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Review

How *Trypanosoma cruzi* deals with oxidative stress: Antioxidant defence and DNA repair pathways

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ABSTRACT

Trypanosoma cruzi, the causative agent of Chagas disease, is an obligatory intracellular parasite with a digenetic life cycle. Due to the variety of host environments, it faces several sources of oxidative stress. In addition to reactive oxygen species (ROS) produced by its own metabolism, *T. cruzi* must deal with high ROS levels generated as part of the host's immune responses. Hence, the conclusion that *T. cruzi* has limited ability to deal with ROS (based on the lack of a few enzymes involved with oxidative stress responses) seems somewhat paradoxical. Actually, to withstand such variable sources of oxidative stress, *T. cruzi* has developed complex defence mechanisms. This includes ROS detoxification pathways that are distinct from the ones in the mammalian host, DNA repair pathways and specialized polymerases, which not only protect its genome from the resulting oxidative damage but also contribute to the generation of genetic diversity within the parasite population. Recent studies on *T. cruzi*'s DNA repair pathways as mismatch repair (MMR) and GO system suggested that, besides a role associated with DNA repair, some proteins of these pathways may also be involved in signalling oxidative damage. Recent data also suggested that an oxidative environment might be beneficial for parasite survival within the host cell as it contributes to iron mobilization from the host's intracellular storages. Besides contributing to the understanding of basic aspects of *T. cruzi* biology, these studies are highly relevant since oxidative stress pathways are part of the poorly understood mechanisms behind the mode of action of drugs currently used against this parasite. By unveiling new peculiar aspects of *T. cruzi* biology, emerging data on DNA repair pathways and other antioxidant defences from this parasite have revealed potential new targets for a much needed boost in drug development efforts towards a better treatment for Chagas disease.

Contents

Abbreviations: ROS, reactive oxygen species; $O_2^{\bullet-}$, superoxide anions; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; $^{\bullet}OH$, hydroxyl radicals; $^{\bullet}NO$, nitric oxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; P5C, D1-pyrroline-5-carboxylate; ROI, reactive oxygen intermediates; RNI, reactive nitrogen intermediates; proPO, prophenoloxidase; PO, phenoloxidase; TAO, alternative oxidase; $T(SH)_2$, trypanothione; GSH, glutathione; TR, trypanothione reductase; TcMPx, mitochondrial tryparedoxin peroxidase; TcCPx, cytosolic tryparedoxin peroxidase; TcTPNI, tryparedoxin; nsGPX, nonselenium glutathione peroxidase; TcAPX, ascorbate peroxidase; SSB, single strand break; DSB, double strand break; AP, apurinic/apirimidinic; NHEJ, non-homologous end joining; HR, homologous recombination; BER, base excision repair; NIR, nucleotide incision repair; NER, nucleotide excision repair; MMR, mismatch repair; TLS, translesion synthesis; dRp, deoxyribose phosphate; SP-BER, short path-base excision repair; LP-BER, long path-base excision repair; $CdCL_2$, cadmium chloride; L-AAO, L-amino acid oxidase.

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1. Introduction

Protozoan parasites from the *Trypanosomatidae* family are unicellular organisms that cause debilitating diseases in many regions of the world, mainly in developing countries with tropical and subtropical climates. Among the members of this family is the etiologic agent of Chagas disease, *Trypanosoma cruzi*. Although there has been a decrease in the global incidence of new infections due to successful programmes of vector control, such as the Southern Cone Initiative initiated in 1991 (aimed to interrupt the vectorial transmission of Chagas disease) [1,2], recent estimates indicate that up to 6–7 million people are infected worldwide, specially in endemic Latin America countries [3], while 10,000 die annually of *T. cruzi* infection [4]. Due to emigration events, the number of diagnosed cases has increased in non-endemic regions such as Europe, North America and Western Pacific regions [5].

Chagas disease spawns a variety of clinical forms. and the possible outcomes of this disease involve an interplay between environmental and genetic factors associated with both the host and the parasite [6–8]. During the initial acute phase of infection parasites invade tissues and multiply, leading to high parasitemia and inflammation. Most individuals then circumvent this acute infection and enter an indeterminate phase consisting of low parasite numbers in the blood and no apparent pathology. However, a chronic phase characterized by low parasitemia but increased tissue injury may follow, resulting in severe digestive and/or cardiac damage that can be lethal if untreated [9].

