Comp Clin Pathol (2008) 17:67–72
DOI 10.1007/s00580-008-0722-6

ORIGINAL ARTICLE

Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in *Trypanosoma brucei*-infected rats

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Received: 26 November 2007 / Accepted: 7 February 2008 / Published online: 14 March 2008

Abstract Lipid peroxidation was assessed in the sera and various organs of rats experimentally infected with *Trypanosoma brucei*. Thirty-six adult albino rats divided into 2 groups of eighteen rats each were used in this study. In experiment one, a group of 18 rats were used and they were divided into three groups (A, B and C) of six rats each. Groups B and C rats were infected with 1.54×10^5 trypanosomes per rat intraperitoneally, whereas group A served as uninfected control. The rats were bled on day 0 and subsequently at 7-day intervals for packed cell volume (PCV), sera peroxidation index and parasitaemia. Also, temperature and weight were taken on day 0 and subsequently at 7-day intervals. In experiment 2, 18 rats were also used. Six rats each were sacrificed on days 0, 14 and 28-postinfection. Five rats each were sacrificed on day 14 and day 28 post-infection (PI) from group B, and their organs were promptly collected and washed with normal saline and used for organ malondialdehyde (MDA) concentration. The infection led to an increase in lipid peroxidation index (MDA concentration) of sera samples. The serum MDA concentration of the infected rat group was significantly ($p<0.01$) higher than in the uninfected group on days 21 and 28 PI. The increase was however reversed by diminazene aceturate (Berenil; Hoechst, Ireland) treatment at the dosage of 7 mg/kg body weight administered on day 14 PI. The organ lipid peroxidation index also increased significantly ($p<0.05$) in the eye, lung and spleen. However, there was no significant ($p>0.05$) increase of lipid peroxidation index in the kidney, heart, liver, testes and brain. Also, the mean weekly MDA concentration increased as the disease progressed, the mean weekly temperature and parasitaemia also increased, but the reverse was the case with the mean weekly body weight and PCV which declined as the disease progressed. The findings are indication that oxidative stress plays an important role in the pathology of trypanosomosis.

Keywords Malondialdehyde · Organs · Rats · Sera · *Trypanosoma brucei brucei*

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Introduction

Lipid peroxidation has been associated in various ways with a number of normal and abnormal physiological processes (Hogg and Kalyanaraman 1999). Under stressed condition, biological molecules are exposed to oxidant species resulting in irreversible oxidation reactions, which lead to chemical modification of biological processes that can lead to cellular dysfunction (Marklund 1988; Ayo et al. 1999). Inflammation and phagocytosis prominent in many disease processes including trypanosomosis (Anosa 1988) lead to increase oxygen consumption by leucocytes, which results in cell toxicity and generation of oxygen-derived free radicals (Bulkley 1983). It was also reported that trypanosomosis infections might be associated with oxidative stress caused by an increased production of pro-oxidants (free radicals) (Igbokwe 1994; Ayo et al. 1999).

The leucocyte-generated oxygen-derived free radicals and metabolites play an important role in host defences, pathogenesis of inflammatory processes and cytotoxic mechanism of tissue damage (Rossi et al. 1985; Bilenko 1989; Levine 1993). The activities of free radicals in living tissues are controlled by protective mechanisms such as the antioxidants (Del Maestro 1980; Bilenko 1989). Commonly

encountered antioxidants are the scavengers (ceruloplasmin, glutathione) or transient metals (manganese, selenium, copper, cobalt and zinc) as well as enzymes (superoxide dismutase, catalase and peroxidase) (Rossi et al. 1985; Bilenko 1989). Cellular damage in diseases has been associated with the overwhelming of the defence mechanisms (antioxidant) by free radicals (Ayo et al. 1999).

Many workers have reported depletion of tissue antioxidant and increased production of pro-oxidants in trypanosome-infected animals (Ayo et al. 1999). Beschin et al. (1998) reported the elicitation of nitric-oxide-dependent and nitric-oxide-independent mechanisms. Also, increased sera nitric oxide in *Trypanosoma brucei rhodensiense*-infected vervet monkey (Maina et al. 1998), increased production of proxidant and a systematic depletion of antioxidants (Igbokwe et al. 1996), including reduced tissue ascorbic acid level (Nyden 1948; Mohamed and Beynen 2002) and decreased liver carotenoid concentration (Ihedioha and Anwa 2001), have been reported. The depletion of these antioxidants strengthens the assertion that oxidative damage of membranes plays a significant role in cellular damage in trypanosomosis. This study was therefore an attempt to assess the level of lipid peroxidation in the sera and organs of *T. brucei*-infected rats with a view to understanding more the role of oxidative stress in pathology of trypanosomosis.

