REVIEW

ROS and *Trypanosoma cruzi*: Fuel to infection, poison to the heart

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Abstract

The activation of macrophage respiratory burst in response to infection with *Trypanosoma cruzi* inflicts oxidative damage to the host's tissues. For decades, the role of reactive oxygen species (ROS) in the elimination of *T. cruzi* was taken for granted, but recent evidence suggests parasite growth is stimulated in oxidative environments. It is still a matter of debate whether indeed oxidative environments provide ideal conditions (e.g., iron availability in macrophages) for *T. cruzi* growth and whether indeed ROS signals directly to stimulate growth. Nitric oxide (NO) and ROS combine to form peroxynitrite, participating in the killing of phagocytosed parasites by activated macrophages. In response to infection, mitochondrial ROS are produced by cardiomyocytes. They contribute to oxidative damage that persists at the chronic stage of infection and is involved in functional impairment of the heart. In this review, we discuss how oxidative stress helps parasite growth during the acute stage and how it participates in the development of cardiomyopathy at the chronic stage.

OPEN ACCESS

Citation: Paiva CN, Medei E, Bozza MT (2018) ROS and *Trypanosoma cruzi*: Fuel to infection, poison to the heart. PLoS Pathog 14(4): e1006928. <https://doi.org/10.1371/journal.ppat.1006928>

Editor: Marc-Jan Gubbels, Boston College, UNITED STATES

Published: April 19, 2018

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Funding: Our work was supported by Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Shifting the old paradigm

It was back in 1968 that the production of reactive oxygen species (ROS) by phagocytes during respiratory burst was proposed as a killing mechanism of pathogens [1]. The respiratory burst of phagocytes quickly became the most well-studied mechanism of microbial killing, and by the late 1970s to the early 1980s, *T. cruzi* was assumed to be killed by ROS from macrophages [2–5], despite contradictory evidence [6–9] that went unnoticed for a decade.

In 2012, *T. cruzi* was found to grow under oxidative conditions both in macrophages and in vivo, and antioxidants were detrimental to its growth [10]. Since then, several publications have followed this idea. Apparently, the killing of *T. cruzi* by ROS from respiratory burst was established as a paradigm in the literature on weak grounds [6, 7, 10]. Here, we bring together important works approaching how ROS production by the host promotes infection and how it contributes to Chagas heart disease at the chronic stage.

A confounding factor in the study of the relationship between ROS and *T. cruzi* is the finding of trypanocidal drugs that cause oxidative stress. The trypanocidal activity of benznidazole, for instance, was credited to benznidazole's capacity to cause oxidative damage to the parasite [11]. However, benznidazole failed to generate ROS in the presence of *T. cruzi* extracts in vitro [12].

Accordingly, benznidazole's trypanocidal actions were assigned to the reduction of its nitro group by trypanosomal type I nitroreductase [13], followed by the covalent linking of reduced benznidazole to internal thiols (e.g., trypanothione) [14] and generation of DNA-toxic glyoxal adducts [15] in an oxygen-insensitive reaction. Although the full mechanism of action of benznidazole remains to be elucidated, these results indicate that ROS are not involved in benznidazole's trypanocidal effects. Benznidazole also activates nuclear erythroid factor-2 (Nrf2) and protects the host from oxidative damage in a delayed time frame [16].

What also has been found confusing is the decreased infectivity found in parasites unable to degrade ROS or repair oxidative damage [17–19]. Unguarded parasites succumb to oxidative damage instead of harvesting the potential benefits of living in an oxidative environment, such as the availability of iron found inside macrophages under oxidative stress, or the proliferation in response to ROS signaling. The antioxidant enzymes expressed by *T. cruzi* are crucial to defending against oxidative damage, allowing the parasite to thrive in oxidative conditions [20–22].

Evidences in favor of ROS as a promoter of *T. cruzi* infection

Increased production of ROS and the resulting oxidative damage have been largely documented during both acute and chronic stages of *T. cruzi* infection with strains from different discrete typing units (DTUs) [23–26], and have been ascribed to NADPH oxidase 2 (NOX2) activation in unstimulated infected macrophages [25, 27]. Thus, protection against oxidative stress has the potential to reduce tissue damage in Chagas disease.

Since even low numbers of parasites cause progressive tissue damage, inducing disease tolerance against oxidative damage was tested as a useful strategy to treat Chagas disease [28, 29]. Since the beginning of infection, mice were treated with cobalt protoporphyrin (CoPP), an antioxidant that induces disease tolerance to malaria mostly by inducing heme oxygenase (HO-1) expression through Nrf2 activation [27, 30]. Even though we expected antioxidants to increase parasite burden, they did not: CoPP actually reduced parasite burden. Parasitemia and macrophages, heart, and skeletal muscle parasitism all reacted similarly to CoPP. No known effects of HO-1 induction [31–33] could explain this reduction of parasitism. What is more, no evidence was found that CoPP affected trypomastigote viability [27].

The first study launched to systematically assess the effects of oxidative stress on the parasite burden during acute *T. cruzi* infection contradicted the paradigm of trypanocidal ROS. Treatment with CoPP reduces the parasite burden of infected macrophages from HO-1-knockout but not from Nrf2-knockout mice, while transfection of THP-1 macrophages with either Nrf2 or HO-1 reduces the parasite burden (Fig 1D); these two observations indicate that the effects of CoPP depend on the redundant action of Nrf2-controlled genes [27]. Incubation of infected macrophages with several antioxidants (NOX2-inhibitor apocynin, glutathione-replenisher N-acetyl-L-cysteine [NAC], polyphenols resveratrol and pterostilbene, biliverdin, bilirubin, catalase-polyethylene glycol [PEG], or superoxide dismutase [SOD]-PEG) reduces parasite burden, while respiratory burst-inducer phorbol 12-myristate 13-acetate (PMA), pro-oxidant paraquat, and H₂O₂ increase it (Fig 1B and 1C). Macrophages taken from *gp91^{phox-/-}* (which lacks NOX2) and infected in vitro have smaller parasitism than their wild-type counterparts [17, 27]. When *gp91^{phox-/-}*-infected macrophages are incubated with H₂O₂, the infection index increases [17], indicating that H₂O₂ supplies an additional proliferative stimulus missing in *gp91^{phox-/-}* macrophages (Fig 1E and 1F). Peritoneal macrophages taken from in vivo infected *gp91^{phox-/-}* mice have decreased parasite burden, and parasitemia was moderately decreased [27]. This latter result, however, was not confirmed in another work [17]. A general picture that emerges from these studies shows that ROS fuel *T. cruzi* infection in macrophages during the early acute stage. In fact, this was not the first work to show that ROS could favor an infection; they do fuel also other types of infection [10].

Fig 1. Oxidative stress fuels *T. cruzi* infection in macrophages, fibroblasts, and cardiomyocytes. (a) Parasite burden of nonactivated C57BL/6 macrophages infected with CL-Brener (type VI) or Y strain (type II) trypomastigotes and cultivated in medium for 48 h. (b) Infected macrophages incubated with H_2O_2 , PMA, or pro-oxidants have increased parasite burden [17, 27]. A similar result is obtained if parasites are treated with H_2O_2 before infection [17]. (c) Infected macrophages incubated with antioxidants have decreased parasite burden. (d) THP-1 human macrophage lineage transfected with an Nrf2- or HO-1-containing plasmid before infection presents smaller parasite burden than cells transfected with an empty expression plasmid [27]. (e) Infected $gp91^{phox-/-}$ macrophages have smaller parasite burden than wild-type macrophages [17, 27]. (f) Trypomastigotes treated with H_2O_2 before infecting $gp91^{phox-/-}$ macrophages give rise to parasite burden similar to that of nontreated trypomastigotes growing in wild-type macrophages, as in (a) [17]. (g) Trypomastigotes treated with H_2O_2 before infecting fibroblasts also give rise to increased parasite burden [50]. (h) Cardiomyocytes infected with the JG strain (type II) respond to CAT-PEG with decreased burden compared to nontreated cells, while cardiomyocytes infected with Col1.7G2 (type I) are unresponsive to CAT-PEG [57]. CAT-PEG, catalase conjugated to polyethylene glycol to permeate the cells; HO-1, heme oxygenase; JG, type II *T. cruzi* strain; Nrf2, nuclear erythroid factor-2; PMA, phorbol 12-myristate 13-acetate.