T. cruzi goes through extensive morphological and biochemical changes during its life cycle, which alternates between mammals and insects hosts. Non-infective epimastigotes proliferate in the midgut of the insect vector, a triatomine hematophagous bug. Once in the insect's hindgut, they differentiate into non-dividing infectious metacyclic trypomastigotes. These are excreted with insect's faeces and infect the mammalian host by passing through mucous membranes or skin lesions during the insect blood meal. These infective metacyclic forms invade host cells, mostly macrophages, where they transform into the replicative intracellular amastigote stage. After multiplying by binary division in the cytoplasm, amastigotes differentiate into infective non-replicative trypomastigotes. Both these forms are released into the bloodstream upon host cell lysis. Subsequently, trypomastigotes penetrate other nucleated cell types, including skeletal and cardiac muscle cells, or are taken up by the insect during a blood meal, starting a new cycle [10].

As an obligatory intracellular parasite *T. cruzi* must withstand its own endogenous toxic metabolites produced as by-products of its aerobic metabolism and also cope with the oxidative burst from the host immune system, which includes the production of superoxide anions ($O_2^{-\bullet}$) and other reactive oxygen species (ROS) [11,12]. However, in contrast with many eukaryotes, it has been reported that trypanosomatids have limited ability to deal with ROS such as O_2^- and various hydroperoxides, based on the absence of catalase and classical selenium-containing glutathione peroxidases [13,14], enzymes capable of metabolizing high levels of hydrogen peroxide (H_2O_2) [15,16]. Whole genome sequencing of *T. cruzi*,*Trypanosoma brucei* and *Leishmania major* [17–20] not only confirms the absence of these enzymes but also suggested the absence of a MutT homologue in *T. cruzi*, which encodes a pyrophosphohydrolase that sanitizes the oxidized nucleotide pool thus preventing mutations caused by spontaneous guanine oxidation [17]. However, it was recently demonstrated that *T. cruzi* encodes a MutT homolog named TcMTH, that was not described in genome project due to sequence misalignment. The TcMTH, is capable of complementing a MutT deficient bacterial strain [21]. The use of ROS to protect against parasite infection could therefore be an adaptation of the host in face of the parasite's limited arsenal of enzymatic defences against these agents. Nevertheless, why should an organism that certainly has to deal with ROS during its life cycle and withstand the ensuing toxic effects in order to survive and establish an infection lack an efficient system to deal with it? Would it be possible that *T. cruzi* could profit from the stress generated from ROS, which may actually provide an evolutionary advantage for the parasite? In this review we will explore the types of oxidative stress *T. cruzi* encounters during its life cycle in its vertebrate and invertebrate hosts, the oxidative stress produced by the parasite itself, the defence mechanisms it possesses to handle those, and the DNA repair pathways involved in the removal of oxidative lesions. We then discuss the possible implications of *T. cruzi* DNA repair response in the generation of parasite genetic variability and, finally, how this knowledge can be used in an alternative treatment against Chagas disease.

2. Dealing with oxidative stress generated from extracellular sources

2.1. Inside the macrophage

For a successful infection, metacyclic trypomastigotes must invade macrophages and survive the particularly harsh oxidative conditions found inside the phagosome. During phagocytosis by macrophages O_2^- is produced as a membrane-associated molecule when NADPH oxidase is activated, contributing to the formation of this oxidative milieu [12,22]. This O_2^- is converted inside the phagosome to toxic effectors such as H_2O_2 (either spontaneously or via superoxide dismutase (SOD)). In fact, cytochemical data revealed that the production of O_2^- starts when the parasite is attached to the macrophage's surface, triggering the activation of NADPH oxidases [23]. Being an uncharged molecule, H_2O_2 can readily diffuse through plasma membranes and oxidise lipids and proteins as well as inhibit membrane transport processes [24]. H_2O_2 can also be converted to hydroxyl radicals ($^\bullet$OH) by the action of transition metals [25]. Due to its large reduction potential, $^\bullet$OH is the most biologically reactive molecule known, reacting with all