Materials and methods

Experimental animals

Thirty-six adult male albino rats weighing between 200 and 230 g were used. They were kept in wire-bottomed rat cages and housed in a fly-proof room and were fed with commercial poultry chick starter mash (Guinea Feed, Bendel Feed and Flour Limited, Nigeria) and given water ad libitum.

Trypanosome

The *T. brucei* used was isolated from a dog that was presented for treatment at the University of Nigeria Veterinary Teaching Hospital. They were identified as *T. brucei* under wet mount and stained blood smear microscopy as described by Soulsby (1983). The trypanosome was maintained in rats and each experimental rat received 0.5 ml of saline-diluted blood of rats containing 1.54×10^5 trypanosomes intraperitoneally (ip).

Trypanocidal drug

The drug used was diminazene aceturate (Berenil; Hoechst, Ireland); Berenil was reconstituted in accordance with the manufacturers' directive and given ip at a dose of 7 mg/kg body weight, respectively.

Experimental design

The experiment was carried out in two phases of 18 rats each. In experiment one, the 18 rats were divided into three groups of six each and were kept in cages A, B and C, respectively. The rats were treated as follows: group A rats served as uninfected control, group B rats were infected whilst group C rats were infected but were treated on day 14 post-infection (PI) with 7 mg/kg body weight of diminazene aceturate (Berenil®; Hoechst, Ireland).

The rats were bled on day 0 (just before infection with trypanosomes) and subsequently at 7-day intervals for determination of serum lipid peroxidation index (malondialdehyde (MDA) concentration). Also, rectal temperature, body weight, packed cell volume (PCV) and parasitaemia were monitored at the same time in group B rats to assess the relationship between these parameters and serum MDA concentration in trypanosome-infected animals.

In experiment two, 18 rats were also used. Twelve rats were infected with trypanosomes whilst six were not infected. The six uninfected rats were sacrificed on day 0 whereas the other 12 rats were infected with *T. brucei* on the same day. On days 14 and 28 PI, six infected rats were sacrificed, respectively. After sacrifice, their organs were promptly collected and washed with normal saline. The organs collected were eyes, testis, brain, spleen, kidneys, liver, lung and heart and were used for determination of organ MDA concentration. All samples were analysed immediately after collection.

Collection of serum samples

About 1 ml of blood was drawn from the nipped tail of each rat into a bijou bottle without anticoagulant and allowed to stand for at least 30 min at room temperature and later centrifuged at 5,000 rpm for 5 min to separate the serum from the cells. The serum was carefully decanted into another sterile bottle.

Preparation of organ homogenates

Rat brain, heart, liver, lung, kidney, spleen, testis and eyes were promptly excised after humane killing of the rats. The organs were carefully washed in normal saline to remove blood. The organs were weighed and homogenised by grinding in a mortar.

Determination of malondialdehyde concentration in serum and organ homogenates

This was performed using the method of thiobarbituric acid which measures MDA-reactive products (Plaser and