<https://doi.org/10.1371/journal.ppat.1006928.g001>

A question still to be answered is whether ROS can reach high concentrations inside invaded macrophages capable of killing or restraining amastigote growth instead of stimulating their proliferation. Although trypomastigotes escape the phagosome, H_2O_2 can diffuse through the cytoplasm and reach low micromolar concentrations [34]. The incubation of infected macrophages with up to 100 μM H_2O_2 promotes a more intensive amastigote proliferation, but the concentrations of H_2O_2 that reach the cytosol are unknown. Taking the cytosolic concentration of H_2O_2 during macrophage respiratory burst as a proxy, it would be within the 1–4 μM range [35, 36]. In fact, when macrophages infected with *T. cruzi* are activated with PMA, they give rise to a greater parasite burden [27], indicating that the concentrations reached during the respiratory burst are stimulatory. When trypomastigotes are directly incubated with H_2O_2 before invading $gp91^{phox/-}$ macrophages, they give rise to an increased amastigote burden at 100 μM , which demonstrates that trypomastigotes not only resist direct exposure to high H_2O_2 concentrations but also differentiate into amastigotes that preserve the memory of this encounter and grow more intensively [17]. However, incubation with H_2O_2 at 300 μM results in reduced parasite burden. Although no data on the effects of this concentration of H_2O_2 on macrophage invasion or parasite differentiation are available [17], we believe that this result is due to parasite elimination by direct oxidative damage. Nevertheless, a concentration of H_2O_2 as high as 300 μM is unlikely to occur in vivo [37], which suggests that the lethal oxidative damage of wild-type *T. cruzi* does not occur in physiological conditions.

In contrast to the early acute phase, by the end of the acute phase, splenic parasitism of $gp91^{phox/-}$ mice infected with Y strain increased [38]. Unlike C57BL/6 wild-type controls, $gp91^{phox/-}$ mice did not survive the infection [27, 38]. Since the extension of the treatment with the antioxidant CoPP until the end of the acute phase was counterproductive to the control of parasite burden and killed wild-type mice [27], we speculate that later, antioxidant therapy might impair the establishment of adaptive immunity. A general impairment of lymphocyte proliferation seems to accompany all ROS-deficient conditions in acute Chagas disease [24, 25, 27], and the establishment of adaptive immunity is fundamental to control parasitemia. The role played by ROS-producing macrophages at the chronic stage of infection has not been evaluated, but it is likely that most of them are activated by interferon gamma ($IFN\gamma$) and producing trypanocidal nitric oxide (NO) and peroxynitrite.

The idea that oxidative stress promotes parasitism of *T. cruzi* may help to explain some results reported in the literature. Cruzipain is an enzyme that increases the susceptibility of macrophages to *T. cruzi* infection [39] and is a major inducer of NOX2 activation during macrophage infection [40]. The genetic absence of signaling lymphocytic activation molecule family member 1 (*Slamf1*), a condition that reduces NOX2 activation in myeloid cells, was found to increase resistance to *T. cruzi* infection [41]. Aryl hydrocarbon receptor (*Ahr*) is a gene known to promote ROS production in response to infections such as listeriosis [42]; its genetic absence decreases *T. cruzi* parasite burden as well as ROS production in infected macrophages [43]. Also, the treatment of infected ES mice with melatonin [44] or curcumin [45], both known to activate antioxidant defenses, reduced parasite burden in blood and heart tissue. These results are all in agreement with the general notion that ROS promotes *T. cruzi* infection, as suggested many years ago [6–9].

Mechanisms underlying *T. cruzi* growth in oxidative environment

The mechanisms by which ROS promote *T. cruzi* infection are still to be fully learned. Antioxidants up-regulated H-ferritin (H-Ft, a cytosolic protein that binds iron) and ferroportin-1 (Fpn-1, a channel that allows iron efflux) expression, decreasing labile iron pool (LIP) in macrophages (Fig 2). Increasing LIP by several means resulted in increased parasite burden, while reducing

Fig 2. LIP is associated with oxidative conditions and parasite growth in macrophages. Treatment of infected macrophages (Y strain) with antioxidants (NAC, apocynin, CoPP) reduces ROS content, LIP, and parasite burden, while reducing the expression of H-Ft and increasing the expression of the iron channel Fpn-1 [27]. CoPP, cobalt protoporphyrin; Fpn-1, ferroportin-1; H-Ft, H-ferritin; LIP, labile iron pool; NAC, N-acetyl-L-cysteine; ROS, reactive oxygen species.

<https://doi.org/10.1371/journal.ppat.1006928.g002>

LIP had the opposite effect. Iron also increased the parasite burden of infected *gp91^{phox-/-}* macrophages to an extent similar to that found in wild-type mice. These results point to the regulation of LIP as the mechanism underlying the increase in macrophage parasitism produced by oxidative stress. However, the research did not study whether the manipulation of LIP could work by interfering with oxidative stress itself, thereby stimulating *T. cruzi* proliferation.

Several research groups have explored the possibility that the oxidative environment is itself a direct stimulus to the growth of *T. cruzi*. Parasites proliferated in response to H₂O₂ [46]. The increased growth of epimastigotes from type I Dm28c clone in response to H₂O₂ involved a calmodulin-dependent protein kinase II (CAMKII)-like enzyme in the pathway to proliferation [47], and the mitochondrial ROS resulting from heme metabolism enhanced the growth [48]. The exposure of epimastigotes to different reduction-oxidation (redox) state environments was evaluated in vitro and in the gut of the insect vector: Antioxidants reduced proliferation and increased metacyclogenesis, while pro-oxidants had the opposite effect [49]. Trypomastigotes responded to incubation with H₂O₂ before the infection, giving rise to greater amastigote burden after they invaded *gp91^{phox-/-}* macrophages (Fig 1F) or fibroblasts [50] (Fig 1G). Altogether, these results indicate that ROS directly affect parasites.

Resistance to oxidative stress confers an advantage to parasites. While the incubation of infected macrophages with catalase-PEG reduces parasitism [17, 18, 27], parasites expressing catalase give rise to a far greater parasite burden in macrophages and in mice than wild-type parasites [18]. Parasites that overexpress an enzyme that hydrolyzes 8-oxo-dGTP (MutT) are more capable of withstanding oxidative damage to their DNA, presenting both an increased growth in macrophages, fibroblasts, and increased overall parasite burden in mice [17, 18, 50], as well as responding with an increased growth to H₂O₂ exposure prior to invading fibroblasts [50]. The incubation with catalase-PEG reduces the growth of these parasites in macrophages more than of wild-type parasites [18]. These results reinforce the idea that the better armed against oxidative stress *T. cruzi* parasites are, the more they reduce their growth in response to a less oxidative environment [50].

We speculate that two possible evolutionary pressures that forced the selection of *T. cruzi* proliferative response were the increased availability of nutrients (such as labile iron) and the decreased activation of efficient trypanocidal mechanisms under oxidative stress; these pressures could have possibly interacted. Also, we emphasize that ROS affect *T. cruzi* growth inside only unstimulated macrophages and thus are more likely to be relevant at the very beginning of infection, when the production of IFN γ is still low and most macrophages are not activated to produce NO.

The case for peroxynitrite as a main trypanocidal species in activated macrophages

Peroxynitrite is a highly lethal weapon used by phagocytes against pathogens. It is formed when there is simultaneous production of NO and ROS [10]. When infected with *T. cruzi*, unstimulated macrophages produce little NO [7, 17, 51]. Radi and colleagues showed that in unstimulated, CL-Brener-infected macrophages, parasites remained well-preserved, although nitroblue tetrazolium was reduced, and superoxide and H₂O₂ predominated within the phagosomes [52]. They detected high amounts of peroxynitrite only when macrophages were infected after activation with lipopolysaccharide (LPS)/IFN γ , indicating that for these species to be present, additional factors must send a signal for NO production. Inside the peroxynitrite-producing macrophages, the authors observed peroxynitrite-dependent protein oxidation and damaged parasites (Fig 3).

