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# Coenzyme $Q_{10}$ prevented full blown splenomegaly and decreased melarsoprol-induced reactive encephalopathy in mice infected with *Trypanosoma brucei rhodesiense*

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## PEER REVIEW

**Peer reviewer**

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**Comments**

The paper systematically addresses the objectives and conclusively determines the possible role of coenzyme $Q_{10}$ in limiting the pathogenesis of trypanosomiasis. The data obtained supports the conclusions reached in this study.

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## ABSTRACT

**Objective:** To establish the modulatory effects of coenzyme $Q_{10}$ on experimental trypanosome infections in mice and evaluate the risk of occurrence and severity of melarsoprol-induced post treatment reactive encephalopathy (PTRE).

**Methods:** Female Swiss white mice were orally administered with 200 mg/kg of coenzyme $Q_{10}$ after which they were intraperitoneally inoculated with *Trypanasoma brucei rhodesiense* (*T. b. rhodesiense*). The resultant infection was allowed to develop and simulate all phases of human African trypanosomiasis and PTRE. Parasitaemia development, packed cell volume, haematological and pathological changes were determined.

**Results:** A histological study in the brain tissue of *T. b. rhodesiense* infected mice demonstrated neuroinflammatory pathology which was highly amplified in the PTRE-induced groups. A prominent reduction in the severity of the neuroinflammatory response was detected when coenzyme-$Q_{10}$ was administered. Furthermore, the mean tissue weight of spleen to body ratio in coenzyme $Q_{10}$ supplemented group was significantly ($P<0.05$) different compared to un-supplemented groups, and clearly indicated that coenzyme $Q_{10}$ prevented full blown splenomegaly pathogenesis by *T. b. rhodesiense*. A significant ($P<0.05$) increase in hemoglobin levels and red blood cells was observed in coenzyme $Q_{10}$ mice compared to those infected and un-supplemented with coenzyme $Q_{10}$.

**Conclusions:** The capacity of coenzyme $Q_{10}$ to alter the pathogenesis of *T. b. rhodesiense* infection in mice and following treatment with melarsoprol, may find application by rendering humans and animals less susceptible to deleterious effects of trypanosome infection such as splenomegaly and melarsoprol-induced PTRE and neurotoxicity.

KEYWORDS

*Trypanasoma brucei rhodesiense*, Encephalopathy, Coenzyme $Q_{10}$, Melarsoprol, Post treatment reactive encephalopathy, Splenomegaly

## 1. Introduction

Human African trypanosomiasis (HAT) is an important neglected protozoan disease that affects man. The causative agents of HAT, protozoan parasites *Trypanosoma brucei gambiense* (West and Central Africa) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) (Eastern and Southern Africa), are transmitted by tsetse flies (*Glossina* spp). Approximately 60 million people in 36 countries in sub-Saharan Africa are threatened by HAT. With over seven

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thousand new cases of people being affected from *gambiense* and *rhodesiense* HAT being reported in 2011[1].

Pathogenesis of trypanosomiasis has been reported to be associated with alteration of multiple organs, especially the spleen being massively enlarged (splenomegaly) and altered haematological parameters[2], with pathological effects of the disease being initiated through the release of cytokines and nitric oxide[3]. Consequently, inflammatory responses are observed in multiple organs. In the central nervous system (CNS), inflammation results from both parasite invasion and post melarsoprol treatment, with microglia and astrocyte activation occurring during the CNS invasion process[4]. Since no vaccine up to date has been developed to protect against HAT, drugs are the only novel method for its management. However, drugs used to treat HAT have multiple limitations, variable efficacy and emergence of drug resistant trypanosome strains[5,6].

Late stage HAT caused by *T. b. rhodesiense* is only treated with melarsoprol (Mel B), a trivalent arsenical, which induces post treatment reactive encephalopathy (PTRE) in 10% of patients resulting in death of up to 5% of patients[7]. Various agents like azathioprine and substance P antagonist (RP-67580) have been shown to prevent an established PTRE, with the latter having profound amelioration effect[8]. Probably such variable efficacy presents the need for agents like coenzyme $Q_{10}$ that can be co-administered with melarsoprol to protect the brain against deleterious effects of PTRE. Use of coenzyme $Q_{10}$ has attracted much interest especially for the treatment of mitochondrial disorders owing to its neuro-protective effect. Potential improvement in both clinical and biochemical mitochondrial disorders have been observed in patients suffering from these disorders[9]. If defects in energy metabolism and oxidative damage play a role in the pathogenesis of neurodegenerative diseases[10], then this implies that coenzyme $Q_{10}$ may exert beneficial therapeutic effects. Therefore, there is an urgent need to develop new effective and safe chemotherapeutic antioxidants to improve the treatment of African sleeping sickness.

It has been observed elsewhere that coenzyme $Q_{10}$ attenuated lesions that were inducted by intrastriatal administration of malonate in an experimental rat model. Similar observations were also depicted in malonate-induced depletions of ATP and up-regulation of lactate concentrations[11]. However, potential role of coenzyme $Q_{10}$ in attenuating pathological CNS lesions due to melarsoprol and *T. b. rhodesiense* infection as the hallmark of pathogenesis of HAT remains to be determined. This study was conducted to establish the modulatory effect of coenzyme $Q_{10}$ on the trypanosome infection process and risk occurrence of melarsoprol induced PTRE in a mouse model.

## 2. Materials and methods

### 2.1. Ethics statement

All protocols and procedures used in the current study were reviewed and approved by the Kenya Agricultural Research Institute-Trypanosomiasis Research Centre and Egerton University Institutional Animal Care and Use Committees.

### 2.2. Experimental animals

Eight weeks old female adult Swiss white mice weighing between 20-30 g from Kabete-Vet Lab (Nairobi Kenya) colony were maintained on mice pellets (Unga Feeds Ltd, Kenya) and water *ad libitum* at a temperature of 21-25 °C. Wood-chippings were provided as bedding material. Prior to the experiment, all mice were treated for ecto and endoparasites using 0.02 mL of evermectin (Ivermectin®, Anupco, Suffolk, England) injected subcutaneously to each mouse and left to acclimatize for two weeks. A total of 60 mice divided into eight groups of six mice each were used. Two donor irradiated mice were obtained from International Livestock Research Institute for multiplication of *T. b. rhodesiense* clone, KETRI 2537.

