Chapter

The Journey of *Trypanosoma cruzi* under the Redox Baton

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Abstract

Trypanosoma cruzi is a protozoan responsible for Chagas disease and has a complex life cycle including vertebrate (mammals) and invertebrate (insects) hosts. The parasite presents proliferative and infective forms that are challenged throughout their cycle as different sources of nutrients, pH, immune system, and levels of reactive oxygen species (ROS). Although ROS cause damage to cells and tissues when their levels are controlled, they are involved in signal transduction pathways involved in cell growth and differentiation. Curiously, the proliferation of epimastigote inside the bug insect is favored by high levels of ROS from the digestion of blood meal, and it is regulated by a cellular signaling mechanism involving heme and CaMKII. On the other hand, the differentiation of epimastigote into metacyclic trypomastigote in the rectum occurs in the reduced state. Interestingly, when the parasite infects the vertebrate, the immune system recognizes this pathogen and macrophages become activated. Thus, NADPH oxidase produces ROS that helps the parasite enter the mammalian cells, improving the infection. The parasite thrives inside the mammalian cells also involving ROS. Thus, the life cycle of *Trypanosoma cruzi* obeys a fine tuning of the redox state, not affecting the host cells and being helpful to the parasite.

Keywords: redox state, reactive oxygen species, redox signaling, host-parasite interaction, iron

1. Introduction

1.1 Chagas disease

Chagas disease was described in 1909 by a Brazilian researcher, Carlos Chagas, who discovered a new trypanosomiasis in Minas Gerais, Brazil, during his work on an anti-malaria campaign [1, 2]. The disease presents three phases: acute, indeterminate, and chronic. The acute phase is asymptomatic and presents nonspecific symptoms and signs, such as inflammatory lesions at the site of entry of the parasite (chagoma) and fever. At this stage, the parasitic load in the blood is high. The indeterminate phase is characterized by the presence of antibodies against *T. cruzi* and absence of clinical manifestations of the disease. The chronic phase of the disease involves the cardiac system, digestive system, or both. The patients at this stage may develop (1) a Chagas' heart disease that compromises the cardiac function by increasing the size of the heart and tissue damage (fibrosis) and (2) chronic inflammation and destruction of parasympathetic neurons leading to progressive enlargement of the esophagus (megaesophagus), sigmoid colon, or rectum (megacolon) [3, 4].

1.2 The biological cycle of *Trypanosoma cruzi*

The causative agent of the disease is a flagellate protozoan that belongs to the order Kinetoplastida and family Trypanosomatidae, the *Trypanosoma cruzi*. Classically, transmission of Chagas disease occurs through insect vectors of the subfamily Triatominae, popularly known as barbers. However, there are other transmission routes such as oral, congenital, blood transfusion, organ transplantation, and laboratory accidents [5].

T. cruzi presents different evolutionary forms that alternate with each other throughout its cycle. Trypomastigote forms present in the blood of infected vertebrate hosts are ingested by the insect vector, where they differentiate into epimastigote replicative forms. These forms undergo another process of differentiation througout the intestine, however this time to infectious and non-replicative forms, the metacyclic trypomastigotes. In turn, these are released with the feces and penetrate into the vertebrate host through the sting of the triatomines or through another portal of entry, such as mucosae. Trypomastigotes are able to invade host cells and differentiate into amastigotes in the intracellular environment. Such forms multiply through binary divisions and are transformed into the infective trypomastigote forms still within the host cell. With the disruption of the plasma membrane of the vertebrate host cell, the trypomastigote forms gain the bloodstream and invade other cells and tissues of the mammal or can be sucked in by new triatomine restarting the cycle [6].

It is known that during its life cycle *T. cruzi* is exposed to different redox environments inside the invertebrate and vertebrate hosts [7, 8] and the ability of *T. cruzi* to adapt to the redox state contributes to the success of the infection [9]. Additionally, in terms of a physiological approach, ROS play a vital role in *T. cruzi*-vector interactions, because heme, a molecule from the insect blood digestion, triggers epimastigote proliferation through a redox-sensitive signaling mechanism [10].

