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The treatment with selenium increases placental parasitismin pregnant Wistar rats infected with the Y strain of *Trypanosoma cruzi*

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ABSTRACT

Selenium (Se) is an essential micronutrient in the diet of mammals and has an important role in the immune function. Selenium is a key element in selenoproteins involved in the in the maintenance of the antioxidant defense. Diet with selenium is beneficial for the treatment of diseases correlated with high levels of oxidative stress, also observed in the Chagas disease. Chagas disease is a neglected disease caused by the protozoan *Trypanosoma cruzi* and several research groups are focused on the illness treatment. Immunomodulation of the infection using microelements is an important tool to avoid deleterious effects of the Chagas disease. Therefore, our objective was to evaluate the effects of selenium supplementation on pregnant Wistar rats infected with *T. cruzi*. Selenium treatment stimulated the weight and length of fetuses and placentas allied to the decrease of blood parasitemia. However, selenium demonstrated a low influence on T cells, diminishing the B cell population ($CD45RA^{+}$). Moreover, the production of pro-inflammatory cytokines was downregulated under selenium administration. Low pro-inflammatory cytokines levels probably are related to the increase in the number of amastigote nests in infected and treated animals. Thus, selenium supplementation during pregnancy could impair the local placental immune response. Further studies are necessary to assess the interaction between selenium and the acute Chagas' disease during pregnancy, which will base future supplementation strategies.

1. Introduction

Chagas disease is a neglected tropical disease caused by different types of the flagellated protozoan *Trypanosoma cruzi*. The disease affects 18 million people and about 20 million are exposed in South and Central America. Every year is estimated that over 10 000 people die due the clinical manifestations of Chagas disease, impacting the economy and life quality (WHO, 2018). The clinical progress of the disease is divided into acute and chronic phases. The acute phase usually demonstrates a parasite proliferation in the bloodstream; however, most of the symptoms are absent or unspecific. The chronic phase of the disease is characterized by cardiac, digestive and/or neurologic alterations. Up to 30% patients develop cardiac arrhythmias and progressive heart failure (WHO, 2018). The parasite is commonly transmitted to humans and other mammals by a *T. cruzi* infected insect from *Triatominae* family. Blood transfusion, organ transplantation, congenital and oral (including breastfeeding) transmission are also related (WHO, 2018). At the same time, the congenital transmission has gained epidemiological importance, due to the lack of treatment strategies on pregnant patients (Blaszkowska and Goralska, 2014; Carlier, 2005). Congenital transmission is related to the "globalization of Chagas disease", afflicting fourteen million people which migrated from endemic areas to North America, Europe, Japan and Australia (Schmunis and Yadon, 2010).

Selenium was described by the Swedish chemist Jöns Jacob Berzelius in 1817 (Mistry et al., 2008; Oldfield, 1987) and recognized as an essential element in 1957 (Small-Howard and Berry, 2005). Selenium is an important component of the selenocysteine amino acid which compose more than 25 selenoproteins, related to a large range of functions (Beckett and Arthur, 2005). Selenium supplementation has been correlated with the endocrine, immune and anti-inflammatory regulation, besides cancer and cardiovascular disease prevention (Hatfield et al., 2006). In pregnancy, the levels of selenium usually decrease due to the plasma dilution, transport of selenium to fetus and

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production of antioxidant enzymes (glutathione peroxidase). The antioxidant defense plays an important role in pregnancy support, controlling oxidative stress associated with perinatal morbidity and mortality (Ferrer et al., 1999; Mihailovic et al., 2000).

In Chagas disease-endemic areas, the population is susceptible to poor nutrition and several studies have correlated the nutritional dysfunction and the infectious course of *T. cruzi* (de Andrade and Zicker, 1995; Rivera et al., 2002). Alimentary supplementation has an influence on the immune system function improving resistance to infections in humans and animals (Moreno-Reyes et al., 1998; Muller and Krawinkel, 2005). Diets based on poor amounts of selenium have been associated with high mortality by *T. cruzi* (de Souza et al., 2010). Following these promising results, the goal of our article evaluated the effects of selenium administration in pregnant Wistar rats infected with *T. cruzi*, observing the immune response, fetal and placental morphology and parasite burden. Our observations will base future strategies related to dietary supplementation on pregnant woman affected by the Chagas disease, concerning the health of mother and fetus.