Alvarez et al. also used a modified parasite that overexpressed a peroxynitrite-degrading enzyme to infect unstimulated macrophages, triparedoxin peroxidase (TcCPX). It grew

Fig 3. In macrophages activated before infection, ROS is detrimental to *T. cruzi* infection. Macrophages activated with LPS and IFN γ before infection eliminate phagocytosed *T. cruzi* CL-Brener by producing peroxynitrite. When the production of peroxynitrite is impaired by inhibiting NOX2-derived ROS with apocynin (right before the infection or after) or when *T. cruzi* expresses TcCPX, an enzyme that degrades peroxynitrite, the resulting parasite burden is increased [52]. IFN γ , interferon gamma; LPS, lipopolysaccharide; ROS, reactive oxygen species; TcCPX, tripartedoxin peroxidase.

<https://doi.org/10.1371/journal.ppat.1006928.g003>

similarly to wild-type parasites [52]. However, it produced an enhanced infection in IFN γ -activated macrophages and in both heart parasitism and the peak of parasitemia in mice, suggesting that when the immune system is activated in vivo, peroxynitrite production is probably an important mechanism of parasite killing.

In general, simultaneous ROS and NO production is associated with *T. cruzi* killing inside activated macrophages [53]. The differences in the role of ROS between unstimulated and activated macrophages mean that the activation of a naïve invaded macrophage is different from the active phagocytosis of *T. cruzi*. We assume that in non-redox-manipulated macrophages, peroxynitrite is the most likely trypanocidal species.

Counterevidence for oxidative stress as a promoter of *T. cruzi* infection

During the course of acute infection with clone Sylvio X10/4, treatment with NOX-inhibitor apocynin increased parasite burden in blood, skeletal muscles, and hearts of mice [24]. Similarly,

in the genetic absence of $p47^{\text{phox}}$, a subunit responsible for NOX2 translocation to the membrane upon activation, Sylvio X10/4 parasites infected a higher percentage of bone marrow macrophages, besides leading to greater parasitemia and heart parasitism of mice [25]. These results greatly differ from those in which peritoneal macrophage parasitism decreased [27] and splenic parasitism decreased [17] in $gp91^{\text{phox-/-}}$ (NOX2-deficient) mice infected with either Y strain and with clone CL-Brener. It is still unclear whether the differences are due to the distinct functions of these NOX2-subunits or to different responses of *T. cruzi* DTU types.

The association between high levels of ROS and reduced parasite burden in infected macrophages from $Nlrp3^{-/-}$ mice was interpreted as an evidence for the contribution of ROS to *T. cruzi* (Sylvio X10/4) killing [54]. Other authors found that ROS were not involved in the control of interleukin 1 beta (IL-1 β) production and that the genetic absence of $Nlrp3^{-/-}$ in macrophages infected with Y strain increased parasitism [55]. The apparent reduction of parasite burden in THP-1 macrophages infected with Sylvio X10/4 and incubated with antioxidants apocynin and NAC seems to argue against the idea that ROS are involved in type I *T. cruzi* killing [54]. Still, no systematic study has been conducted that would help us understand the role of ROS in type I parasite proliferation/elimination.

Heart parasite burden under different redox status

Heart parasite burden may vary after treatment with antioxidants. Assessing the effects of oxidative stress on heart parasite burden is difficult because the published studies are based on different animal models, different stages of infection, and different *T. cruzi* strains. Neither BALB/c mice chronically infected with type I Colombian strain and treated with the SOD-mimetic antioxidant tempol [56] nor rats infected with type I Sylvio X10/4 strain and treated since the beginning of infection until the chronic phase with ROS-scavenger phenyl- α -tert-butyl (PBN) [23] presented any changes in heart parasite burden. However, BALB/c chronically infected with Colombian strain and treated with resveratrol had reduced heart parasite burden [56]. C57BL/6 mice acutely infected with type II Y strain and treated with CoPP had reduced parasite burden [27], while C3H/HeN mice infected with Sylvio X10/4 and treated with apocynin until chronic stage had increased parasite burden [24]. To explain these results, we should be able to distinguish between different effects of antioxidants at different stages of the disease or different behaviors of type I versus II *T. cruzi*.

Recently, Andrade and colleagues showed that the parasite burden of cardiomyocytes infected with type II (JG strain) decreased after treatment with antioxidant enzyme catalase (conjugated to PEG in order to permeate cells), while that of cardiomyocytes infected with type I (Col1.7G2, a clone obtained from Colombian strain), did not [57] (Fig 1H). These results could explain the differences in heart parasite burden after treatment of different infections with antioxidants [25, 27, 56]. Also, resveratrol's trypanocidal action [58, 59] rather than its antioxidant action may be responsible for the reduction of the parasite burden resveratrol induces at the chronic [56] and acute stages [27].

The role of oxidative stress in chronic phase cardiomyopathy

Chagas heart disease develops many years after the vector infects the host in about 30%–40% of the patients. A connection between heart disease and infection is still elusive. Most researchers believe Chagas heart disease occurs after cumulative, irreversible tissue destruction. They disagree, however, about the reasons for this tissue damage. Recently, the BENEFIT clinical trial of benznidazole at the chronic stage of Chagas disease has revealed that once established, not even a parasitological cure could prevent the progression of heart disease [60], demonstrating that direct parasite-mediated damage is not responsible for progression.

A series of works succeeded in preventing [61, 62] and even reversing [56] established functional heart disease with antioxidant therapy in mice, independently of the therapy's effects on heart parasite burden. These results suggest that Chagas cardiomyopathy is, at least partially, a ROS-dependent pathology [63] (Figs 4 and 5). In cardiomyocytes, ROS elicits production of tumor necrosis factor (TNF) and IL-1 β [64], cytokines that may affect heart function [65–67]. ROS may also represent a funnel to which the pathways of several injury-causing factors (such as autoantibodies with adrenergic activities [68–71]) converge to disturb the myocardium physiology, possibly through CAMKII oxidation [69, 72, 73].

Activities of mitochondrial respiratory complexes are depressed in chagasic patients, a phenomenon associated with increased ROS production [74]. Superoxide and H₂O₂ are intensively produced in the mitochondria of mice chronically infected with Sylvio X10/4, a process resulting from electron leakage at the respiratory chain [75]. Conversely, treatment with the ROS-scavenger antioxidant PBN improved respiratory chain function and reduced electron leakage in the myocardium of infected mice [76]. In fact, mitochondrial oxidative metabolism was preserved in the hearts of chronically infected mitochondrial SOD (SOD2) superexpressing mice [77]. Altogether, these data indicate that ROS production and mitochondrial dysfunction intertwine in a positive feedback loop in Chagas disease.

Because of oxidative stress, oxidative adducts are found in the heart tissues of both the experimental animals and the chagasic patients [23, 62, 78]. The genetic superexpression of the antioxidant SOD2 preserves mitochondrial structure, abrogates oxidative damage, and inhibits fibrosis and the infiltration of mononuclear cells into heart tissue in the Sylvio X10/4 model [79]. These processes demonstrate that mitochondrial ROS are involved in several of the pathological events of heart disease.

Acute *T. cruzi* infection of mice with Sylvio X10/4 clone gave rise to oxidized patterns in myocardium, which could be prevented by treatment with NOX-inhibitor apocynin since day 0 of infection [24] (Fig 4), but the treatment actually increased parasite burden at the acute phase. When the treatment was extended to the chronic phase, no increase was observed in heart weight, cardiomyocyte size, hypertrophy markers, lipid content, and fibrosis. Based on these results, the authors proposed that NOX-derived ROS are responsible for inflammation/cardiac remodeling and for the control of parasite burden in Chagas disease. On the other hand, treatment of infected rats with ROS-scavenger PBN since day 0 of infection did not interfere with chronic parasite burden and only slightly decreased inflammatory infiltrates, but it prevented the formation of oxidized molecular patterns, fibrosis, hypertrophy, and the loss of left-ventricle function [23] (Fig 4). The different effects of apocynin and PBN might result from the different sources of ROS these two antioxidants inhibit, an inconclusive claim for the simple reason that one study used mice [24] while the other used rats [23].

