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Trypanosoma evansi: Cholinesterase activity in acutely infected Wistar rats

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ABSTRACT

The aim of this study was to evaluate cholinesterase activity during the early acute phase of *Trypanosoma evansi* infection in rats. Fifteen male Wistar rats were randomly distributed into three groups ($n = 5$ animals per group): two trypanosome-infected groups (T3 and T5) and uninfected controls (C). The animals were inoculated intraperitoneally with 10^6 trypanosomes. The blood was collected by cardiac puncture on the 3rd (T3) or 5th day post-infection (T5 and C). Cerebrum and cerebellum were removed for the evaluation of acetylcholinesterase (AChE) activity. AChE activity was also evaluated in whole blood and butyrylcholinesterase activity (BUChE) in plasma samples. Parasitemia were progressive increase and parasites were observed in the peripheral blood of all infected animals one day post-inoculation. AChE activity was not altered in cerebrum and cerebellum tissues. AChE activity in blood significantly decreased in the T3 and T5 groups (26.63 and 25.86 mU/l mol Hb) compared with the control (37.84 mU/l mol Hb). In addition BUChE activity in plasma was lower in the T3 (7.01 μmol BTC hydrolyzed/h/mL) than the T5 and C groups (9.84 and 12.00 μmol BTC hydrolyzed/h/mL). This study therefore, shows that reductions in the activity of cholinesterase occur in acute infection by *T. evansi* in rats and this demonstrates an important change occurring in animals infected by the protozoan and may indicate a potential role the enzymes play in the mechanism of disease.

1. Introduction

Trypanosome infections are among the most serious parasitic diseases in humans and animals in Africa and in some parts of Asia and South America. *Trypanosoma evansi* has a wide range of hosts and is pathogenic to most domestic and laboratory animals, causing Surra disease. Several species including horse, camel, cattle, water buffalo, and pig are infected in most enzootic areas, the infection in these animals being usually devoid of clinical symptoms and seldom fatal (Biswas et al., 2001). Consequently these animals serve as reservoirs for *T. evansi* in enzootic areas, especially in India, the Far East, and in Central and South America. *T. evansi* is fatal to various laboratory animals, causing severe disease manifestations (Misra and Choudhury, 1975).

Animals infected with *T. evansi* develop anemia (Wolkmer et al., 2007) and clinical signs of neurological disorder in the final stage of the disease (Tuntasuvan et al., 1997, 2000; Rodrigues et al., 2005). In the first report of *T. evansi* infection in humans, signs of sensory deficit, disorientation, agitation and aggression were described (Joshi et al., 2005). The signs of neurological disorder may be the result of necrotizing panencephalitis (Rodrigues et al., 2005), leading to alteration in the actions of neurotransmitters. Recently we reported that chronic infection by *T. evansi* influenced cholinesterases of cats seen as changes in the responses of the cholinergic system (Da Silva et al., in press). These changes in enzymes could be related with the neurological disorder of animals infected with *T. evansi*.

This finding is important because acetylcholinesterase (AChE: EC 3.1.1.7) and butyrylcholinesterase (BUChE: EC 3.1.1.8) coupled with the acetylcholine (ACh) receptor, are responsible for the transmission of action potentials across nerve and neuromuscular synapses, a key process in the regulation of the cholinergic neurotransmission (Darvesh et al., 2003). However, the biological role of AChE is not limited to cholinergic transmission. AChE has been implicated in several non-cholinergic actions including cell proliferation (Appleyard, 1994), neurite extension and responses to various insults including oxidative stress (Grisaru et al., 1999). The first evidence of additional functions of AChE protein was based

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on the high levels of this enzyme observed in non-neuronal tissues such as blood cells, notably erythrocytes and lymphocytes (Kawashima and Fujii, 2000).

Therefore, considering the importance of AChE the aim of this study was to investigate the activity this enzyme in rats acutely infected with *T. evansi*. In addition, since *T. evansi* may be involved in disorders in the central nervous system (CNS), we also investigated the effect of AChE activity in whole blood in order to determine whether the activity of this enzyme in blood reflects its status in the CNS.

2. Materials and methods

2.1. Animals

The experiment was conducted using 15 adult male Wistar rats (*Rattus norvegicus;* 250–290 g) that were housed in a room with controlled temperature (23 °C) and relative humidity (70%) and fed with commercial rat pellets with continuous access to water. The rats were randomly divided into three groups ($n = 5$ animals per group), 2 trypanosome-infected groups (T3 and T5) and an uninfected control group (C). The number that follows the group's name refers to the day after infection on which cardiac blood samples were collected (day 0 = day of treatment).

