ORIGINAL ARTICLE

Rescue Therapy with Nifurtimox and Dipyridamole for Severe Acute Chagas Myocarditis with Congestive Heart Failure in NMRI Albino Mice

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Abstract

Background: Chagas disease is a global health problem; therefore, the development of new therapeutic protocols is necessary. Our group recently demonstrated that nifurtimox associated with dipyridamole has curative effects in mice with acute Chagas disease. In this study, we assess the effect of this therapeutic protocol in chagasic mice with heart failure.

Objective: To evaluate whether nifurtimox and dipyridamole are useful to rescue mice with severe acute chagasic myocarditis with heart failure.

Methods: 42 mice with acute chagasic myocarditis and congestive heart failure were divided into three groups: control chagas (n = 11), Nif-Dip treated with nifurtimox and dipyridamole (n = 14) and Nif-Dip-heart failure treated with nifurtimox and dipyridamole associated with digoxin, furosemide, and captopril (n = 17). Nifurtimox and dipyridamole doses were 40 and 30 mg/kg/day, respectively, for 6 weeks. Mice underwent clinical, electrocardiographic, hemoparasitological and histopathological assessments.

Results: Lower mortality in Nif-Dip (28.57%; n = 4) compared to control chagas (54.54%; n = 6) and Nif-Dip-heart failure (52.9%; n = 9) was observed. Clinically, nifurtimox and dipyridamole-treated mice increased body weight and improved heart failure without splenomegaly. In these groups, parasitemia and tissue parasites were eradicated; fibrosis, myocytolysis, inflammatory cell infiltrate and mast cells decreased. Repolarization disorders, prolonged QRS and QT intervals, increase of S wave amplitude and atrioventricular dissociation were reversed by the treatment.

Conclusion: Nifurtimox with dipyridamole can rescue NMRI mice from severe acute chagas disease, as nifurtimox showed trypanocidal activity and dipyridamole potentiated its effect. Dipyridamole would be useful in chagasic heart failure. (Int J Cardiovasc Sci. 2017;30(2):145-156)

Keywords: Chagas Disease; Chagas Cardiomyopathy; Heart Failure; Mice; Nifurtimox; Dipyridamole.

Introduction

Chagas Disease (ChD) is a global public health problem due to a high residual 1.06% prevalence in endemic countries, where reemergence has been reported as oral transmission outbreaks and because of the disease globalization, as a product of the migration of people from endemic countries to developed countries; it has resulted in a 4.2% prevalence in immigrant populations in Europe.[1-4]

Symptomatic acute ChD cases have been associated with patent parasitemia and severe acute myocarditis, with high morbidity and mortality.[5] Severe heart damage would reflect parasite pathogenic action; therefore, it would be mandatory to conduct aggressive trypanocidal

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therapeutic interventions, to decrease parasitic load, morbidity, and mortality.

Currently, the accepted treatment protocols for acute ChD are nifurtimox and benznidazole. The use of this protocol, even when the best results are obtained, shows these results are not fully satisfactory. In Venezuela, studies using nifurtimox and/or benznidazole have been disappointing, with more than 70% of the cases with proven presence of parasites in tissues, parasite genome or its antigens (positive serology) after using these drugs.[5,6]

Thus, it would be useful to create therapeutic schemes where these drugs will be combined with others, which could increase nifurtimox and benznidazole efficacy and decrease their side effects. Ideally, these drugs should have trypanocidal effect, promote cardiac functionality, and counteract pathophysiological phenomena resulting from infection, inflammation, and free-radical generation.

According with the microvascular theory, dipyridamole could be seen as the adjunctive drug, because it induces coronary vasodilation through nitric oxide (NO), improving blood flow; it has antiplatelet effect preventing thrombi formation; it increases extracellular levels of adenosine, improving cardiac function in patients with HF; it promotes an anti-inflammatory effect suppressing free radical formation and it improves the redox state of cells undergoing inflammatory phenomena[7,8].

Recently, our group demonstrated that dipyridamole has a trypanocidal effect *in vitro* with an IC_{50} of 372 μM and potentiates the effect of subtherapeutic doses of nifurtimox, providing curative effects on NMRI mice with acute ChD.[9] For this reason, in this study we consider whether a therapeutic rescue in acute chagasic myocarditis (AChM) animals with severe CHF can be performed with nifurtimox and dipyridamole, allowing a reduction in mortality and cardiac functional sequelae.

Materials and methods

Sample

The sample consisted of 48 male NMRI adult mice, weighing between 30-50 g, obtained from the *animal* research *facilities at Center-West* Lisandro Alvarado University (Barquisimeto, Venezuela), which were infected intraperitoneally with blood trypomastigotes suspended in 0.9% NaCl, at a dose of 100 trypomastigotes per gram of body weight. The strain of TcI lineage used has been registered in the WHO bank under the name MHOM/VEcepa792/2-92-YBM.[9] The assayed parasites were obtained from blood of infected mice in the acute phase of ChD and maintained in vector/mouse cycles; vectors were *Rhodnius prolixus* stage III nymphs. At week 2 of inoculation, confirmation of parasitemia was performed, and only positive mice were included in the study.

Of the 48 infected mice, 6 (12.50%) died during the first six weeks of infection, the remaining 42 mice with AChM with clinical signs of CHF were electrocardiographically studied under anesthesia, then divided in three groups and received treatment during 6 weeks. The Control Chagas group (CC; n = 11) were treated with vehicle; the Nif-Dip group (n = 14) were treated with nifurtimox (40 mg/kg) associated with dipyridamole (30 mg/kg) and Nif-Dip-CHF group (n = 17) were treated with nifurtimox (40 mg/kg) and dipyridamole (30 mg/kg) associated with drugs for CHF treatment (captopril 5 mg/kg, digoxin 8 μg/kg and furosemide 2 mg/kg, the latter two administered for only for 1 week).

The mice were distributed in stainless steel cages, measuring 29x30x14 cm, with 5 to 8 animals per cage, with free access to water and food (Perrarina®, Protinal, Venezuela), with a 12-hour light/dark cycle and an average 27°C temperature.

