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Immunomodulatory effects of zinc and DHEA on the Th-1 immune response in rats infected with *Trypanosoma cruzi*

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Abstract

Chagas' disease is considered the sixth most important neglected tropical disease worldwide. Considerable knowledge has been accumulated concerning the role of zinc on cellular immunity. The steroid hormone dehydroepiandrosterone (DHEA) is also known to modulate the immune system. The aims of this paper were to investigate a possible synchronization of their effects on cytokines and NO production and the resistance to *Trypanosoma cruzi* during the acute phase of infection. It was found that zinc, DHEA or zinc and DHEA supplementation enhanced the immune response, as evidenced by a significant reduction in parasitemia levels. Zinc and DHEA supplementation exerted additive effects on the immune response by elevation of macrophage counts, and by increasing concentrations of IFN-γ and NO.

Keywords: *Trypanosoma cruzi*; Zinc; DHEA; Peritoneal macrophages; Interferon gamma (IFN-γ); Nitric oxide (NO)

Introduction

Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, is considered the sixth most important neglected tropical disease worldwide. Globally, this illness is associated with 14,000 deaths per year and 0.7 million disability-adjusted life years (Hotez et al. 2006). Chagas' disease remains a serious obstacle to health and economic development in Latin America, especially for the rural poor, but as the trend for global migration increases, the scope of Chagas disease threatens to expand exponentially, from rural to urban areas, and from endemic to non-endemic regions (WHO—World Health Organization 2002; The Lancet 2006). According to Maguire (2006) approximately 90 million individuals remain at risk of contracting the illness.

The infection is rarely lethal in immunocompetent hosts and, after a characteristic period of acute parasitemia, is usually controlled by a potent immune response. The rat provides a particularly useful experimental model of the immune response to *T. cruzi* infection (Brazão et al. 2009) because of its ability to mimic the human disease, including a prominent parasitemia, heart parasitism, and myocarditis (Machado and Ribeiro 1989; Melo and Machado 1998).

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Symptoms of acute infection may last up to a few weeks or months, and parasites may be found in the blood during this stage. After a short acute phase and a very long asymptomatic phase, 25–40% of infected individuals progress to chronic disease with cardiac, digestive, or neurological manifestations (WHO—World Health Organization 2002; Dias et al. 2002), which, if left untreated, are severely debilitating, and, in many cases, fatal (Prata 2001). Individual differences in specific clinical manifestations could depend on genetic differences in the host and/or parasite. Furthermore, immunosuppression (e.g., due to HIV/AIDS or chemotherapy) can cause reactivation, leading to abundant parasitemia in the blood and tissues.

$CD4^+$ Th1 lymphocytes are largely responsible for induction of partially protective immunity against *T. cruzi* (Hoft et al. 2000). $CD8^+$ lymphocytes, IFN-γ and macrophages are important elements controlling parasite replication during the acute phase of infection. Macrophages activated by IFN-γ and/or TNF-α synthesize nitric oxide (NO), which in the mouse is considered the major effector of intracellular amastigote killing (Vespa et al. 1994; Silva et al. 1995). Mice deficient in the receptor for IFN-γ (IFN-γR−/−) or the inducible NO synthase (iNOS−/−) (Gazzinelli et al. 1992) are highly susceptible to infection. These alterations lead to an exacerbation of parasitemia and mortality due to defective macrophage activation and NO production (Hölscher et al. 1998).

Considerable knowledge has been accumulated concerning the role of zinc on cellular immunity (Bach 1981; Shankar and Prasad 1998). It is essential for the activities of more than 300 enzymes (Chang et al. 2006). Zinc-deficient persons develop increased susceptibility to infections because of immune disorders (Prasad 1998; Prasad et al. 2006). Zinc deficiency has been described to influence the balance of Th1/Th2 T helper cell subsets (Prasad 2000, 2004), and has been specifically shown to have potent immunomodulatory effects (Petanova et al. 2000).

In a previous study, we verified that zinc supplementation increases resistance to experimental infection by *T. cruzi* by up-modulating the host's immune response (Brazão et al. 2008b).

Dehydroepiandrosterone (DHEA) plays a vital role in regulating the immune responses in some infectious diseases. DHEA has been shown to protect mice from a variety of infections such as *Cryptosporidium parvum* (Rasmussen et al. 1993, 1995), *Plasmodium falciparum* (Kurtis et al. 2001; Leenstra et al. 2003) and *T. cruzi* (Dos Santos et al. 2005).

The aims of this paper were to investigate a possible additive effect of zinc and DHEA supplementation on cytokines and NO production and the resistance to *T. cruzi* during the acute phase of infection.

Material and methods

Animals

Male Wistar rats (120 animals) 4 weeks old, weighing 90–100 g were used. Rats were obtained from the Facility House of the Universitary Campus of Ribeirão Preto. Animals were divided in groups of $n = 5$ per group/day of infection: Non-infected males-without-zinc and DHEA supplementation (MWZDNI), non-infected males-zinc supplemented (MZNI), non-infected males-DHEA supplemented (MDNI), non-infected males-zinc and DHEA supplemented (MZDNI), infected males-without-zinc and DHEA supplementation (MWZDI), infected males-zinc supplemented (MZI), infected males-DHEA supplemented (MDI), infected males-zinc and DHEA supplemented (MZDI). Rats were separated in number of 5 in plastic cages and commercial rodent diet and water were available *ad libitum*. Rat pad was changed 3 times/week to avoid concentration of ammonia from urine. The protocol of this study was approved by the local Ethics Committee protocol number 06.1.427.53.2.

Parasites and experimental infection

Rats were intraperitoneally (i.p.) inoculated with 1×10^5 blood tripomastigotes of the Y strain of *T. cruzi* (Silva and Nussenzweig, 1953). The experiments were performed in duplicate on 7, 14 and 21 days after infection. Parasitaemia was determined by Brener's method (Brener 1962). It is important to emphasize that since Wistar rats are normally resistant to most *T. cruzi* strains, we found it necessary to use relatively high inoculums (1×10^5 blood trypomastigotes), which resulted in a more intense pathological response such as enhanced parasitemia and tissues lesions.

Zinc and DHEA supplementation

Rats were orally supplied with zinc sulfate (Sigma Chemical Co., MO, USA), at a dose of 20 mg/kg body weight, dissolved in 0.1 mL of distilled water (gavage), once a day over the course of the experiment (Brazão et al. 2009) and were treated with 0.1 mL of DHEA (Sigma Chemical Co.), which had been dissolved in absolute ethanol (0.05 mL) and then diluted with an equal volume of distilled water (0.05 mL). DHEA was administered subcutaneously at a dose of 40 mg/kg body weight once a day over the course of the experiment (Dos Santos et al. 2005). Treatment of the infected group started 48 h before infection. Control groups were supplied as prior described.