

Cardiorenal interaction during the acute phase of experimental *Trypanosoma cruzi* infection: the influence of aldosterone and the AT₁ receptor on mortality

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Abstract

Introduction: *Trypanosoma cruzi* infection is a serious public health problem in Latin America. Despite positive results from programs directed to the interruption of vectorial transmission, there are still millions of people infected, and a large number, at the risk of infection. Chagas disease is typically associated with cardiac complications, and chronic symptoms include heart failure and megaesophagus development.

Objective: In this study, we aimed to evaluate the importance of the cardiorenal axis and renin-angiotensin-aldosterone system (RAAS) activation in experimental *T. cruzi* infection. We also evaluated the influence of aldosterone and the angiotensin II receptor type 1 (AT₁R) on the mortality of infected mice.

Methods: BALB/c mice infected with the Y strain of *T. cruzi* were treated with spironolactone or losartan. We assessed parasitemia, mortality, and renal and cardiac function by using non-invasive methods.

Results: Our data show that AT₁R promotes an important (and necessary) inotropic positive effect in the heart and minimizes acute kidney injury. Conversely, aldosterone increases the release of K⁺ and aggravates heart failure. It also has associated effects on the renal, cardiovascular, and cardiac electrical conduction systems. Consequently, the aldosterone antagonist reduced the mortality rate by 50%, whereas the AT₁R antagonist increased it by 20%.

Conclusions: The RAAS connects the cardiorenal systems, and its components differentially affect the mortality of animals experimentally infected by *T. cruzi*.

Key words:

Acute kidney injury;
Acute myocarditis;
RAAS; *Trypanosoma cruzi*

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Introduction

In Latin America, transmission of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, has steadily declined through a series of multinational initiatives aimed at interruption of vector transmission (by *Triatoma infestans*) and at promotion of blood donors screening [1-3]. The incidence of Chagas disease has dropped from 700,000 to 40,000 new cases per year, and the annual number of deaths has dropped from more than 45,000 to 12,500 [4]. However, the epidemiology of this disease has become more complex due to the presence of multiple vectors and reservoirs and to the added effects of geopolitical, economic, and ecological upheaval [5-7].

The classic description of Chagas disease includes a short acute phase characterized by mild, non-specific symptoms [4, 8], in which approximately 10% of all patients have severe

myocarditis, with 90% of these having an unfavorable prognosis [9]. Notably, an unknown pathogenesis recently emerged in the Amazon owing to *T. cruzi* infection. Despite treatment with benznidazole, this pathogenesis was associated with fatal myocarditis, renal failure, and cardiac tamponade in 3 patients [10]. Recent results from our laboratory have suggested that acute kidney injury occurs in *T. cruzi*-infected mice before the parasitemia peak (*i.e.*, not related to the parasite load within the tissue) and the onset of inflammatory myocardial damage [11]. Kidney damage seems to be associated with local production of pro-inflammatory mediators (*e.g.*, nitric oxide, tumor necrosis factor- α , and interferon- γ), leading to interstitial inflammation and vascular congestion, alterations in renal perfusion, and loss of cell integrity [12].

The term “cardiorenal syndrome” (CRS) includes a vast array of interrelated complications, highlighting the bidirectional nature of the interactions between the heart and kidneys [13]. Neurohormonal activation is a very important contributor to CRS pathophysiology, leading to exaggerated abnormalities in the activation of the renin-angiotensin-aldosterone system (RAAS) [14].

The use of RAAS blockers in experimental infection with *T.cruzi* allows an interesting approach to pathological studies. For instance, captopril, an angiotensin-converting enzyme (ACE) inhibitor, can ameliorate the progression of necrosis and fibrosis in infected mice [15]. In fact, in highly vascularized tissues such as kidney and lung parenchyma, abundant expression of ACE [16] has reduced the invasion of *T.cruzi* [17]. Similarly, spironolactone, the aldosterone antagonist, attenuates myocardial remodeling during the chronic symptomatic phase of the infection, reducing the severity of chagasic dilated cardiomyopathy by decreasing inflammatory infiltration [18]. Moreover, in patients with chronic cardiomyopathy derived from *T.cruzi* infection, treatment with an ACE inhibitor, enalapril, and spironolactone (and the subsequent addition of the β/α 1 blocker carvedilol) ameliorates the impairment in cardiac function and improves their clinical condition [19].

