Experimental Parasitology 133 (2013) 357–364

Contents lists available at SciVerse ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Iron metabolism and its relationship to anemia and immune system in *Trypanosoma evansi* infected rats

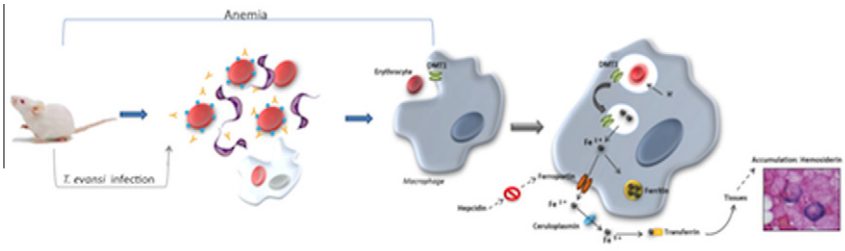
Cássia Bagolin da Silva [a,*], Patrícia Wolkmer [b], Francine Chimelo Paim [a], Aleksandro Schafer Da Silva [c], Lucas Carvalho Siqueira [d], Camila Lopes de Souza [a], Raqueli Teresinha França [a], Guilherme Lopes Dornelles [a], Marta Maria Medeiros Frescura Duarte [e], Silvia Gonzalez Monteiro [f], Cinthia Melazzo Mazzanti [a], Sonia Terezinha dos Anjos Lopes [a]

[a] Department of Small Animals, Universidade Federal de Santa Maria, Brazil
[b] Department of Chemistry, Universidade Federal de Santa Maria, Brazil
[c] Department of Animal Science, Universidade do Estado de Santa Catarina, Brazil
[d] Laboratory of Animal Reproduction, Universidade de Cruz Alta, Brazil
[e] Lutheran University of Brazil – ULBRA, Brazil
[f] Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil

HIGHLIGHTS

- *T. evansi* infection can cause erythrophagocytosis.
- Accumulate iron in the bone marrow of infected animals.
- Reduction of serum levels of iron and prohepcidin.
- Increase in serum levels of transferrin and ferritin.
- Changes in iron metabolism related to the immune response and anemic status of rats infected.

GRAPHICAL ABSTRACT



During the infection with *T. evansi* occurs erythrophagocytosis. Antigens of trypanosomes adhere to the membrane of RBC, making them susceptible to phagocytosis by macrophages. Erythrocytes are degraded in phagolysosomes of macrophages, releasing the heme group. The iron from degradation of erythrocytes can be released or stored as hemosiderin in bone marrow. Infected animals revealed an increase in iron accumulation at bone marrow.

ARTICLE INFO

Article history:
Received 5 August 2012
Received in revised form 14 December 2012
Accepted 15 December 2012
Available online 25 December 2012

Keywords:
Trypanosomiasis
Iron
Transferrin
Ferritin
Prohepcidin

ABSTRACT

The aim of this study was to evaluate biochemical parameters of iron metabolism in rats experimentally infected with *Trypanosoma evansi*. To this end, 20 rats (Wistar) were intraperitoneally inoculated with blood containing trypomastigotes 10^6 (Group T) and 12 animals were used as negative control (Group C) and received saline (0.2 mL) through same route. Blood samples were collected by cardiac puncture on day 5 (C5, T5) and 30 (C30, T30) post-inoculation (pi) to perform complete blood count and determination of serum iron, transferrin, ferritin, total and latent iron fixation capacity, transferrin saturation and prohepcidin concentration. Also, bone marrow samples were collected, to perform Pearls staining reaction. Levels of iron, total and latent iron binding capacity and prohepcidin concentration were lower ($P < 0.05$) in infected rats (T5 and T30 groups) compared to controls. On the other hand, levels of transferrin and ferritin were higher when compared to controls ($P < 0.05$). The transferrin saturation increased on day 5 pi, but decreased on day 30 pi. The Pearls reaction showed a higher accumulation of iron in the bone marrow of infected animals in day 5 pi ($P < 0.01$). Infection with *T. evansi* in rats caused anemia and changes in iron metabolism associated to the peaks of parasitemia. These results suggest that changes in iron metabolism may be related to the host immune response to infection and anemic status of infected animals.

* Corresponding author. Address: Departamento de Pequenos Animais da UFSM, Faixa de Camobi – Km 9, Campus Universitário, 97105-900, Hospital Veterinário, Sala 103, Santa Maria – RS, Brazil. Fax: +55 55 3220 8958.
E-mail address: cassiabagolin@hotmail.com (C.B. da Silva).

0014-4894/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.exppara.2012.12.010

1. Introduction

Trypanosoma evansi is a protozoan digenetic, etiologic agent of the disease known as *Mal das cadeiras* or *Surra* in horses (Silva et al., 2002; Herrera et al., 2004). This organism has a wide geographical distribution and is commonly found parasitizing several species of domestic and wild animals (Silva et al., 2002).

Anemia is a common and possibly the most important feature in infections caused by *T. evansi* (Silva et al., 1995; Wolkmer et al., 2009), but the mechanisms leading to it are considered multifactorial and still not fully understood (França et al., 2011; Paim et al., 2011; Wolkmer et al., 2009). It is believed that hemolysis, as a result of immune-mediated erythrophagocytosis, and depression of erythropoiesis through iron uptake by macrophages, are involved in the process of anemia (Seed and Hall, 1985; Silva et al. 1995; Connor and Van Den Boosche, 2004). This reduction of iron also is associated with the inflammatory process, causing resistance to infection and improving the inflammatory condition (Knutson and Wessling-Resnick, 2003). During inflammatory anemia, cytokines and cells from the reticuloendothelial system can interfere on erythropoiesis, leading to anemia. During this process, INF-γ is related to the reduction of ferroportin. A recent study shows that the blockage of iron mobilization by hepcidin is the most evident mechanism to explain inflammatory and infection-induced anemia (Weiss, 2005).

