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Effects of iron supplementation on blood adenine deaminase activity and oxidative stress in *Trypanosoma evansi* infection of rats

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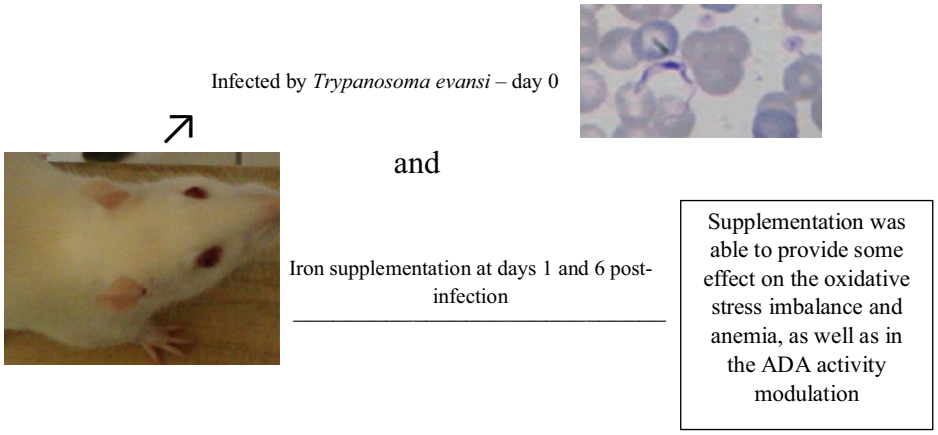
HIGHLIGHT

- *Trypanosoma evansi* is the etiological agent of the disease known as "*Surra*" or "*Mal das cadeiras*".
- *T. evansi* infection plays an important role in the anemia, iron levels, ADA activity and oxidative stress.
- Iron supplementation was able to provide some effect on the oxidative stress imbalance, as well as in the ADA modulation.
- Iron supplementation helped to diminish the parasitemia, and indirectly reduced the anemic process.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to assess the effects of iron supplementation on oxidative stress and on the activity of the adenosine deaminase (ADA) in rats experimentally infected by *Trypanosoma evansi*. For this purpose, 20 rats were divided into four experimental groups with five animals each as follows: groups A and B were composed by healthy animals, while animals from groups C and D were infected by *T. evansi*. Additionally, groups B and D received two subcutaneous doses of iron (60 mg kg^{-1}) within an interval of 5 days. Blood samples were drawn on day 8 post infection in order to assess hematological and biochemical variables. Among the main results are: (1) animals from group C showed reduced erythrogram (with tendency to anemia); however the same results were not observed for group D; this might be a direct effect of free iron on trypanosomes which helped to reduce the parasitemia and the damage to erythrocytes caused by the infection; (2) iron supplementation was able to reduce NO_x levels by inhibiting iNOS, and thus, providing an antioxidant action and, indirectly, reducing the ALT levels in groups B

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and D; (3) increase FRAP levels in group D; (4) reduce ADA activity in serum and erythrocytes in group C; however, this supplementation (5) increased the protein oxidation in groups B and D, as well as group C (positive control). Therefore, iron showed antioxidant and oxidant effects on animals that received supplementation; and it maintained the activity of E-ADA stable in infected/supplemented animals.

1. Introduction

Trypanosoma evansi is the etiological agent of the disease known as "*Surra*" or "*Mal das cadeiras*" in horses. It shows a wide geographical distribution, being found parasitizing various species of domestic and wild animals (Silva et al., 2002). Rapid weight loss, anemia, swollen limbs, progressive weakness, neurological signs and paralysis of the hind limbs (in chronic cases) are the clinical signs usually observed in trypanosomosis (Herrera et al., 2004; Rodrigues et al., 2005). The disease in its acute form is characterized by hematological changes, progressive anemia, anorexia, leading in some cases to death (Omer et al., 2007).

T. evansi infection is able to cause an inflammatory response in the host (Paim et al., 2011). Considering that inflammation is a stereotypical physiological response to infections and tissue injury, which leads to pathogen killing as well as tissue repair processes, helping to restore homeostasis at infected or damaged sites (Calder et al., 2009), it is important to consider on this conditions, may changes in the plasma level of certain trace metals (e.g., a reduction in plasma iron) can be observed (Weinberg, 1978). Iron is an essential nutrient for biochemical processes, such as electron transfer and regulation of cell growth, transport oxygen. It is also essential for cellular homeostasis, DNA synthesis, energetic metabolism and synthesis of heme group (Iannotti et al., 2006; Smith, 2002). Iron redox enzymes are essential for cell respiration, DNA synthesis, and free radical-scavenging mechanisms, but in the presence of an inflammatory process, we observe a lack of free iron (Doherty, 2007).