The loss of antioxidant defenses during Chagas disease might underlie the increased oxidative stress. Alternatively, the exhausting oxidative stress during Chagas disease might produce the loss of antioxidant enzymes. Selenium is a cofactor of the antioxidant enzymes glutathione peroxidase (GPX) and thioredoxin reductase (TrxR). Selenium supplementation to mice chronically infected with Brazil strain prevented the prolongation of ECG P wave and the cardiac dilation of the right chamber [80]. Nrf2 is a transcriptional factor that controls gene expression of antioxidant enzymes under oxidative conditions [81]. The declines in left-ventricle function and in the expression of Nrf2, HO-1, and gamma-glutamylcysteine synthetase (GCS) were prevented in the hearts of SOD2 (MnSOD) superexpressing transgenic mice [77] (Fig 4). These data indicate that mitochondrial ROS can both inhibit Nrf2-dependent antioxidant defenses in Chagas disease and impair ventricular function.

The sirtuin 1 (SIRT1) pathway is activated in response to a variety of stressors, such as fasting, and activates a myriad of cell functions by deacetylating key proteins. SIRT1 is connected

Fig 4. Antioxidants improve cardiac mechanical function in Sylvio X10/4 clone chronically infected animals. Sprague-Dawley rats were infected with Sylvio X10/4 clone and treated with ROS-scavenger PBN from the beginning and throughout infection, improving heart mechanical function [61]. Infected C57BL/6 mice were treated with the antioxidants SRT1720 and resveratrol, resulting in improved heart mechanical function [62].

Infected C57BL/6-MnSOD overexpressing transgenic mice presented healthier mechanical heart function than wild-type mice at the chronic stage of infection [77, 79]. The effects of the antioxidant treatment or genetic modification on ROS, oxidative damage, parasitism, inflammatory infiltrates, and fibrosis are shown for each condition, together with the exact timing of infection and treatment. CO, cardiac output; EF, left ventricle ejection fraction; PBN, phenyl- α -tert-butyl; ROS, reactive oxygen species; Mn-SOD, manganese superoxide dismutase.

<https://doi.org/10.1371/journal.ppat.1006928.g004>

to the energy-sensing adenosine monophosphate-activated protein kinase (AMPK) pathway. The two pathways seem to activate each other, after which they act together to activate key proteins, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) [82]. PGC1 α is a transcriptional coactivator that regulates mitochondrial biogenesis, energy expenditure, oxidative phosphorylation (OXPHOS), and antioxidant defenses [83]. The activation of SIRT1 and AMPK pathways has many cardioprotective capacities [84, 85] in ROS-dependent cardiomyopathies, such as diabetes.

Garg and colleagues considered the activation of SIRT1 a potential means to restore mitochondrial respiratory chain activity and OXPHOS capacity as well as to induce mitochondrial biogenesis, processes they found earlier [61, 62] to be impaired in the myocardium of chronically infected rodents. They treated mice chronically infected with type I Sylvio X10/4 clone during the late acute stage with SIRT1 agonist SRT1720 and assessed heart mechanical function during chronic infection [62] (Fig 4). During the infection, SIRT1 activity was depressed in the heart, and SIRT1 agonist treatment improved its activity along with left-ventricular function, although it did not significantly affect cardiac remodeling or interstitial fibrosis. Despite increasing acetylation of PGC1 α , the treatment did not improve compromised mitochondrial biogenesis or the expression of mitochondrial DNA-encoded proteins. The authors credited the improvement in cardiac function to the decreased ROS production, oxidative damage, and increased Nrf2 expression they found in the hearts of SRT1720-treated mice; these results indicate that chronic chagasic cardiomyopathy is a ROS-dependent pathology. What must be emphasized, however, is that the authors intervened during the disease development, achieving the long-term prevention, and not reversion, of the disease.

Resveratrol is a multitarget drug with cardioprotective properties exerted through Nrf2, AMPK, SIRT1, SIRT3, and PGC1 α [85]. As an attempt to induce tolerance to heart disease, we infected highly susceptible BALB/c mice with the DTU type I Colombian strain and treated them with resveratrol after the disease was established [56]. Treatment reversed individual electrocardiography abnormalities and improved left-ventricular function. The effects of resveratrol could be maintained for long periods of continuous treatment and could be achieved, though smaller, even late after the disease was established. No significant changes in inflammatory infiltrates or interstitial fibrosis were observed in heart tissues. The treatment activated AMPK phosphorylation and increased the expression of mitochondrial SOD2 (MnSOD), in that way reducing heart extravascular ROS, but it did not interfere with SIRT1 expression/activity. Accordingly, treatment with AMPK-activator metformin or with SOD-mimetic tempol were able to mimic resveratrol's effects on heart function. These results point to a physiological heart dysfunction in Chagas disease, which can be reversed with the appropriate antioxidants and AMPK activators, thus opening a new avenue for the development of specific drugs.

Garg and colleagues also administered resveratrol to mice infected with Sylvio X10/4 [62] (Fig 4). The treatment improved cardiac output, stroke volume, and end systolic volume but did not improve left-ventricle ejection fraction. The difference between two studies were likely due to the different dosages and timing of intervention [56]. We found some improvement in oxidative damage at the chronic stage, but Garg and colleagues did not [56]. Since resveratrol's half-life is very short, and its effects are usually transient, it is likely that the combination of

Fig 5. Antioxidants improve cardiac mechanical and electrical function in Colombian strain chronically infected mice. BALB/c mice were infected with *T. cruzi* Colombian strain; their heart function was analyzed individually by electro and echocardiography [56]. The mice were treated with antioxidant resveratrol for a month after cardiac disease detection, and the reversal of heart systolic and electrical dysfunction, including arrhythmias, was verified. The treatment with resveratrol or control vehicle was prolonged for an additional month, or performed late after infection, with successful results. The treatment with antioxidant tempol for a month after heart disease detection also improved heart function. The effects of the antioxidant treatment on ROS, oxidative damage, parasitism, inflammatory infiltrates, and fibrosis are shown for each condition, together with the exact timing of infection and treatment. CO, cardiac output; EF, left ventricle ejection fraction; ROS, reactive oxygen species.

<https://doi.org/10.1371/journal.ppat.1006928.g005>

late intervention, late function assessment, and the low oral dose they used impaired the observation of pronounced, long-lasting effects.

The association between parasite burden and functional chronic heart disease has been challenged by many studies [60]. In fact, in murine experimental Chagas disease, the prevention/reversion of cardiac dysfunction was accompanied by unchanged [56], increased [86], or decreased parasite burden [56]. Therefore, varying results corroborate the idea that at the chronic stage of infection, parasite burden is no longer responsible for heart dysfunction. Also, the association between inflammatory infiltrates and cardiac dysfunction in Chagas heart disease is far looser than one might expect [87]. Treatments successful in preventing/reversing cardiac dysfunction have been found to either reduce [86] or not interfere [23, 56] with heart inflammatory infiltrates and fibrosis in murine experimental Chagas disease.

The evidence presented in this session suggests oxidative stress is an important pathogenic factor in Chagas cardiomyopathy. The inhibition of oxidative stress seems to be critical to both improving and preventing heart dysfunction from developing. The interference with parasite burden, interstitial fibrosis, inflammatory infiltrates, and cardiac hypertrophy may either accompany functional improvement or not [56, 62, 80, 86]. A first antioxidant tested as a therapy to Chagas disease was vitamin E. It reversed oxidative damage [88] and, along with benznidazole, prevented oxidative damage and reduced the incidence of premature ventricular contraction (PVC) [89]. Selenium is being tried as a therapy [90], and we expect that soon indirect antioxidants that act through Nrf2 or SIRT1 pathways should find their way to clinical trials.

Outstanding questions for further scrutiny

The above analysis of the subject literature led us to formulate the following challenging questions to answer:

- How is it possible that, unlike other microorganisms, *T. cruzi* has evolved to thrive in oxidative conditions?
- Can preconditioning against oxidative stress prevent the initial spread of infection?
- Do all ROS work in the same way? Does *T. cruzi* grow in response to ROS in all cell types?
- Do ROS act directly on the parasite by signaling to enhance its growth, and/or do they act by transforming the environment in which amastigotes grow?
- Do *T. cruzi* from different DTUs behave differently in response to ROS? Are conflicting results in the literature due to different strains used?
- What can explain the unchanged or even increased parasite burden found after treatment with antioxidants at the chronic stage, unlike treatment applied at the acute stage?
- Can we inhibit ROS production without interfering with *T. cruzi* elimination by activated macrophages? Is peroxynitrite indispensable to the killing of *T. cruzi* by activated macrophages in vivo, or does NO alone suffice to kill it?
- Are ROS involved in both physiological, reversible heart dysfunction and cumulative, progressively destructive cardiomyopathy?
- Can antioxidant therapy prevent the progression of human Chagas heart disease or even reverse it?