### 2.3. Trypanosomes

Cryopreserved *T. b. rhodesiense* isolate (KETRI 2537) was obtained from Trypanosomiasis Research Centre trypanosome bank. The parasites were propagated and maintained in donor irradiated Swiss white mice that were obtained from International Livestock Research Institute for multiplication of the parasite.

### 2.4. Experimental design and drugs

After two weeks of acclimatization, mice were randomly selected and divided broadly into three experimental groups. Experiment (I) employing control and infected mice; Experiment (II) employing trypanosome infected animals challenged with Mel B 21 d post infection with or without the intervention of coenzyme $Q_{10}$ and Experiment (III) infected animals employed for PTRE studies. These animals were employed to study severe late CNS stage infection.

#### 2.4.1. Infected untreated groups

Experimental animals were subdivided into three groups each consisting of six. Mice in the second and third groups were infected with *T. b. rhodesiense* for 21 and 57 d post infection (dpi) respectively, while those in the first group were uninfected and served as health control for the second

and third group. Animals in the third group were projected to survive for 60 dpi after which they were sacrificed at termination of experiment.

#### 2.4.2. Infected melarsoprol groups

Experimental animals in this experiment were randomly divided into four groups each consisting six mice. Mice in the second, third and fourth group were infected with *T. b. rhodesiense* for 21 d and subsequently treated for 4 d with 3.6 mg/kg melarsoprol. While those in the fourth group were treated but had orally been treated with 200 mg/kg coenzyme $Q_{10}$ on every other day from 7 d prior to infection with *T. b. rhodesiense* KETRI 2537 and continued up to the termination of the experiment. Mice in the first group were uninfected health control.

#### 2.4.3. Infected PTRE-induced groups

Experimental animals were subdivided into three groups each consisting of six mice. The test antioxidant, coenzyme $Q_{10}$ was administered orally at a dosage of 200 mg/kg body weight after every second day using a gavage needle to one group of mice. Administration of coenzyme $Q_{10}$ commenced 10 d prior to infection to allow concentration of the antioxidants in the mice tissues including the brain and was continued until the last day of the experiment. After 10 d, animals were inoculated intraperitoneally with approximately $10^4$ trypanosomes diluted with ESG buffer pH 8.0. The infection was allowed to progress with or without antioxidant intervention until 21 dpi when animals were treated sub-curatively with 5 mg/kg diminazene aceturate (DA) for 3 d. Treatment with DA clears the parasites from the extravascular compartment but leaves residual trypanosomes organisms in the CNS (Jennings and Gray, 1983) and thus is used to induce severe late CNS infection that closely mirrors PTRE in human subjects. Thereafter, mice were monitored for relapse of parasitaemia after which they were treated with melarsoprol at a dosage of 3.6 mg/kg for 4 d and sacrificed 24 h post the last dosage to obtain brain samples. The last group consisted of uninfected mice and served as a control for infected mice with or without antioxidant.

### 2.5. Parasitaemia, spleen tissue weight to body weight ratio, blood sampling and determination of packed cell volume

The parasitaemia level for each mouse in every group was determined every day. To estimate the number of circulating parasites in infected mice, two methods were used: the rapid "matching" method by Herbert and Lumsden[12], when parasites were seen by direct microscopy and the buffy coat technique as described by Murray *et al.* when parasites could not be seen by direct microscopy[13]. The live body weight and spleen tissue were determined using the analytical electronic balance (Mettler PM34, DoltaRange®) during the euthinization period of each individual infected mouse and those not infected. Skewed tissue weight were first transformed (square root+1) before further statistical analysis. At one week interval, blood was taken from each mouse by tail snip into 100 μL microhaematocrit capillary tubes for PCV determination by the method of Woo[14]. After blood collection, the capillary tubes were sealed with plastic at one end and centrifuged in a haematocrit centrifuge (Hawksley House England) at 10 000 revolutions per minute for 5 min. PCV was then read using a micro-haematocrit reader and expressed as a percentage (%) of the total blood volume.

### 2.6. Determination of haematological values and brain tissue processing

Blood was collected directly from the heart by euthanization of the mice in a 1 mL syringe flashed with 5% EDTA and transferred to bijou bottles. Blood samples were analyzed using automated Bechman Coulter counter and a complete blood haemogram of each experimental mouse, giving hemoglobin (Hb) levels, haematocrit, red blood cell (RBC) count and white blood cell (WBC) count generated. The brains from all groups of mice were isolated and then fixed in 10% buffered formalin solution, pH 7.2. The material was processed for paraffin embedding, cut into 5 μm thick section and these sections were then stained with hematoxylin and eosin method; hippocampus was examined for vacuolar pathology to determine lesion severity and distribution as described by Fraser and Dickinson[15].

### 2.7. Statistical analyses

The statistical analysis was carried out using One-way ANOVA and post-hoc Bonferronis' test for multiple comparisons with significance level set at $P<0.05$. The statistical analyses were done using GraphPad Prism version 5 Software Inc. (San Diego, CA).

## 3. Results

### 3.1. Modulatory effect of coenzyme $Q_{10}$ on splenomegaly

Effects of coenzyme $Q_{10}$ on the spleen of mice which were intraperitoneally inoculated with *T. b. rhodesiense* at different stages and following treatment are presented in Figure 1. The mean spleen weight to body ratio of mice at 21 dpi with *T. b. rhodesiense* was 14-fold greater than in un-infected control mice. This difference was statistically significant ($P<0.05$). The ratio increased to 2-fold for mice in which the infection was allowed to progress to the terminal stage significantly higher than in the 21 dpi ($P<0.05$) (Figure 1a).