At 6 weeks of treatment, the animals were clinically evaluated (weight, piloerection, and clinical signs of CHF, such as dyspnea and/or cyanosis at rest or under anesthesia), as well as submitted to parasitological (parasitemia) and electrocardiographic assessments. They were subsequently sacrificed by exsanguination via cardiac puncture under anesthesia, followed by autopsy, when organs were removed and weighed, and samples for histopathology were collected.

Bioethics

All experimental procedures performed in this study were based on the principles established in the bioethics and biosafety manual of the National Fund for Science and Technology, Ministry of Popular Power for Science and Technology, Venezuela.

Electrocardiography

Electrocardiographic studies were performed under anesthesia with sodium pentobarbital 25 mg/kg and ketamine 25 mg/kg of body weight ip, in bipolar configuration, using 3 needle-type electrodes (one positive, one negative and one neutral or reference), positioned in the subcutaneous tissue. We worked with 4 lead-ECG configurations: in DI, a positive electrode was placed together with the reference electrode on the left shoulder joint, while the negative electrode was positioned on the right shoulder joint; in DII, a positive electrode was positioned on the xiphoid process, while a negative one was placed on the right shoulder joint and the reference on the left shoulder joint; in DIII, both the negative electrode and the reference electrode were placed on the left shoulder joint and a positive electrode was maintained on the xiphoid process, and in AVF lead, a negative electrode was positioned on the cervical midline above the suprasternal fossa, while a positive electrode was placed on the xiphoid process and a reference one remained on the left shoulder joint.

Electrodes were connected to a BioAmp amplifier (AD Instruments, New Zealand), and analog signals were converted to digital signals through a Powerlab/8sp interphase system (AD Instruments, New Zealand), displayed, recorded, and analyzed on a personal computer using the Chart v4.2.1 software (AD Instruments New Zealand). The signal capture was performed at a 1000 events/sec frequency and filtered at 60 Hz.

Histopathology

Right ventricular and cardiac apex samples were fixed with 4% paraformaldehyde solution in PBS, pH 7.4 for 2 hours. Fixed tissue samples were embedded successively in 10, 15, 20, 25 and 30% sucrose solutions until samples migrated to the container bottom in each solution; then samples were stored at -70°C until used. Samples were thawed and embedded in Optimal Cutting Temperature (OCT) compound, and again frozen with liquid nitrogen, cryostat-cut obtaining 5 μm-sections, put on microscope slides and stained with hematoxylin-eosin or toluidine blue stains. Additionally, another group of samples was fixed with 10% formalin in PBS pH 7.4, embedded in paraffin, cut with a microtome and stained with hematoxylin-eosin.

Data analysis

Data is expressed as absolute values, percentages or mean ± SEM. Paired or unpaired Student's *t* test was used to analyze the significance of observed differences before and after treatment in a group or to compare two unrelated groups, respectively; while ANOVA followed by Tukey's post-test was used to analyze significance of observed differences between the three experimental groups. A value of $p < 0.05$ was considered significant. GraphPad Prism Software 5.0 was used for the statistical analysis.

Results

All mice became infected with an average parasitemia of 4.19 ± 0.74 x 10^6 parasites/mL on the 6th week post-infection before treatment protocol began. In all experimental treated groups, parasites were not detected at the 7th week, while in the CC group, parasitemia disappeared between the 8th and 9th week; all groups had 0 parasitemia at sacrifice.

During the post-anesthetic period (0-48 hours), the CC group had 45.45% (n = 5), Nif-Dip 21.42% (n = 3) and Nif-Dip-CHF 41.17% (n = 7) of mortality rate, respectively. During the following six weeks, 1 animal of each CC and Nif-Dip groups and 2 from Nif-Dip-CHF group died, resulting in a total mortality of 54.54% (n = 6), 28.57% (n = 4) and 52.9% (n = 9), respectively.

In Figure 1, clinical parameters observed at the end of the experimental protocol are shown. We can observe that treated groups significantly increased body weight; while spleen weight and CHF clinical signs decreased significantly in these groups as compared with the CC group.

Quantitative and qualitative electrocardiographic parameters are shown in Tables I and II, respectively, before and after starting the therapeutic protocol. Altered electrocardiographic signs found before treatment included decreased heart rate, cardiac conduction disturbances, characterized as an increase in QRS and QT intervals values; alterations in ventricular repolarization, characterized by disorders in amplitude, axis direction and T wave decay (see Table I and Figures 2 and 3). These signs were reversed by the therapeutic protocol; however, R and S wave amplitudes decreased (See Table I and Figures 2 and 3). Regarding qualitative data disorders, nifurtimox-dipyridamole-based therapeutic protocol tended to decrease atrioventricular dissociation and post-depolarization disorders; however, rhythm disorders (nodal and ventricular extrasystoles) tended to increase (see Table II).

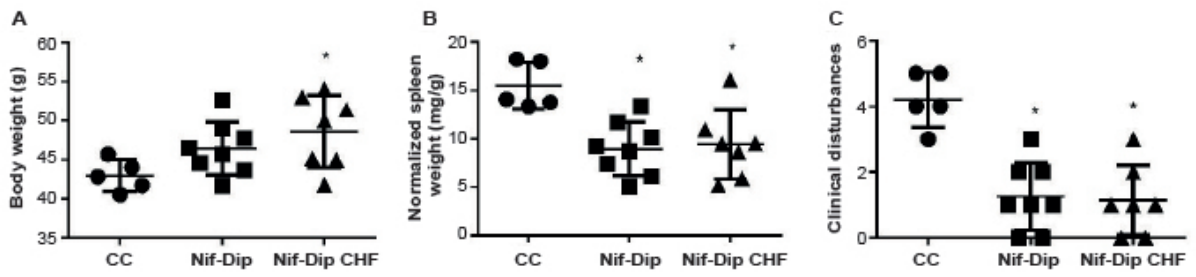

Figure 1 – *Clinical parameters quantified in mice with AChD treated or not with nifurtimox and dipyridamole.*
*In A, body weight is shown; mice submitted to the treatment protocol increased weight. In B, normalized spleen weight based on body weight is shown; splenomegaly decreased in mice submitted to the treatment protocol. In C, a summative clinical analysis based on the presence (1) or absence (0) of the following clinical signs were performed in each mouse: dyspnea and cyanosis at rest or under anesthesia and piloerection; mice submitted to the treatment protocol had decreased signs of CHF or infection. * means $p < 0.05$ when comparing treated groups in relation to Control Chagas non-treated group, using ANOVA test followed by Tukey's post-test.*