The aim of this study was to evaluate the importance of the cardiorenal axis and RAAS activation in experimental infection with *T.cruzi*. We also aimed to evaluate aldosterone and angiotensin II receptor type 1 (AT₁R) involvement in kidney and heart function upon *T.cruzi* infection, through the use of spironolactone and losartan and blocking action, respectively, using noninvasive methods. We also examined their influence on the mortality rate in infected mice. Our results clearly show that components of the cardiorenal system interact in experimental *T.cruzi* infection to directly influence the mortality of animals in the absence of trypanocidal treatment.

Materials and methods

Mice

Eight-week-old specific pathogen-free male BALB/c mice were obtained from the FIOCRUZ animal facility (CECAL). The animals were housed for at least 1 week before parasite infection at the Animal Experimentation Division of the Cellular Biology Laboratory/IOC (SEA/LBC) under environmental conditions and sanitation that

conformed to the “Guide for the Care and Use of Laboratory Animals” (DHEW Publication No. [NIH] 80-23, revised 1985). This project is registered by the protocol number 29/08 at the FIOCRUZ Committee of Ethics in Research. The number of animals used in each experiment is stated in the figure legends.

Parasites and infection

T.cruzi Y strain was maintained by *in vivo* passages in nonsyngeneic Swiss Webster mice. Blood trypomastigote forms were isolated as previously described [20]. Parasites were then diluted in saline and counted using a hemocytometer to adjust the inoculum to 1×10^3 parasites per 200 μ L for intraperitoneal (i.p.) injection.

Aldosterone and AT₁R blockade

On the day before infection and during 30 consecutive days the animals were separated into 3 groups: The *Sp group* was treated with spironolactone (EMS S/A, Sao Paulo, Brazil) in drinking water, with a calculated dose of 20 mg/kg per animal [21]; the *Lo group* was treated with losartan (EMS), administered in doses of 200 mg/L of drinking water [22]; and the *Nb group* was infected but not treated. We also evaluated the effects of both drugs in uninfected animals for all time and identified no toxicity at their individual dosage.

Parasitological parameters

Parasitemia was determined daily in the infected animals between 5 and 15 day post infection (dpi) by the Pizzi-Brener method [20]. Cumulative mortality was determined by counting animals daily from 1 to 30 dpi. We also evaluated the average gain weight of mice in each group in this period.

Non-invasive methods

Markers of renal function and electrolytes concentration

We evaluated serum levels of creatinine (CREA), urea (URE), and potassium (K⁺) as indicators of renal function and determined electrolyte concentration in blood samples collected from tail snips on 0, 6, 14, 21, and 30 dpi. Ten microliters of serum was collected and diluted in 1 mL of 7% bovine serum albumin in phosphate-buffered saline in accordance with the manufacturer’s guidelines, using VITROS[®] 750XRC Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, NY, USA).

Locomotor activity (ergometric test)

To characterize the spontaneous activity of the mice, we used the video-tracking tool Noldus EthoVision XT6 (Noldus Information Technology,

Leesburg, the Netherlands). The arena was divided into 12 rectangles, defined as lateral and central areas. Each rectangle was calibrated to be of equal area to ensure consistency in the detection of transitional movements. Locomotor activity was thus measured as the total distance covered (in cm) in 5 min. Locomotor activity was recorded on 0, 7, 14, 21, and 30 dpi. Video was recorded with a camera placed 1 m away from the observation arena.

Blood pressure

Before blood pressure was evaluated, mice were manipulated and adapted daily for 7 days and a tail sphygmomanometer was fitted for 3 consecutive readings until stabilization. Caudal artery blood pressure was individually recorded in non-sedated animals on 0, 7, 14, 21, and 30 dpi by using an LE 5001 Pressure meter® (PanLab Instruments, Barcelona, Spain). Values of systolic (SP), diastolic (DP), and mean pressure were calculated as indicated by the manufacturer.

