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Tissue-specific oxidative imbalance and mitochondrial dysfunction during *Trypanosoma cruzi* infection in mice

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Abstract

In this study, we examined the tissue specificity of inflammatory and oxidative responses and mitochondrial dysfunction in mice infected by *Trypanosoma cruzi*. In acute mice, parasite burden and associated inflammatory infiltrate was detected in all tissues (skeletal muscle > heart > stomach > colon). The extent of oxidative damage and mitochondrial decay was in the order of heart > stomach > skeletal muscle > colon. In chronic mice, a low level of parasite burden and inflammation continued in all tissues; however, oxidant overload and mitochondrial inefficiency mainly persisted in the heart tissue (also detectable in stomach). Further, we noted an unvaryingly high degree of oxidative stress, compromised antioxidant status, and decreased mitochondrial respiratory complex activities in peripheral blood of infected mice. A pair-wise log analysis showed a strong positive correlation in the heart-versus-blood (but not other tissues) levels of oxidative stress markers (malonyldialdehyde, glutathione disulfide), antioxidants (superoxide dismutase, MnSOD, catalase), and mitochondrial inhibition of respiratory complexes (CI/CIII) in infected mice. *T. cruzi*-induced acute inflammatory and oxidative responses are widespread in different muscle tissues. Antioxidant/oxidant status and mitochondrial function are consistently attenuated in the heart, and reflected in the peripheral-blood of *T. cruzi*-infected mice. Our results provide an impetus to investigate the peripheral-blood oxidative responses in relation to clinical severity of heart disease in chagasic human patients.

Keywords: Chagas' disease; Mitochondrial dysfunction; Oxidant/antioxidant status; Tissue specificity; *Trypanosoma cruzi*

1. Introduction

Trypanosoma cruzi is the etiologic agent of Chagas' disease, a major human health problem in the southern parts of the American continent. Several decades after the initial infection, >30% of infected individuals develop chronic cardiomyopathy that may lead to congestive heart failure and sudden death [1].

Mitochondria are the prime source of energy, and many of the body functions, including cardiac metabolic and contractile activities, require the mitochondrial generation of ATP. Studies in mice have shown that the altered expression of the transcripts for mitochondrial function-related proteins and

Abbreviations: CAT, catalase; CI, NADH ubiquinone oxidoreductase; CIII, ubiquinol cytochrome *c* oxidoreductase; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; HNE, 4-hydroxy-2-nonenal; MDA, malonyldialdehyde; 3NT, 3-nitrotyrosine; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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increased oxidation of these proteins occur in chagasic hearts [2,3], and are associated with an impaired activity of the respiratory complexes [4,5]. The functional effect of the respiratory complex inhibition was noted to be reduced oxidative phosphorylation and ATP levels in the myocardium of chagasic mice [6].

Besides reduced energy output, mitochondrial defects of the respiratory chain can result in increased electron leakage and production of reactive oxygen species (ROS) that are deleterious to mitochondrial and cellular components [7]. Additionally, chagasic patients are exposed to reactive nitrogen species (RNS) and ROS of inflammatory origin [8]. These reactive oxidants, though important for the control of *T. cruzi*, may also elicit toxicity to host cellular components.

In this study, we investigated whether oxidative cellular damage and mitochondrial dysfunction of the respiratory chain are specific to the heart during *T. cruzi* infection and disease development. For this, we compared the antioxidant/oxidant status and mitochondrial function in tissue from the heart, skeletal muscle, colon, and stomach of *T. cruzi*-infected mice. We chose to study muscle tissues because *T. cruzi* exhibits myotropic behavior, and muscle cells are the preferred site for infection and parasite replication. Our data show that oxidative damage in acute mice was a bystander effect of parasite-induced inflammatory responses and was widespread in different tissues. The inflammatory processes persisted at a low level in all tissues after the control of acute parasite burden. The finding of persistent oxidative responses in the heart and peripheral blood of chronically infected mice suggest the pathologic importance of these responses in Chagas' disease, and provide an impetus to pursue human studies investigating whether changes in blood correlate with the degree of clinical presentation in the heart of chagasic patients.

2. Materials and methods

2.1. Mice and parasites

We infected 6- to 8-week-old male C3H/HeN mice with *T. cruzi* trypomastigotes (SylvioX10/4, 10,000/mouse) and euthanized them during the acute phase (20–35 days post infection (dpi)) corresponding to peak parasitemia and the chronic (150–180 dpi) phase of disease development [9].

2.2. Mitochondria isolation

Freshly harvested tissues (~50 mg) were homogenized, and mitochondria isolated by differential centrifugation [6]. Red blood cells (RBCs) were removed by hypotonic lysis, and the remaining white blood cells (WBCs) were used for mitochondria isolation. Mitochondrial preparations were >96% pure (<3% glucose-6-phosphatase (ER-marker) and acid-phosphatase (peroxisome-marker) activities).

2.3. Biochemical activities

Superoxide dismutase (SOD) activity$_{420nm}$ in tissue and blood homogenates and MnSOD activity in isolated mitochondria was measured by a decrease in $O_2^{\cdot-}$-dependent pyrogallol oxidation. Catalase (CAT) activity$_{240nm}$ was measured by H_2O_2 reduction. Glutathione peroxidase (GPx) activity$_{340nm}$ was measured, using *tert*-butyl-hydroperoxide substrate, by GSH oxidation coupled to NADPH utilization by glutathione reductase [10]. CI-complex activity$_{340nm}$ in isolated mitochondria was monitored by NADH (200 μM) oxidation with 80 μM 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DB) electron acceptor (±6.35 μM rotenone). Complex-CIII activity$_{550nm}$ was quantitated by 60 μM DBH_2 (60 μM) oxidation with 50 μM cytochrome *c* electron donor (±3.75 μM antimycin) [5].

2.4. Oxidants/antioxidants

We measured malonyldialdehyde (MDA) levels by a TBARS assay [11,12]. The 2,4-dinitrophenylhydrazine (DNP)-derivatized protein carbonyls and 3-nitrotyrosine (3NT) contents were detected by Western blotting (WB) using an anti-DNP and anti-3NT antibody, respectively. Glutathione (GSH, GSSG) content was determined by a DTNB-GSSG reductase recycling assay [10].

2.5. Parasite detection

Tissues (50 mg) were subjected to Proteinase K lysis, and total DNA was isolated by phenol:chloroform extraction and ethanol precipitation method. Total DNA (50 ng) was used as a template in a PCR reaction with *T. cruzi 18SrRNA*-specific oligonucleotides. Amplicons were resolved/visualized by agarose gel electrophoresis. Murine *GAPDH* was amplified as a control [9].

2.6. Histology

Tissues were fixed in 10% formalin, embedded in paraffin, and hematoxylin/eosin-stained sections (5 μm) visualized by light microscopy [9]. Tissues were graded for type/distribution of inflammatory infiltrate, amount of inflammation (rare–mild–moderate–severe), parasite foci (absent–rare–scattered–abundant), and other pathologic changes (e.g., calcification, necrosis, fibrosis, vascular thickening).

2.7. Data analysis

Data are expressed as mean ± SD ($n \geq 9$ animals/group). ANOVA and Student's *t*-tests were employed to determine the significance ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, infected versus normal).