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Trypanosoma evansi: Effects of zinc and copper in experimentally infected rats

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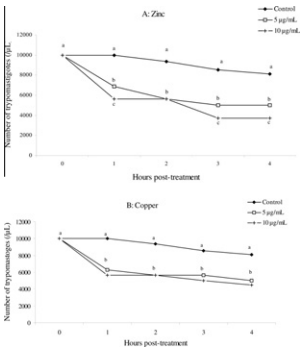
HIGHLIGHTS

- *Trypanosoma evansi* is the etiologic agent of a disease known as *Mal de cadeiras*.
- Zinc is an essential trace element for all organisms.
- Zinc can be considered a tool in the modulation of immune response.
- Copper is an essential nutrient to iron metabolism and activation of enzymes.
- The treatment with these metals is capable of controlling the infection by *T. evansi*.

GRAPHICAL ABSTRACT

Dose–response effect of zinc (A) and copper (B) on the viability of *Trypanosoma evansi in vitro* compared to control group.



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ABSTRACT

The aim of this study was to evaluate the effects of a treatment using injectable zinc and copper in rats infected with *Trypanosoma evansi*. 48 rats were divided into eight groups of six animals each. Group A was composed of uninfected animals. Animals from groups B–H were inoculated at the 5th day of experiment with 1.2×10^6 trypanosomes. Group B was used as a positive control. The infected groups received prophylactic (C, D and E) and therapeutic (F, G and H) treatments with the zinc and copper, both at a dose of 5 mg kg^{-1}. The effectiveness of treatment was confirmed by negative blood smears and Polymerase Chain Reaction (PCR) at the end of study. All treated animals had their prepatent period and survival prolonged when compared with control group (group B). Treatment efficacy was 17% (C: zinc), 33% (D: copper), 50% (E: zinc + copper), 0% (F: zinc), 50% (G: copper) and 50% (H: zinc + copper). Thus, we can conclude that treatment with zinc and copper are capable of controlling and/or curing *T. evansi* infection in rats, delaying the parasitemia and prolonging their survival.

1. Introduction

Trypanosoma evansi (Kinetoplastida: Trypanosomatidae) is the etiologic agent of a disease known as "*Mal de cadeiras*", considered endemic in Brazilian Pantanal, where the parasite is found in horses, dogs, cattle, buffaloes, goats, pigs, elephants, capybaras,

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coatis, deer, wild rodents (Silva et al., 2002) and rarely in humans (Joshi et al., 2005). The transmission of this parasite is mainly mechanical, where trypomastigotes are transferred directly from one mammal to another by blood-sucking insects (e.g. flies of the families Tabanidae and Stomoxydae), vampire bats (Hoare, 1972) or artificially by needles contaminated with infected blood (Rodrigues et al., 2005). This disease may cause different degrees of anemia, subcutaneous widespread edema, fever, lethargy, loss of weight, abortion, cutaneous plaque formation, nasal and ocular bleeding, and a peculiar stiffness of pelvic members, being quickly fatal in rodents, dogs, horses and camels (Silva et al., 2002; Herrera et al., 2004).

Diminazene aceturate is commonly used in the treatment of trypanosomosis in domestic animals (Peregrine and Mamman, 1993) . However, single doses are not effective for horses, mules and dogs (Tuntasuvan et al., 2003; Colpo et al., 2005). The return of parasitemia after treatment may be related to failure of the drug to cross the blood–brain barrier, which creates a refuge for the trypanosomes (Kaminsky and Brun, 1998). Therefore, new therapeutic approaches need to be tested. The administration of zinc in mice infected with *Trypanosoma cruzi* has shown promising results (Brazão et al., 2009).

Zinc is an essential trace element for all organisms and modulates the immune response, influencing cellular growth and affecting the development and integrity of immune system (Dardene, 2002). Zinc is involved in nucleic acid and carbohydrate metabolism, protein synthesis, production, storage and secretion of hormones, as well as in the activation of receptors and in organic responses (McDowell, 1992). It has a broad impact on key immunity mediators, explaining the paramount importance of zinc status on the regulation of lymphoid cell activation, proliferation and apoptosis (Brazão et al., 2008). In addition, copper is known as an essential nutrient for iron metabolism and activation of some enzymes such as superoxide dismutase, which protects the cells from the toxic effects of oxygen metabolism, and cytochrome c oxidase, an important enzyme for electron transport chain during aerobic metabolism (Berger, 2002). Moreover, researchers have reported the importance of copper in the antibody generation process (mainly IgG), in the cellular immunity and in the inflammatory response (Saker, 2006). Based on these considerations, the aim of this study was evaluate the effects of treatment using zinc and copper in injectable form in rats infected with *T. evansi*.