Electrocardiographic studies

Electrocardiographic parameters were evaluated in non-sedated mice by using transducers carefully placed under the skin in accordance with chosen preferential derivation (DII). Traces were recorded using a digital system (Power Lab 2/20) connected to a bio-amplifier at 2 mV for 1 second (PanLab Instruments). Filters were standardized between 0.1 and 100 Hz, and traces were analyzed using Scope software for Windows V3.6.10 (PanLab Instruments). We measured cardiac frequency (beats per minute, bpm), duration of the PR, QRS, and QT intervals in milliseconds (ms) on 0, 7, 14, 21, and 30 dpi. We individually assessed the relationship between the QT interval and RR interval. To obtain physiologically relevant values for the heart rate-corrected QT interval (QTc) giving a normalized RR interval ($RR_{100} = RR_0/100$ ms). Next, the value of the exponent (y) in the formula $QT_0 = QTc \times RR^y_{100}$ was assessed, where QT_0 is the observed QT and both QT and QTc are in ms. Taking the natural logarithm of each side of the formula $(QT_0) = \ln(QTc) + y \ln(RR_{100})$, the slope of the linear relationship between the log-transformed QT and RR_{100} thus defined the exponent to which the RR interval ratio should be raised to correct QT for heart rate [23].

Statistical analysis

We used Mann-Whitney non-parametric tests to compare the groups (Software SPSS version 8.0); p values are presented in the figure legends.

Results

With regard to parasitological assessments (Fig.1), our results show the maximum number of parasites (parasitemia peak) occurred at 8 dpi for all groups (Fig.1A). The *Sp* and *Lo* groups had similar parasite loads (242 ± 84 and 311 ± 58 parasites $\times 10^4/mL$, respectively), which were lower than the parasite load of the untreated group (*Nb*: 409 ± 54 parasites $\times 10^4/mL$). The mortality of animals (Fig.1B) was directly related to the antagonist used.

The *Nb* group had 80% mortality during the acute phase (*i.e.*, by 30 dpi). Aldosterone blockage (*Sp* group) significantly reduced mortality by 50%, whereas the use of losartan (*Lo* group) increased this parameter by 20%. All infected mice lost weight during the acute phase of the infection. However, the *Sp* group showed a lower weight loss (-4.0 g) compared with the *Nb* (-5.9 g) and *Lo* (-5.7 g) groups. Uninfected animals (*NI*) gained approximately 3.5 g during the same period of time (data not shown).

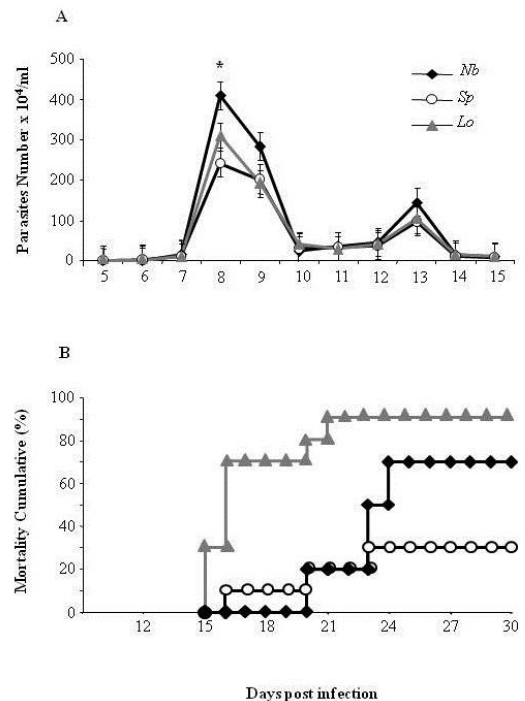


Figure 1. Parasitological parameters: (A) concentration of trypanomastigotes in the bloodstream after *T. cruzi* infection. Counting was performed using the Pizzi-Brenner method between 5 and 15 dpi; (B) percent mortality rate at 30 dpi in untreated infected mice (*Nb*; black diamonds), mice treated with an aldosterone antagonist (*Sp*; white circles), and mice treated with an AT_1R antagonist (*Lo*; gray triangles). Values are mean \pm SD of 3 independent experiments performed with 10 mice per group. * $p < 0.05$ between *Nb* and other groups.