Such knowledge could help reconcile conflicting literature on the role of ROS in *T. cruzi* infection, potentially helping develop new therapies.

Concluding remarks

The recent literature has pointed toward oxidative stress as a villain in Chagas disease: It helps parasites to grow during the acute stage of infection, and it participates in the generation of cardiomyopathy at the chronic stage. Although the reduction of parasite burden seems useless at the chronic stage, a plethora of data suggest antioxidant therapy as a candidate treatment to chronic Chagas disease cardiomyopathy. We still need to learn a lot about the effects of antioxidant therapy on infection by different *T. cruzi* strains. Crucial knowledge could be gained by research that would tightly control parasite burden, thanks to which therapy's efficiency could be tuned up and patients' safety could be ensured. Even despite these gaps in the knowledge, however, antioxidants are—for the moment—the most promising road to a specific treatment in Chagas disease.

References

1. Baehner RL, Karnovsky ML. Deficiency of reduced nicotinamide-adenine dinucleotide oxidase in chronic granulomatous disease. *Science*. 1968; 162(3859):1277–9. PMID: [4387010](#).
2. Nathan C, Nogueira N, Juangbhanich C, Ellis J, Cohn Z. Activation of macrophages in vivo and in vitro. Correlation between hydrogen peroxide release and killing of *Trypanosoma cruzi*. *J Exp Med*. 1979; 149(5):1056–68. PMID: [376774](#); PubMed Central PMCID: PMCPMC2184875.
3. Tanaka Y, Kiyotaki C, Tanowitz H, Bloom BR. Reconstitution of a variant macrophage cell line defective in oxygen metabolism with a H₂O₂-generating system. *Proc Natl Acad Sci U S A*. 1982; 79(8):2584–8. PMID: [7045866](#); PubMed Central PMCID: PMCPMC346244.
4. Murray HW. Pretreatment with phorbol myristate acetate inhibits macrophage activity against intracellular protozoa. *J Reticuloendothel Soc*. 1982; 31(6):479–87. PMID: [6288939](#).
5. Locksley RM, Klebanoff SJ. Oxygen-dependent microbicidal systems of phagocytes and host defense against intracellular protozoa. *J Cell Biochem*. 1983; 22(3):173–85. <https://doi.org/10.1002/jcb.240220306> PMID: [6365936](#).
6. McCabe RE, Mullins BT. Failure of *Trypanosoma cruzi* to trigger the respiratory burst of activated macrophages. Mechanism for immune evasion and importance of oxygen-independent killing. *J Immunol*. 1990; 144(6):2384–8. PMID: [2155965](#).
7. Metz G, Carlier Y, Vray B. *Trypanosoma cruzi* upregulates nitric oxide release by IFN-gamma-preactivated macrophages, limiting cell infection independently of the respiratory burst. *Parasite Immunol*. 1993; 15(12):693–9. PMID: [7877845](#).
8. Russo M, Starobinas N, Ribeiro-Dos-Santos R, Minoprio P, Eisen H, Hontebeyrie-Joskowicz M. Susceptible mice present higher macrophage activation than resistant mice during infections with myotropic strains of *Trypanosoma cruzi*. *Parasite Immunol*. 1989; 11(4):385–95. PMID: [2528710](#).
9. Guevara AG, Guilvard E, Borges MM, Cordeiro da Silva A, Ouassii A. N-Acetylcysteine and glutathione modulate the behaviour of *Trypanosoma cruzi* experimental infection. *Immunol Lett*. 2000; 71(2):79–83. PMID: [10714433](#).
10. Paiva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens? *Antioxid Redox Signal*. 2014; 20(6):1000–37. <https://doi.org/10.1089/ars.2013.5447> PMID: [23992156](#); PubMed Central PMCID: PMCPMC3924804.
11. Pedrosa RC, De Bem AF, Locatelli C, Pedrosa RC, Geremias R, Wilhelm Filho D. Time-dependent oxidative stress caused by benznidazole. *Redox Rep*. 2001; 6(4):265–70. <https://doi.org/10.1179/135100001101536328> PMID: [11642718](#).
12. Moreno SN, Docampo R, Mason RP, Leon W, Stoppani AO. Different behaviors of benznidazole as free radical generator with mammalian and *Trypanosoma cruzi* microsomal preparations. *Arch Biochem Biophys*. 1982; 218(2):585–91. PMID: [6297399](#).
13. Wilkinson SR, Taylor MC, Horn D, Kelly JM, Cheeseman I. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *Proc Natl Acad Sci U S A*. 2008; 105(13):5022–7. <https://doi.org/10.1073/pnas.0711014105> PMID: [18367671](#); PubMed Central PMCID: PMCPMC2278226.
14. Trochine A, Creek DJ, Faral-Tello P, Barrett MP, Robello C. Benznidazole biotransformation and multiple targets in *Trypanosoma cruzi* revealed by metabolomics. *PLoS Negl Trop Dis*. 2014; 8(5):e2844. <https://doi.org/10.1371/journal.pntd.0002844> PMID: [24853684](#); PubMed Central PMCID: PMCPMC4031082.