1.3 Redox signaling

Cells generate ROS endogenously and constitutively when oxygen is partially reduced in mitochondria-producing oxidants, the so-called reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [11]. To maintain their hemostasis, cells adopt strategies called antioxidant defense. ROS participate in signal transduction pathways involved in cell growth and differentiation [12]. However, when oxidant levels are high, the oxidative/antioxidant balance within the cells disrupts the redox signaling and the redox control, which can lead to cellular damage [13–16]. This exacerbation of the endogenous production of ROS is known as oxidative stress. These oxidant species can lead to lipid peroxidation, affecting membrane integrity, DNA damage, and oxidation of sugars and protein thiols [14, 15]. On the other hand, controlled ROS increase leads to a temporary imbalance that represents the physiological basis for redox regulation [16, 17]. Indeed, redox processes have fundamental implications in biology.

In addition to ROS, other reactive species have notable impacts on redox biology, including the reactive nitrogen species (RNS), such as nitric oxide, nitrogen dioxide (both free radicals), peroxynitrite, and nitrite/nitrate. Besides these, forms of cysteine, methionine, and some low-molecular-mass compounds such as glutathione and trypanothione are called reactive sulfur species (RSS). Another group of reactive species is the reactive carbonyl species (RCS) including various forms of metabolically generated aldehydes and electronically excited (triplet) carbonyls. Finally, reactive selenium species (RSeS) include low molecular mass such as selenocysteine and selenomethionine residues in proteins [17].

2. ROS and *Trypanosoma cruzi*

2.1 The journey inside the bug insect

2.1.1 The epimastigotes and redox environment

Evidence in the literature indicates that the interaction between *T. cruzi* and triatomines is essential for the successful spread of Chagas disease [18] and several factors and molecules have been shown to be important in establishing the infection. After feeding, the insect vector digests the blood in the midgut, where hemoglobin protein degradation occurs and a large amount of heme is released. Heme is a molecule known to increase the formation of reactive oxygen species (ROS) and is able to alter membrane selectivity and permeability [19, 20]. These reactive species can also be generated as a by-product of aerobic metabolism of the parasite [7, 21]. Therefore, the former region of the midgut represents an environment rich in nutrients, but it is potentially an oxidative environment as well. Then, *Trypanosoma cruzi* needs to deal with high concentrations of heme and ROS while inhabiting the midgut of the vector. The epimastigote form, present in this environment, is the replicative and non-infective form that is able to increase its rate of proliferation in the presence of heme in a dose-dependent manner [22], and this heme-induced *T. cruzi* growth is associated with calcium-calmodulin-dependent kinase II (CaMKII) activity [23].

Besides heme, ROS have been shown to trigger proliferation of the epimastigote forms of *Trypanosoma cruzi* [10]. According to these authors, the growth of the parasites in the presence of these molecules is regulated by a cellular signaling mechanism involving CaMKII and the redox status, since the antioxidants, such as urate and GSH, inhibited heme-induced ROS and parasite proliferation. In addition, Myr-AIP, a specific CaMKII inhibitor, extinguishes heme-induced ROS in epimastigotes, decreasing parasite growth. To exclude the possibility of other molecules similar to heme being able to induce a potent proliferative effect on *T. cruzi*, tests were carried out with protoporphyrin IX (PPIX), mesoporphyrin IX (MPIX), Fe mesoporphyrin IX (Fe-MPIX), Sn protoporphyrin IX (SnPPIX), and Zn protoporphyrin IX (ZnPPIX), and only heme showed a potent proliferative effect [10]. These data show that the parasite had to adapt to high concentrations of ROS in order to establish itself in such an oxidizing environment.

Heme and two classical oxidants, H_2O_2 and the well-known superoxide generator, paraquat, are able to promote the growth of epimastigotes in vitro [24]. This effect was reversed in the presence of other reductive molecules (GSH, a thiol-based antioxidant found in the hemolymph of triatomines; urate, an important antioxidant rich in the urine of these insects [25], and n-acetylcysteine (NAC), a classic antioxidant) suggesting a competition between these molecules of antagonistic redox status. An important physiological molecule present in the midgut is hemozoin, a crystal composed of heme dimers [26, 27] that *Rhodnius prolixus*, a Chagas disease vector, uses as an efficient detoxification pathway of heme. The addition of this crystal to an epimastigote culture does not produce an increase in the proliferation of these cells [24].

