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Research Brief

Leishmania chagasi: Effect of the iron deficiency on the infection in BALB/c mice

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ABSTRACT

Iron deficiency and visceral leishmaniasis are serious problems of public health. The aim of this study was to evaluate the effect of iron deficiency, induced by the iron chelator desferrioxamine, on the course of the infection by *Leishmania chagasi* in BALB/c mice. Our data show that the iron chelator caused significant reduction in hemoglobin concentration of treated mice and reduction in parasite load in spleen and liver. Significant differences were not observed in the production of IFN-gamma and IL-4 among the experimental groups. In conclusion, the data reported in this paper suggest that iron deficiency may favor the host. If there is not enough iron available to the parasite, its multiplication may be reduced and infection attenuated.

1. Introduction

Currently, both iron deficiency and infection caused by *Leishmania* parasites are considered serious problems of public health which affect millions of people. Visceral leishmaniasis (VL) has an annual incidence of 500,000 new cases (WHO, 2005), and recently, an increase in the number of cases has been observed in many areas where there is a high prevalence of iron deficiency.

Iron deficiency, according to the World Health Organization (WHO), is the most prevalent nutritional disorder in the world affects 1.62 billion people, (24.8% of the world population) (WHO, 2008) and affects mainly children under four years old, breastfeeding and pregnant women and women of fertile age. In relation to the genus *Leishmania* (Protozoa: Kinetoplastida), only a few studies have evaluated the effect of iron deficiency on the infection (Huynh et al., 2006; Huynh and Andrews, 2008; Das et al., 2009; Carvalho et al., 2009 and Jacques et al., 2010). These authors have demonstrated the importance of elemental iron for the replication of pathogens in the host.

However, very little is known about the mechanisms involved in the relationship between infection by *L. chagasi* and iron deficiency. As discussed by Malafaia (2008a), studies concerning iron and infection have presented results which are frequently contradictory, some showing that iron shortage increases susceptibility to infectious processes whereas others show that iron excess is much more harmful to the human host and that the iron shortage could even play a protective role in certain infections. In the case of infection by *Leishmania*, the capture of iron ions required for antioxidizing functions and other metabolic reactions seems to be crucial for their survival and multiplication (Marquis and Gros, 2007; Huynh and Andrews, 2008; Malafaia, 2008b; Das et al., 2009).

Thus, the present work aimed to evaluate the effect of iron deficiency induced by the iron chelator desferrioxamine on *L. chagasi* infection in BALB/c mice. Since few works have dealt with the existing association between iron deficiency and VL caused specifically by *L. chagasi*, this study may help to elucidate the complex

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relationships that govern this micronutrient deficiency and the course of VL.

2. Materials and methods

2.1. Animals, division of groups and experimental design

Female BALB/c mice (3–5 weeks old) were used and were randomly divided into three experimental groups: uninfected and not treated with desferrioxamine (DFO) (UNT) group, infected with *L. chagasi*, but not treated (INT) group and infected and treated (IT) group. The data presented in this study are from two experiments performed independently (n = 8 mice/per group/per experiment).

Initially, all animals of the treated groups received an intraperitoneal (ip) injection of 10 mg of DFO (Desferal®, Novartis, Basel, Switzerland) in 100 μL of PBS (3 doses per week). The treatment protocol was based on Arantes et al. (2007), regarding the route of inoculation and concentration of the drug administered in each animal (double the concentration used by Arantes et al. (2007) was used in this study). The animals of the groups not treated with DFO received the same amount of PBS over the same period.

After two weeks of treatment, animals in the infection groups were infected with *L. chagasi* promastigotes, given intravenously by lateral tail vein. In order to do this, promastigote forms of *L. chagasi* strain (MHOM/BR/1974/M2682) were used. *L. chagasi* was cultured at 26 °C in Grace's Insect Medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Cripion, Andradina, SP, Brazil), 2 mM-glutamine (Gibco BRL) and 100 U/mL penicillin G potassium (USB Corporation, Cleveland, OH, USA), pH 6.5 at 26 °C. Infectivity was maintained by serial passage in BALB/c mice. For the inoculation, promastigotes of *L. chagasi* were harvested from late-log-phase cultures by centrifugation and washed three times in PBS. For the experimental infection, 1×10^7 promastigotes were suspended in 200 μL RPMI, pH 7.2.

