The beneficial effect of dietary zinc supplementation on anaemia and immunosuppression in Trypanosoma brucei infected rats

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HIGHLIGHTS

• Increased antibody titre.
• Increased total leucocyte count.
• Improved erythrocyte count.
• Improved haemoglobin concentration.
• Reduced parasitaemia level.

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Abstract

Zinc is an essential trace element crucial for normal development and function of cells mediating non-specific immunity and protects bio-molecules from oxidative damage. This study was designed to assess the effects of dietary zinc supplementation on anaemia and immunity of trypanosome-infected rats. Thirty rats, divided into five groups (A–E) of 6 each, were used for the study. Parameters used to assess the effect of the supplementation are antibody response to Sheep RBC using direct haemagglutination test, parasitaemia using the rapid matching method, WBC count using the improved Neubauer haemocytometer method, haemoglobin concentration using the cynomethaemoglobin technique while PCV was determined using the microhaematocrit method. The pre-infection supplementation did not prolong the pre-patent period significantly (p > 0.05). However, it significantly (p < 0.05) increased the packed cell volume (PCV), haemoglobin (Hb) concentration, leucocyte count, and antibody titre by day 7 on the supplementation (OTS). Following infection on day 7 OTS, the PCV and Hb decreased but remained significantly (p < 0.05) higher than the infected not supplemented (INS) group, while on day 14 OTS, they maintained a significantly (p < 0.05) higher antibody titre as compared to other groups. On day 21 OTS, the
1. Introduction

Animal and human trypanosomosis remains responsible for substantial global morbidity and mortality in tropical and subtropical regions (Espuelas et al., 2012). Animal trypanosomosis causes direct losses to livestock production, with more than 20 million American dollars spent per annum on trypanocidal drugs and indirect losses related to the opportunity cost of land and other resources currently not used for livestock production due to the presence of tsetse fly (FAO, 2005). Also, approximately 70 million people distributed over a surface of 1.55 million km² are estimated to be at different levels of risk of contracting Human African trypanosomosis (Simarro et al., 2012). Major modifications of immune system and erythrocytic indices have been observed, thus making immunosuppression and anaemia the major features in African trypanosomosis.

In the absence of safe and efficient vaccines, chemotherapy, together with vector control, remains the most important measure to control the disease. Nevertheless, the current chemotherapeutic treatments are clearly inadequate because of their toxic effects, generation of resistance as well as routine and schedule of administration not well adapted to the field condition (Espuelas et al., 2012). Also, trypanosomes, especially the Trypanosoma brucei sub-groups, are known for their antigenic variation and subsequent escape from immune clearance. The qualitative and quantitative aspects of the host immune response play an important role in the disease process and seem to be essential for the control of the early parasite replication, which is associated with host resistance (Trishmann, 1986).

In view of the above, many researchers have used immunostimulants or immunomodulatory agent such as vitamins and micronutrients in the management of African trypanosomosis (Eze and Ochike, 2007; Eze et al., 2011; Toma et al., 2008). The immune system is a highly proliferative, complex and integrated network of cells and organs, and therefore can be strongly influenced by these micronutrients and vitamins (Wellinghausen and Rink, 1998).

Zinc biology is a rapidly developing field, and recent research reveals zinc’s strategic role in most organ systems. Zinc, an essential trace element, is required by all organisms and modulates the immune response, influencing cellular growth and affecting the development and integrity of immune system (Dardene, 2002). Also, zinc functions as an antioxidant and is involved in many critical biochemical reactions. Zinc deficiency results in dysfunction of plasma membrane proteins, which present with some pathological features (Sharifian et al., 2012; Wellinghausen and Rink, 1998). It has been found that zinc deficiency is associated with alteration of the immune response, anaemia, increased erythrocyte fragility, pellagrous dermatitis, diarrhoea, alopecia, and mental disturbances (Shankar and Prasad, 1998).

Zinc levels may be an influential factor determining susceptibility or resistance of West African cattle to trypanosomiasis (Traoré-Leroux et al., 1985). In an investigation, decrease in zinc levels coincided with the onset of T. brucei gambiense in peripheral blood of rabbit (Mwangi et al., 1995). Zinc-deficient animals showed three times the number of trypanosomes as that of the complete and pair-fed mice (Lee et al., 1983).

This study was therefore undertaken to investigate the effect of zinc supplementation in T. brucei brucei infected rats and the possible protective effects on anaemia and immune response.

2. Materials and methods

2.1. Experimental animals

Thirty (30) growing rats were used for this study. The rats were obtained from the Department of Veterinary Medicine laboratory animal unit and kept in rat cages in a fly proof departmental experimental room. The rats were fed and given water ad libitum. They were allowed 7 days for acclimatisation.

2.2. Trypanosome isolate

The Trypanosoma brucei brucei used for the experiment was isolated from a naturally infected dog presented at the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The isolate was properly characterised and maintained as UNVTH 007 through serial passage in rats.