The mean spleen weight to body ratio of control mice was 15-fold less compared to infected melarsoprol treated mice which was significantly higher ($P<0.05$) (Figure 1b). In contrast, for mice that were supplemented with coenzyme $Q_{10}$ before infection with *T. b. rhodesiense* (treated exactly the same as group six except for coenzyme $Q_{10}$ supplementation), the mean spleen weight to body ratio was only 6.1-fold in comparison with un-infected, indicating that coenzyme $Q_{10}$ prevented full blown splenomegaly. The same pattern was witnessed for the group of mice which were supplemented with coenzyme $Q_{10}$, where the infected mice treated with melarsoprol alone showing enhanced splenomegaly as compared to the coenzyme $Q_{10}$ treated ($P<0.05$) (Figure 1b). In addition, results show a significantly higher mean spleen weight to body ratio in infected, PTRE-induced and melarsoprol treated mice than control ($P<0.05$) (Figure 1c). Similarly, the mean weight of the spleen to body ratio in infected mice in which PTRE was induced and treated with melarsoprol was significantly higher than the group of mice that received the same treatment but supplemented with coenzyme $Q_{10}$ ($P<0.05$). Overall, splenomegaly was more pronounced in either *T. b. rhodesiense* infected with or without treatment with melarsoprol than in control and infected coenzyme $Q_{10}$ supplemented group.

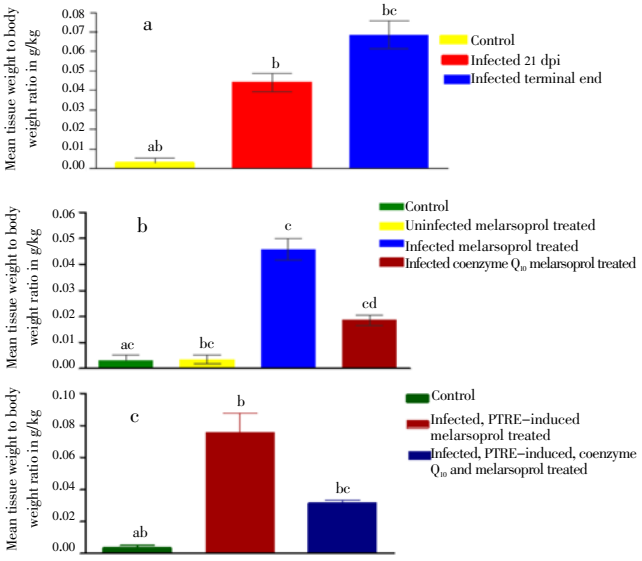


**Figure 1.** Effect of HAT infection, coenzyme $Q_{10}$, PTRE-induced on mean spleen to body weight ratio of mice.

a: Un-infected controls, infected sacrificed 21 dpi and sacrificed at the terminal end 57 dpi; b: (i) Control, (ii) Uninfected mice treated with melarsoprol, infected with *T. b. rhodesiense* treated with melarsoprol and *T. b. rhodesiense* infected mice that were treated with melarsoprol and supplemented with coenzyme $Q_{10}$; c: Healthy control, *T. b. rhodesiense* infected, PTRE-inducted and after relapse treated with melarsoprol and same as group nine but orally supplemented with coenzyme $Q_{10}$.

### *3.2. Haematoxylin and eosin stained sections of the hippocampal brain region prepared from T. b. rhodesiense infected and uninfected mice*

The neuropathological evidence shows an exacerbation of inflammation in the trypanosome infected mice in this study. As the infection progressed, there was an increase in development of perivascular cuffing (Figure 2), inflammatory cellular infiltration, encephalitis, loss of distinct cellular structure and lining, reactive gliosis and proliferation of microglia cells as the infection progressed to the terminal end of the disease. These lesions were more pronounced and aggravated in the infected mice that were euthanized after reaching the terminal end compared to those that were euthanized at 21 dpi (Figure 2). Detachment of the choroid plexus (choroidosis) was observed only in the terminal group. A reactive encephalopathy was observed in uninfected mice treated with melarsoprol. Additionally, perivascular cuffing and cellular infiltration were evident but these lesions were more marked in infected mice treated with melarsoprol at 21 dpi. The observed lesions were mild in infected mice that were given coenzyme $Q_{10}$ and administered with melarsoprol. For this group of mice, the hippocampus region showed distinctly normal layers. Treatment of infected mice with DA at 21 dpi to induce and/or simulate PTRE and administration of melarsoprol following relapse resulted in severe meningitis, prominent perivascular cuffing by lymphocytes and macrophages, reactive gliosis, hemorrhage (RBC present in the parenchyma), encephalitis and marked increase in the cellularity infiltration. On the other hand, infected mice orally administered with coenzyme $Q_{10}$ treated with DA and melarsoprol after relapse showed less of these lesions (Figure 2).

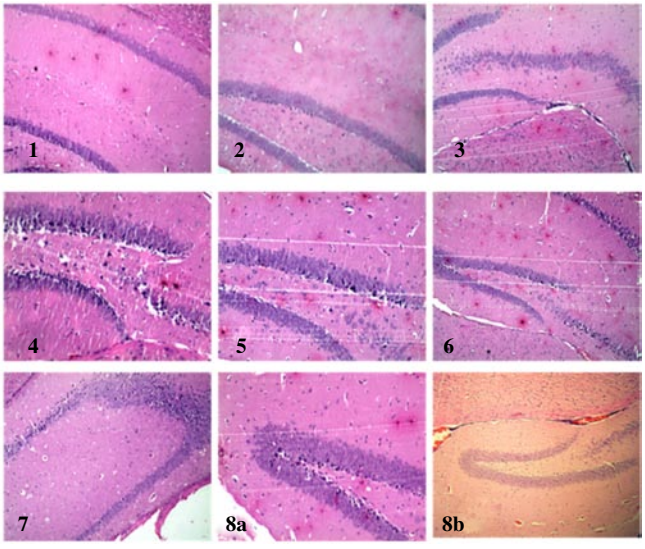


**Figure 2.** Haematoxylin and eosin stained sections through the hippocampal brain region.

The sections show different neuro-inflammation at different stages of the disease, and also with different treatments: (1) uninfected (control), (2) early-CNS stage (21 dpi), (3) severe late-CNS stage, (4) uninfected melarsoprol treated, (5) infected melarsoprol treated 21 dpi, (6) infected $CoQ_{10}$ melarsoprol treated 21 dpi, (7) infected $CoQ_{10}$, PTRE-induced and melarsoprol treated with less marked cellular infiltration and perivascular cuffing, and infected, PTRE-induced and melarsoprol treated, (8a & 8b) increasing development of the perivascular cuffs, cellular infiltration and encephalitis as demonstrated.