Histopathological analysis (see Table III) of the right ventricular sections from the CC mice group displayed thickened epicardium, myocytolysis and diffuse mononuclear inflammatory infiltrate; while in the Nif-Dip mice group, only reduced focal inflammatory infiltrate was observed and epicardial thickness was diminished; fibrosis degree was similar in both groups. In the cardiac apex sections, fibrosis, myocytolysis, epicardium thickness and inflammatory infiltrate were lower in mice treated with nifurtimox-dipyridamole when compared to the CC group. In both sections, in 60% of untreated mice, intact and/or broken parasitic nests were present, while in sections of nifurtimox-dipyridamole treated mice amastigotes and/or parasite nests were not observed (see Figure 4). Additionally, in 60% (n = 3) of CC and in 50% (n = 2) of Nif-Dip group mice, mast cells were observed in cardiac apex sections; however, the number of mast cells per field was higher in the CC group (10.60 ± 7.53) than in the Nif-Dip group (1.00 ± 0.82); (see Figure 5 and Table III).

Discussion

In this study, we demonstrate that the combination of nifurtimox with dipyridamole was therapeutically useful in mice with AChM with CHF, since, compared with untreated control mice, it reduced mortality, decreased or eradicated infection and, furthermore clinical, and histopathological signs of severe AChM were reversed. To our knowledge, this is the first study carried out in animal models to evaluate nifurtimox-dipyridamole-based therapeutic rescue in mice with severe AChM.

The trypanocidal effect of nifurtimox treatment was tested in the present study by demonstrating absence of parasitemia after the first week of treatment and the absence of amastigotes or parasite nests in cardiac right ventricular and apex histopathology sections. These results concur with those reported by Santeliz et al.[9] who demonstrated that the nifurtimox (40 mg/kg) and dipyridamole (30 mg/kg) combination eradicates tissue and hematic parasites in mice with acute ChD without CHF. The trypanocidal effect shown in the present work could depend solely on the effect of nifurtimox at a 40 mg/kg dose, as this dose has been reported to be curative (16); however, several studies in murine models about the use of nifurtimox in ChD treatment have been controversial (see below).

Studies on nifurtimox effect in experimental animals have used high doses of the drug, obtaining conflicting results; while Bustamante et al.[10] working with C57BL/6 (Ly5.2 +) mice obtained a 95-100% cure rate with doses of 100 mg/kg, during 40 days of continuous or 13 days of intermittent treatment, Wong-Baeza et al.[11] working with NIH albino mice, obtained a slight parasitemia decrease, with similar doses using a 50-day therapeutic protocol. This discordance observed between the aforementioned studies and our work can be explained by mouse strains used; however,

response to nifurtimox treatment may depend also on the tested *T. cruzi* strain, as demonstrated in the study by Andrade et al.[12], who found that Type I strain (high and early parasitemia, macrophage tropism) showed high sensitivity (56 ± 16% cure), type II strains (high and late parasitemia, heart muscle tropism) showed medium to high sensitivity (52 ± 11% cure) and type III strains (low parasitemia and skeletal muscle tropism) showed low sensitivity (0.45±0.45% cure) to therapeutic regimens based on nifurtimox 200 mg/kg for 4 days, followed by 50 mg/kg for 5 days a week, up to a total of 90 days. Additionally, Faúndez et al.[13] found parasitemia decreases using doses of 2.5 and 10 mg/kg, with 25 and 100% of survival, respectively.

Table 1 – Electrocardiographic quantitative parameters in mice with acute chagasic cardiomyopathy with heart failure before and after starting the therapeutic protocol

Parameter	Experimental groups			
	Nif-Dip		Nif-Dip- CHF	
	Pre-treatment	Post-treatment	Pre-Treatment	Post-treatment
HR (bpm)	373.40 ± 22.08	431.40 ± 24.35	382.20 ± 39.34	416.10 ± 22.04
PR	57.37 ± 2.87	52.59 ± 3.72	54.70 ± 3.73	58.07 ± 3.94
QRS (ms)	13.83 ± 0.21	11.50 ± 0.74*	14.71 ± 0.69	11.13 ± 0.45*
QT (ms)	101.50 ± 11.54	68.31 ± 12.81*	105.50 ± 11.33	90.01 ± 6.20
QTc (ms)	244.90 ± 22.16	175.00 ± 29.03*	252.30 ± 21.94	230.80 ± 11.50
P (μV)	56.77 ± 10.65	65.57 ± 8.02	34.07 ± 12.74	65.96 ± 4.18*
R (μV)	680.30 ± 82.25	577.40 ± 83.88	755.10 ± 83.82	631.80 ± 82.50
S (μV)	-363.90 ±62.56	-204.20 ± 40.44*	-252.10 ± 45.81	-40.10 ± 40.48*
T (μV)	218.20 ± 46.28	270.00 ± 41.68	225.60 ± 49.06	255.50 ± 38.40
τ1	5.19 ± 0.71	7.82 ± 1.07	6.30 ± 0.78	8.41 ± 2.63
τ1 %	40.63 ± 8.19	79.25 ± 4.21*	47.72 ± 6.91	74.04 ± 6.25*
τ2	67.16 ± 11.69	104.50 ± 23.29	69.88 ± 6.57	67.94 ± 5.07
τ2 %	59.70 ± 9.28	23.32 ± 3.76	30.12 ± 6.57	32.06 ± 5.07
D_5	73.78 ± 2.33	62.24 ± 4.39*	71.47 ± 3.51	59.78 ± 4.55*
D_{10}	48.69 ± 4.98	30.98 ± 5.46	49.41 ± 4.78	38.56 ± 4.49
D_{20}	39.06 ± 3.80	20.78 ± 3.01*	38.21 ± 3.97	24.22 ± 3.72*
D_{40}	37.27 ± 2.85	17.26 ± 3.16*	33.84 ± 3.38	23.43 ± 3.50*
D_{60}	20.33 ± 3.09	11.64 ± 2.44*	22.69 ± 2.88	14.46 ± 3.04*
QRS axis	43.80 ± 21.17	46.90 ± 15.85	70.28 ± 12.26	78.86 ± 6.47
P axis	53.17 ± 18.53	53.38 ± 9.87	15.40 ± 18.69	58.32 ± 12.45
T axis	68.00 ± 16.83	79.78 ± 5.31*	59.49 ± 17.08	74.61 ± 6.89