According to Wolkmer et al. (2007) and Da Silva et al. (2009) *T. evansi* infection plays an important role in anemia. In this sense, the mechanism of anemia in trypanosomosis is greatly associated with the generation of free radicals and super oxides following lipid peroxidation. These oxidative products generally attack the cellular integrity of erythrocytes during trypanosomosis (Anosa and Kaneko, 1983; Igbokwe, 1994). Additionally, it is well known that the main adenosine deaminase (ADA) substrate, adenosine, is involved in various functions of *T. evansi* (Suswam et al., 2003), as well as in the pathogenesis of anemia (Zhang and Xia, 2012; Zhang et al., 2011).

Therefore, considering the inflammatory response generated after *T. evansi* infection and its mechanism to cause anemia, the aim of this study was to assess the effects of iron supplementation on oxidative stress and on the ADA activity in rats experimentally infected by *T. evansi*.

2. Materials and Methods

2.1. Experimental design

Twenty male rats (Wistar), 120-day-old, weighing an average of 420 ± 24 g were kept in cages (five rats each) throughout the experiment. They were housed in an experimental room on a light/dark cycle of 12 h, under controlled temperature and humidity (23 ± 2 °C; 70%, respectively), fed with commercial feed and water *ad libitum*. All animals were submitted to a period of 10 days of adaptation before the beginning of the experiment.

The animals were divided into four groups (A–D – Table 1). Groups A and B were composed of healthy rats, representing the uninfected group. Animals of Groups C and D were subcutaneously inoculated with 0.1 mL of blood cryopreserved, containing 4.7×10^5 of *T. evansi* (on Day 0). Thus, Groups A and C represented the negative and positive controls of infection (without iron treatment), respectively; while rats of Groups B and D received two doses (60 mg kg^{-1}) of iron (Ferrodex/Agroline®, Campo Grande, Mato Grosso, Brazil), with an interval of 5 days (Sampaio, 2012), i.e., the iron was administered subcutaneously on days 1 and 6 post-infection (PI), in both groups.

This protocol was approved by the Animal Welfare Committee of Universidade do Estado de Santa Catarina, under number 1.61.13.

2.2. Trypanosoma evansi isolate

The cryopreserved isolate of *T. evansi* used in this experiment was firstly obtained from a naturally infected dog (Colpo et al., 2005). According to the literature (Tavares et al., 2011), the use of inoculum cryopreserved of *T. evansi* is indicate when a slow pattern of infection is required, since the cryopreservation helps to keep the parasitemia lower. This procedure prevents animal death by acute disease. Therefore, blood containing *T.* evansi under cryopreservation was thawed, and immediately inoculated in the animals.

2.3. Level of parasitemia

The evolution of parasitemia and the effect of the treatment were daily monitored through fresh blood smears microscopically examined during 8 days PI. Each slide was mounted with blood collected from the distal region of the tail vein, stained by the panoptic method, and visualized at a magnification of 1000× (Da Silva et al., 2006). The technique requires the observation of 25 fields, and the mean parasitemia was estimated for each animal.

2.4. Sample collection

Blood was collected on day 8 PI (Table 1). Part of the blood (2 mL) was stored in EDTA tubes for hemogram (1 mL), and erythrocytes separation to E-ADA analysis (1 mL). The remaining aliquot (6 mL) was stored without anticoagulant in order to obtain serum for assessment of E-ADA activity, as well as to assess the levels of iron and other biochemical variables.

2.5. Hemogram

Total erythrocyte count, hematocrit (Ht), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total leukocytes were evaluated. The determination of hematocrit was performed according to the technique described by Feldman et al. (2000). Erythrocyte and leukocytes count, and hemoglobin concentration were carried out using an automatic counter (BC 2800 Vet, Mindray, Curitiba, Paraná, Brazil). Blood smears were prepared and stained according to *Romanowski's* method, allowing the evaluation of cell morphology and leukocytes differentiation.