### 3.3. Packed cell volume

The changes in the PCV levels for the various treatment groups are presented in Figure 3a, b and c. There was a gradual decline in the mean PCV levels following infection with *T. b. rhodesiense* 21 dpi which was significantly ($P$<0.05) different from the control (Figure 3a). Analysis with $t$-test revealed the means±SEM of 10.200±2.083 with the 95% confidence interval 5.396 to 15.000. Significant improvement in the PCV levels in 200 mg/kg orally administered *T. b. rhodesiense* infected mice was witnessed comparable to *T. b. rhodesiense* infected 21 dpi coenzyme $Q_{10}$ un-supplemented group (Figure 3c). Similarly, trend was observed in groups of mice where PTRE was induced to study severe late stage of HAT (Figure 3b). The rate of PCV fall was higher in infected mice in which PTRE had been induced, and treated with melarsoprol after relapse compared to mice that received similar treatment but were orally supplemented with coenzyme $Q_{10}$ (Figure 3b). However, the healthy (controls) mice did not show any significant drop in the PCV during the period of study ($P$<0.05).

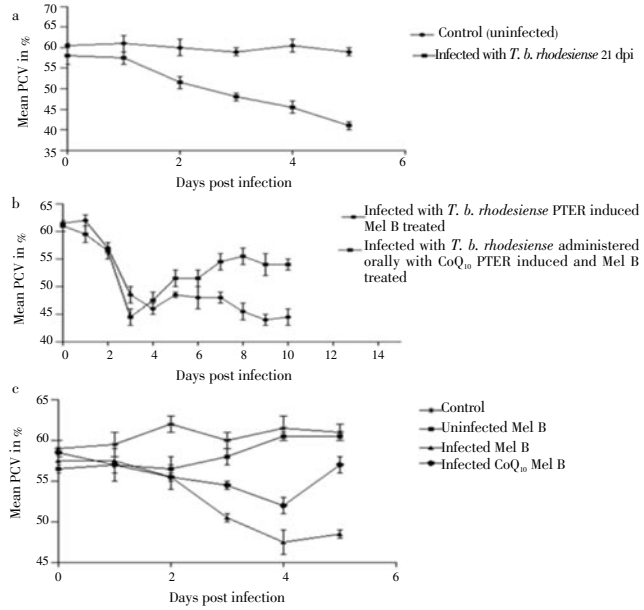


**Figure 3.** PCV changes in female Swiss white mice infected with *T. b. rhodesiense* infection, $CoQ_{10}$, PTRE-induced.
(a) Un-infected controls, infected 21 dpi and infected terminal end 57 dpi; (b) *T. b. rhodesiense* infected mice, PTRE-induced treated with melarsoprol after relapse and *T. b. rhodesiense* infected mice, PTRE-induced, $CoQ_{10}$ administered and treated with melarsoprol. There was significant ($P$<0.05) difference in mean packed cell volume of control and *T. b. rhodesiense* infected mice at different time intervals; (c) Control, uninfected mice treated with melarsoprol, *T. b. rhodesiense* infected mice treated with melarsoprol at 21 dpi *T. b. rhodesiense* infected mice $CoQ_{10}$ administered and melarsoprol treated 21 dpi.

### 3.4. Effects of T. b. rhodesiense, coenzyme $Q_{10}$, melarsoprol and PTRE induction on haematological profile (Hb, WBC and RBC)

Results of the mean haematological profile levels for Hb, RBC and WBC in (i) PTRE-induced, (ii) melarsoprol and (iii) coenzyme $Q_{10}$ treated mice, intraperitoneally inoculated with *T. b. rhodesiense* mice are presented in Figures 4, 5 and 6. One way ANOVA revealed that there was a significant difference in levels of Hb in *T. b. rhodesiense* infected minus treatment ($P$<0.05) or *T. b. rhodesiense* infection treated with melarsoprol and coenzyme $Q_{10}$ treatment ($P$<0.05) or *T. b. rhodesiense* infection/PTRE-induced/melarsoprol/coenzyme $Q_{10}$ ($P$<0.05) treatment in responses to infection and treatment. Bonferroni's multiple comparison test indicated significantly higher Hb levels in *T. b. rhodesiense* 21 dpi relative to the infected mice that went to the terminal end ($P$<0.05) (Figure 4c). Infected mice treated with melarsoprol had significantly lower Hb levels compared to the infected melarsoprol and coenzyme $Q_{10}$ treated mice ($P$<0.05) (Figure 4a). Similarly, Bonferroni's *post hoc* test comparison of the means revealed significantly lower levels of Hb in infected mice (PTRE-induced) treated with melarsoprol than in control mice ($P$<0.05). While in infected mice (PTRE-induced) treated with melarsoprol alone, Hb was significantly lower relative to (PTRE-induced), coenzyme $Q_{10}$ and melarsoprol treated mice ($P$<0.05) (Figure 4b). Analysis of RBC using ANOVA revealed that there was a significant difference in levels of RBC in *T. b. rhodesiense* infected mice (minus treatment) compared to the control ($P$<0.05). Bonferroni's multiple comparison test indicated significantly higher RBC levels in *T. b. rhodesiense* 21 dpi relative to the control ($P$<0.05) (Figure 5a). Infected mice treated with melarsoprol had significantly lower RBC levels compared to the control ($P$<0.05). While infected mice treated with melarsoprol had significantly lower RBC levels relative to infected melarsoprol and coenzyme $Q_{10}$ treated mice ($P$<0.05) (Figure 5b). Similarly, Bonferroni's *post hoc* test comparison of the means revealed significantly lower levels of RBC in infected mice PTRE-induced treated with melarsoprol than in control mice ($P$<0.05). Infected mice (PTRE-induced) treated with melarsoprol alone, RBC was significantly lower relative to (PTRE-induced) coenzyme $Q_{10}$ and melarsoprol treated mice ($P$<0.05) (Figure 5c).