From our evaluation of renal function markers and electrolyte concentrations (Fig. 2), we observed marked acute kidney injury in all groups at 6 dpi (Fig.2A). However, CREA concentrations were lower in groups *Sp* and *Lo* (1.94 ± 0.05 and 2.15 ± 0.08 mg/dL, respectively) than in *Nb* (2.57 ± 0.04 mg/dL). In addition, we found that antagonism of the AT₁R maintained CREA levels higher than in the other groups at 14 (1.36 ± 0.05 vs 0.30 ± 0.02 and 0.3 ± 0.04 mg/dL) and 21 dpi (0.62 ± 0.04 vs 0.26 ± 0.01 and 0.28 ± 0.02 mg/dL).

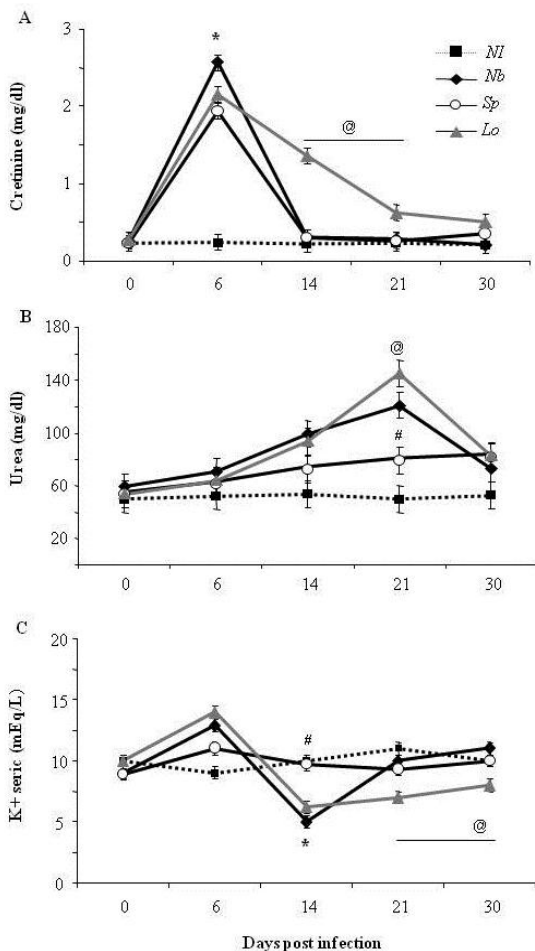


Figure 2. Renal function and potassium concentrations: based on the serum CREA (mg/dL) (A), it was possible to determine the presence of acute kidney injury at 6 dpi and gradually increasing levels of URE (mg/dL) (B) until 21 dpi in infected and no block (black squares), untreated infected mice (black diamonds), mice treated with an aldosterone antagonist (*Sp*; white circles), and mice treated with an AT₁R antagonist (*Lo*; gray triangles). Potassium levels (mEq/L) were also measured (C). Values are mean \pm SD of 3 independent experiments performed with 10 mice per group. * $p < 0.05$ between *Nb* and other groups; # $p < 0.05$ between *Sp* and other groups; @ $p < 0.05$ between *Lo* and other groups.

During the acute phase of *T.cruzi* experimental infection, we observed a significant reduction in K⁺ levels (Fig.2C) at 14 dpi in both the *Nb* (5 ± 0.5 mEq/L) and *Lo* groups (6.2 ± 0.3 mEq/L) compared to uninfected animals (10 ± 0.4 mEq/L). Furthermore, the K⁺ levels in the *Lo* group remained low at 21 (7 ± 0.3 mEq/L) and 30 dpi (8 ± 0.2 mEq/L). Interestingly, the use of spironolactone significantly inhibited such K⁺ drop, particularly at 14 dpi (9.7 ± 0.2 mEq/L), when compared with the untreated infected animals (5 ± 0.5 mEq/L). There were no significant disturbances in uninfected animals.