Thus, the redox environment is considered to be very important for *Trypanosoma cruzi*. Furthermore, the parasite needs ROS for growth inside the vector. If this hypothesis is correct, the disturbance of ROS levels in vivo would lead to differences in the levels of epimastigote proliferation within the intestine. In fact, the heme molecule and ROS are examples of important relationships between parasite and vector because they are capable of promoting the proliferation of the epimastigote forms, but when the insect is fed with blood and antioxidants, such as NAC and urate, the proliferation in vivo decreases as demonstrated in vitro [10, 24].

Observing the effect of the physiological molecules (heme, hemozoin, and urate), it is possible to confirm that there is a modulation between molecules of antagonistic redox status, indicating an inhibitory role of reductive molecules on epimastigote proliferation and confirming the requirement of an oxidant signal to promote the growth of these parasites. Furthermore, in 2017, Nogueira and collaborators showed that heme affects the mitochondrial function of *T. cruzi* epimastigotes and, as a consequence, mitochondrial ROS production is increased, triggering parasite proliferation [28].

2.1.2 Differentiation of epimastigotes into metacyclic trypomastigotes

Still on its journey inside the vector, *Trypanosoma cruzi* reaches the rectum of the bug. This region greatly favors metacyclogenesis, and one important factor is the reductive environment promoted by the high concentration of urate. The levels of metacyclic trypomastigotes are increased in the presence of urate and other antioxidants both in vitro and in vivo. On the other hand, the proliferation of epimastigotes decreases in reductive environments [10, 24].

When the blood meal is supplemented with antioxidants, there is a shift in the redox status of the gut compartments (anterior midgut, posterior midgut, and rectum), increasing differentiation of the parasites in an unusual midgut region and greatly favoring metacyclogenesis in the bug rectum. Notably, contrary to proliferation, the differentiation process appears to be favored by reductive environments [24].

A *Trypanosoma cruzi* eIF2α kinase (TcK2) was characterized by Augusto and collaborators [29] as a transmembrane protein located in organelles that accumulate nutrients in the proliferative forms. The heme molecule has been shown to bind specifically to the catalytic domain of the kinase, inhibiting its activity. On the other hand, in the absence of heme, TcK2 is activated, preventing cell growth and inducing the differentiation of epimastigote forms into infectious and nonproliferative forms. Parasites without TcK2 lose this differentiation ability, and heme is not stored in reserve organelles, as demonstrated by Lara and collaborators [21], remaining in the cytosol. Furthermore, if ROS levels are not controlled in TcK2 null, they cause damage to the parasite, including death. Thus, in wild cells, heme has been shown to be a key factor for growth control and differentiation by regulating an unusual type of eIF2α kinase in *T. cruzi* [29].

As demonstrated by science, the coevolution between parasites and their insect vectors has promoted an elegant strategy for the development and maintenance of the protozoa in the invertebrate vector.

2.2 The transmission of the disease: metacyclic trypomastigotes infect the vertebrate hosts—a new journey

2.2.1 The participation of NADPH oxidase in the infection

The immune system of the higher vertebrates is able to recognize pathogens and respond through their innate immune responses. ROS is an important component of this response produced by phagocytes and can be highly toxic. Macrophages are one of the first lines of defense in mammals, especially against pathogens [30], and become activated facing such challenges.