2. Material and methods

2.1. Animals

Female Wistar rats (five animals/group) weighing 180–200 g were used in all experiments. Rats were obtained from the Facility House of the University Campus of Ribeirão Preto. Animals were randomized into the following groups: pregnant control (PC), pregnant control treated with selenium (PCS), pregnant infected without treatment (PI), pregnant infected treated with selenium (PIS). The animals were housed two to a cage. One male Wistar rat was introduced into each cage and was allowed to mate with the females. The period of appearance of a vaginal plug was designated as day 1 of gestation. The protocol of this study was approved by the local Ethics Committee (protocol number 01.193.53.4).

2.2. Experimental infection

Three days after confirmation of pregnancy, rats were intraperitoneally (i.p.) inoculated with 1×10^5 blood trypomastigotes of the *T. cruzi* Y strain (da Costa et al., 2013). Parasitemia was determined by Brener's method at 8^{th} and 15^{th} days after infection.

2.3. Treatment

Sodium selenate was purchased from Sigma-Aldrich (catalogue number: S8295). The groups were orally supplied with 0.2 mg selenium/kg body weight/day (de Souza et al., 2002) diluted in water from the 4^{th} day of pregnancy until the end of the experiment (18^{th}). Selenium was administered every day in the morning.

2.4. Euthanasia

On 18^{th} of pregnancy, which corresponded to the 15^{th} day after infection, animals were euthanized by decapitation after anesthesia using tribromoethanol 2.5%. The euthanasia was carried out at the end of gestation when the placenta and fetuses were totally developed.

2.5. Morphology of placentas and fetuses

Placentas and fetuses were weighed throughout the course of the experiment using an analytic balance. Placental and fetal lengths were measured with a digital caliper.

2.6. Peritoneal cells counting

Peritoneal cells were harvested after injection of 5 ml of cold RPMI 1640 medium into the peritoneal cavity. The cells were centrifuged at 410 *g* for 15 min and the pellet suspended in 1 ml of RPMI 1640 medium (Sigma-Aldrich, USA) and diluted (1:10) with Turk's solution (15 ml glacial acetic acid; 0.2 ml gentian violet 2%; 500 ml distilled water). Peritoneal cells were counted in a hemocytometer.

2.7. Flow cytometry assay

The cell surface phenotype analysis was performed according to (da Costa et al., 2018). Briefly, cells from spleen were mechanically disaggregated by extrusion using a 100 mm nylon cell strainer (Falcon, USA) and washed with a hypotonic buffer (160 mM NH_4Cl, 10 mM Tris-HCl, pH = 7.4). Cells were suspended in RPMI (2×10^7 cells/mL) and the viability verified by trypan blue assay (Sigma-Aldrich, USA).

The cells (2×10^6 cells/tube) were resuspended in 12 × 75 mm round-bottomed polystyrene tubes (Falcon, USA) in staining buffer (BSA) (BD-Pharmingen, San Diego, USA) and blocked using Fc receptor blocking (anti-CD32). The cells were incubated with specific monoclonal antibodies for 30 min at 4 °C in the dark (da Costa et al., 2017), followed by analysis using a FACS Canto flow cytometer (BD Biosciences, California, USA) equipped with the FACSDiva software. The conjugated monoclonal antibodies (anti-$TCD3^+$/APC, anti-$TCD8^+$/PE, anti-CD161/FITC, anti-CD45RA/PE) were obtained from BD Biosciences Pharmingen (CA, USA).

2.8. Cytokine quantification

The serum obtained from 18 days post pregnancy animals was used for cytokine assays. Concentrations of IL-2, TNF-α and IFN-γ were measured by specific two-site enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications and using reference standard curves. All kits were purchased from R&D Systems (Minneapolis, MN, USA). The samples were processed individually (five animals/group), assayed in duplicate and plates read at 450 nm in an ELISA reader (Sunrise Tecan).

2.9. Histopathology

Hearts, placentas and fetuses were harvested and subsequently immersed in buffered 10% formaldehyde. Paraffin-embedded tissue was cut horizontally into 6 µm sections and stained with hematoxylin-eosin for evaluation of inflammation, fibrosis and presence of amastigote nests by optical microscopy. Parasite burden was estimated in sections separated at 70 µm intervals to avoid recounting amastigote nests. For each tissue fragment, all microscopic fields were analyzed at a magnification of 400× and all amastigote nests were counted in each field.

2.10. Statistical analysis

All statistical analyses were performed using the program Graph Pad PRISMA 5.0 (Graph Pad, USA). The data were analyzed using ANOVA followed by Bonferroni test and the results were expressed as mean with standard deviation. In all analyzes, the differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Parasitemia

The parasitemia from selenium infected/treated animals (PIS) was lower compared to the infected group (PI) at 8^{th} and 15^{th} days after *T. cruzi* inoculation (Fig. 1).

3.2. Morphology of placentas and fetuses

Table 1 shows the number of fetuses and placentas in all