Effect of Cyanocobalamin on Antioxidant Enzymes of Male Wistar Albino Rats Infected with *Trypanosoma brucei brucei*

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Abstract  The study was undertaken to check the effects of cyanocobalamin on antioxidant enzymes of male Wistar albino rats infected with *T. b. brucei*. Fifty-four (54) male Wistar albino rats were divided into six groups of three rats each which were replicated three times. The rats were marked and kept in stainless wire cages labeled A-F. Groups A, B, and C were normal, negative and standard control respectively. Groups D, E and F were infected with 1.0 x 10⁶ trypanosomes and treated with 0.2mg/kg (low-dose), 0.3mg/kg (enriched-dose), and 0.4mg/kg (high-dose) body weight of vitamin B₁₂ respectively. The experiment lasted for twenty-one days from the day *T. b. brucei* infection was established. A sample of heart tissue homogenate was collected weekly across the groups and subjected to biochemical determination of catalase, superoxide dismutase (SOD), glutathione reductase, and glutathione peroxidase concentrations. There were significant differences in the effect of cyanocobalamin on the concentrations of cardiac tissue antioxidant enzymes which were also dependent on the duration of the experiment. At post-treatment, catalase, superoxide dismutase, and glutathione reductase levels differed significantly (p<0.05) from the negative control. There were significant reductions in the levels of catalase, glutathione reductase, glutathione peroxidase, and rise in the SOD level as infections grow. The result, however, showed that cyanocobalamin caused a significant elevation in the catalase and glutathione reductase levels, and no change in the level of glutathione peroxidase following treatments with cyanocobalamin.

Keywords  *Trypanosoma brucei brucei*, SOD, Cyanocobalamin, Catalase, Glutathione Reductase, Glutathione Peroxidase

1. Introduction

Human African Trypanosomiasis (HAT) is one of the tropical diseases that are widespread in Africa (Welburn and Maudlin, 2012). The primary reason for the diagnosis of HAT is to enable researchers to devise an appropriate means of therapeutic and prophylactic management. The diagnosis involves three steps: screening, diagnostic confirmation and staging. HAT manifests in two stages; the first (haemolymphatic) stage, where parasites are found in the blood and lymphatic tissues (Brun et al., 2010). The last (meningoencephalitic) stage is characterized by the presence of trypanosomes in the central nervous system (Brun et al., 2010). HAT is a dreadful disease with severe pathological consequence (Jamonneau et al., 2012). The transmission of HAT is mostly cyclical, which is dependent on the interactions of pathogenic parasites, tsetse flies, and mammalian reservoirs (WHO, 2013). Oxidative stress is a common mediator in the pathogenicity of established cardiovascular risk factors (Edwin et al., 2013). The sera of camels infected with trypanosomes recorded a significant decrease in SOD concentration (El-Deeb and Esmoslemany, 2015). The reduced SOD level might be a result of its depletion during oxidative stress caused by *T. envasi*. Catalase is an enzyme found in animals exposed to oxygen. It is used by cells to metabolize
the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water (Chelikani et al., 2004). It is an important enzyme that protects the cell from damage caused by reactive oxygen species (ROS). Glutathione reductase (GR) is an enzyme that catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), thereby maintain the cell integrity by reducing the oxidative damage caused by ROS (Deponte, 2013). Glutathione peroxidase (GPx) is a cytosolic enzyme that maintains cellular redox equilibrium and protects the organism from oxidative damage. The biological function is to reduce free hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Kannan et al., 2011). Vitamin B12 in the form of cyanocobalamin can be given parenterally as a prescriptive medication, usually by intramuscular injection (Andres et al., 2007). Cyanocobalamin functions in red blood cell formation, neurologic, and cognitive regulations (Koury et al., 2011). Its role has been established in subjects suffering from diabetic neuropathy (Sun et al., 2005). It also functions in nervous regulation and for the metabolism of carbohydrates, protein, fat, and maintenance of fertility. Intramuscular injection of cyanocobalamin has proven effective in increasing sperm counts for men undergoing intracytoplasmic sperm injection (ICSI) procedure (Bioxmeer et al., 2007). The deficiency of vitamin B12 is associated with the pathogenesis of megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, weight loss, and subacute combined degeneration of the spinal cord (Lansa et al., 2009).

