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Trypanosoma cruzi: Effects of repetitive stress during the development of experimental infection

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Abstract

Activation of the hypothalamus–pituitary–adrenal axis plays a major role in the suppression of the immune system. We have investigated the effects of repetitive stress on Wistar rats infected with the Y strain of *Trypanosoma cruzi* and a control group that underwent stressor stimuli by exposure to ether vapor for one minute twice a day. Repetitive stress resulted in an elevated number of circulating parasites accompanies by deep tissue disorganization, and cardiac histopathological alterations. The infected and stressed group displayed a decrease in body weight, and an increased parasite burden in heart tissue, and adrenal glands. Histological analysis of the heart also showed a moderate to severe diffused mononuclear inflammatory process. These results suggest that repetitive stress could be considered an important factor during development of experimental Chagas' disease, enhancing pathogenesis through disturbance of the host's immune system.

Keywords: *Trypanosoma cruzi*; Chagas' disease; Stress; Histopathology; Parasitemia; Rats

1. Introduction

American trypanosomiasis, a chronic disease caused by the hemoflagellate protozoan *Trypanosoma cruzi*, affects about 20 million people in Central and South Americas (WHO, 1991). The acute phase is normally characterized by patent parasitemia and intense proliferation of amastigotes in several tissues including heart. The cardiac pathogenesis of Chagas' disease is characterized by myocarditis and inflammatory processes. The host immune response to *T. cruzi* has been studied in different experimental models, including sylvatic rodents (Prado et al., 1999), mice (Bustamante et al., 2003), and rats (Melo and Machado, 2001) due to their ability to mimic the human disease.

Infection by pathogenic agents leads to an imbalance in the immune response, creating conditions favorable to the parasite's establishment. Suppression of the immune response can be heightened in humans and animals by both acute and chronic stress (Bohus et al., 1991; Chrousos and Gold, 1992). Many stressors have been shown to affect both cellular and humoral immune function (Khansari et al., 1990). In recent years, several studies have documented that many cytokines can influence the secretory activity of the hypothalamic–pituitary–adrenal (HPA) axis. During inflammation, cytokines stimulate the HPA axis through direct and indirect actions on the central nervous system and pituitary and adrenal glands, which in turn, generally inhibit or modulate inflammation through immunosuppressive effects of glucocorticoids (Turnbull and Rivier, 1999).

Although the hormones of the HPA axis also play a role in regulating the establishment and maturation in

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models of parasite disease (Morales-Montor et al., 2001), there is as yet no strong evidence relating the activity of the HPA axis to experimental Chagas' disease. Ether vapor as a stress stimulus can be used to increase the HPA axis activity, increasing corticosterone plasma levels within a few minutes (Caldeira and Franci, 2000).

Immune suppression resulting from AIDS, malignant tumors, or glucocorticoid therapies can affect the development of *T. cruzi* infection (Oz et al., 2002). The importance of immune suppression in *T. cruzi* pathogenesis is clear, and this area has received considerable attention in recent years (Rassi et al., 1997). Since stress is known to significantly influence the activity of immune system, it is important to understand its effects on the course of infection by *T. cruzi*.

Profound societal changes have caused humans to be subjected to new kinds of stress. Modern life exposes people to conflict, anxiety, and emotionally trying situations unknown to our ancestors (Khansari et al., 1990). Stress can trigger a worsening of several pathologies, including Chagas' disease (Leite de Moraes et al., 1991). The effects of these kinds of stimuli have been studied by comparing the parasitaemias in males and females kept singly or in groups. It was shown that both genders, when kept alone, develop a lower parasitaemia than when kept together; suggesting that social stress can affect the course of *T. cruzi* infection (Schuster and Schaub, 2001). Other authors have also described the influences of social environment as a source of stress, not only affecting glucocorticoid levels, but also testosterone in males (Koolhaas et al., 1997).

We have studied the effects of repetitive stress during the development of Chagas' disease using male Wistar rats infected with the Y strain of *T. cruzi*. We focused on the hypothesis that stress can affect the course of Chagas' disease, based on the histopathological alterations of heart and adrenal tissues.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n = 60$) weighing 90–100 g were used. The animals were divided in four groups: Non-Stressed Non-Infected, Stressed Non-Infected, Non-Stressed Infected, and Stressed Infected. Rats were kept five to a cage with commercial rodent chow and water available ad libitum. Animals were weighed daily during the experiments using an analytical balance.

2.2. Infection

The rats were intraperitoneally (i.p.) inoculated with 2×10^5 blood trypomastigotes of the Y strain of *T. cruzi* (Silva and Nussenszweig, 1953). The experiments were performed on 7, 14, and 21 days after infection. Daily individual parasitemia was determined by Brener's Method (Brener, 1962).

2.3. Stress stimuli

Over the period of the study, the animals were exposed to ether vapor for one minute twice a day as a stressor stimulus. The stress exposure was performed in an isolated room separate from other animals and laboratory personnel and the rats were introduced to a closed container with an ether-soaked rag placed inside. The sealed ether bottle $(C_2H_5)_2O$ was stored in a cold room to avoid vapor release to the environment, and was only opened when required.

2.4. Euthanasia

The animals were killed by decapitation to prevent additional stress and enhanced corticosterone plasma levels. All procedures were conducted in compliance with the requirements of the Brazilian Committee on Animal Experimentation (COBEA).

2.5. Histopathology

Heart and adrenal glands were harvested, weighed in an analytical balance, and subsequently immersed in a 10% ALFAC solution (alcohol, formaldehyde, and acetic acid) and embedded in paraffin. Six-micrometer thick tissue sections were stained with hematoxylin–eosin. Parasite density was estimated in sections separated at 70 μm intervals to avoid recounting amastigote nests. For each tissue fragment, 50 microscopic fields were analyzed at a magnification of 400× and all amastigote nests were counted in each field (Castro and Brener, 1985).

2.6. Karyometry

To determine the changes in nuclear size of myocardial cells karyometric analysis consisting of measuring the longest and shortest axes of nuclei from cardiac cells (Yan et al., 1999) was used. This was conducted to determine whether stress could trigger effects on the morphology of cardiac nuclear cells that resulted in nuclear size alterations. Tissue sections were evaluated using a computer image capture system. Fifty nuclei per section were identified (by circling) and a total of 250 nuclei per animal were measured. The parameters analyzed were mean diameter, perimeter, volume/area, shape coefficient, and contour and eccentricity index (Sala et al., 1994).

2.7. Statistical analysis

The results were expressed as means ± SD. Statistical significance between the groups was determined by