*"HR"-heart rate; "bpm" - beats per minute; T wave decay was analyzed by an exponential decay equation for two components. obtaining values and time constants τ1 and τ2 with their respective percentage contribution to decay; additionally, T wave amplitude percentage decay at 5. 10. 20. 40 and 60 ms after T peak was quantified; * means p < 0.05 comparing values before and after treatment using a paired Student's t test.*

Table 2 – Electrocardiographic qualitative parameters recorded in mice with acute chagas cardiomyopathy with heart failure before and after starting the therapeutic protocol

Parameters	Experimental groups			
	Nif-Dip		Nif-Dip-CHF	
	Before n (%)	After n (%)	Before n (%)	After n (%)
AV dissociation	5 (50)	2 (20)	0 (0)	2 (25)
Ventricular extrasystoles	0 (0)	3 (30)	1 (12.5)	1 (12.5)
Nodal extrasystoles	0 (0)	5 (50)	0 (0)	1 (12.5)
Atrial fibrillation	4 (40)	3 (30)	0 (0)	2 (25)

"AV"- atrioventricular

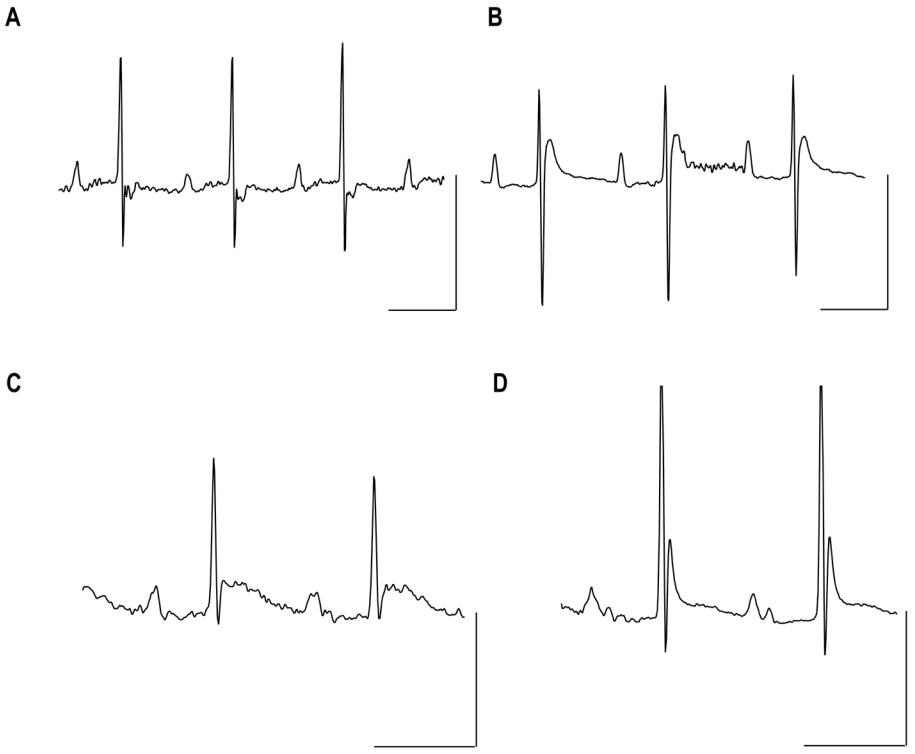

Figure 2 – *Electrocardiographic changes observed before and after nifurtimox and dipyridamole therapeutic protocol was applied, associated with drugs for heart failure. Lead-ECG DIII, electrocardiographic records of two individuals are shown, before starting treatment (left) or after treatment (right). In record A, ischemic disorders are shown, characterized by inversion of T wave and J point below the isoelectric line, which were reversed by the therapeutic protocol with restitution of T wave morphology and amplitude (record B); additionally, the treatment induced a recovery of P wave amplitude. In record C, a decrease in the QRS complex and T wave amplitudes can be observed; note that T wave has a prolonged plateau; after treatment (record D) recovery of the QRS complex and T wave amplitude can be observed, which almost recovered normal morphology. Vertical bars indicate 400 μV, horizontal ones indicate 100 ms.*