Iron is a vital mineral for the synthesis of heme group. It is essential for cellular homeostasis, oxygen transport, DNA synthesis and energetic metabolism. Iron is also an important cofactor for enzymes of the mitochondrial respiratory chain and nitrogen fixation (Smith 2002; Wijayanti et al., 2004). Iron deficiency is defined as a reduction in total body iron to an extent that iron stores are fully exhausted and some degree of tissue iron deficiency is present (Wijayanti et al., 2004). Since the distribution of iron has a unique dynamic, this element may occupy different compartments (stock, transport and functional), which are interconnected, but can didactically be evaluated separately. These compartments are affected sequentially as the body iron deficit progresses. Initially there is a decrease on iron stock, followed by deficiencies in transport and, finally, reduction in the erythroid or functional compartment (Krishnamurthy et al., 2007; Oates, 2007). To assess iron metabolism tests such as serum iron, serum ferritin, hepcidin concentration, transferrin saturation, transferrin and total capacity of iron binding to transferrin may be used. (Frazer and Anderson, 2003; Knutson and Wessling-Resnick, 2003).

Due to iron involvement in hematopoiesis (Smith, 1997), its deficiency could plays an important role in anemia caused by trypanosomiasis. (Da Silva et al., 2009a; França et al., 2011; Wolkmer et al., 2012). In recent studies it was observed that infection with *T. evansi* in cats and rats decreases serum iron (Da Silva et al., 2009b; França et al., 2011; Wolkmer et al., 2012), but the mechanism that causes the reduction of this mineral was not established. Considering that anemia caused by *T. evansi* is an important aggravating factor in this infection and iron deficiency may be one of several causes of anemia, it is relevant to determine the effect of *T. evansi* infection on serum iron availability, forms of storage and transport, and bone marrow storage-level.

2. Material and methods

2.1. Animals

The experiment was conducted using 32 adult male Wistar rats (*Rattus norvegicus*; 250–290 g). The animals were kept in cages with five animals and housed in a room with controlled temperature (25 °C) and relative humidity (70%). They were fed with commercial ration and water ad libitum. The procedure was approved by the Committee on Ethics in Animal Experimentation of Federal University of Santa Maria (protocol number: 23081.018369/2010-00).

2.2. Groups and trypanosome infection

The experimental groups were formed according to the time of infection and the degree of parasitemia. Rats were divided into four groups as follows: controls (C5 and C30) each group composed of six non-inoculated animals; and tests groups (T5 and T30), inoculated with *T. evansi*, formed of 10 animals each.

The isolate of *T. evansi* obtained from a naturally infected dog (Colpo et al., 2005) and stored in liquid nitrogen, was used. The animals from group T were inoculated intraperitoneally with blood (0.2 mL) containing 10^6 trypomastigotes, from an experimentally infected rat. The number of trypanosomes per mL was determined using a hemocytometer. The control animals received 0.2 mL of sterile saline (0.9% NaCl) by the same route.

2.3. Parasitemia

The presence and degree of parasitemia were estimated for each animal daily by blood smear examination. A drop of blood was collected from the tail vein and placed on a slide, and a thin blood smear was manually prepared. The blood films were stained with Romanovsky stain and then examined under a microscope, the parasites being counted in 10 fields at 1000× magnification.

2.4. Samples

At day 5 and 30 post-inoculation (pi), the rats were anesthetized with isoflurane in a gas chamber for collection of blood by cardiac puncture. For complete blood count, 0.5 mL of blood from each rat was placed in tubes containing 10% ethylene diamine tetraacetic acid (EDTA 10%). The remainder was transferred into tubes without anticoagulant and centrifuged to obtain the serum to perform specific tests of iron metabolism. After blood collection the animals were euthanized and the left femur of the rats was removed to obtain the bone marrow.

2.5. Hematology

Red blood cells (RBC) and hemoglobin (Hb) determinations were performed using an automated cell counter (Vet Auto Hematology Analyzer, model BC 2800). The packed cell volume (PCV) was obtained by centrifugation using a microcentrifuge (Sigma) at 18,600*g* for 5 min. Blood smears were prepared and stained with Diff-Quick commercial kit and used for the morphological evaluation of erythrocytes. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Lassen and Weiser (2004).

2.6. Evaluation of iron metabolism

The quantification of iron metabolism in serum was performed using commercial kits of ferrozine and cromazurol iron, ferritin, transferrin and latent capacity iron-binding (BioTécnica, Minas Gerais, Brazil), following the manufacturer protocols on a semi-automatic analyzer Bio-2000 (BioPlus Ltda, São Paulo, Brazil). The total iron binding capacity (TIBC) was calculated by summing the values of serum iron values of latent iron binding capacity (LIBC) of each animal (TIBC (μg/dL) = serum iron + LIBC) and the index of transferrin saturation (ITS) was calculated from the values of serum iron and total capacity of iron-binding by the equation ITS (%) = (serum iron × 100)/TIBC. All equipment used in analysis was left in 10%