Two, four and six weeks after the infection with *L. chagasi*, the infected mice were sacrificed and spleen and liver parasite loads were determined. In this case, the parasite load was determined by quantitative limiting dilution culture as described by Titus et al. (1985) and modified by Marques-da-Silva et al. (2005), with some modifications. Fragments of spleen and liver were obtained and weighed separately for parasite quantification. In addition we decided to assess some immunological parameters including production of cytokines IFN-gamma and IL-4, and the production of NO by splenocytes. Single-cell suspensions of spleen were obtained by tissue grinder homogenization and processed as described previously (Marques-da-Silva et al., 2005) and the production of IFN-gamma and IL-4 was determined in these supernatants by ELISA (Afonso and Scott, 1993). The production of nitric oxide (NO) was determined by the Greiss method (Green et al., 1982). To prepare the *L. chagasi* antigen, parasites were disrupted by three rounds of freezing and thawing (freeze–thawed antigen), protein content was estimated by the Lowry method (Lowry et al., 1951) and the preparation was frozen at −20 °C until use.

2.2. Levels of hemoglobin and physical parameters

To determine if the treatment with DFO was capable of reducing the blood levels of hemoglobin (Hb) (which indicates a reduction in the levels of iron), its concentration was determined in blood samples, collected from the ocular plexus, using a commercial assay procedure (Labtest Kit catalogue No. 43). Evaluations were performed in the second, fourth and sixth week after infection, on the day of sacrifice.

To evaluate the effect of infection *by L. chagasi* and of DFO on physical parameters, we measured the body weight and liver and spleen mass of the mice. The assessment of body weight was performed weekly until the sacrifice of animals. The evaluations of organ weight were performed on the day of animals' sacrifice (2, 4 and 6 weeks after infection).

2.3. Statistical analyses and ethics issues

All data were analyzed by Kolmogorov–Smirnov normality test. Data with a normal distribution were analyzed by Student's t test (data of body weight, organ weight and blood hemoglobin levels). Data whose distributions were not considered normal were submitted to non-parametric Mann–Whitney's test (data of parasite load, cytokines and NO). Differences with a p value <0.05 were considered statistically significant.

All animal procedures were approved by the Committee on Ethics in Research of the Universidade Federal de Ouro Preto-MG, Brazil, and followed the guidelines for the use and care of animals for research published by the Canadian Council on Animal Care (1980, 1984).

3. Results

3.1. Effect of treatment with DFO on total body weight and liver and spleen mass

No difference in the total body weight between the experimental groups was observed (data not shown). In addition, no significant difference between the liver mass of the INT and IT groups measured in the second, fourth and sixth weeks after infection were observed. A significant difference was observed between UNT and INT groups and UNT and IT groups only in the sixth week of evaluation (Fig. 1A).

There was no difference in spleen mass between the INT and IT groups in the second, fourth and sixth weeks after infection. However, a significant difference was observed between UNT and INT groups and UNT and IT groups also only in sixth week of evaluation (Fig. 1B).

3.2. Levels of hemoglobin

We did not observe significant differences in hemoglobin between UNT and INT groups measured in the second, fourth and sixth experimental weeks. However, significant differences between INT and IT groups and UNT and IT groups were observed 4 and 6 weeks after infection (Fig. 2).

3.3. Parasite load in spleen and liver

In order to study the influence of iron deficiency on *L. chagasi* infection in BALB/c mice, this study evaluated the parasite load in spleen and liver. Our data show that the IT group had a significantly smaller splenic parasitic load compared to INT group 6 weeks after infection (Fig. 3A). Regarding hepatic parasitic load, a significant difference in number of parasites between the INT and IT groups was observed when the evaluations were carried out after 4 and 6 weeks of infection (Fig. 3B).

3.4. Determination of production of cytokine and NO

After mice were killed, spleen cells were harvested and incubated in the presence or absence of freeze–thawed *L. chagasi* antigen (50 μg/mL), in order to determine the cytokine levels (IFN-gamma and IL-4) in culture supernatants. It was observed that the treatment with DFO did not influence the production of IFN-gamma and IL-4. Although the *Leishmania* antigen was able