2.3. Zinc oxide

Zinc as zinc oxide was used for the study and was produced by Zinc National Monterrey, NL, Mexico.

2.4. Sheep red blood cells (SRBC)

Fresh sheep blood was obtained from sheep in the animal house of the Department of Veterinary Parasitology and Entomology, University of Nigeria, through their jugular vein. Before use, the red blood cells were washed three times with 1 part of blood to 9 parts of phosphate-buffered saline (PBS), pH 7.2, by centrifugation at 3000 rpm for 10 min on each occasion. After the final wash, the SRBCs were suspended in PBS as a 2% suspension (based on packed cell volume) for the serological tests and as a 10% suspension for

weight of 8 ppm and not infected not supplemented (NINS) groups was significantly (p < 0.05) higher but the relative organ weight of their liver and spleen was significantly (p < 0.05) lower than 2 ppm, 4 ppm and INS groups. On day 21 OTS, the parasitaemia levels of INS group was significantly (p < 0.05) higher than the supplemented groups. From the results, dietary zinc supplementation can be useful in the management of anaemia and immunosuppression caused by trypanosomes in rats.
immunization of the rats. A 1 ml amount of the 2% suspension contained approximately $5 \times 10^8$ red blood cells.

2.5. Experimental design

The rats were randomly assigned into five groups (A–E), with each group having six (6) rats each. Groups A, B and C rats were fed with feed supplemented with 2, 4 and 8 parts per million (ppm) of zinc. The supplemented groups and group D rats were infected with $5.34 \times 10^5$ trypanosomes per mouse on day 7 on the supplementation. Group E served as uninfected control.

The parameters used to assess the effect of the supplementation were as follows: change in body weight, level of parasitaemia, antibody titre, haemoglobin concentration, packed cell volume, total white blood cell count and relative organ weight. The parameters were taken on day 0 and every other 7th day. The rats were humanely sacrificed on day 21 on the supplementation after sample collection, and their liver, spleen and heart carefully dissected out for determination of relative organ weight (ROW). Animal studies were in compliance to the ethical procedure of the Animal Use and Care Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, which corresponds with NIH guidelines (NIH, 1996).

2.6. Detection of parasites

Following infection of rats with T. brucei, the parasitaemia were monitored in the rat blood using the wet mount and haematocrit centrifuge methods on a daily basis until all rats were positive.

2.7. Blood sample collection

Blood sample was collected from the retro-bulbar plexus of the median canthus of the eyes of the rats using microhaematocrit capillary tube. The blood was collected into two different sample bottles, one containing anticoagulant (EDTA) and the other without anticoagulant for haematology and serology respectively.

2.8. Determination of antibody titre

Sheep RBCs (0.1 ml of 10% sheep RBC) were used to immunise the rats by intraperitoneal injection and challenged by similar IP injection of the same amount in day 5 post immunization (PI). On the 7th day post challenge or day 0 (supplementation started) and subsequent 7 days, the antibody response was determined using haemagglutination test as described by Nelson and Mildenhall (1967). Booster doses of the sheep RBC were given every other 14th day following challenge.

2.9. Determination of PCV, haemoglobin concentration, total leucocyte count and parasitaemia

The packed cell volume and Hb concentration were determined by the microhaematocrit methods (Jain, 1986) and cyanomethaemoglobin method (Jain, 1986) using SP6-500UV spectrophotometer (PYE UNICAM, England) respectively. The total leucocyte count were carried out manually using the improved Neubauer haemocytometer method, as described by Jain (1986). Parasitaemia was estimated using the matching method of Herbert and Lumsden (1976).

2.10. Determination of body weight and relative organ weight

The weight of individual rat was determined using electronic weighing balance. Whereas the relative organ weights (ROW) of different organs were calculated using the formula:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Whole animal weight}}$$

2.11. Statistical analysis

The data collected were subjected to analysis of variance (ANOVA). Variant means were then separated using Duncan’s multiple range tests. Differences between means were considered significant at $p < 0.05$.

