic needle. The maximum injection volume was 100 μL.

Electrocardiographic recordings and data analysis

Mice were previously anesthetized with 40 mg/kg of intraperitoneal thiopental (sodium salt). Electrocardiography (ECG) was performed using a bipolar system in which the electrodes were placed subcutaneously at the xiphoid cartilage (positive electrode), right shoulder (negative), and left shoulder (reference). Electrodes were connected to a Bioamp amplifier (AD instruments ®, Bella Vista, Australia) and were digitalized through an A/D converter PowerLab 8sp (AD instruments ®). Digital recordings were analyzed with Chart software for Windows v7.3.1 (AD Instruments ®). Events were registered to 4 K/s and were filtered to 60 Hz. ECG recordings were obtained for five minutes prior to infection (healthy) and at 7 and 14 days after infection (before and after treatment, respectively).

Heart rate variability (HRV) was determined using the Chart for Windows HRV module. Each recording was carefully revised and QRS complexes were classified as normal complexes, artifacts, or premature ventricular beats to create a temporal series and calculate the R–R intervals and the standard deviation of the R–R interval (SDNN). In addition, the P–R, QRS, and QTc intervals and the P and R amplitudes were calculated using the Chart for windows ECG analysis module, with a normal 10-ms QRS murine complex used as a reference. The QT interval was adjusted to heart rate using the Bazzet method[11]. Moreover, ECG wave morphology was qualitatively evaluated using ECG recordings obtained from the same animal when it was healthy as a reference. Finally, T-wave amplitude and slope were calculated using Chart Peak Analysis (v7)®. The peak detection threshold was adjusted to a 5% increasing basal line voltage.

Data are expressed as means ± standard deviations and were compared using a paired Student's t-test. P values of < 0.05 were considered statistically significant. Survival curves were compared using the Gehan–Breslow–Wilcoxon test. All analyses were performed using Prism5® (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Survival was determined during the experimental acute phase in both the treated and untreated groups. As shown in Figure 1, the treated group exhibited a significant increase in survival compared with the untreated group. Survival began to decrease quickly 20 days after infection in the treated group and 11 days after infection in the untreated group.

To evaluate cardiac function during the acute phase, ECG recordings were obtained prior to the infection and 14 days after infection (after treatment). Figure 2A shows that voltage in the QRS complex and P waves was significantly lower in the treated group than in the untreated group (Table 1). In addition, the treated group exhibited a nonsignificant tendency of increase in P–R and R–R intervals (Table 1, Figure 2B). Values for the remaining measured parameters measured are shown in Table 1.

Finally, we observed that dispersion in Poincare plots at 14 days after infection was lower for treated animals than for untreated animals (Figure 3). HRV represents a comparison between a given R–R interval value and the successive R–R interval, which is an indirect evaluation of cardiac autonomic regulation. Autonomic dysfunction is reflected in altered heart rate regulation capacity in response to physiological and pathological variations. In this study, we observed a decrease in SDNN, as presented in Table 1.