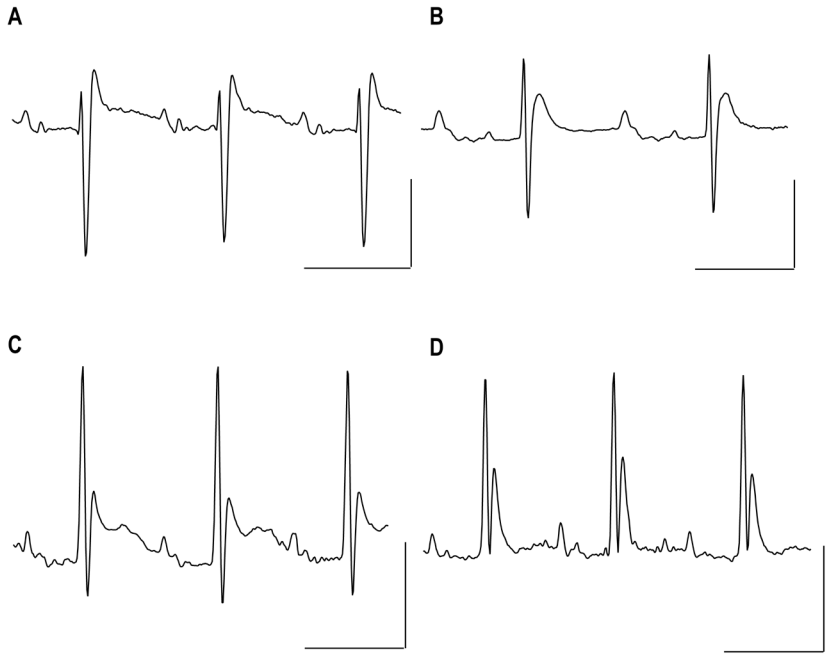

Figure 3 – *Electrocardiographic abnormalities observed before and after the nifurtimox and dipyridamole therapeutic protocol was applied. Lead-ECG DIII electrocardiographic records of two animals are shown, before starting treatment (left) or after treatment (right). In record A, low amplitude of bimodal P wave, decreased R wave amplitude, deep S wave, prolonged QRS complex and T wave second extended component, which does not reach the isoelectric line are shown; after treatment (record B) P, R and S wave amplitudes are recovered and a T wave amplitude of the second component is decreased. In record C, it is clearly shown that the second component of T wave forms an upward deflection, compatible with post-depolarization phenomenon, which was totally reversed by the treatment protocol (record D).Vertical bars indicate 400 μV, horizontal ones indicate 100 ms..*

Table 3 – Histopathological analysis in right ventricle and cardiac apex sections from mice with acute chagas cardiomyopathy with heart failure treated or not with nifurtimox and dipyridamole

Parameter	Experimental groups			
	Control Chagas		Nif-Dip	
	RV	Apex	RV	Apex
Parasitic nests	6.00 ± 2.47	3.00 ± 1.91	0*	0*
Fibrosis	1.40 ± 0.50	1.75 ± 0.47	1.40 ± 0.50	0.25 ± 0.25*
Myocytolysis	2.00 ± 0.44	1.50 ± 0.28	1.40 ± 0.24	0.25 ± 0.25*
Epicardium thickness	1.80 ± 0.20	1.75 ± 0.25	1.20 ± 0.20	0.75 ± 0.25*
Inflammatory infiltrate	2.80 ± 0.20	2.00 ± 0.57	1.80 ± 0.37*	0.50 ± 0.28
Mast cells	--	10.60 ± 7.53	--	1 ± 0.82

*"RV": right ventricle; * means $p < 0.05$ when comparing the values given for the same cardiac region with and without nifurtimox and dipyridamole treatment by an unpaired Student's t test.*

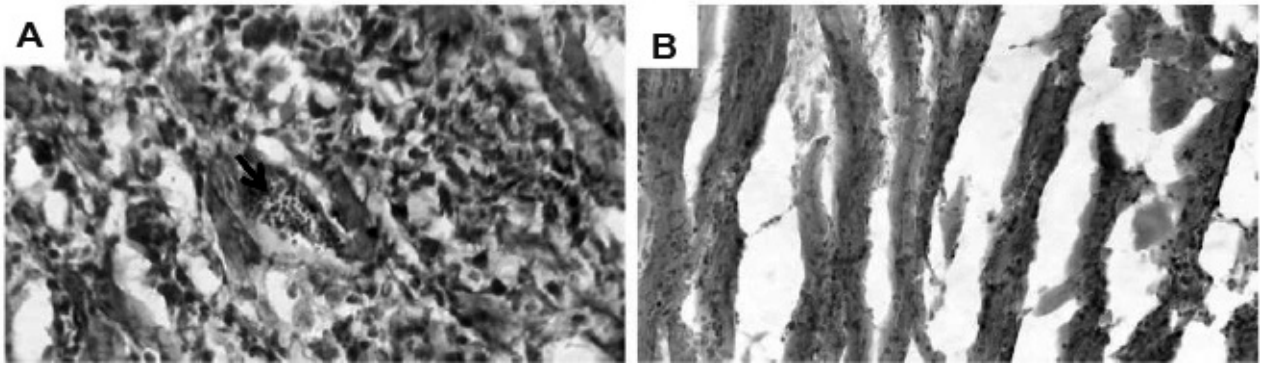

Figure 4 – *Heart tissue histopathological characteristics of mice with acute Chagas disease treated or not with nifurtimox and dipyridamole. Tissue sections fixed with 4% paraformaldehyde, cryopreserved with 30% sucrose, embedded in OCT, and stained with hematoxylin-eosin are shown. In A, a representative Control Chagas heart section is displayed, where amastigotes nests (arrow), a mononuclear diffuse infiltrate and myocytolysis (1000X magnification) can be observed. In B, a representative heart section in mice treated with nifurtimox and dipyridamole is shown, disclosing muscle fiber integrity and a very discrete mononuclear cell infiltrate; fiber separation is a product of the cryopreservation technique (400X magnification).*