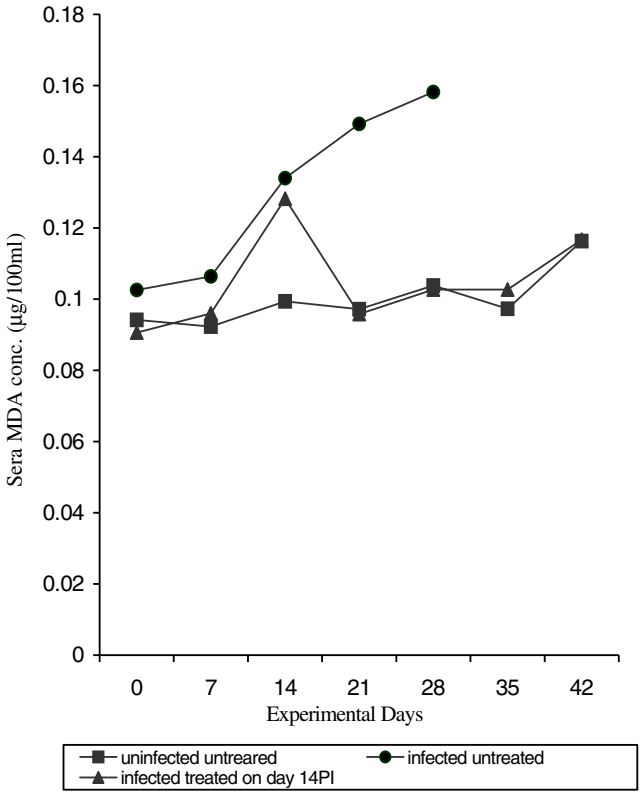

Fig. 1 Graph of weekly mean MDA concentration of *T. b. brucei*-infected rat groups

Cushman 1966) as described by Todorova et al. (2005). Mix 0.5 ml serum or organ homogenates, 0.5 ml physiological solution and 0.5 ml 25% trichloroacetic acid and centrifuge at 2,000 rpm for 20 min. A 1 ml of protein-free supernatant was mixed with 0.25 ml 0.5% thiobarbituric acid and heated at 95°C for 1 h. After cooling, the intensity of pink colour of the end fraction product was determined at 532 nm. The MDA concentration was calculated according to the following formula:

$$1\ \mu\text{mol}/1\ \text{MDA} = \frac{\text{OD}_{532} \times 1.75}{0.156}$$

OD_{532}^{-} optic density in λ=532 and extinction=$1.56 \times 10^5\ \text{M}^{-1}\ \text{cm}^{-1}$.

Determination of other parameters

The rectal temperature was determined by inserting a clinical thermometer into the rectum for 2 min and reading the mercury level. The body weight was determined using a simple weighing balance. The PCV was done as described by Coles (1968) whereas the parasitaemia was estimated as described by Herbert and Lumsden (1976).

Statistical analysis

The statistical analysis was done using the analysis of variance method. Means were compared using the least significant difference method (Steel and Torrie 1960).

Result

In experiment one, the MDA concentration (lipid peroxidation index) increased progressively (Fig. 1). By days 21 and 28 PI, the MDA concentration in group B (infected untreated) was significantly higher ($p<0.05$) than in groups A and C. The increase in the MDA concentration in infected rats was reversed following treatment with Berenil (Hoechst, Ireland) in group C. All rats in group B died before day 35 PI.

Table 1 Mean organ MDA concentration (nmol MDA/g wet weight) taken on days 0, 14 and 28 post-infection

Organ	Day 0, *N*=6	Day 14, *N*=6	Day 28, *N*=6
Kidney	3.5570 [a] (0.3455)***	4.9543 [a] (1.4400)***	4.7094 [a] (1.4045)***
Heart	4.0047 [a] (0.5261)***	4.3690 [a] (0.8432)***	4.9526 [a] (1.0195)***
Liver	3.4390 [a] (0.2150)***	3.8655 [a] (0.4952)***	4.1806 [a] (0.7566)***
Testis	5.0323 [a] (0.6439)***	6.1063 [a] (0.9049)***	6.1026 [a] (1.0048)***
Eye	5.8210 [a] (1.0203)*	7.3590 [a, b] (1.3679)*	7.8374 [b] (0.4933)*
Lungs	3.8647 [a] (0.1501)*	4.9313 [b] (0.8502)*	4.7936 [b] (0.7876)*
Spleen	2.9123 [a] (0.1197)**	4.2620 [c] (0.2738)**	4.481 [c] (0.8839)**
Brain	3.0500 [a] (0.1701)***	3.3273 [a] (0.5999)***	

Results are presented as means with standard deviation in parentheses.
Different letters in brackets in a row indicate significant difference between the means, while similar letters in brackets in a row indicate lack of significant difference between the means.
*$p<0.05$; **$p<0.01$; ***$p>0.05$