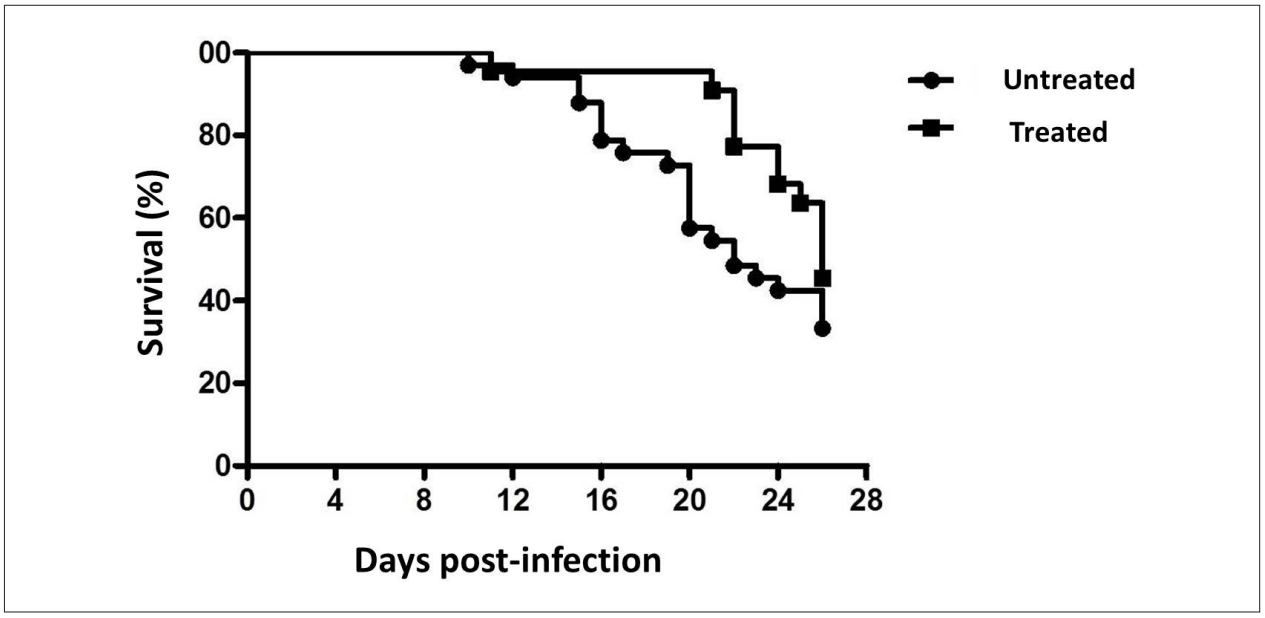

Figure 1 - *The survival rates of treated (filled boxes, n = 8) and untreated (filled circles, n = 8) groups are displayed. The p value as obtained by the Gehan-Breslow-Wilcoxon test is 0.0477 (statistical significance, p < 0.05).*

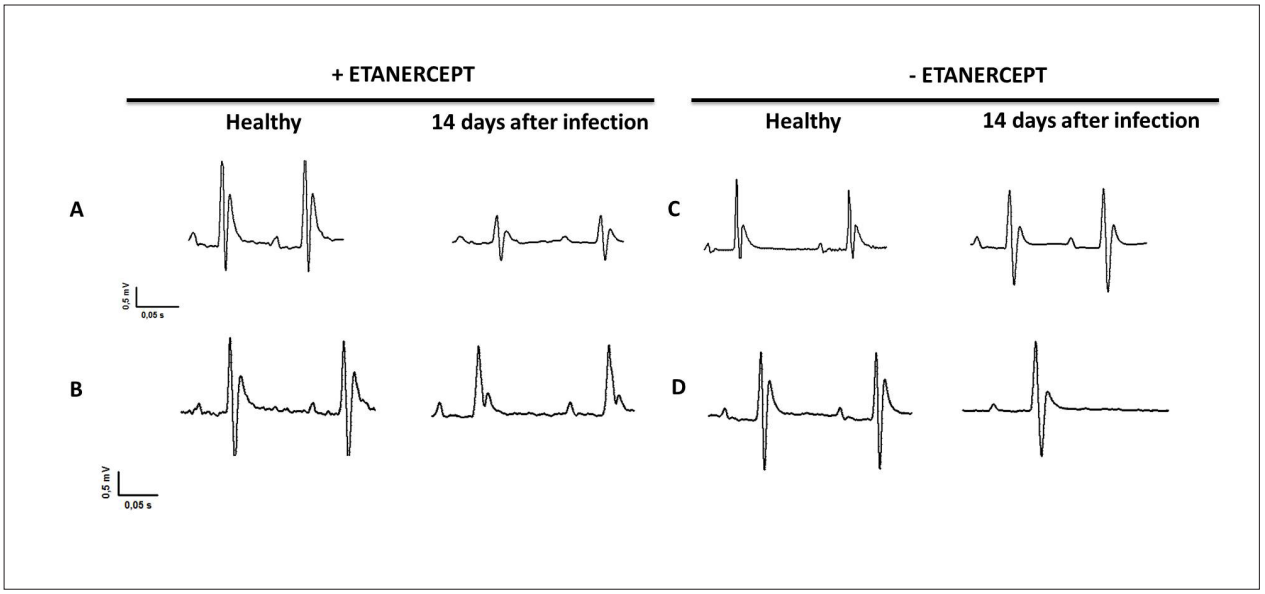

Figure 2 - *Electrocardiography recordings for the treated (+ etanercept, left panel) and untreated groups (−etanercept, right panel) are displayed. Each register identified with letters (A–D) corresponds to different animals. Each panel shows paired recordings obtained before infection (healthy) and 14 days after infection (infected).*