2. Materials and Method

2.1. Animal Model and Experimental Protocol

Fifty-four (54) male albino Wistar rats (Rattus norvegicus) aged 3 months, weighing between 180-220g were procured, housed and allowed to acclimatize for two weeks at the Pharmacy-Animal House, Madonna University Elele, Rivers state. The rats were grouped into six (6) cages labeled A-F and each cake has three (3) rats that were replicated three (3) times from each group. The animals were kept under normal room temperature with ad libitum access to feed and water. The cages were cleaned daily to prevent infection of the animals and to minimize extraneous variables. The groups (A-F) were as thus: Group A (Normal Control) were neither infected with trypanosomes nor treated with vitamins; Group B (Negative Control) were infected with 1.0x10^6 trypanosomes but not treated; Group C (Standard Control) were infected with 1.0x10^6 trypanosomes and treated with 0.2 mg/kg Diminazene aceturate body weight; Group D (Low-dose of vitamin B12) were infected with 1.0x10^6 trypanosomes and treated with 0.2 mg/kg body weight of vitamin B12; Group E (Enriched dose of vitamin B12) were infected with 1.0x10^6 trypanosomes and treated with 0.3 mg/kg body weight of vitamin B12; Group F (High dose of vitamin B12) were infected with 1.0x10^6 trypanosomes and treated with 0.4 mg/kg body weight of vitamin B12. The experiment lasted for twenty-one days after Trypanosoma brucei brucei infection was established. A sample of cardiac tissue homogenate was collected weekly from the three (3) rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka for biochemical determination of catalase, SOD, GR, and GPx concentrations.

2.2. Procurement and Inoculation of Trypanosomes

Trypanosoma brucei brucei was obtained from an experimentally infected rat previously inoculated with the parasite from the Faculty of Veterinary Medicine, UNN. Each experimental rat was administered 0.1ml of infected blood in 0.3ml normal saline containing 1x10^6 trypanosomes using the rapid matching method (Herbert and Lumbsden, 1976) to determine the level of parasitemia. Inoculation was done intraperitoneally.

2.3. Determination of Parasitaemia

Wet blood preparation was covered with a coverslip on a slide and examined under X40 and oil immersion using a microscope at X100 magnification. The identification of parasites was done using morphological description (Van-Wyk and Mayhew, 2013).

2.4. Formulation and Administration of Vitamin B12

Vitamin B12 (cyanocobalamin) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria in a powdered bottle. The working concentrations were determined at the Department of Biochemistry, Madonna University, Elele. From the result of acute oral toxicity (LD50) test of vitamin B12 as thus:

- Mild dose: 0.2 mg/kg b.w.d.
- Enriched dose: 0.3 mg/kg b.w.d.
- High dose: 0.4 mg/kg b.w.d.

The working concentrations were administered via intubation using 2ml of distilled water as a vehicle.

2.5. Standard Drug

Diminazene aceturate was procured from the Faculty of Veterinary Medicine Clinic, University of Nigeria, Nsukka, Nigeria in a 2.36g granules. The working dosage was 0.2m/kg. The administration was intravenous.

2.6. Preparation of Cardiac Tissues Homogenate

The heart was weighed and homogenized with a potter-Elvenhjem tissue homogenizer in a 10 mM potassium phosphate buffer (pH 7.4). The crude tissue homogenate was centrifuged at 10,000 revolutions per
minute, for 15 minutes in a cold centrifuge, and the resultant supernatant was used for the different estimations of proteins.

2.6.1. Catalase Activity

Catalase activity was determined in erythrocyte lysate using Aebi’s method (Aebi, 1984).

2.6.2. Determination of Superoxide Dismutase (SOD)

The method illustrated by McCord and Fridovich (1969) was used for the determination of superoxide dismutase (SOD).

2.6.3. Glutathione Reductase (GR) Assay

The method illustrated by Kakkar et al. (1984) was followed in the determination of glutathione reductase.

2.6.4. Glutathione Peroxidase (GPx)

The procedure according to Wood (1970) was used in the estimation of glutathione peroxidase concentration.

3. Result

Table 1-3 show the effects of cyanocobalamin on cardiac tissue while Table 4 shows the effects of Vitamin B12 on cardiac tissue. Generally, there are significant differences on the effects of cyanocobalamin and Vitamin B12 across the six groups of rat but there was no significant differences with respect to the weeks.