15. Hall BS, Wilkinson SR. Activation of benznidazole by trypanosomal type I nitroreductases results in glyoxal formation. *Antimicrob Agents Chemother*. 2012; 56(1):115–23. <https://doi.org/10.1128/AAC.05135-11> PMID: 22037852; PubMed Central PMCID: PMC3256028.
16. Rigalli JP, Perdomo VG, Ciriaci N, Frances DE, Ronco MT, Bataille AM, et al. The trypanocidal benzimidazole promotes adaptive response to oxidative injury: Involvement of the nuclear factor-erythroid 2-related factor-2 (Nrf2) and multidrug resistance associated protein 2 (MRP2). *Toxicol Appl Pharmacol*. 2016; 304:90–8. <https://doi.org/10.1016/j.taap.2016.05.007> PMID: 27180241; PubMed Central PMCID: PMC4930729.
17. Goes GR, Rocha PS, Diniz AR, Aguiar PH, Machado CR, Vieira LQ. Trypanosoma cruzi Needs a Signal Provided by Reactive Oxygen Species to Infect Macrophages. *PLoS Negl Trop Dis*. 2016; 10(4):e0004555. <https://doi.org/10.1371/journal.pntd.0004555> PMID: 27035573; PubMed Central PMCID: PMC4818108.
18. Freire ACG, Alves CL, Goes GR, Resende BC, Moretti NS, Nunes VS, et al. Catalase expression impairs oxidative stress-mediated signalling in Trypanosoma cruzi. *Parasitology*. 2017; 144(11):1–13. <https://doi.org/10.1017/S0031182017001044> PMID: 28653592.
19. Machado-Silva A, Cerqueira PG, Grazielle-Silva V, Gadelha FR, Peloso Ede F, Teixeira SM, et al. How Trypanosoma cruzi deals with oxidative stress: Antioxidant defence and DNA repair pathways. *Mutat Res Rev Mutat Res*. 2016 Jan-Mar; 767:8–22. <https://doi.org/10.1016/j.mrrev.2015.12.003> PMID: 27036062.
20. Hugo M, Martinez A, Trujillo M, Estrada D, Mastrogiovanni M, Linares E, et al. Kinetics, subcellular localization, and contribution to parasite virulence of a Trypanosoma cruzi hybrid type A heme peroxidase (TcAPx-CcP). *Proc Natl Acad Sci U S A*. 2017; 114(8):E1326–E35. <https://doi.org/10.1073/pnas.1618611114> PMID: 28179568; PubMed Central PMCID: PMC5338437.
21. Piacenza L, Alvarez MN, Peluffo G, Radi R. Fighting the oxidative assault: the Trypanosoma cruzi journey to infection. *Curr Opin Microbiol*. 2009; 12(4):415–21. <https://doi.org/10.1016/j.mib.2009.06.011> PMID: 19616990.
22. Piacenza L, Peluffo G, Alvarez MN, Martinez A, Radi R. Trypanosoma cruzi antioxidant enzymes as virulence factors in Chagas disease. *Antioxid Redox Signal*. 2013; 19(7):723–34. <https://doi.org/10.1089/ars.2012.4618> PMID: 22458250; PubMed Central PMCID: PMC3739954.
23. Wen JJ, Gupta S, Guan Z, Dhiman M, Condon D, Lui C, et al. Phenyl-alpha-tert-butyl-nitron and benznidazole treatment controlled the mitochondrial oxidative stress and evolution of cardiomyopathy in chronic chagasic Rats. *J Am Coll Cardiol*. 2010; 55(22):2499–508. <https://doi.org/10.1016/j.jacc.2010.02.030> PMID: 20510218; PubMed Central PMCID: PMC2887697.
24. Dhiman M, Garg NJ. NADPH oxidase inhibition ameliorates Trypanosoma cruzi-induced myocarditis during Chagas disease. *J Pathol*. 2011; 225(4):583–96. <https://doi.org/10.1002/path.2975> PMID: 21952987; PubMed Central PMCID: PMC34378678.
25. Dhiman M, Garg NJ. P47phox^{-/-} mice are compromised in expansion and activation of CD8⁺ T cells and susceptible to Trypanosoma cruzi infection. *PLoS Pathog*. 2014; 10(12):e1004516. <https://doi.org/10.1371/journal.ppat.1004516> PMID: 25474113; PubMed Central PMCID: PMC4256457.
26. Wen JJ, Nagajyothi F, Machado FS, Weiss LM, Scherer PE, Tanowitz HB, et al. Markers of oxidative stress in adipose tissue during Trypanosoma cruzi infection. *Parasitol Res*. 2014; 113(9):3159–65. <https://doi.org/10.1007/s00436-014-3977-7> PMID: 24948102; PubMed Central PMCID: PMC34374356.
27. Paiva CN, Feijo DF, Dutra FF, Carneiro VC, Freitas GB, Alves LS, et al. Oxidative stress fuels Trypanosoma cruzi infection in mice. *J Clin Invest*. 2012; 122(7):2531–42. <https://doi.org/10.1172/JCI58525> PMID: 22728935; PubMed Central PMCID: PMC3386808.
28. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science*. 2012; 335(6071):936–41. <https://doi.org/10.1126/science.1214935> PMID: 22363001; PubMed Central PMCID: PMC3564547.
29. Soares MP, Teixeira L, Moita LF. Disease tolerance and immunity in host protection against infection. *Nat Rev Immunol*. 2017; 17(2):83–96. <https://doi.org/10.1038/nri.2016.136> PMID: 28044057.
30. Pamplona A, Ferreira A, Balla J, Jeney V, Balla G, Epiphanio S, et al. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med*. 2007; 13(6):703–10. <https://doi.org/10.1038/nm1586> PMID: 17496899.
31. Tzima S, Victoratos P, Kranidioti K, Alexiou M, Kollias G. Myeloid heme oxygenase-1 regulates innate immunity and autoimmunity by modulating IFN-beta production. *J Exp Med*. 2009; 206(5):1167–79. <https://doi.org/10.1084/jem.20081582> PMID: 19398754; PubMed Central PMCID: PMC2715044.
32. Yoon SJ, Kim SJ, Lee SM. Overexpression of HO-1 Contributes to Sepsis-Induced Immunosuppression by Modulating the Th1/Th2 Balance and Regulatory T-Cell Function. *J Infect Dis*. 2017; 215(10):1608–18. <https://doi.org/10.1093/infdis/jix142> PMID: 28368519.

33. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol Life Sci*. 2016; 73(17):3221–47. <https://doi.org/10.1007/s00018-016-2223-0> PMID: 27100828; PubMed Central PMCID: PMC4967105.
34. Winterbourn CC, Kettle AJ. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid Redox Signal*. 2013; 18(6):642–60. <https://doi.org/10.1089/ars.2012.4827> PMID: 22881869.
35. Imlay JA. Oxidative Stress. *EcoSal Plus*. 2009; 3(2). <https://doi.org/10.1128/ecosalplus.5.4.4> PMID: 26443771.
36. Slauch JM. How does the oxidative burst of macrophages kill bacteria? Still an open question. *Mol Microbiol*. 2011; 80(3):580–3. <https://doi.org/10.1111/j.1365-2958.2011.07612.x> PMID: 21375590; PubMed Central PMCID: PMC3109634.
37. Huang BK, Sikes HD. Quantifying intracellular hydrogen peroxide perturbations in terms of concentration. *Redox Biol*. 2014; 2:955–62. <https://doi.org/10.1016/j.redox.2014.08.001> PMID: 25460730; PubMed Central PMCID: PMC4215397.
38. Santiago HC, Gonzalez Lombana CZ, Macedo JP, Utsch L, Tafuri WL, Campagnole-Santos MJ, et al. NADPH phagocyte oxidase knockout mice control *Trypanosoma cruzi* proliferation, but develop circulatory collapse and succumb to infection. *PLoS Negl Trop Dis*. 2012; 6(2):e1492. <https://doi.org/10.1371/journal.pntd.0001492> PMID: 22348160; PubMed Central PMCID: PMC3279332.
39. Stempin CC, Garrido VV, Dulgerian LR, Cerban FM. Cruzipain and SP600125 induce p38 activation, alter NO/arginase balance and favor the survival of *Trypanosoma cruzi* in macrophages. *Acta Trop*. 2008; 106(2):119–27. <https://doi.org/10.1016/j.actatropica.2008.02.004> PMID: 18359001.
40. Guinazu N, Carrera-Silva EA, Becerra MC, Pellegrini A, Albesa I, Gea S. Induction of NADPH oxidase activity and reactive oxygen species production by a single *Trypanosoma cruzi* antigen. *Int J Parasitol*. 2010; 40(13):1531–8. <https://doi.org/10.1016/j.ijpara.2010.05.012> PMID: 20637209.
41. Calderon J, Maganto-Garcia E, Punzon C, Carrion J, Terhorst C, Fresno M. The receptor Slamf1 on the surface of myeloid lineage cells controls susceptibility to infection by *Trypanosoma cruzi*. *PLoS Pathog*. 2012; 8(7):e1002799. <https://doi.org/10.1371/journal.ppat.1002799> PMID: 22807679; PubMed Central PMCID: PMC3395606.
42. Kimura A, Abe H, Tsuruta S, Chiba S, Fujii-Kuriyama Y, Sekiya T, et al. Aryl hydrocarbon receptor protects against bacterial infection by promoting macrophage survival and reactive oxygen species production. *Int Immunol*. 2014; 26(4):209–20. <https://doi.org/10.1093/intimm/dxt067> PMID: 24343818.
43. Barroso A, Gualdron-Lopez M, Esper L, Brant F, Araujo RR, Carneiro MB, et al. The Aryl Hydrocarbon Receptor Modulates Production of Cytokines and Reactive Oxygen Species and Development of Myocarditis during *Trypanosoma cruzi* Infection. *Infect Immun*. 2016; 84(10):3071–82. <https://doi.org/10.1128/IAI.00575-16> PMID: 27481250; PubMed Central PMCID: PMC4503804.
44. Santello FH, Frare EO, dos Santos CD, Toldo MP, Kawasse LM, Zucoloto S, et al. Melatonin treatment reduces the severity of experimental *Trypanosoma cruzi* infection. *J Pineal Res*. 2007; 42(4):359–63. <https://doi.org/10.1111/j.1600-079X.2007.00427.x> PMID: 17439552.
45. Nagaiyothi F, Zhao D, Weiss LM, Tanowitz HB. Curcumin treatment provides protection against *Trypanosoma cruzi* infection. *Parasitol Res*. 2012; 110(6):2491–9. <https://doi.org/10.1007/s00436-011-2790-9> PMID: 22215192; PubMed Central PMCID: PMC3556637.
46. Finzi JK, Chiavegatto CW, Corat KF, Lopez JA, Cabrera OG, Mielniczki-Pereira AA, et al. *Trypanosoma cruzi* response to the oxidative stress generated by hydrogen peroxide. *Mol Biochem Parasitol*. 2004; 133(1):37–43. PMID: 14668010.
47. Nogueira NP, de Souza CF, Saraiva FM, Sultano PE, Dalmau SR, Bruno RE, et al. Heme-induced ROS in *Trypanosoma cruzi* activates CaMKII-like that triggers epimastigote proliferation. One helpful effect of ROS. *PLoS ONE*. 2011; 6(10):e25935. <https://doi.org/10.1371/journal.pone.0025935> PMID: 22022475; PubMed Central PMCID: PMC3191175.
48. Nogueira NP, Saraiva FMS, Oliveira MP, Mendonca APM, Inacio JDF, Almeida-Amaral EE, et al. Heme modulates *Trypanosoma cruzi* bioenergetics inducing mitochondrial ROS production. *Free Radic Biol Med*. 2017; 108:183–91. <https://doi.org/10.1016/j.freeradbiomed.2017.03.027> PMID: 28363600.
49. Nogueira NP, Saraiva FM, Sultano PE, Cunha PR, Laranja GA, Justo GA, et al. Proliferation and differentiation of *Trypanosoma cruzi* inside its vector have a new trigger: redox status. *PLoS ONE*. 2015; 10(2):e0116712. <https://doi.org/10.1371/journal.pone.0116712> PMID: 25671543; PubMed Central PMCID: PMC4324650.
50. Aguiar PH, Furtado C, Repoles BM, Ribeiro GA, Mendes IC, Peloso EF, et al. Oxidative stress and DNA lesions: the role of 8-oxoguanine lesions in *Trypanosoma cruzi* cell viability. *PLoS Negl Trop Dis*. 2013; 7(6):e2279. <https://doi.org/10.1371/journal.pntd.0002279> PMID: 23785540; PubMed Central PMCID: PMC3681716.