Discussion

The role of TNF-α in *T. cruzi* infection control and the pathological changes that occur during CC has been a matter of debate in recent years. Initially, TNF-α and IFN-γ were thought to be related to macrophage activation and resistance to infection in mice that were experimentally infected with *T. cruzi*[12,13]. However, later studies established that although TNF-α plays a protective role during early infection, high levels promote cachexia and increase mortality during the acute phase of infection[14]. From this perspective, TNF-α blockade ameliorates experimental acute myocarditis[8]. During the chronic phase, TNF-α blockade with infliximab decreases histopathological damage in experimentally infected rats[9]. Alternatively, high serum TNF-α levels have been shown to be related to chronic CC[15]. However, other authors have demonstrated that etanercept treatment during the chronic phase promotes ventricular

Table 1 - Electrocardiography parameters

	Untreated	Treated	p value
P–R interval	1.097 ± 0.05828	1.101 ± 0.03453	0.9441
QTc interval	1.149 ± 0.1081	1.038 ± 0.03163	0.3529
P amplitude	1.171 ± 0.1714	0.7593 ± 0.09617	0.0381
R amplitude	0.8724 ± 0.05804	0.6322 ± 0.07338	0.0275
T amplitude	0.8455 ± 0.1585	0.6901 ± 0.1182	0.4398
T slope	0.9740 ± 0.1701	0.6904 ± 0.1397	0.2361
SDNN	1.268 ± 0.3947	0.4315 ± 0.1016	0.0170

The means represent the ratio of values obtained 14 days after infection to values obtained before infection. Statistically significant results are indicated in bold.