Similarly, analysis of WBC using ANOVA revealed that there was a significant difference in levels of WBC in *T. b. rhodesiense* infected mice that were not treated ($P$<0.05) or *T. b. rhodesiense* infection 21 dpi melarsoprol/coenzyme $Q_{10}$ treatment ($P$<0.05) or *T. b. rhodesiense* infection/PTRE-induced/ melarsoprol/ coenzyme $Q_{10}$ ($P$<0.05) treatment in response to infection and treatment. Bonferroni's multiple comparison test indicated significantly higher WBC levels in *T. b. rhodesiense* 21 dpi relative to the mice at the terminal stage ($P$<0.05) (Figure 6c). Infected mice treated with Mel B had significantly higher WBC levels compared to the control ($P$<0.05) (Figure 6a).

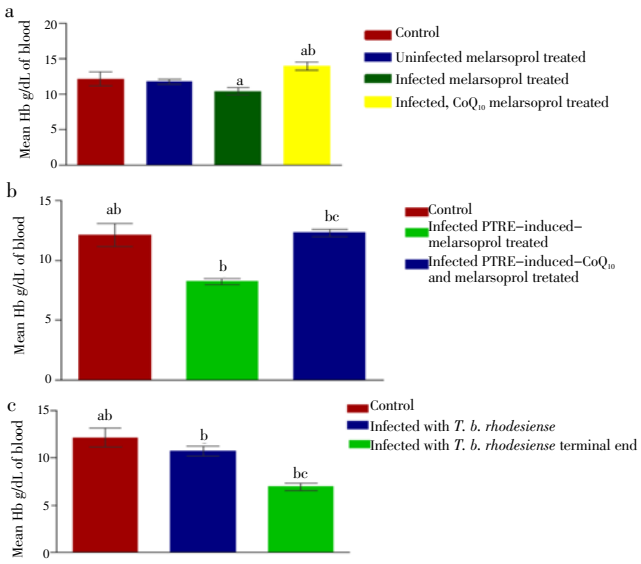


**Figure 4.** Effects of *T. b. rhodesiense* infection, $CoQ_{10}$, PTRE-induced and melarsoprol treatment on Hb.

(a) (i) Control, (ii) *T. b. rhodesiense* infected mice treated with melarsoprol, (iii) uninfected mice treated with melarsoprol and (iv) *T. b. rhodesiense* infected mice $CoQ_{10}$ administered and treated with melarsoprol. ab indicates significant difference ($P<0.05$) between (iii) and (iv); (b) (i) Control (ii) *T. b. rhodesiense* infected mice PTRE-induced and treated with melarsoprol at relapse and (iii) *T. b. rhodesiense* infected mice $CoQ_{10}$ administered, PTRE-induced treated with melarsoprol at relapse. ab represent significant difference ($P<0.05$) between ii and i while bc represent significant difference ($P<0.05$) between (ii) and (iii); (c) (i) Control (ii) *T. b. rhodesiense* 21 dpi and (iii) *T. b. rhodesiense* infected mice 57 dpi (terminal end). ab represent significant difference ($P<0.05$) between (i) and (ii) while bc represent significant difference ($P<0.05$) between (ii) and (iii).

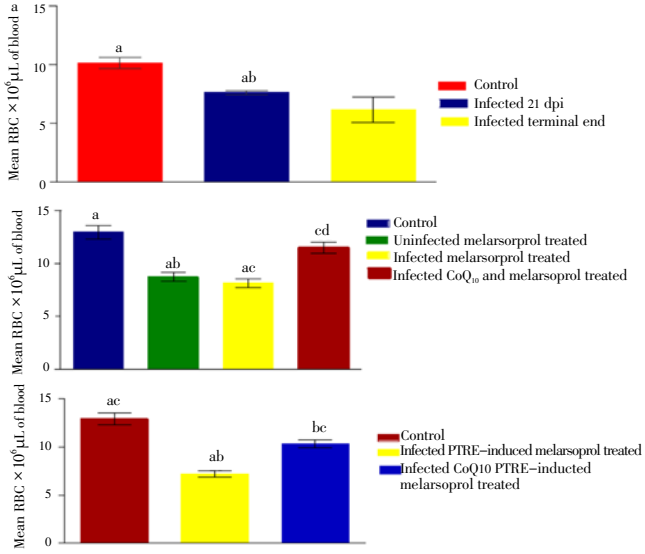


**Figure 5.** Effects of *T. b. rhodesiense* infection, $CoQ_{10}$, PTRE-induced and melarsoprol treatment on RBC concentration.

(a) (i) Control (ii) *T. b. rhodesiense* infected 21 dpi, (iii) *T. b. rhodesiense* infected mice 57 dpi (terminal end). ab represents significant ($P<0.05$) different between (i) and (ii); (b) (i) Control (ii) uninfected melarsoprol treated mice, (iii) *T. b. rhodesiense* infected melarsoprol treated and (iv) Infected $CoQ_{10}$ administered and melarsoprol treated. ab represents RBC concentration significantly ($P<0.05$) different between (i) and (ii), ac represents significant ($P<0.05$) different between (iii) and (i) and cd represents significant ($P<0.05$) different between (iii) and (iv); (c) (i) Control (ii) *T. b. rhodesiense* infected mice, PTRE-induced and treated with melarsoprol at relapse and (iii) *T. b. rhodesiense* infected mice, PTRE-induced $CoQ_{10}$ administered and melarsoprol treated after relapse. The letters bc indicates the RBC concentration is significantly ($P<0.05$) different between (ii) and (iii) and ab represents significant ($P<0.05$) different between (i) and (ii).