The $O_2^{\bullet-}$ production after NADPH oxidase activation in macrophages is converted inside the phagosome to H_2O_2 (spontaneously or via superoxide dismutase), and this ROS production, termed the "oxidative burst" of activated phagocytic cells, usually kills the pathogens. In order to infect the vertebrate host, *T. cruzi* metacyclic trypomastigotes invade macrophages and overcome the highly oxidative conditions

generated inside the phagosome. Then, biochemical changes occur [9, 31, 32] including antioxidant enzyme activities [33], and, curiously, *Trypanosoma cruzi* depends on ROS involved in this activation process to establish the infection in the vertebrate host [8]. The NADPH oxidase (Phox) activation and this $O_2^{\bullet-}$ production are directly involved in increased infection of macrophages by *T. cruzi* since mice deficient in the $gp91^{phox}$ (Phox KO), subunit of NADPH oxidase, macrophages present reduced parasitism [8, 34].

Peroxynitrite is also highly lethal and used by phagocytes against pathogens. It is formed when nitric oxide (NO) and $O_2^{\bullet-}$ react with each other. Thus, the production of peroxynitrite is decreased by the inhibition of ROS or NO production [35]. Paiva and collaborators, in 2012, showed that macrophages infected with T. cruzi and activated with the burst inducer phorbol 12-myristate 13-acetate (PMA) have stimulated the parasite load [36]. In conclusion, the generation and the regulation of the ROS level can help these parasites thrive in an oxidative environment [8, 35–37].

2.2.2 Murine models of Chagas disease and ROS

After the infective metacyclic forms invade host cells, macrophages, or cardiac cells, for example, they are transformed into the replicative intracellular amastigote form [6]. In response to infection, Chagas hearts present increased mitochondrial ROS [38, 39] because during *T. cruzi* infection an inefficient electron transport for ATP synthesis occurs in mitochondria [39]. Also, when deficient superoxide dismutase (SOD2 or MnSOD) mice are infected with *Trypanosoma cruzi,* the loss of the mitochondrial function increases the oxidative damage of the myocardium in Chagas cardiomyopathy and shows the importance of ROS-level regulations [40]. Moreover, ROS mobilizes intracellular iron which is essential as a cellular factor for amastigote division [30, 36]. ROS, including mitochondrial ROS, contribute to oxidative damage that persists during the chronic stage of infection and is involved in the functional impairment of the heart [40–42]. Some studies show that cardiac parasite load may vary after treatment with antioxidants but depend on the animal model and the strain used [42–44]. In fact, Gupta and collaborators [45] demonstrated that *T. cruzi* infection increases ROS production in cardiomyocytes and this effect is augmented by the pro-inflammatory cytokines. The authors argue that the ROS production by cardiomyocytes is not a defense response against *T. cruzi*. Instead, the infection promotes a mitochondrial dysfunction, including ROS production. Thus, ROS also participates in the successful infection in mammals.

3. Conclusion

Several groups have carried out research on the influence of the oxidative environment on the growth and differentiation of *Trypanosoma cruzi* in both vertebrate and invertebrate hosts. As we have learned in this chapter, the epimastigote, the non-infective and proliferative form, has its growth stimulated in the presence of oxidative compounds. Conversely, in the presence of antioxidants, or in a reductive environment, its proliferation becomes compromised. The regulated ROS levels also influence, in an orchestrated way, the differentiation of epimastigotes into metacyclic trypomastigotes (the infective form). The reductive environment increases differentiation, while ROS dramatically decreases its transformation into the infective forms. These same metacyclic forms that are formed in the rectum of the vector insect invade the vertebrate host by subverting the logic of the phagocytes that, by activation of NADPH oxidase, exacerbate the concentration of ROS in the intention to kill the pathogens. In fact, the trypomastigote forms of *T. cruzi*

resist ROS and establish themselves in the cells of the vertebrate host differing into amastigotes that in cardiomyocytes coexist with increased levels of ROS when compared to uninfected hearts. However, these levels of ROS cannot decrease or increase indiscriminately.

Thus, we have followed the journey of the parasite *Trypanosoma cruzi*, both in the invertebrate and in the vertebrate hosts, that occurs under adverse redox conditions, as if in an orchestra of ROS and antioxidants, and furthermore we can observe that its journey through the intestine of the insect, along the mammalian bloodstream, and its entry and lodging in mammalian cells are finely and elegantly ruled by a redox baton.

Conflict of interest

There is no conflict of interest.

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