This study was approved by the Committee of Ethics and Animal Welfare of the Rural Science Center of the Federal University of Santa Maria (CCR/UFSM), No. 23081.005984/2008-23 in accordance with existing legislation and the Ethical Principles published by the Brazilian College of Animal Experiments (COBEA).

2.2. Trypanosome infection

Animals of trypanosome-infected groups were inoculated intraperitoneally with 0.2 mL of rat blood containing approximately 10^6 trypanosomes. *T. evansi* was originally isolated from a naturally infected dog (Colpo et al., 2005) and maintained by passages through Wistar rats. This blood stabilate induces an acute infection in rats with a survival period of 4–6 days post-infection (dpi) (Da Silva et al., 2009b). The control group received 0.2 mL of saline solution by intraperitoneal injection.

2.3. Parasitemia estimation

The presence and degree of parasitemia were determined daily for each animal by blood film examination. A drop of blood was collected from the tail, placed on a slide and a thin blood smear was prepared manually. The blood films were stained (Panótico Rápido LB stain, Laborclin, Brazil) and then examined under a light microscope, counting 10 fields at 1000× magnification. The parasitemia after euthanasia was also estimated using a Neubauer chamber.

2.4. Collection and preparation of blood and brain samples

The animals were anesthetized with diethyl ether and a blood sample collected by cardiac puncture on the 3rd (T3), and 5th (TC, T5) dpi. Progression of the disease was monitored by hemogram. For determination of AChE activity, the cerebrum and cerebellum were removed, weighed and homogenized (1:10, w/v) in 10 mM Tris–HCl buffer, pH 7.2 with 160 mM sucrose. Blood samples were collected in tubes with sodium heparin and the samples were diluted 1:50 (v/v) in lysis solution (0.1 mmol/L potassium/sodium phosphate buffer containing 0.03% Triton X-100) to determine AChE activity in blood. For plasma BUChE assays, blood samples were conditioned in tubes containing heparin and the plasma was obtained by blood centrifugation at 2000*g* during 10 min. The samples were frozen at −20 °C until analysis.

2.5. Hematology

The packed cell volume (PCV) was measured using the standard microhematocrit method (Centimicro mod. 1–15-Sigma, Germany) according to Schalm et al. (1975). The red blood cell (RBC) count and hemoglobin (Hb) concentration were determined by an automated blood cell counter (CC-550-Celm, São Paulo, Brazil). Red cell indices such as the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using a standard formula (Schalm et al., 1975). Blood smears were prepared for microscopic examination.

2.6. Enzyme assay

The activity of AChE in the cerebrum and cerebellum was determined by the method described by Ellman et al. (1961) as modified by Pereira et al. (2004). The mixture assay containing 5,5′-Dithiobis(2-Nitrobenzoic Acid) (DTNB) 1.04 mM, potassium phosphate buffer 24 mM pH 7.2 and 25 μL of enzymatic material was pre-incubated for 2 min at 30 °C and the reaction was started with the addition of acetylthiocholine (ATC) 0.83 mM. The product of thiocholine reaction with DTNB was determined at 412 nm in 2 min. The specific activity is expressed as μmol ATC hydrolyzed/h/mg of protein.

The AChE enzymatic assay in whole blood was determined by the method described by Ellman et al. (1961) as modified by Worek et al. (1999). The incubation system was composed of sodium phosphate buffer 0.063 mM pH 7.4, DTNB 0.316 mM and 0.5 mL of the hemolyzed blood. The increase in absorbance was registered over 2 min at 436 nm. The specific activity of whole blood AChE was calculated from the quotient between AChE activity and hemoglobin concentration and the results were expressed as mU/l mol Hb.

The plasma BUChE activity was determined as described by Ellman et al. (1961). The BUChE activity was assayed in a medium containing sodium phosphate buffer 0.1 mM, pH 7.4, DTNB 0.30 mM and 50 μL of plasma. After 3 min of pre-incubation at 30 °C, the reaction was started with butyrylthiocoline (BTC) 1 mM. The increase in absorbance was registered over 2 min at 412 nm. The specific activity was expressed in μmol of BTC hydrolyzed/h/mL of plasma.

2.7. Protein determination

Protein concentrations of the homogenates of cerebrum, cerebellum and blood were determined by the Coomassie blue method (Bradford, 1976) using bovine serum albumin as the standard.

2.8. Statistical analysis

All samples were run in triplicate. The results are presented as the mean (±SEM). Differences among the means were compared by the one-way analysis of variance (ANOVA) followed by Duncan's multiple range test when appropriated, using the SPSS version 11.5 software program. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Parasitemia and course of the infection

Parasites were observed in the peripheral blood of all infected animals one day post-inoculation, with a progressive increase in parasitemia over time (Fig. 1). The mean parasite count was 10^4