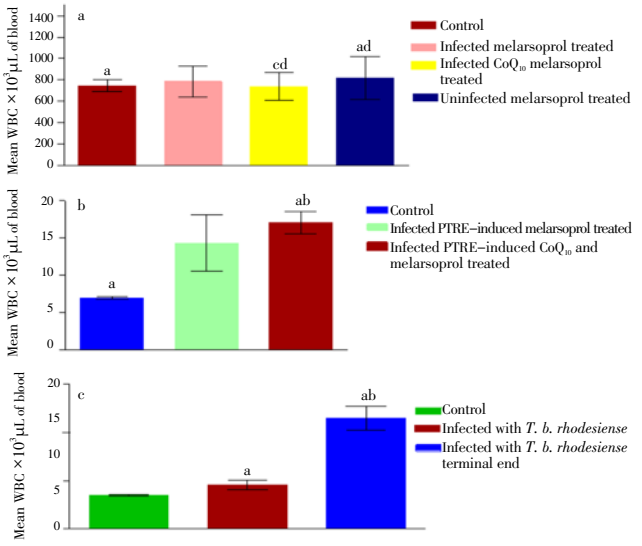


**Figure 6.** Effects of *T. b. rhodesiense* infection, $CoQ_{10}$, PTRE-induced and melarsoprol on WBC concentration.

(a) (i) control, (ii) *T. b. rhodesiense* infected, melarsoprol treated mice, (iii) *T. b. rhodesiense* infected $CoQ_{10}$ administered and melarsoprol treated and (iv) uninfected melarsoprol treated. Letters ad indicates that WBC concentration is significantly different between (i) and (iv) while cd indicates that WBC concentration is significantly different between (iii) and (iv) ($P<0.05$); (b) (i) Control), (ii) Infected mice, PTRE-induced and treated with v after relapse and (iii) Infected mice $CoQ_{10}$ administered, PTRE-induced and melarsoprol treated after relapse. ab represents significant difference ($P<0.05$) between (i) and (iii); (c) (i) Control, (ii) Infected mice 21 dpi and (iii) Infected terminal stage (57 dpi). There was significant ($P<0.05$) difference between (iii) and (ii) (ab).

## 4. Discussion

This study has shown that coenzyme $Q_{10}$ alters the pathogenesis of *T. b. rhodesiense* in mice and protects the mice brain from melarsoprol neurotoxicity. This is evidenced by blockage of full blown splenomegaly and prolonged relapse time following treatment with 5 mg/kg diminazene aceturate to induce PTRE. Additionally, coenzyme $Q_{10}$ boosted Hb and RBC levels. Typical clinical signs and symptoms of HAT due to *T. b. rhodesiense* are manifested in both early and late stage of the disease, with the early stage beginning one to three weeks after infection. The early stage is normally characterized by fever, lymphadenopathy, hepatomegaly, splenomegaly, skin rash, pruritis, tachycardia, weight loss, general malaise, and weakness[16].

It is noteworthy that, splenomegaly in trypanosomiasis occurs as a result of activated macrophages accumulating in splenic sinusoids that lead to high inflammation. For their optimal function, cells and tissues like heart, liver and spleen that have specific roles in immune function are energy-dependent and therefore require constant supply of coenzyme $Q_{10}$. Immune-enhancing effects of coenzyme $Q_{10}$ have been studied in experimental infections with increased phagocytic activity of macrophages and increased proliferation of granulocytes being observed[17,18]. It is plausible that in an experimental animal model in which Friend Leukemia virus was inoculated. It was

observed that coenzyme $Q_{10}$ levels in the blood and spleen decreased optimally[19]. Interestingly, in a similar study, it was observed that administration of coenzyme $Q_{10}$ to the same infected animals resulted in extension of survival rate with concomitant decrease in the severity of hepatomegaly and splenomegaly[20]. This observation is in agreement with the present study in which infected mice treated with melarsoprol and orally administered with coenzyme $Q_{10}$ showed a marked and significant decrease in severity of splenomegaly as demonstrated by mean spleen to body weight ratios. We therefore postulate that this observation is an indicative of the antioxidant capacity of coenzyme $Q_{10}$ in response to oral administration that resulted in its optimal function that was well tolerated by the spleen.

Previously it has been demonstrated that extracellular supply of coenzyme $Q_{10}$ through the circulation influences coenzyme $Q_{10}$ levels in blood cells[21]. This may indicate an underlying possibility that coenzyme $Q_{10}$ prevents splenomegaly through other mechanism other than its antioxidant capabilities. It is necessary to investigate this possibility in our future studies.

Elevation of WBC in *T. b. rhodesiense* infected mice that were given coenzyme $Q_{10}$ and treated with 3.6 mg/kg at 21 dpi was unusual. Similarly, mycotoxins result in elevation of white cell count in *T. b. rhodesiense* infected mice[22]. Thus, this observations suggest that coenzyme $Q_{10}$ is immunostimulatory rather than immunosuppressive. Such stimulation of the immune system components would be beneficial in any infection as is the case with trypanosomiasis[23]. However, reduced leucocytosis in *T. b. rhodesiense* infected mice indicates trypanosome-induced suppression of lymphoproliferative responses. Reduced number of WBC at severe late stage could be due to severe suppression of lymphocyte proliferation in response to increased burden of *T. b. rhodesiense* infection.

African trypanosomes are extracellular parasites that survive in the blood stream and they are capable of generating reactive oxygen species (ROS). During oxidative metabolism, ROS has the ability to inflict damage on all classes of macromolecules and can ultimately lead to cell death, more so in the brain. Indeed, ROS are implicated in a number of human neurodegenerative diseases[24]. One of the effects of *T. b. rhodesiense* is the reduction of RBCs due to increase in the oxidative effect on the RBC membranes. This stress is most acute in RBCs because the membranes have a high content of polyunsaturated lipids and a rich oxygen supply making them vulnerable to lipid peroxidation[25]. RBCs lack mitochondria and hence have no alternative means of generating reducing power. The major role of NADPH in the RBCs is to reduce the disulfide form of glutathione to the sulfhydryl form.

Remarkably, in the present study, all groups of mice that were orally administered with 200 mg/kg of coenzyme $Q_{10}$ registered high levels of RBC compared to un-supplemented one. This clearly indicates that coenzyme $Q_{10}$ protected or enhanced RBCs, perhaps by curtailing lipid peroxidation due to its potent antioxidant capability.

Anemia observed in trypanosome infected un-supplemented mice in this study was expected since anemia in *T. b. rhodesiense* infection is well documented in various animal models including vervet monkeys[26]. In the present study, oral administration of coenzyme $Q_{10}$ in *T. b. rhodesiense* infected mice shows that coenzyme $Q_{10}$ has a beneficial effect on RBCs that can prevent anemia as indicated by high levels of Hb and RBC. Similar studies have shown that administration of vitamin E reduced cadmium toxicity on the hematological processes with potent protective role in anemia[27]. It is evident from this study that coenzyme $Q_{10}$ attenuated anemia in mice and that such a phenomenon in humans could have profound implications in the treatment and management of trypanosomiasis and other blood disorders. The mechanism by which coenzyme $Q_{10}$ attenuates pathogenesis of anemia in the present study could be multi-factorial perhaps involving up-regulation of erythrogenin thus increasing erythroporetin activities. All these hypothesis merits further investigation, to determine the putative role of coenzyme $Q_{10}$ in elevation of WBCs, RBCs and Hb.

