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Relationship between DNA damage in liver, heart, spleen and total blood cells and disease pathogenesis of infected rats by *Trypanosoma evansi*

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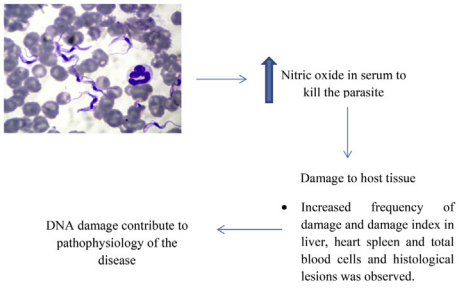
HIGHLIGHTS

- *Trypanosoma evansi* infection cause increased frequency damage using comet assay.
- *T. evansi* infection cause increased damage index using comet assay.
- Histopathological alterations in liver, spleen and heart were observed.
- *T. evansi* infection causes genotoxicity due to the production of NO.

GRAPHICAL ABSTRACT



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ABSTRACT

Trypanosoma evansi is an important pathogen that causes changes in nitric oxide (NO) levels and antioxidant enzymes, as well as oxidative stress. The present study evaluated the *in vivo* effect of *T. evansi* infection on frequency and index of DNA damage in liver, heart, spleen and total blood of rats. Twenty rats were assigned into two groups with ten rats each, being subdivided into four subgroups (A1 and A2, 5 animals/group; and B1 and B2, 5 animals/group). Rats in the subgroups A1 and A2 were used as control (uninfected) and animals in the subgroups B1 and B2 were inoculated with *T. evansi* (infected). NO in serum and the comet assay were used to measure DNA damage index (DI) and damage frequency (DF) in liver, heart, spleen and total blood of infected rats. Increased NO levels on days 3 and 9 post-infection (PI) was observed ($P < 0.001$). Also, it was verified an increase on DI and DF in the evaluated organs on days 3 and 9 PI ($P < 0.001$). Our data show that *T. evansi* infection causes genotoxicity due to the production of NO, causing not only the death of the protozoan, but also inducing DNA damage in the host.

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1. Introduction

Trypanosoma evansi is a microscopic unicellular protozoan that belongs to the family Trypanosomatidae. It is widely distributed as blood parasites in wild and domestic animals and rarely in humans (Robinson et al., 2002). *T. evansi* causes an important disease in horses called "Surra" or "*Mal das Cadeiras*", characterized by anemia, immunosuppression, severe neurological signs, hepatospleenomegaly, oxidative stress and death of untreated animals (Silva et al., 1995; Herrera et al., 2005; Baldissera et al., 2014a). According to Wolkmer et al. (2009), the *T. evansi* infection in rats is related to the oxidative stress due to oxidative damage in their erythrocyte membranes. It has also been reported that there was disorder of oxidant/antioxidant levels in the blood of horses when infected by *T. evansi* (Ranjithkumar et al., 2011). It has been suggested that trypanosomosis causes oxidative stress in liver, heart, total blood and serum of infected animals (Saleh et al., 2009; Paim et al., 2011; Baldissera et al., 2014a; El-Deeb and Elmoslemany, 2015).

Oxidative DNA damage is a major source of mutation load in living organisms, with more than one hundred oxidative DNA adducts (purine, pyrimidine, deoxyribose backbone) (Lu et al., 2001). Oxidative stress has been suggested as one of the mechanisms of the disease pathophysiology (Baldissera et al., 2014a). This results of the fact that infection by *T. evansi* generates a variety of toxic free radical species, including superoxide radical, hydrogen peroxide and hydroxyl radical. These metabolites have the potential to harm critical biomolecules such as DNA, proteins and lipids, and to cause cell death (Jenner, 2003). Therefore, these metabolites not only affect the etiology of diseases as trypanosomosis, but the resulting DNA damage may also be a direct contributor to deleterious biological consequences (Girard and Boiteux, 1997).