3. Results

The supplementation did not prolong the pre-patent period significantly ($p > 0.05$), with the pre-patent period being $5.32 \pm 0.36$, $5.64 \pm 0.61$, $6.02 \pm 0.83$ and $4.88 \pm 0.44$ days for 2 ppm, 4 ppm, 8 ppm, infected not supplemented (INS) and not infected not supplemented (NINS) groups respectively. The pre-infection supplementation made no significant changes on the packed cell volume (PCV) in all the groups (Fig. 1). However, on day 14 on the supplementation (OTS), 4 ppm, 8 ppm and NINS groups were significantly ($p < 0.05$) higher than 2 ppm and INS groups. Also, on day 21 OTS, NINS group was highly significantly ($p < 0.01$) higher than 2 ppm, 4 ppm and INS groups, but significantly ($p < 0.05$) higher than 8 ppm group. Also from Fig. 2, the pre-infection supplementation with zinc increased the haemoglobin concentration of 8 ppm group significantly ($p < 0.05$) on day 7 OTS when compared with other groups. Following infection on day 7 OTS, the Hb concentration on days 14 and 21 OTS decreased in all the infected groups, with INS group being significantly ($p < 0.05$) lower than other groups. From Fig. 3, the pre-infection supplementation significantly ($p < 0.05$) increased the WBC count of the supplemented groups when compared with the unsupplemented groups on day 7 on the supplementation (OTS). On day 14 OTS, INS and NINS groups were significantly ($p < 0.05$) lower than 2, 4 and 8 ppm groups. However, on day 21 OTS, NINS group was significantly ($p < 0.05$) higher than other groups. The supplementation led to increase in antibody titre of the supplemented groups (Fig. 4). The antibody titre increased significantly ($p < 0.05$) in the supplemented groups when compared to unsupplemented groups on day 7. Following infection, the supplemented groups maintained significantly ($p < 0.05$) higher antibody titre when compared with other groups on days 14 OTS. However, on day 21 the antibody response declined in all supplemented groups but not to the level of pre-supplementation values, with 8 ppm group being significantly ($p < 0.05$) higher than other groups. Figure 5 shows

![Fig. 1. Bar chart of mean packed cell volume (%) of Trypanosoma brucei infected fed diet supplemented with different levels of zinc.](image)
the relative organ weights of the liver and spleen of the supplemented and control groups. The relative organ weight of the liver of 2 ppm, 4 ppm and INS groups were significantly higher than 8 ppm and NINS groups. On the other hand, the relative organ weight of the spleen showed that NINS group was significantly lower than groups A, B and D, but not with group C. The supplementation did not cause any significant change between the infected groups on day 14 OTS (Fig. 6). However, on day 21 OTS the parasitaemia levels of INS group had significantly (p < 0.05) higher parasitaemia than other infected groups. From Fig. 7, the supplementation with zinc before infection with *Trypanosoma brucei brucei* did not significantly (p > 0.05) affect the weight of the different groups. However, following infection, the weight of 8 ppm and NINS groups was significantly (p < 0.05) higher than other groups on day 21 OTS.

4. Discussion

The pre-patent period was not significantly increased following pre-infection supplementation with zinc. This differs with the report that zinc supplementation prolonged the pre-patent period of supplemented groups when compared with the control in *Trypanosoma evansi* infected rats (Dalla Rosa et al., 2012). The variation could be attributed to the difference in species, route of administration or dosage and duration of supplementation. Packed cell volume and haemoglobin concentration were used to assess the anaemia in this study. Increase in PCV values and haemoglobin concentrations were recorded before infection. Zinc plays an important role in haemoglobin synthesis by activating delta-aminolevulinic acid (ALA) dehydrogenase, an enzyme essential for the formation of porphobilinogen from two ALA molecules (Akhtar et al., 2003; Jaffe and Lawrence, 2013). Also, the increase in PCV values can be attributed to the anti-oxidative effect of zinc. Zinc concentrations in cell membranes appear to be important in preserving their integrity (Bray and Bettger, 1990) and, together with glutathione peroxidase and superoxide dismutase, plays a crucial role in the
antioxidant system and thus protects biomolecules from oxidative damage (Mustafa et al., 2010; Prasad, 2014).

The PCV and haemoglobin values of the supplemented groups remained significantly higher than the infected un-supplemented group but did not differ with the un-infected control following infection. This result is consistent with the works of Silva et al. (2006) and Polat (2011). This could be attributed to the beneficial effect of zinc as an antioxidant and immunostimulant. Oxidative stress of the red blood cells (RBC) and their subsequent life span reduction are suggested to play an important role in the development of anaemia in African trypanosomiasis (Eze et al., 2008; Igbohke and Mohammed, 1992; Taiwo et al., 2003). Zinc is a component of dozens of vital enzymes within the body and in these enzymes the zinc molecule acts directly as an anti-oxidant, protecting the biochemical structure of the enzyme from free radical attack. Secondly, zinc acts to stabilise proteins which may otherwise react with highly unstable minerals, particularly iron and copper, to form free radicals. Zinc deficiency has been found to be associated with anaemia, increased erythrocyte fragility, as well as some other bodily functional abnormalities (Akhtar et al., 2003; Jaffe and Lawrence, 2013). Therefore, it is expected that zinc supplementation would reduce oxidative stress, prolong RBC survival, and thus maintain high PCV and haemoglobin levels.

In conclusion, zinc supplementation enhanced the immune response and reduced anaemia in Trypanosoma brucei infected rats.
Conflict of interest

There were no conflicts of interests related to this work.

References