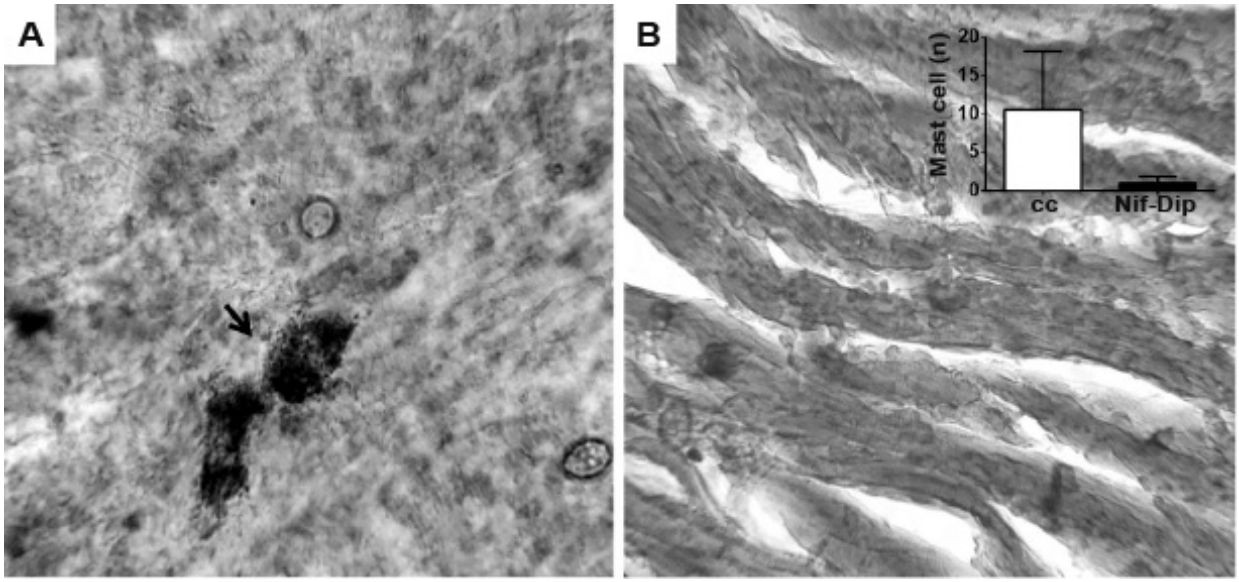

Figure 5 – *Mast cell expression in heart tissue of mice with acute Chagas disease, treated or not with nifurtimox and dipyridamole. Tissue sections that were fixed with 4% paraformaldehyde, cryopreserved with 30% sucrose, embedded in OCT, and stained with toluidine blue are shown. In A, a representative Control Chagas heart section is shown, where two mast cells with degranulation in process can be seen (see arrow, 1000x magnification). In B, a representative heart section from a mouse treated with nifurtimox and dipyridamole is shown, where mast cells are not present (400Xmagnification). In the upper right corner, a bar graph is shown with the average number of mast cells in 10 fields and SEM values obtained in heart sections of the Chagas Control (white bar) and Nif-Dip (black bar) groups.*

The trypanocidal effect of dipyridamole should also be considered, as this effect has been reported on epimastigotes in axenic culture with an IC_{50} of 372 μM; this effect was partially reproduced while working with NMRI mice, where this drug decreased, but not cleared parasitemia.[9] However, we propose that dipyridamole most beneficial effect is functional and it is related to its action mechanism.

The microvascular theory suggests that Chagas myocardiopathy development reflects endothelium damage in the microcirculation, with the formation of platelet and blood thrombi, causing diffuse ischemia in cardiac tissue, which then causes necrosis, cardiac remodeling, and fibrosis, leading to the development of dilated cardiomyopathy with CHF.[14] Because dipyridamole is an antiplatelet agent, it prevents platelet thrombus formation and, consequently, hematic ones; also by increasing cGMP and adenosine levels, it induces coronary vasodilation mediated by NO production, improving ischemic phenomena in the chagasic myocardium. Similarly, by increasing levels of adenosine acting on A1- and A3-receptors, it has a cardioprotective effect that allows better management of the failing heart's energy demands and avoids early apoptotic phenomena, respectively.[15]

Likewise, it has been reported that endogenous adenosine acting on A1-receptors generate a chronotropic and dromotropic negative effect, reducing the incidence of ventricular arrhythmias caused by ischemia-reperfusion in isolated beating rat hearts,[16] and when acting on A2-receptors it has an antiarrhythmic effect in acute myocardial ischemia, thus reducing the frequency of ventricular fibrillation;[17] therefore it could prevent lethal arrhythmias observed in Chagas cardiomyopathy. Also in this line of thought, it has been reported that long-term oral administration of dipyridamole improves physical and cardiac status of patients with mild to moderate CHF, resulting in echocardiographic improvements of ejection fraction, left ventricular systolic diameter, and plasma B-type natriuretic peptide level.[18]

Recently, Ramakers et al.[19] reported that dipyridamole treatment increases the levels of IL-10, which is considered an anti-inflammatory cytokine that can induce TNF-α and IL-6 decrease. Increased levels of TNF-α and IL-6 have been associated with ChD advanced stages and negatively correlated with cardiac function;[20,21] while high levels of IL-10 have been associated with cardiac function recovery, ejection fraction improvement and a reduction in left ventricular diastolic diameters, therefore being a cardioprotective factor. In the indeterminate phase of ChD, elevated levels of IL-10 have been reported, whereas in patients who develop CChM these levels are decreased.[22]

Evidence suggests that during *T. cruzi* infection, the myocardium is exposed to lesions caused by continuous oxidative stress through the production of reactive oxygen species, which are released continuously as a result of mitochondrial injuries during Chagas cardiomyopathy progression.[23] The molecular structure of dipyridamole allows it to accept electrons, thus functioning as a free radical scavenger, with a greater capacity than α-tocopherol and ascorbic acid, suggesting that dipyridamole beneficial effect observed in the present study is associated with its antioxidant capacity.[7]

In this study, the early mortality observed in all experimental groups can be explained by cardiac functional impairment caused by AChM, which was aggravated by subjecting the mice to general anesthesia. In vigil state, mice usually have a heart rate of around 580 bpm, while under anesthesia with pentobarbital-ketamine, as reported in the present study, it ranges between 373 and 382 bpm. Anesthesia-induced bradycardia decreases cardiac output and aggravates HF, thereby increasing death risk, which was evidenced in the CC group mortality. In contrast, mice treated with nifurtimox and dipyridamole had the lowest mortality, which could be related to those previously described dipyridamole effects (see above). Unexpectedly, the Nif-Dip-CHF group had a higher mortality than the Nif-Dip group; this can be explained considering that the administration of drugs such as digoxin to a vulnerable host, the risk of cardiac arrhythmias and sudden death is increased, as it has been shown in humans by Madelaire et al.,[24] who reported that digoxin use was associated with increased risk of death in patients with chronic heart failure.