The alkaline single-cell gel electrophoresis assay is a rapid, simple, and reliable biochemical technique for evaluating DNA strand breaks in mammalian cells. It was reported that strand breaks arise from DNA damage caused by oxidative stress (Rojas et al., 1999). Nitric oxide (NO) is used for evaluation of oxidative stress, since it reacts with oxygen species and biological molecules, such as superoxide anion and nitrites/nitrates (Tatsch et al., 2011). Changes in its concentrations are commonly found in natural and experimental infections by *T. evansi* (Saleh et al., 2009; Ranjithkumar et al., 2011). Studies have demonstrated that infection of BALB/c mice with *Trypanosoma cruzi* and *Leishmania chagasi* induces DNA damage in heart and spleen cells (Ribeiro et al., 2007) and peripheral blood and spleen cells (De Oliveira et al., 2011), respectively. Considering the strong evidences linking NO and oxidative stress to DNA damage, the aim of this study was to evaluate if *T. evansi* induces DNA damage in the liver, heart, spleen and total blood.

2. Material and methods

2.1. Trypanosoma evansi isolate and animals

The *T. evansi* isolate was originally isolated from a naturally infected dog (Colpo et al., 2005), maintained cryopreserved under controlled laboratory conditions. Initially, one rat was infected intraperitoneally with blood containing 10^6 parasites in order to obtain a major number of parasites for the infected experimental groups of this study. Twenty outbread strain of adult female Wistar rats (180–200 g) were maintained under controlled environment and light conditions (12:12 h light–dark cycle, 23 ± 1 °C, 70% relative humidity) with free access to food and water. All animals were subjected to a period of 15 days for adaptation to the laboratory.

2.2. Experimental design and estimation of parasitemia

The 20 animals were assigned into two groups (A and B − 10 animals/group). Groups A (uninfected) and B (infected) were then divided in four subgroups (A1 and A2 − 5 animals/subgroup; and B1 and B2 − 5 animals/subgroup). Animals in the subgroups B1 and B2 were inoculated subcutaneously with 0.1 mL of blood from a rat (R1) containing 10^4 trypanosomes (Day 0). Animals in the subgroups A1 and A2 received physiological solution instead, undergoing the same procedure as the subgroups B1 and B2.

The peripheral blood from the tail of the rats was examined daily for evaluation of the degree of parasitemia. Each slide was prepared with fresh blood collected from the tail coccygeal vein, stained by the Romanowski method (commercial kit: *Panotico Rápido*®), and visualized at 1,000x magnification, according to the method described by Da Silva et al. (2006). For evaluation of the degree of parasitemia, an average of parasites observed in 10 fields at a 100× magnification in an optical microscope was performed.

The procedures were approved by the Animal Welfare Committee of Federal University of Santa Maria under number 2249060515.

2.3. Samples collection

The sample collections were performed on day 3 post infection (PI) for subgroups A1 and B1, and day 9 PI for subgroups A2 and B2. The animals have been euthanized by decapitation, using isoflurane as anesthetic. After decapitation, the liver, the heart and the spleen were immediately removed and dissected on a glass dish placed over ice. The tissues were kept on ice and protected from light until the comet assay was carried out. Blood was drawn through Vacutainer® system in tubes containing anticoagulant solution (EDTA) on the days 3 and 9 PI in order to allow the comet assay in total blood. Serum was obtained from total blood collected without anticoagulant after centrifugation at 3500 x*g* for 10 min and stored at − 20 °C.

2.4. Measurement of NO_x

NO levels in serum of rats infected with *T. evansi* were analyzed indirectly by nitrite/nitrate (NOx) quantifying according to the technique described in detail by Tatsch et al. (2011). Therefore, the NO_x was measured by the modified Griess method using the Cobas Mira automated analyzer. 50 μL of sample was pipetted into the reaction cuvette and 50 μL of vanadium (III) chloride (VCl_3) was added to reduce nitrate to nitrite after 25 s. Thus, 50 μL of Griess reagent was added. The mixture sample/VCl_3/Griess reagent was incubated for 20 min and read at 550 nm. Results were expressed as μmol/L.

2.5. Comet assay

The alkaline comet assay was performed as described by Tice et al. (2000), with modifications. Liver, heart and spleen cells (washed and minced in cold PSB solution) were added to 0.75% (w/v) low melting point agarose and the mixture was placed in a microscope slide pre-coated with 1.5% (w/v) normal melting point agarose and covered with a coverslip. The slide was briefly placed on ice, aiming the solidification of the agarose, and the coverslip was carefully removed. The slide was then immersed in lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5) containing freshly added 1% solution of triton X-100 and 10% solution of dimethyl sulfoxide (DMSO) for the least 1 h at 4 °C. Subsequently, the slides were incubated in fresh alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 25 min, leading to the

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