2. Material and methods

2.1. Compounds

Copper and zinc were acquired from Laboratórios Agroinsumos (Buenos Aires, Argentina). Both minerals are pure, sold in commercial formulation.

2.2. Isolated

This study used an isolate of *T. evansi* (LPV-2005) originating from a naturally infected dog (Colpo et al., 2005). First, two rats (R_1 and R_2) were infected intraperitoneally with blood cryopreserved in liquid nitrogen containing 1×10^6 parasites/animal. This procedure was performed to obtain a large amount of parasites to inoculate the experimental groups.

2.3. In vitro tests

When parasitemia of Rat_1 reached 34 parasites per microscopic field at 1000× magnification, the animal was anesthetized with isoflurane for blood collection. Blood was diluted in PBS-glucose (1/1 v) and then distributed in tubes (1 mL) for *in vitro* tests (Da Silva et al., 2009a). The susceptibility of *T. evansi* trypomastigotes to copper and zinc was assessed at the concentrations of 0, 5 and 10 μg mL^{-1}. After 1, 2, 3, 4 and 12 h, live trypanosomes were counted using a Neubauer chamber, according to the methodology described by Da Silva et al. (2009a).

2.4. Animals

48 rats (female) aged 60 days and weighing an average of 210 ± 17 grams were used. The animals were kept in cages with six animals each in an experimental room with temperature and humidity controlled (25 °C; 70%). They were fed with commercial ration and water *ad libitum*. All animals were submitted to a period of 15 days for adaptation.

The procedure was approved by the Animal Welfare Committee of Ethics in Animal Experimentation of Federal University of Santa Maria (UFSM), number 87/2010.

2.5. Experimental design

The rats were divided into eight groups of six animals each. Group A was composed by healthy, uninfected animals (negative control). Animals in groups B–H were inoculated intraperitoneally with 0.1 mL of blood from a rat (R_2) containing 1.2×10^6 trypanosomes at the 5th day of experiment. Group B was used as a positive infection control and not-treated. Groups C, D and E received prophylactic (day 0) and therapeutic (days 5 and 10) treatment and the groups F, G and H received only therapeutic treatment (days 5 and 10). Rats from groups C and F were treated with three and two doses of zinc (5 mg kg^{-1}), respectively. Rodents of groups D and G were treated with three and two doses of copper (5 mg kg^{-1}), respectively. Animals from groups E and H were treated with an combination of zinc and copper, at a dose of 5 mg kg^{-1} of each drug. Zinc and copper were administered subcutaneously with a 1 mL syringe. The dose of 5 mg kg^{-1} for zinc and copper followed the manufacturer's recommendations for other animal species, being adjusted to rats in a pilot study according to the literature (Cheng et al., 2002; Chung et al., 2009). The rats were observed for up to 40 days.

2.6. Estimation of parasitemia

Parasitemia was estimated daily by microscopic examination of smears. Each slide was mounted with blood collected from the tail vein, stained by the panoptic method, and visualized at a magnification of 1000× (Da Silva et al., 2006).

2.7. DNA extraction and PCR detection of T. evansi in brain and blood of rats

After 40 days, the remaining animals in the infected groups were anesthetized to collect blood, stored in tubes with anticoagulant and ethanol (1 v/v), and then the animals were euthanized for removal of brain which was preserved in ethanol. In blood and brain tissue investigated the presence of *T. evansi* DNA was investigated by PCR.

For preparation of DNA templates, a small section (0.4 × 0.4 mm) of each brain and 0.2 ml of blood were removed, transferred to sterile tubes, and washed three times (5 min each) in bi-distillated water under shaker. Then, brain were cut in small segments, incubated with lysis buffer (1% SDS, 100 mM EDTA pH 8.0, 20 mM Tris–HCl, pH 8.0, and 350 mg/ml of proteinase K) at 37 °C for 18 h, centrifuged at 14000*g* for 5 min, and DNA purified using Wizard Purification Systems (Promega, USA). Purified DNA samples from brain and blood were used as templates for PCR