Experimental results obtained in the present study confirmed that all NMRI mice with acute ChD showed repolarization abnormalities, which were improved by the therapeutic scheme. Alvarado et al.[25] stated that repolarization disorders are characteristic signs of AChM in humans and NMRI mice, which gives them a diagnostic value for acute ChD, clearly demonstrated in the present work. Ventricular repolarization disorders have been associated with ischemia and ventricular overload.[26]

As nifurtimox has a trypanocidal effect potentiated by dipyridamole, it would quickly eradicate the infection, which would reverse the inflammatory phenomena related to parasitism, thus improving cardiac function; however, the adjuvant effect of dipyridamole must be crucial, because it reverses the functional ischemic phenomena, causing coronary vasodilation and improving the energy balance of cardiomyocytes, allowing the transmembrane ionic equilibrium to be restored and thus normalizing the repolarizing electrical phenomena.

Coincident with repolarization disorders, an increase in the S wave amplitude was observed in the present study, which was reversed with the therapeutic scheme. Few studies have defined the S wave electrogenic substrate; however, in humans, the S wave reflects a late depolarization of the diaphragmatic surface or Purkinje cell depolarization.[27] Nonetheless, in mice, given the high density of early repolarizing currents, it is proposed that S wave also reflects an early repolarizing component,[28] and thus, as repolarization disorders improves, the S wave amplitude decreases, as shown in the present study.

On the other hand, slowed intraventricular conduction reflected in QRS and QT values improved significantly after the therapeutic scheme was applied; however, atrioventricular (AV) conduction values reflected by PR length did not show improvement. Improvement of intraventricular conduction may reflect an inflammation and blood flow restoration in a reversible morphofunctional substrate, while irreversible AV conduction disorders, including arrhythmias such as atrial fibrillation, which seems to be a reflection of an irreversible damage, product of intense inflammation, remodeling and fibrosis of the atria, usually observed in ChD.[29]

Likewise, the R wave amplitude was unchanged, which might be due to irreversible damage to the myocardial structure or it could reflect a net pharmacological effect due to dipyridamole-induced negative inotropic effect mediated by adenosine acting on A1 receptors.[9]

Mast cell density in heart tissue histological sections was higher in the CC group when compared to the Nif-Dip group. Mast cells are inflammatory cells that increase and trigger a series of responses to inflammatory and infectious stimuli involved in myocarditis and dilated cardiomyopathy pathogenesis; a correlation between the degree of fibrosis and mast cell density in hearts of patients with dilated cardiomyopathy with CHF has been reported.[30] In this regard, Rork et al.[31] (2008) reported that mast cell degranulation contributes to myocardial injury observed in ischemia-reperfusion protocols and adenosine receptors (A2A) activation reduced the infarct area.

Conclusion

In conclusion, this study showed that treatment with nifurtimox (40 mg/kg) and dipyridamole (30 mg/kg) has a beneficial effect on NMRI albino mice with acute ChD and CHF. The effect of nifurtimox is related to their trypanocidal activity, while the effect of dipyridamole can be related to its ability to potentiate nifurtimox trypanocidal effect and to its capacity to reverse the pathophysiological phenomena related to AChM, such as microvascular ischemia, free radical generation, immunomodulation of inflammation and cardiac overload. Dipyridamole safety, experience based on its use and wide therapeutic window constitute a valuable advantage for its inclusion in future ChD therapeutic protocols in humans.

Author contributions

Conception and design of the research: Cabarcas RB. Acquisition of data: Aparicio DY, González-Hernández M, Hernández-Forero G, Guédez-Ortiz M, Santeliz S, Goncalves L, Cabarcas RB. Analysis and interpretation of the data: Aparicio DY, González-Hernández M, Santeliz S, Goncalves L, Cabarcas RB. Statistical analysis: Aparicio DY, González-Hernández M, Santeliz S, Cabarcas RB. Obtaining financing: Cabarcas RB. Writing of the manuscript: Aparicio DY, González-Hernández M, Hernández-Forero G, Guédez-Ortiz M, Santeliz S, Goncalves L, Cabarcas RB. Critical revision of the manuscript for intellectual content: Aparicio DY, González-Hernández M, Hernández-Forero G, Guédez-Ortiz M, Santeliz S, Goncalves L, Cabarcas RB. Supervision: Santeliz S, Goncalves L, Cabarcas RB.