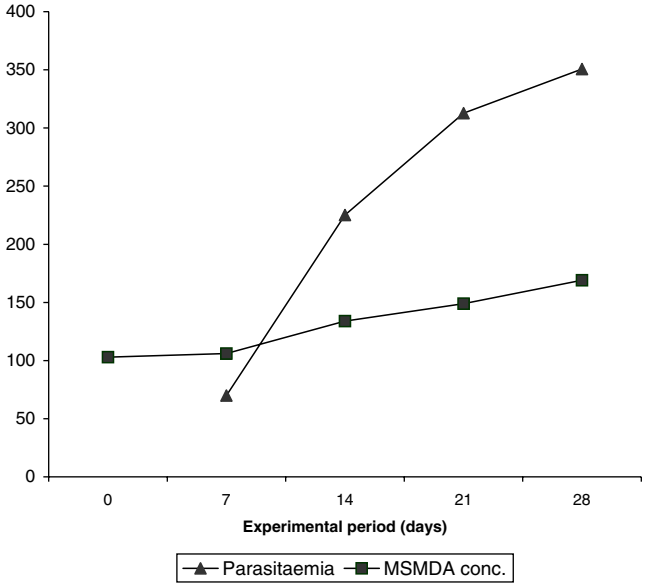

Fig. 2 Weekly mean parasitaemia (trypanosomes/ml of blood) and serum malondialdehyde concentration (10^{-3}) of *T. brucei*-infected rats

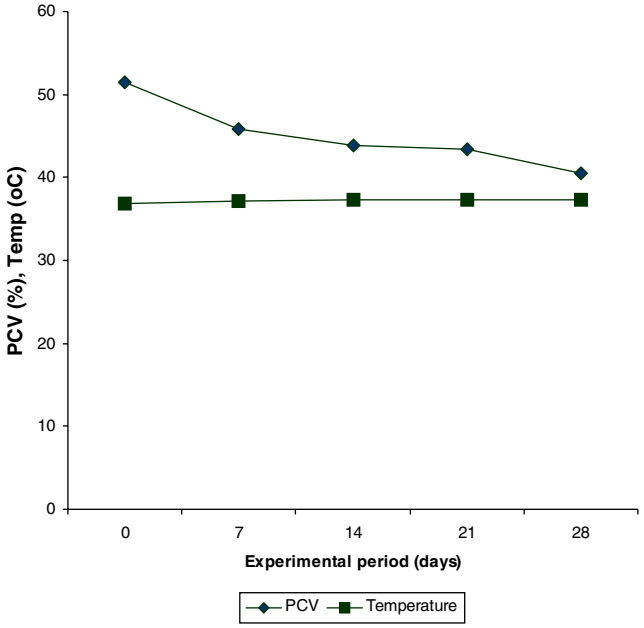

Fig. 4 Weekly mean PCV (%) and temperature (°C) of *T. brucei*-infected rats

All the organs showed some increases in lipid peroxidation (MDA concentration) within 14 days following trypanosome infection (Table 1). The increases however were not statistically significant in some organs such as the kidney, heart, liver, testis and brain. The eye had a statistically significant ($p<0.05$) increase in lipid peroxidation by day 28 PI. The spleen and lungs also showed significantly higher MDA concentration ($p<0.05$) by days 14 and 28 PI.

Figures 2, 3 and 4 also showed that the mean weekly MDA concentration increased as the disease progressed; the mean weekly temperature and parasitaemia also increased, but the reverse was the case with the mean weekly body weight and PCV which declined as the disease progressed. The parasitaemia and the MDA concentration peaked on day 28 PI.