In relation to serum URE levels (Fig.2B), we observed a progressive increase in all groups until 21 dpi, with a tendency to return to normal values by 30 dpi. However, aldosterone blockage resulted in URE levels that were lower than those in the *Nb* group at 21 (72.6 ± 12 vs 99.4 ± 8.5 mg/dL) and 14 dpi (79.1 ± 10 vs 120.8 ± 14 mg/dL). On the other hand, the use of losartan promoted a significant increase in URE levels at 21 dpi compared to the levels in the untreated infected animals (145 ± 16 vs 120.8 ± 14 mg/dL).

The use of noninvasive methods for cardiovascular evaluation (Figs.3&4) revealed, in all aspects, a more severe pathophysiology after blocking the AT₁R. Ergonomic tests (Fig.3A) showed reduced locomotor activity in all infected groups. However, the group *Lo* group showed diminished activity at 14 (700 ± 10 cm), 21 (100 ± 1 cm), and 30 dpi (520 ± 10 cm), than the *Sp* (1200 ± 12 , 500 ± 10 , and 1140 ± 14 cm, respectively).

The mean arterial pressure of the *Lo* group (Fig.4B) was also significantly decreased at 14 (55 ± 12 mmHg) and 21 dpi (40 ± 10 mmHg) when compared to that of the *Nb* (78 ± 10 and 60 ± 12 mmHg) and *Sp* groups (100 ± 14 and 85 ± 12 mmHg). There were no significant disturbances in the uninfected animals.

We also evaluated the cardiac electrical conduction (Table 1) and observed that the *Lo* group showed a significant increase in the PR interval compared to *Nb* at 14 (43 ± 4 vs 35.4 ± 1.0 ms) and 21 dpi (55 ± 3 vs 46 ± 4 ms). In addition, we observed a reduced cardiac frequency in both the *Lo* and *Nb* groups (470 ± 19 vs 603 ± 21 bpm) at 14 dpi. The QTc interval significantly increased with losartan treatment (39.4 ± 2 vs 32 ± 2 ms) at 21 dpi in comparison with the QTc interval of the untreated infected mice. However, aldosterone blockage preserved the cardiac electrical conduction, particularly maintai-

ning the heart rate (7 dpi: 644 ± 30 ; 14 dpi: 568 ± 36 ; 21 dpi: 568 ± 39 and 30 dpi: 624 ± 40 bpm) and QTc interval (7 dpi: 22.4 ± 3 ; 14 dpi: 26.1 ± 2 ; 21 dpi: 26.1 ± 2 and 30 dpi: 23 ± 2 ms). These remained close to normal range throughout the studied period, as can be observed by the electrocardiogram traces (Fig.4). The uninfected mice showed no disturbances in cardiac electrical conduction (Fig.4A). The experimental

infection with *T.cruzi* was the most severe at 21 dpi. At this time, we observed that infected animals had atrioventricular blockage (BAV) and decreased heart rate (Fig.4B). The blockage of AT₁R increased the incidence of BAV, sinus bradycardia, and the QTc interval (Fig.4C). However, the use of spironolactone improved heart rate and the ventricular repolarization interval represented by QTc (Fig.4D).