Potential Conflict of Interest

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

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Study Association

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References

1. World Health Organization. (WHO). Chagas disease in Latin America: An epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015;90(6):33-43.
2. Requena A, Aldasoro E, de Lazzari E, Sicuri E, Brown M, Moore D, et al. Prevalence of Chagas disease in Latin-American migrants living in Europe: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2015;9(2):1-15.
3. Forsyth C, Hernandez S, Olmedo W, Abuhamidah A, raina M, Sanchez D, et al. Safety Profile of Nifurtimox for Treatment of Chagas Disease in the United States. Clin Infect Dis. 2016;63(8):1056-62.
4. Toso A, Vial F, Galanti N. Oral transmission of Chagas' disease. Rev Méd Chile. 2011;139(2):258-66.
5. Noya B, Díaz Z, Colmenares C, Ruiz R, Mauriello L, Muñoz A, Noya O. Update on oral Chagas disease outbreaks in Venezuela: epidemiological, clinical and diagnostic approaches. Mem Inst Oswaldo Cruz. 2015;110(3):377-86.
6. Inglessis I, Carrasco H, Añez N, Fuenmayor C, Parada H, Pacheco J, et al. Clinical, parasitological and histopathologic follow-up studies of acute Chagas patients treanted with benznidazole. Arch Inst Cardiol Mex. 1998;68(5):405-10.
7. Kim H, Liao J. Translational therapeutics of dipyridamole. Arterioscler Thromb Vascular Biol. 2008;28(3):e39-e42.
8. Schaper W. Dipyridamole, an underestimated vascular protective drug. Cardiovasc Drugs Ther. 2005;19(5):357-63.
9. Santeliz S, Caicedo P, Giraldo E, Alvarez C, Yustiz MD, Rodriguez-Bonfante C, et al. Dipyridamole potentiated trypanocidal effect of nifurtimox and improved cardiac function in NMRI mice with acute chagasic myocarditis. Mem Inst Oswaldo Cruz. 2016 [In Press].
10. Bustamante JM, Craft JM, Crowe BD, Ketchie SA, Tarleton RL. New, combined, and reduced dosing treatment protocols cure Trypanosoma cruzi infection in mice. J Infect Dis. 2014;209(1):150-62.
11. Wong-Baeza C, Nogueda-Torres B, Serna M, Meza-Toledo S, Baeza I, Wong C. Trypanocidal effect of the benzyl ester of N-propyl oxamate: a bi-potential prodrug for the treatment of experimental Chagas disease. BMC Pharmacol Toxicol. 2015;16:10.
12. Andrade SG, Magalhães JB, Pontes A. Evaluation of chemotherapy with benznidazole and nifurtimox in mice infected with Trypanosoma cruzi strains of different types. Bull World Health Organ.1985;63(4):721-6.
13. Faúndez M, López-Muñoz R, Torres G, Morello A, Ferreira J, Kemmerling U, et al. Buthionine sulfoximine has anti-Trypanosoma cruzi activity in a murine model of acute Chagas' disease and enhances the efficacy of nifurtimox. Antimicrob Agents Chemother. 2008;52(5):1837-9.
14. Rossi M, Tanowitz H, Malvestio L, Celes M, Campos E, Blefari V, et al. Coronary microvascular disease in chronic Chagas cardiomyopathy including an overview on history, pathology, and other proposed pathogenic mechanisms. PLoS Negl Trop Dis. 2010;4(8):e674.
15. Hussain A, Gharanei A, Nagra A, Maddock H. Caspase inhibition via A3 adenosine receptors: a new cardioprotective mechanism against myocardial infarction. Cardiovasc Drugs Ther. 2014;28(1):19-32.
16. Lee Y, Chern J, Yen M. Antiarrhythmic effects of BN-063, a newly synthesized adenosine A1 agonist, on myocardial ischaemia in rats. Br J Pharmacol. 1994;112(4):1031-6.
17. Schreieck J, Richardt G. Endogenous adenosine reduces the occurrence of ischemia-induced ventricular fibrillation in rat heart. J Mol Cell Cardiol. 1999;31(1):123-34.
18. Sanada S, Asanuma H, Koretsune Y, Watanabe K, Nanto S, Awata N, et al., Long-term oral administration of dipyridamole improves both cardiac and physical status in patients with mild to moderate chronic heart failure: a prospective open-randomized study. Hypertens Res. 2007;30(10):913-9.
19. Ramakers B, Riksen N, Stal T, Heemskerk S, van den Broek P, Peters W, et al. Dipyridamole augments the antiinflammatory response during human endotoxemia. Critical Care. 2011;15(6):R289.
20. López L, Arai K, Giménez E, Jiménez M, Pascuzo C, Rodríguez-Bonfante C, C-reactive protein and interleukin-6 serum levels increase as Chagas disease progresses towards cardiac failure. Rev Española Cardiol. 2006;59(1):50-6.
21. D'Angelo Mendoza E, Rodríguez Bonfante C, Camacho I, Martínez J, Perdomo T, Cabrera A, et al. [Patients suffering dilated chagasic cardiopathy or non chagasic cardiopathy show an increased levels of tumor necrosis factor alpha]. Invest Clin. 2005;46(3):229-40.
22. Sousa GR, Gomes JA, Fares RC, Damásio MP, Chaves AT, Ferreira KS, et al. Plasma cytokine expression is associated with cardiac morbidity in Chagas disease. PLoS One.2014;9(3):e87082.
23. Vyatkina G, Bhatia V, Gerstner A, Papaconstantinou J, Garg N. Impaired mitochondrial respiratory chain and bioenergetics during chagasic cardiomyopathy development. Biochim Biophys Acta. 2004;1689(2):162-73.
24. Madelaire C, Schou M, Nelveg-Kristensen K, Schmiegelow M, Torp-Pedersen C, Gustafsson F, et al. Use of digoxin and risk of death or readmission for heart failure and sinus rhythm: a nationwide propensity score matched study. Int J Cardiol. 2016;221:944-50.
25. Alvarado-Tapias E, Miranda-Pacheco M, Rodríguez-Bonfante C, Velásquez G, Loyo J, Gil-Oviedo M, et al. Electrocardiography repolarization abnormalities are characteristic signs of acute chagasic cardiomyopathy. Invest Clin. 2012;53(4):378-94.
26. De Micheli A, Medrano G. On the electrophysiopathological concept and ECG manifestations of ischemia, injury and necrosis. Arch Cardiol Mex. 2009;79(1):2-4.
27. Ashley E, Niebauer J. Cardiology explained. London: Remedica; 2004. Chap. 4. [Cited in 2016 Feb 10]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2204/
28. Boukens B, Rivaud M, Rentschler S, Coronel R. Misinterpretation of the mouse ECG: ''musing the waves of Mus musculus''. J Physiol. 2014;592(21):4613-26.
29. Novaes R, Penitente A, Gonçalves R, Talvani A, Peluzio M, Neves C, et al. Trypanosoma cruzi infection induces morphological reorganization of the myocardium parenchyma and stroma, and modifies the mechanical properties of atrial and ventricular cardiomyocytes in rats. Cardiovasc Pathol. 2013;22(4):270-9.
30. Batlle M, Pérez-Villa F, Lázaro A, Garcia-Pras E, Ramirez J, Ortiz J, et al. Correlation between mast cell density and myocardial fibrosis in congestive heart failure patients. Transplant Proc. 2007;39(7):2347-9.
31. Rork T, Wallace K, Kennedy D, Marshall M, Lankford A, Linden J. Adenosine A2A receptor activation reduces infarct size in the isolated, perfused mouse heart by inhibiting resident cardiac mast cell degranulation. Am J Physiol Heart Circ Physiol. 2008;295(5):H1825-33.