The effects of selenium and tocopherol supplementation on the efficacy of diminazene aceturate in reversing *T. brucei*-induced anemia in rats

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ABSTRACT

This study investigated the separate influence of selenium or tocopherol supplementation on the efficacy of diminazene aceturate in reversing the anemia caused by *Trypanosoma brucei* infection in rats. Changes in PCV, hemoglobin and RBC on day 7 post infection and days 7, 14 and 21 post treatment with diminazene aceturate at 7 mg/kg BW were evaluated. Hemoglobin concentration attained the highest level in the diminazene/tocopherol group by day 7 PT compared to the diminazene/selenium and diminazene groups. The mean PCV in the diminazene/tocopherol and diminazene/selenium groups were significantly higher than the PCV recorded in the diminazene group on day 7 PT. Hematological parameters (PCV, Hb and RBC) maintained upward trends in the diminazene and diminazene/selenium groups with the values of PCV, Hb and RBC becoming higher in the diminazene/tocopherol group by day 14 and 21 PT. The higher levels of hematomatological parameters in the antioxidant supplemented groups within the first week showed that antioxidant supplementation led to the more rapid return of these parameters to normal. The results indicate that a trypanocide/antioxidant combination may have significant therapeutic application in trypanosomosis.

Key words: diminazene, tocopherol, selenium, *Trypanosoma brucei*, rat, antioxidant, supplementation

Introduction

Anemia is a major clinical and laboratory finding in trypanosomosis and is characterized by a pronounced decrease in packed cell volume, hemoglobin, red blood cell, and white blood cell levels (LOSOS and IKEDE, 1972). The cause of anemia in trypanosomosis is multifactoral. Haemadilution, erythropagocytosis, haematopoietic response, haemolytic factor and bone marrow dyserythropoiesis have all been cited (ANOSA and ISOUN, 1976; OSOS and IKEDE, 1972). The cause of anemia in trypanosomosis is multifactoral.
DARGIE et al., 1979; MURRAY and DEXTER 1988). Recently, oxidative stress has been implicated in the anemia observed in trypanosomosis (IGBOKWE, 1994; UMAR et al., 2000). It is hypothesized that the large amounts of peroxides and free radicals generated by trypanosome and activated mononuclear phagocytes predispose erythrocytes to early ageing and fragmentation.

Several reports claim that vitamin E (α-tocopherol) is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation, thus stabilizing them and maintaining their permeability (HERRERA and BARBAS, 2001; TRABER and ATKINSON, 2007). A number of studies have also demonstrated that dietary vitamin E supplement elevates the activities of antioxidant enzymes (AMMOUCHE et al., 2002; KIRON et al., 2004). Selenium, an essential component of the enzyme glutathione peroxidase, is a natural antioxidant (YU, 1994) and immunostimulant (KIREMIDJIAN-SCHUMACHER et al., 1994; BECK et al., 2003; BROOME et al., 2004).

Diminazene aceturate (Berenil®) was introduced into trypanosomosis chemotherapy in the 1950s and has been widely used as a curative trypanocide. The chemotherapy of trypanosomosis is currently characterized by drug toxicity and resistance of trypanosomes to the drugs. This situation is complicated by the absence of new drugs. To ensure that the current drugs perform their therapeutic best, improve efficacy or reduce toxicity, trypanocides have been used in combination with adjuvant drugs (ZWEYGARTH and ROTTCHER, 1987; ONYEYILI and ANIKA, 1990; ABATAN, 1991; KAMINSKY and ZWEYGARTH, 1991).