Many studies have shown that a decline in PCV is one of the key features in the pathogenesis of HAT that contributes to morbidity and mortality[28]. Infected mice orally given coenzyme $Q_{10}$ in the present work registered higher levels in PCV compared to un-supplemented mice. This remarkable observation could be due to many beneficial effects imparted by coenzyme $Q_{10}$. One possibility is increased resistance of RBCs to haemolysis. This finding provides compelling evidence to demonstrate that coenzyme $Q_{10}$ protects erythrocytes from haemolysis *in vivo* due to its antioxidant potency. ROS generated during infection leads to oxidative stress that can attack erythrocyte membrane causing its oxidation and consequently haemolysis[29]. It is plausible that the antioxidant capability of coenzyme $Q_{10}$ led to the reduction in the susceptibility of erythrocytes to membrane oxidative damage. Our findings have potential health implication in the management of HAT, which is a neglected disease.

The neuropathological evidence shows an exacerbation of inflammation in the trypanosome infected mice in this study. As the infection progressed to the terminal end, there was increase in development of perivascular cuffing, inflammatory cellular infiltration, encephalitis, loss of distinct cellular structure and lining, and reactive gliosis. Proliferation of microglia cells was aggravated with the progression of the infection. More pronounced brain pathological effects were observed in the terminal stage (57 dpi) of the disease compared to those that were euthanized at

21 dpi. The reported lesions have been reported previously with activation of the astrocytes and microglia[30]. The severe inflammatory cell infiltration in the infected mice (terminal/CNS stage) could have also resulted from the increased number of necrotic cells[31].

During the terminal phase of CNS infection, histological evidence of meningoencephalitis is evident[23]. In this study, coenzyme $Q_{10}$ ameliorated the severe brain damage due to the parasites and melarsoprol neurotoxicity. However, further studies may shed some light on this observation. Perivascular cuffing, cellular infiltration and reactive encephalopathy were observed in uninfected mice treated with melarsoprol. Although the lesions were more marked in infected mice treated with Mel B at 21 dpi. Similarly, trends have been observed where neurotoxicity and reactive encephalopathy were inducted due to melarsoprol administration[24]. The observed lesions were mild in infected mice given coenzyme $Q_{10}$ and melarsoprol treated mice in which the hippocampus region showed distinct layers. In this study, it is evident that coenzyme $Q_{10}$ may reduce neurotoxicity of melarsoprol and a reactive encephalopathy (PTRE) while at the same time reducing the inflammation due to the trypanosomes and the drug.

Infected mice in which PTRE was induced at 21 dpi and thereafter treated with melarsoprol after relapse; severe meningitis, prominent perivascular cuffing by lymphocytes and macrophages, reactive gliosis, hemorrhage (RBC present in the parenchyma), encephalitis and marked increase in the cellularity infiltration were observed. When the drug DA is administered, it leads to the production of an experimental PTRE that closely mimics the disease following melarsoprol in humans and persists after relapse of the parasitaemia[6]. Treatment with melarsoprol following relapse resulted in exacerbation of the inflammatory processes. In contrast, the group of mice that were infected with *T. b. rhodesiense*, (PTRE-induced), orally administered with coenzyme $Q_{10}$ and treated with melarsoprol after relapse showed less encephalitis, minimal perivascular cuffing and meningitis, very few inflammatory cells in the choroid tissue, less cellular infiltration and a marked reduction in loss of distinct structures. These results demonstrate that coenzyme $Q_{10}$ can significantly ameliorate the CNS inflammatory responses and reduce PTRE. Factors underlying this neuroprotection of brain lesions and their actual and specific roles in the established PTRE are not obvious and require further studies. However, coenzyme $Q_{10}$ deficiency may be common following HAT and that supplementation with coenzyme $Q_{10}$ may improve immune function and reduce the severity of haemolypmhatic and neurological pathogenesis of the disease. Consequently, in the near future, it will be critical to determine endogenous coenzyme $Q_{10}$ levels in HAT and following melarsoprol treatment.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### *Background*

Trypanosomiasis is a disease of economic importance especially in sub-Saharan Africa where about 60 million people are threatened by HAT. Complications resulting from Mel B treatment is highlighted and hence there is a need for strategies that could limit the effects of treatment.

### *Research frontiers*

The paper addresses an important topic that may contribute significantly to the management of trypanosomiasis through co-administration of coenzyme $Q_{10}$ with the drug melarsoprol.

### *Related reports*

The use of coenzyme $Q_{10}$ in treatment of mitochondrial disorders has been documented due to its neuro-protective effect. Potential improvement in both clinical and biochemical mitochondrial disorders have been observed in patients suffering from these disorders.

### *Innovations and breakthroughs*

The study clearly demonstrates that coenzyme $Q_{10}$ can significantly ameliorate the CNS inflammatory responses, reduce PTRE and prevent full brown splenomegaly.

### *Applications*

The results from this paper may be applied in the management of trypanosomiasis. Coenzyme $Q_{10}$ may exert beneficial therapeutic effects following administration of the drug choice, melarsoprol.

### *Peer review*

The paper systematically addresses the objectives and conclusively determines the possible role of coenzyme $Q_{10}$ in limiting the pathogenesis of trypanosomiasis. The data obtained supports the conclusions reached in this study.

## References


[1] Simarro PP, Franco JR, Diarra A, Ruiz Postigo JA, Jannin J. Diversity of human African trypanosomiasis epidemiological settings requires fine-tuning control strategies to facilitate disease elimination. *Res Rep Trop Med* 2013; **4**: 1-6.

[2] Poltera AA, Hochmann A, Lambert PH. A model for cardiopathy induced by *Trypanosoma brucei brucei* in mice. A histologic and immunopathologic study. *Am J Pathol* 1980; **99**: 325-352.

[3] Tiberti N, Hainard A, Lejon V, Courtioux B, Matovu E, Enyaru JC, et al. Cerebrospinal fluid neopterin as marker of the meningo-encephalitic stage of *Trypanosoma brucei gambiense* sleeping sickness. *PLoS One* 2012; **7**: 40909.