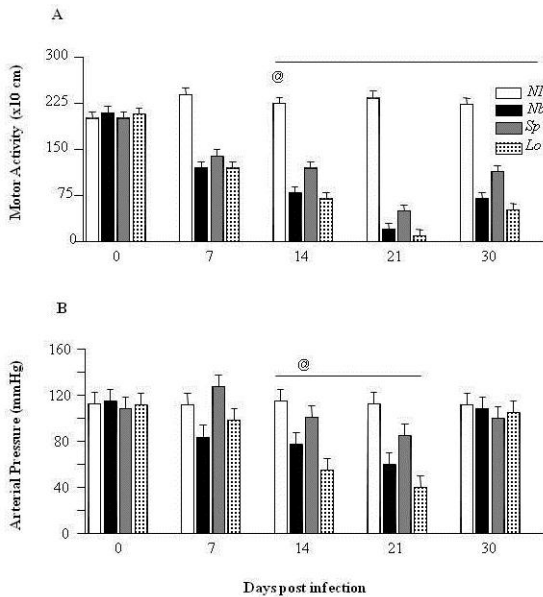


Figure 3. Evaluation of cardiovascular function: noninvasive ergonomic testing (A) describing the spontaneous motor activity as the distance traveled by each animal (cm), and mean arterial pressure (mmHg) (B) in untreated infected mice (Nb; black bars), mice treated with an aldosterone antagonist (Sp; gray bars), and mice treated with an AT₁R antagonist (Lo; dotted bars) before infection and until 30 dpi. We used uninfected mice as a positive control (NI; white bars). Values are mean \pm SD of 3 independent experiments performed with 10 mice per group. [@]p < 0.05 between Lo and other groups.

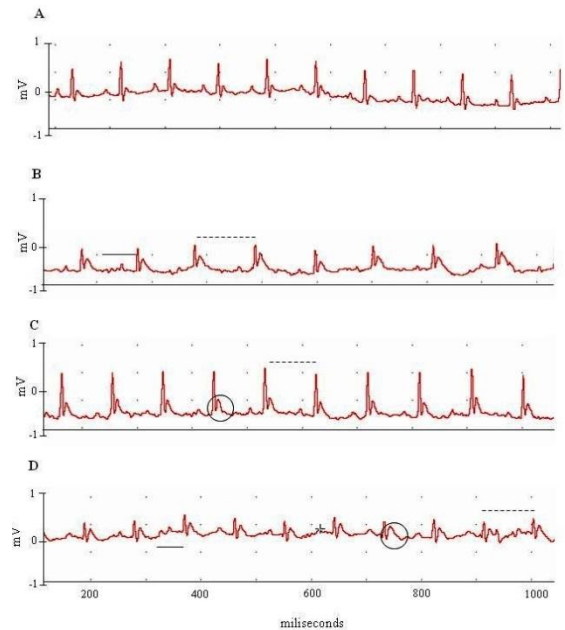


Figure 4. Electrocardiogram traces: the major changes in the electrical conduction system at 21 dpi. Representative first- and second-degree atrioventricular blockage (BAV) (black line), sinus bradycardia (dotted line), and increased QT interval (circle) in animals without infection (A), untreated infected mice (B), mice treated with an aldosterone antagonist, (C) and mice treated with an AT₁R antagonist (D). This result is relative a 3 independent experiments performed with ten mice/group.

Table 1. Electrocardiogram intervals and cardiac frequency

Group	dpi 7			dpi 14			dpi 21			dpi 30		
	Nb	Esp	Los	Nb	Esp	Los	Nb	Esp	Los	Nb	Esp	Los
PR	30.4 ± 2	29.7 ± 3	28.6 ± 3	35.4 ± 1	32 \pm 2	43 ± 4 [@]	46 \pm 4	35.7 ± 4	55 ± 3 [@]	38.9 ± 3	32 \pm 5	40.4 ± 2
QRS	9 ± 1	8.8 ± 2	9.8 ± 1	9.4 ± 2	9.1 ± 2	10.0 ± 1	10.3 ± 2	9.1 ± 1	10.8 ± 2	9.9 ± 2	9.8 ± 1	9.4 ± 2
QTc	20.4 ± 2	22.4 ± 2	29.7 ± 3	23.7 ± 2	26.1 ± 2	25.9 ± 4	32 \pm 2	26.1 ± 2 [#]	39.4 ± 2 [@]	41 \pm 3	23 ± 2 [#]	35.6 ± 3
bpm	681 ± 55	644 ± 30	635 ± 52	603 ± 21	568 ± 36	470 ± 19 [@]	459 ± 23	568 ± 39 [#]	420 ± 37	540 ± 51	624 ± 40	578 ± 25

Values are mean \pm SD; [@]Lo compared to Nb or Sp at the same time (p < 0.05), [#]Sp compared to Nb or Lo at the same time (p < 0.05). [dpi, day post infection; bpm, beat per minute]

Discussion

Chagas disease is typically associated with cardiac symptoms. Kidney disturbances due to *T.cruzi* infection are, however, poorly described in the literature. Some studies have described the presence of the parasite in the kidney, albeit in small numbers, but none have addressed the consequences of infection in this vital organ [17, 24]. Most studies associating the kidney with Chagas disease point to renal transplantation as the main issue [25, 26].