51. Gazzinelli RT, Oswald IP, Hieny S, James SL, Sher A. The microbicidal activity of interferon-gamma-treated macrophages against *Trypanosoma cruzi* involves an L-arginine-dependent, nitrogen oxide-mediated mechanism inhibitable by interleukin-10 and transforming growth factor-beta. *Eur J Immunol*. 1992; 22(10):2501–6. <https://doi.org/10.1002/eji.1830221006> PMID: 1396957.
52. Alvarez MN, Peluffo G, Piacenza L, Radi R. Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized *Trypanosoma cruzi*: consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. *J Biol Chem*. 2011; 286(8):6627–40. <https://doi.org/10.1074/jbc.M110.167247> PMID: 21098483; PubMed Central PMCID: PMC3057850.
53. Cutrullis RA, Petray PB, Corral RS. MIF-driven activation of macrophages induces killing of intracellular *Trypanosoma cruzi* dependent on endogenous production of tumor necrosis factor, nitric oxide and reactive oxygen species. *Immunobiology*. 2017; 222(2):423–31. <https://doi.org/10.1016/j.imbio.2016.08.007> PMID: 27591076.
54. Dey N, Sinha M, Gupta S, Gonzalez MN, Fang R, Endsley JJ, et al. Caspase-1/ASC inflammasome-mediated activation of IL-1beta-ROS-NF-kappaB pathway for control of *Trypanosoma cruzi* replication and survival is dispensable in NLRP3-/- macrophages. *PLoS ONE*. 2014; 9(11):e111539. <https://doi.org/10.1371/journal.pone.0111539> PMID: 25372293; PubMed Central PMCID: PMC4221042.
55. Goncalves VM, Matteucci KC, Buzzo CL, Miollo BH, Ferrante D, Torrecilhas AC, et al. NLRP3 controls *Trypanosoma cruzi* infection through a caspase-1-dependent IL-1R-independent NO production. *PLoS Negl Trop Dis*. 2013; 7(10):e2469. <https://doi.org/10.1371/journal.pntd.0002469> PMID: 24098823; PubMed Central PMCID: PMC3789781.
56. Vilar-Pereira G, Carneiro VC, Mata-Santos H, Vicentino AR, Ramos IP, Giarola NL, et al. Resveratrol Reverses Functional Chagas Heart Disease in Mice. *PLoS Pathog*. 2016; 12(10):e1005947. <https://doi.org/10.1371/journal.ppat.1005947> PMID: 27788262; PubMed Central PMCID: PMC4508285.
57. Dias PP, Capila RF, do Couto NF, Estrada D, Gadelha FR, Radi R, et al. Cardiomyocyte oxidants production may signal to *T. cruzi* intracellular development. *PLoS Negl Trop Dis*. 2017; 11(8):e0005852. <https://doi.org/10.1371/journal.pntd.0005852> PMID: 28832582.
58. Campo VA. Comparative effects of histone deacetylases inhibitors and resveratrol on *Trypanosoma cruzi* replication, differentiation, infectivity and gene expression. *Int J Parasitol Drugs Drug Resist*. 2017; 7(1):23–33. <https://doi.org/10.1016/j.ijpddr.2016.12.003> PMID: 28038431; PubMed Central PMCID: PMC5199159.
59. Valera Vera EA, Saye M, Reigada C, Damasceno FS, Silber AM, Miranda MR, et al. Resveratrol inhibits *Trypanosoma cruzi* arginine kinase and exerts a trypanocidal activity. *Int J Biol Macromol*. 2016; 87:498–503. <https://doi.org/10.1016/j.ijbiomac.2016.03.014> PMID: 26976067.
60. Morillo CA, Marin-Neto JA, Avezum A, Sosa-Estani S, Rassi A Jr., Rosas F, et al. Randomized Trial of Benznidazole for Chronic Chagas' Cardiomyopathy. *N Engl J Med*. 2015; 373(14):1295–306. <https://doi.org/10.1056/NEJMoa1507574> PMID: 26323937.
61. Wen JJ, Garg NJ. Mitochondrial complex III defects contribute to inefficient respiration and ATP synthesis in the myocardium of *Trypanosoma cruzi*-infected mice. *Antioxid Redox Signal*. 2010; 12(1):27–37. <https://doi.org/10.1089/ARS.2008.2418> PMID: 19624257; PubMed Central PMCID: PMC2821147.
62. Wan X, Wen JJ, Koo SJ, Liang LY, Garg NJ. SIRT1-PGC1alpha-NFkappaB Pathway of Oxidative and Inflammatory Stress during *Trypanosoma cruzi* Infection: Benefits of SIRT1-Targeted Therapy in Improving Heart Function in Chagas Disease. *PLoS Pathog*. 2016; 12(10):e1005954. <https://doi.org/10.1371/journal.ppat.1005954> PMID: 27764247; PubMed Central PMCID: PMC45072651.
63. Tada Y, Suzuki J. Oxidative stress and myocarditis. *Curr Pharm Des*. 2016; 22(4):450–71. PMID: 26696256.
64. Ba X, Gupta S, Davidson M, Garg NJ. *Trypanosoma cruzi* induces the reactive oxygen species-PARP-1-RelA pathway for up-regulation of cytokine expression in cardiomyocytes. *J Biol Chem*. 2010; 285(15):11596–606. <https://doi.org/10.1074/jbc.M109.076984> PMID: 20145242; PubMed Central PMCID: PMC2857037.
65. Pereira IR, Vilar-Pereira G, Silva AA, Lannes-Vieira J. Severity of chronic experimental Chagas' heart disease parallels tumour necrosis factor and nitric oxide levels in the serum: models of mild and severe disease. *Mem Inst Oswaldo Cruz*. 2014; 109(3):289–98. <https://doi.org/10.1590/0074-0276140033> PMID: 24937048; PubMed Central PMCID: PMC4131780.
66. Pereira IR, Vilar-Pereira G, Silva AA, Moreira OC, Brito C, Sarmento ED, et al. Tumor necrosis factor is a therapeutic target for immunological unbalance and cardiac abnormalities in chronic experimental Chagas' heart disease. *Mediators Inflamm*. 2014; 2014:798078. <https://doi.org/10.1155/2014/798078> PMID: 25140115; PubMed Central PMCID: PMC4130030.
67. Monnerat G, Alarcon ML, Vasconcellos LR, Hochman-Mendez C, Brasil G, Bassani RA, et al. Macrophage-dependent IL-1beta production induces cardiac arrhythmias in diabetic mice. *Nat Commun*.