Table 1. Effect of cyanocobalamin on cardiac tissue catalase level (u/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>49.423±0.190&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>50.155±0.481&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>51.412±1.237&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>42.252±0.064&lt;sup&gt;b1&lt;/sup&gt;</td>
<td>40.510±0.346&lt;sup&gt;b2&lt;/sup&gt;</td>
<td>38.345±0.191&lt;sup&gt;b3&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>48.131±0.017&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>49.435±0.156&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>49.527±0.507&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>44.833±0.059&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.682±0.117&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.571±0.038&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>44.980±0.173&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.780±0.114&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.674±0.118&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>45.483±0.117&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.824±0.118&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>43.751±0.116&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

Table 2. Effect of cyanocobalamin on cardiac tissue SOD level (u/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.444±0.095&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>3.526±0.162&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>3.460±0.167&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>3.216±0.136&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>4.972±0.399&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>5.002±0.096&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>3.485±0.057&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>3.657±0.071&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>3.635±0.090&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>3.992±0.005&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>4.044±0.065&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>4.111±0.003&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>3.978±0.007&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>3.977±0.035&lt;sup&gt;a2&lt;/sup&gt;</td>
<td>4.071±0.032&lt;sup&gt;a2&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>3.949±0.029&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>4.001±0.066&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>4.001±0.002&lt;sup&gt;a1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

4. Discussion and Conclusions

4.1. Discussion

The results of the present study indicated that *Trypanosoma brucei brucei* caused a significant reduction in the level of antioxidant catalase in the heart tissues of Wistar albino rats (Table 1). This observation is in agreement with the work of Matheus et al. (2016) who reported a significant reduction of enzymatic activities of catalase in the heart of rats infected with *T. evansi*. They attributed these decreased enzymatic activities to cardiac injury. However, the present work conflicts with the observation of Atalay and Laaksonen (2002), who reported that under the condition of oxidative stress, the activity of catalase enzyme increases. The levels of catalase enzyme in the groups infected and treated with the antioxidant vitamin B12 significantly increased the trypanosome-induced catalase reduction. The result of the
The present study also agreed with the observation of Eze et al. (2016) who reported that vitamin E supplementation significantly increases catalase activities of T. b. brucei-infected rats. The present experiment showed a higher level of superoxide dismutase (SOD) in the heart of T. b. brucei infected rats from day 7-21 post-infection when compared to the normal control (Table 2). This is in line with the work of Atalay and Laaksonen (2002) who observed that under the condition of oxidative stress, the activity of antioxidant superoxide dismutase increases. In the infected and treated groups, there is a statistically significant difference in the activity of SOD when compared to normal control. The result of the present experiment contradicts the observation of Eze et al. (2016) who reported that antioxidant vitamin supplementation enhances SOD activities of T. b. brucei-infected rats. The present study showed that the proliferation of trypanosomiasis caused a reduction in the levels of glutathione reductase in the experimental group (Table 3). Previous studies measuring glutathione reductase activity showed a decrease in the parameter in chronic lymphocytic leukemia patients (Bakan et al., 2003). In the groups infected and treated with the intervention, the glutathione reductase significantly increased. The observed elevation of glutathione reductase in the treated groups is indicative of the antioxidant and trypanocidal properties of cyanocobalamin. This is consistent with the result of Verhagen et al. (2006) who reported that antioxidant vitamin can prevent the damaging effects of reactive oxygen species. The experiment showed that the proliferating parasites caused reductions of GPx in the rats (Table 4). This aligns with the works of Anschau et al. (2013) who reported decreased glutathione peroxidase activity in the blood of rats infected with T. evansi. In the groups infected and treated with vitamin B12, there are no significant differences in the heart tissue GPx between the treated and untreated groups. This is consistent with the findings of Clarke et al. (2007) which reported that cyanocobalamin does not affect the principal cardiovascular events.

4.2. Conclusions

The findings of this study indicated that the pathogenesis of T. b. brucei brucei caused a significant increase in the level of SOD, and a profound reduction in the concentrations of tissue catalase, glutathione reductase, and glutathione peroxidase (GPx). However, the administration of the rats with vitamin B12 caused dose-dependent reduction in the concentrations of SOD and increased cardiac tissue catalase and glutathione reductase, while GPx showed no significant change.

REFERENCES