Experimental infection by *T.cruzi* causes acute kidney injury in the early stages of infection, before peak parasitemia and acute myocarditis [11]. This renal injury is not related to the presence of parasite, but to reduced blood flow partially compromising the proximal tubules, causing congestion and capilar microhemorrhages [11]. Recently, we found that mesangial cells infected with *T.cruzi* have high levels of nitric oxide and proinflammatory cytokines in the culture supernatants. These cells are less susceptible to infection, but their integrity and viability are impaired. We believe that nitric oxide, a known potent microbicide, has an antagonistic role in protecting the infected cell from parasite invasion and the associated cell damage [12].

The present study clearly showed the importance of the cardiorenal interconnection and revealed a direct influence of aldosterone and AT₁R on the mortality rate of mice infected with *T.cruzi*. Both aldosterone and AT₁R blockage promote a slight decrease in parasite load as compared to the parasite load in untreated animals. However, it is evident that the RAAS may act antagonistically on the survival of an animal. The use of spironolactone reduced mouse mortality by up to 50%, whereas the use of losartan increased this same parameter by 20%.

Leon and coworkers described ameliorated myocarditis in acute experimental Chagas disease after treatment with captopril [15]. Therapeutic doses of spironolactone also reduced the extent of myocardial remodeling during the chronic symptomatic phase, attenuating the severity of chagasic dilated cardiomyopathy by reducing inflammatory infiltration [18]. Our results show that blockage of the AT₁R prolongs and enhances the elevation of serum CREA and UREA, thereby aggravating the acute kidney injury in infected mice. Aldosterone blockage significantly minimizes the loss of K⁺ during the acute phase of infection. Blockage of the AT₁R with losartan also reduces

the physical activity levels and mean arterial pressure of animals. Binding of angiotensin II to the AT₁R mediates its classical physiologic actions [27], such as sympathetic nervous system activation, resulting in reinforced vasoconstriction and increased rate and force of heart contraction [27]. These effects can be deleterious in situations, such as left ventricular hypertrophy, however during the acute phase of experimental infection they are beneficial.

Furthermore, disturbance in the cardiac electrical conduction by BAV, presence of sinus bradycardia, and increased QT interval clearly show that the absence of functional AT₁R worsens heart failure in *T.cruzi* infection. Antagonism of aldosterone, on the other hand, preserves cardiovascular function and cardiac electrical conduction, thereby minimizing the effects of heart failure and the morbidity of the animals.

Our results demonstrate a new aspect of the pathophysiology of infection with *T.cruzi*. The study of renal injury, the cardio/renal connection and new associated therapy applications can increase quality of life and decrease side effects of trypanocidal agents used especially in children patients highly susceptible to the etiological treatment. We emphasize that the possible limitations of this study is associated with the need for further preclinical trials to evaluate the similarity of the importance of cardiorenal axis between murine models and humans. Furthermore, we need to deepen this study during the chronic phase in which is the greatest social impact of this disease in patients.

The aim of this study was to show the importance of the cardiorenal axis and the involvement of the RAAS [28]. We clearly observed that, unlike other experimental models (cardiac ischemic/reperfusion lesion) which increased the incidence of arrhythmias as well as mortality after myocardial infarction [29], the AT₁R and its positive inotropic effects are needed during the course of an acute of parasitic infection. On the other hand, aldosterone promotes K⁺ loss, and worsening of heart failure that can directly influence mortality. Inflammatory factors and toxemia cause the release of nitric oxide to promote vasodilatation [30, 31]. Consequently, this disturbance activates the RAAS, increasing the absorption of Na⁺ and water and K⁺ release from the proximal renal tubules [32]. Aldosterone interrupts this mechanism, causing increased retention of K⁺ and reduction of cardiovascular complications. We conclude that the cardiorenal

interconnection is dependent upon the RAAS and acts to both exacerbate and attenuate symptoms with experimental *T.cruzi* infection. Furthermore, aldosterone and AT₁R appear to be crucial in determining the mortality of infected mice.

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