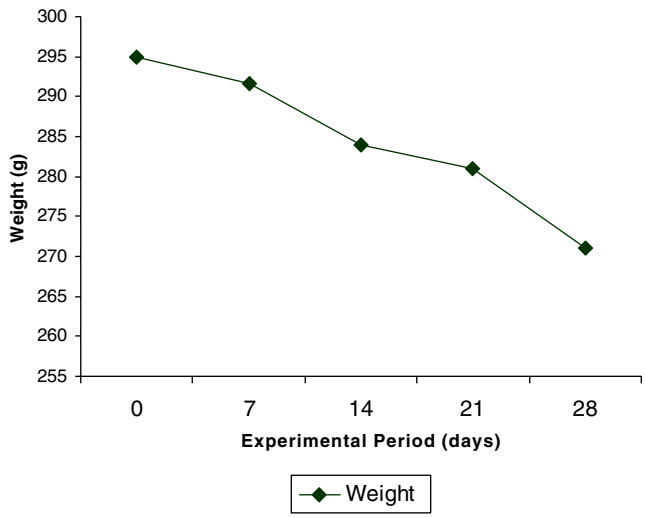

Fig. 3 Weekly mean changes in weight (g) of *T. brucei*-infected rats

Discussion

The infection led to an increase in the mean sera MDA concentration (lipid peroxidation index). The increase was reversed in the treated group when Berenil (Hoechst, Ireland) treatment cleared the infection. This is an indication that the infection with trypanosomes was responsible for the increase. Rogers et al. (1995) reported that stimulation of oxygen consumption and oxidant production is known to be evident only after addition of microbes or soluble stimulants.

Also, the mean organ MDA concentration (lipid peroxidation index) increased as the infection progressed (Table 1). Organs such as the eye, lung and spleen showed significant ($p<0.05$) increase by days 14 and 28 PI, whereas increases in the kidney, heart, liver, testis and brain were not significant. The increase in mean sera and organ lipid peroxidation may not be surprising as trypanosomes have been reported to elicit increased production of peroxidants (free radicals) such as nitric oxide (Beschin et al. 1998; Maina et al. 1998) and a systemic depletion of antioxidants, e.g. tissue ascorbic acid (Nyden 1948), decreased liver carotenoid concentration (Ihedioha and Anwa 2001) and reduced vitamin C level (Mohamed and Beynen 2002). The depletion in systemic antioxidants has been attributed to consumption of these substances in the

neutralisation of the large amount of free radicals being generated (Niki 1983).

Trypanosomes are normally located in the blood and cerebrospinal fluid of the host; but organisms of the *T. brucei* subgroup, unlike other trypanosomes, in addition are found in the body organs (Losos and Ikede 1972; Ikede 1974; Murray et al. 1974; Anosa 1983). It is this tissue localisation and the subsequent multiplication at these sites that lead to inflammatory cell infiltration and the subsequent tissue damage. Morrison et al. (1981) reported that the most severe tissue lesions occur in dog, with the heart, the central nervous system and the eye always being most severely affected. Also, anaemia occurring in trypanosomosis has multifactorial aetiology that includes haemolytic factors produced by the trypanosomes, immunologic mechanism, fever and disseminated intravascular phagocytic system (Banks 1980; Anosa 1983; Anosa and Isoun 1983). Increase in oxygen consumption by leucocytes during phagocytosis in disease processes result in cell toxicity and generation of oxygen-derived free radicals (Bulkley 1983; Marklund 1988; Kogan and Kudrin 1999).

Increase in sera MDA concentration has been reported in *Plasmodium* (malaria) infection (Eze 1992), sickle cell anaemia patient and plasmodium-infected sickle cell anaemia patient and normal infection (Uzoegwu 2001). Generation of reactive oxygen species which led to peroxidation has been reported in more than 100 diseases from malaria and haemorrhagic shock to acquired immunodeficiency syndrome with their increased formation accompanying tissue injury in the host (Alho and Leinonen 1999). Also, trypanosomosis has been mentioned as one of the diseases in which leucocyte-generated free radicals may play a significant role (Bulkley 1983; Ayo et al. 1999; Igbokwe et al. 1992).

The increase in mean sera MDA concentration in trypanosomosis may have a direct relationship with mean temperature, PCV, parasitaemia and weight (Fig. 2). The mean sera MDA increased as the parasitaemia and temperature increased and as the PCV and weight decreased. Maina et al. (1998) reported that, in vervet monkey infected with *T. b. rhodensiense*, sera nitric oxide increased rapidly with a peak corresponding to the peak of parasitaemia, low PCV and high body temperature. The pyrexia in trypanosomiasis results from haemolytic crisis, which enhances RBC damage and destruction leading to anaemia (Stephen 1986; Anosa 1988). Anene (1997) also reported the concurrence of febrile peaks with period of high parasitaemia and a fall in red cell values.

It could therefore be concluded that trypanosomes caused increase in MDA concentration, an index of lipid peroxidation. Also, the increase in MDA concentration was reversed by Berenil (Hoechst, Ireland) treatment and was directly related to increase in temperature and parasitaemia and decrease in PCV and weight. The increase in MDA concentration may be associated with tissue damage in trypanosomosis.

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