- 2016; 7:13344. <https://doi.org/10.1038/ncomms13344> PMID: 27882934; PubMed Central PMCID: PMC5123037.
68. Maciel L, Pedrosa RC, Campos de Carvalho AC, Nascimento JH, Medei E. Ventricular arrhythmias are related to the presence of autoantibodies with adrenergic activity in chronic chagasic patients with preserved left ventricular function. *J Card Fail*. 2012; 18(5):423–31. <https://doi.org/10.1016/j.cardfail.2012.02.007> PMID: 22555274.
69. Sepulveda M, Gonano LA, Viotti M, Morell M, Blanco P, Lopez Alarcon M, et al. Calcium/Calmodulin Protein Kinase II-Dependent Ryanodine Receptor Phosphorylation Mediates Cardiac Contractile Dysfunction Associated With Sepsis. *Crit Care Med*. 2017; 45(4):e399–e408. <https://doi.org/10.1097/CCM.0000000000002101> PMID: 27648519.
70. Tinker A. The mechanisms of ventricular arrhythmia in Chagas disease. *Int J Cardiol*. 2017; 240:372–3. <https://doi.org/10.1016/j.ijcard.2017.03.094> PMID: 28606682.
71. Jimenez MAV, Nascimento JHM, Monnerat G, Maciel L, Paiva CN, Pedrosa RC, et al. Autoantibodies with beta-adrenergic activity from chronic chagasic patients induce cardiac arrhythmias and early after-depolarization in a drug-induced LQT2 rabbit hearts. *Int J Cardiol*. 2017; 240:354–9. <https://doi.org/10.1016/j.ijcard.2017.02.066> PMID: 28320606.
72. Luczak ED, Anderson ME. CaMKII oxidative activation and the pathogenesis of cardiac disease. *J Mol Cell Cardiol*. 2014; 73:112–6. <https://doi.org/10.1016/j.yjmcc.2014.02.004> PMID: 24530899; PubMed Central PMCID: PMC4048820.
73. Kohler AC, Sag CM, Maier LS. Reactive oxygen species and excitation-contraction coupling in the context of cardiac pathology. *J Mol Cell Cardiol*. 2014; 73:92–102. <https://doi.org/10.1016/j.yjmcc.2014.03.001> PMID: 24631768.
74. Wen JJ, Yachelini PC, Sembaj A, Manzur RE, Garg NJ. Increased oxidative stress is correlated with mitochondrial dysfunction in chagasic patients. *Free Radic Biol Med*. 2006; 41(2):270–6. <https://doi.org/10.1016/j.freeradbiomed.2006.04.009> PMID: 16814107.
75. Wen JJ, Garg NJ. Mitochondrial generation of reactive oxygen species is enhanced at the Q(o) site of the complex III in the myocardium of Trypanosoma cruzi-infected mice: beneficial effects of an antioxidant. *J Bioenerg Biomembr*. 2008; 40(6):587–98. <https://doi.org/10.1007/s10863-008-9184-4> PMID: 19009337.
76. Wen JJ, Dhiman M, Whorton EB, Garg NJ. Tissue-specific oxidative imbalance and mitochondrial dysfunction during Trypanosoma cruzi infection in mice. *Microbes Infect*. 2008; 10(10–11):1201–9. <https://doi.org/10.1016/j.micinf.2008.06.013> PMID: 18675934; PubMed Central PMCID: PMC2613841.
77. Wen JJ, Porter C, Garg NJ. Inhibition of NFE2L2-Antioxidant Response Element Pathway by Mitochondrial Reactive Oxygen Species Contributes to Development of Cardiomyopathy and Left Ventricular Dysfunction in Chagas Disease. *Antioxid Redox Signal*. 2017. <https://doi.org/10.1089/ars.2016.6831> PMID: 28132522.
78. Dhiman M, Zago MP, Nunez S, Amoroso A, Rementeria H, Dousset P, et al. Cardiac-oxidized antigens are targets of immune recognition by antibodies and potential molecular determinants in chagas disease pathogenesis. *PLoS ONE*. 2012; 7(1):e28449. <https://doi.org/10.1371/journal.pone.0028449> PMID: 22238578; PubMed Central PMCID: PMC3251564.
79. Dhiman M, Wan X, Popov VL, Vargas G, Garg NJ. MnSODtg mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease. *J Am Heart Assoc*. 2013; 2(5):e000302. <https://doi.org/10.1161/JAHA.113.000302> PMID: 24136392; PubMed Central PMCID: PMC3835234.
80. Souza AP, Jelicks LA, Tanowitz HB, Olivieri BP, Medeiros MM, Oliveira GM, et al. The benefits of using selenium in the treatment of Chagas disease: prevention of right ventricle chamber dilatation and reversal of Trypanosoma cruzi-induced acute and chronic cardiomyopathy in mice. *Mem Inst Oswaldo Cruz*. 2010; 105(6):746–51. PMID: 20944987.
81. Sies H, Berndt C, Jones DP. Oxidative Stress. *Annu Rev Biochem*. 2017; 86:715–48. <https://doi.org/10.1146/annurev-biochem-061516-045037> PMID: 28441057.
82. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, et al. AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab*. 2010; 298(4):E751–60. <https://doi.org/10.1152/ajpendo.00745.2009> PMID: 20103737; PubMed Central PMCID: PMC2853213.
83. Rowe GC, Jiang A, Arany Z. PGC-1 coactivators in cardiac development and disease. *Circ Res*. 2010; 107(7):825–38. <https://doi.org/10.1161/CIRCRESAHA.110.223818> PMID: 20884884; PubMed Central PMCID: PMC2955978.
84. Salt IP, Hardie DG. AMP-Activated Protein Kinase: An Ubiquitous Signaling Pathway With Key Roles in the Cardiovascular System. *Circ Res*. 2017; 120(11):1825–41. <https://doi.org/10.1161/CIRCRESAHA.117.309633> PMID: 28546359; PubMed Central PMCID: PMC5447810.

85. Xia N, Daiber A, Forstermann U, Li H. Antioxidant effects of resveratrol in the cardiovascular system. *Br J Pharmacol*. 2017; 174(12):1633–46. <https://doi.org/10.1111/bph.13492> PMID: 27058985; PubMed Central PMCID: PMC5446570.
86. Wen JJ, Wan X, Thacker J, Garg NJ. Chemotherapeutic efficacy of phosphodiesterase inhibitors in chagasic cardiomyopathy. *JACC Basic Transl Sci*. 2016; 1(4):235–50. <https://doi.org/10.1016/j.jacbts.2016.04.005> PMID: 27747306; PubMed Central PMCID: PMC5065248.
87. Eickhoff CS, Lawrence CT, Sagartz JE, Bryant LA, Labovitz AJ, Gala SS, et al. ECG detection of murine chagasic cardiomyopathy. *J Parasitol*. 2010; 96(4):758–64. <https://doi.org/10.1645/GE-2396.1> PMID: 20738200; PubMed Central PMCID: PMC3150498.
88. Macao LB, Wilhelm Filho D, Pedrosa RC, Pereira A, Backes P, Torres MA, et al. Antioxidant therapy attenuates oxidative stress in chronic cardiopathy associated with Chagas' disease. *Int J Cardiol*. 2007; 123(1):43–9. <https://doi.org/10.1016/j.ijcard.2006.11.118> PMID: 17328977.
89. Barbosa JL, Thiers CA, de Braganca Pereira B, do Nascimento EM, Ribeiro Frazon CM, Budni P, et al. Impact of the Use of Benznidazole Followed by Antioxidant Supplementation in the Prevalence of Ventricular Arrhythmias in Patients With Chronic Chagas Disease: Pilot Study. *Am J Ther*. 2016; 23(6): e1474–e83. <https://doi.org/10.1097/MJT.000000000000137> PMID: 25461962.
90. Alvarenga Americano do Brasil PE, Pereira de Souza A, Hasslocher-Moreno AM, Xavier SS, Lambert Passos SR, de Fatima Ramos Moreira M, et al. Selenium Treatment and Chagasic Cardiopathy (STCC): study protocol for a double-blind randomized controlled trial. *Trials*. 2014; 15:388. <https://doi.org/10.1186/1745-6215-15-388> PMID: 25284194; PubMed Central PMCID: PMC4197263.