[4] Maloba F, Kagira JM, Gitau G, Ombui J, Hau J, Ngotho M. Astrocytosis as a biomarker for late stage human African trypanosomiasis in the vervet monkey model. *Sci Parasitol* 2011; **12**(2): 53-59.

[5] Brun R, Don R, Jacobs RT, Wang MZ, Barrett MP. Development of novel drugs for human African trypanosomiasis. *Future Microbiol* 2011; **6**: 677-691.

[6] Mäser P, Wittlin S, Rottmann M, Wenzler T, Kaiser M, Brun R. Antiparasitic agents: new drugs on the horizon. *Curr Opin Pharmacol* 2012; **12**: 562-566.

[7] Rodgers J, Jones A, Gibaud S, Bradley B, McCabe C, Barrett MP, et al. Melarsoprol cyclodextrin inclusion complexes as promising oral candidates for the treatment of human African trypanosomiasis. *PLoS Negl Trop Dis* 2011; **5**: e1308.

[8] Kennedy PG, Rodgers J, Jennings FW, Murray M, Leeman SE, Burke JM. A substance P antagonist, RP-67,580 ameliorates a mouse meningoencephalitic response to *Trypanosoma brucei brucei*. *Proc Natl Acad Sci U S A* 1997; **94**: 4167-4170.

[9] Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL. Elucidation of molecular mechanism involved in neuroprotective effect of coenzyme $Q_{10}$ in alcohol-induced neuropathic pain. *Fundam Clin Pharmacol* 2013; **27**: 603-622.

[10] Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E. Mitochondria, oxidative stress and neurodegeneration. *J Neurol Sci* 2012; **322**: 254-262.

[11] Yang L, Calingasan NY, Wille EJ, Cormier K, Smith K, Ferrante RJ, et al. Combination therapy with coenzyme $Q_{10}$ and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's Diseases. *J Neurochem* 2009; **109**: 1427-1439.

[12] Herbert WJ, Lumsden WH. *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitaemia. *Exp Parasitol* 1976; **40**: 427-431.

[13] Murray M, Murray PK, McIntyre WI. An improved parasitological technique for the diagnosis of African trypanosomosis. *Trans R Soc Trop Med Hyg* 1977; **71**: 325-326.

[14] Woo PT. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop* 1970; **27**: 384-386.

[15] Bruce ME, Boyle A, Cousens S, McConnell I, Foster J, Goldmann W, et al. Strain characterization of natural sheep scrapie and comparison with BSE. *J Gen Virol* 2002; **83**: 695-704.

[16] Kennedy PG. Human African trypanosomiasis-neurological aspects. *J Neurol* 2006; **253**: 411-416.

[17] Nikbakht H, Shokouhyar S, Ghazalian F. The effects of coenzyme $Q_{10}$ supplementation on footsal competition-induced changes in immune system in male players. *Eur J Exp Biol* 2013; **3**(3): 370-375.

[18] Hekimi S. Enhanced immunity in slowly aging mutant mice with high mitochondrial oxidative stress. *Oncoimmunology* 2013; **2**: e23793.

[19] Bliznakov E, Casey A, Kishi T, Kishi H, Folkers K. Coenzyme Q deficiency in mice following infection with Friend leukemia virus. *Int J Vitam Nutr Res* 1975; **45**: 388-395.

[20] Bliznakov EG. Effect of stimulation of the host defense system by coenzyme $Q_{10}$ on dibenzpyrene-induced tumors and infection with Friend leukemia virus in mice. *Proc Natl Acad Sci U S A* 1973; **70**: 390-394.

[21] Franke AA, Morrison CM, Bakke JL, Custer LJ, Li X, Cooney RV. Coenzyme $Q_{10}$ in human blood: native levels and determinants of oxidation during processing and storage. *Free Radic Biol Med* 2010; **48**: 1610-1617.

[22] Bondy GS, Pestka JJ. Immunomodulation by fungal toxins. *J Toxicol Environ Health B Crit Rev* 2000; **3**: 109-143.

[23] Islam MR, Roh YS, Cho A, Kim J, Kim JH, Eo SK, et al. Immune modulatory effects of the food borne contaminant citrinin in mice. *Food Chem Toxicol* 2012; **50**: 3537-3547.

[24] Martin LJ. Biology of mitochondria in neurodegenerative diseases. *Prog Mol Biol Transl Sci* 2012; **107**: 355-415.

[25] Thuita JK, Wang MZ, Kagira JM, Denton CL, Paine MF, Mdachi RE, et al. Pharmacology of DB844, an orally active aza analogue of pafuramidine, in a monkey model of second stage human African trypanosomiasis. *PLoS Negl Trop Dis* 2012; **6**(7): e1734.

[26] Thuita JK, Kagira JM, Mwangangi D, Matovu E, Turner CM, Masiga D. *Trypanosoma brucei rhodesiense* transmitted by a single tsetse fly bite in vervet monkeys as a model of human African trypanosomiasis. *PLoS Negl Trop Dis* 2008; **2**: e238.

[27] Eteng MU, Onwuka FC, Akpanyung EO, Osuchukwu NC, Bassey SC, Nwankpa P. Reversal of cadmium induced toxicity following dietary supplementation with garlic, ginger and cabbage in male Wistar rats. *J Nat Prod Plant Resour* 2012; **2**: 169-174.

[28] Ngotho M, Kagira JM, Kariuki C, Maina N, Thuita JK, Mwangangi DM, et al. Influence of trypanocidal therapy on the haematology of vervet monkeys experimentally infected with *Trypanosoma brucei rhodesiense*. *Acta Trop* 2011; **119**: 14-18.

[29] La Greca F, Magez S. Vaccination against trypanosomiasis: can it be done or is the trypanosome truly the ultimate immune destroyer and escape artist? *Hum Vaccin* 2011; **7**: 1225-1233.

[30] Maloba FC, Ngotho M, Rajesh K. Glial cells response during human african trypanosomiasis infection. *Sci Parasitol* 2012; **13**: 145-152.

[31] De Sousa KP, Atouguia JM, Silva MS. Induced cytokine network during experimental African trypanosomiasis. *Int J Infereron Cytokine Mediator Res* 2011; **3**: 71-78.