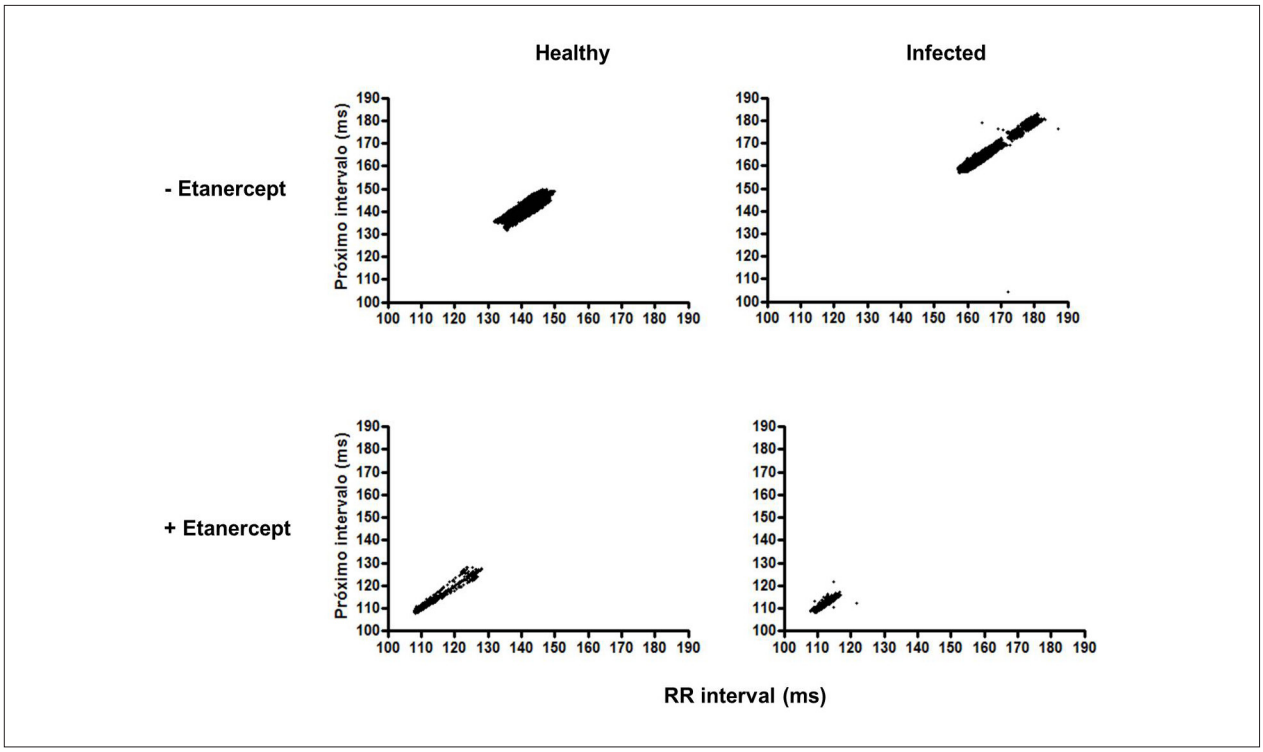

Figure 3 - *Poincare plots show R–R interval variability during an entire electrocardiography recording. The upper and lower panels show paired recordings obtained from the untreated group (−etanercept) and the treated group (+etanercept), respectively.*

dysfunction; therefore, the controversy regarding the role of TNF-α in CC pathophysiology remains unresolved[10]. Low-voltage QRS complexes have been related to acute myocarditis in patients with Chagas disease[16]. Similarly, our results suggest that TNF-α blockade may be associated with a deterioration in ventricular conduction. Another interesting fact is that our results showed an increased survival rate in the treated group (Figure 1), suggesting that ECG changes do not have a predictive value during the acute phase. Therefore, TNF-α increases survival rates despite the worsening electrocardiographic indicators of myocarditis. Some authors have reported that acute infection with Venezuelan wild-type strains in a mouse model generated a generalized inflammatory response, particularly in the central nervous system[17]. We can speculate that systemic inflammation related to TNF-α plays a key role in acute mortality in experimental models. Results obtained by our group (unpublished results) indicate that treatment with etanercept decreases allodynia associated with visceral inflammation and improves the general condition of mice infected with the same wild strain used in the present work. On the basis of these results, we can assert that the regional response to TNF-α (in the heart and abdominal viscera) can vary during the acute phase of infection.

Finally, we observed a decrease in the HRV parameters of the animals treated with etanercept (Figure 3). A decrease in HRV is reportedly characterized by autonomic dysfunction and ventricular dysfunction in patients with Chagas disease[18]. Llaguno et al[18] demonstrated autonomic dysfunction in patients with chronic Chagas disease and high cardiovascular risk, but they failed to relate this condition with serum cytokine levels[19]. Our results suggest that TNF-α blockade induces autonomic dysfunction during the acute phase of infection in mice with Chagas disease and that cardiovascular function worsens after treatment. In contrast, Yu et al[20] reported that etanercept administration in dogs subjected to coronary ligation could decrease ventricular tachyarrhythmia and myocardial necrosis via processes related to kinase-mediated β-adrenergic desensitization[20]. In Chagas disease, however, it has been demonstrated that autoantibodies directed against β_1 adrenergic receptors during the acute phase of infection are able to activate L-type calcium channels in a desensitization-independent process[21,22]. In addition, Chung et al[23] demonstrated that cytokines released by inflammatory cells inhibit the adrenergic stimulation caused by specific agonists, inducing AMPc accumulation due to signaling interruption through the sarcolemma[23].

Conclusion

These results suggest that TNF-α blockade induces an increase in survival despite deteriorating ECG parameters, opening a discussion about whether mortality in experimental infection is related to a TNF-α-induced systemic inflammatory response rather than worsening heart function. Further studies in different animal models will be necessary to validate the results obtained, particularly in regard to survival rate, and discard the fact that mortality occurrs mainly by chance and not by the direct effects of treatment. To the best of our knowledge, this is the first report that associates TNF-α blockade with myocarditis characterized by ECG changes and autonomic dysfunction during the acute phase of infection in mice with experimental Chagas disease. However, it remains unclear whether there is a delicate equilibrium between parasite replication control and heart function preservation. These findings also highlight the need to assess the potential therapeutic role of TNF-α blockade during the acute and chronic phases of *T. cruzi* infection.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Rodríguez-Ângulo H, Mijares A; Acquisition of data: Rodríguez-Ângulo H, García O, Castillo E, Cárdenas E; Statistical analysis: Rodríguez-Ângulo H; Writing of the manuscript: Rodríguez-Ângulo H, Marques J, Mijares A; Critical revision of the manuscript for intellectual content: Marques J, Mijares A.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any post-graduation program.

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