With the allusion that oxidative stress has a role to play in the pathogenesis of trypanosomosis, some workers have reported the efficacy of antioxidant supplements alone in trypanosomosis therapy (UMAR et al., 2000; EZE and OCHIKE, 2007; TOMA et al., 2008). Results from these earlier studies have not been encouraging. Antioxidant supplementation alone has been ineffective in trypanosome treatment even though reports indicate less severe signs (UMAR et al., 2000; EZE and OCHIKE, 2007). This failure can be explained. While antioxidants can aid the reversal of pathological damage due to trypanosome infection and cause rapid healing, they cannot eliminate the parasites. There is a dearth of data on the efficacy of trypanocides in combination with antioxidants. Hence, this study was carried out to elucidate the efficacy of combining diminazene, a trypanocide with antioxidants, tocopherol and selenium, in experimental Trypanosoma brucei infection.

**Materials and methods**

*Experimental animals.* Albino rats of Wistar strain, mixed sexes and weighing 80 - 100g were used in this study. The breeding stock of the animals was originally obtained from The Nigerian Institute for Trypanosomiasis Research, Vom. The animals were then allowed to breed locally. They were housed in standard rat cages (International Biological Laboratories, Haryana, India) with white plastic solid bottom and wire tops. Wood
shavings were used as beddings. The cages were accommodated in a well ventilated fly proof house. Animals were humanely cared for in compliance with The Principles of Laboratory Animal Care. Animals were fed ad libitum with commercially formulated 8 mm pelletized mouse cubes (Ladokun Feeds, Ibadan). Water was provided ad libitum using plastic bottles equipped with sipper tubes. Excess feed and water were removed and replaced with fresh ones daily.

The parasite. The Federe strain of Trypanosome brucei which had been stabilized and maintained in liquid Nitrogen at the Nigerian institute for Trypanosomosis Research, Vom, Nigeria was used in this study. During the study the parasites were maintained in rats by serial passage.

Experimental procedures. Five (5) uninfected rats of mixed sexes were used to generate baseline hematology data. These represented the uninfected control. The rats were screened for trypanosomes and were certified free of the parasite before collection of blood.

Thirty five (35) rats were used to evaluate the effects of treatment. The rats were randomly put in seven (7) groups (1 to VII) of five rats per group. They were infected with T brucei by intraperitoneal injection of approximately 1.5 x 10^6 trypanosomes obtained from the blood of rat with heavy parasitemia. The infective blood was diluted in normal saline to obtain 1.5 x 10^6 trypanosomes in 0.1 mL of the final dilution. The number of infective trypanosomes was determined using the rapid matching method of HERBERT and LUMSDEN (1976). Seven days after infection (DOT), all animals were treated intramuscularly with diminazene aceturate at 7.0 mg/kg body weight (Trypadim®, Merial, France). In addition to diminazene treatment, groups II, III and IV received daily supplement of 100-, 200- and 400- mcg/kg sodium selenite (Sigma-Aldrich) respectively. Similarly, animals in groups V, VI and VII received 150-, 300- and 450 mg/kg dl-α- tocopherol acetate (Sigma-Aldrich) respectively. Tocopherol and selenium were administered daily in drinking water for twenty one days beginning from the day of diminazene treatment. Daily tocopherol and selenium supplements were dispensed in small volumes of drinking water. Fresh water was given to the animals after the drug solutions were exhausted. Animals in group I received no antioxidant supplement.

Collection of blood samples and determination of haematological parameters. Blood samples were obtained from each rat in all groups on days 7, 14 and 21 post diminazene treatment. Blood was withdrawn from the median canthus with capillary tubes into sample bottles containing Na,EDETA while the rats were under chloroform (BDH Laboratory Reagents, England) sedation. The red blood cells (RBC) were estimated using the improved Neubauer counting chamber as described by WALTERS et al. (1986). The hemoglobin (Hb) concentration was determined by the cyanmethemoglobin method and the packed cell volume (PCV) was determined by the microhaematocrit method, also as described by WALTERS et al. (1986).
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**Analysis of results.** The differences in the means of all parameters were analyzed statistically with Instat® software (GraphPad Inc., USA) using one-way analysis of variance (ANOVA) (for ≥3 means) and two-tailed P value (for 2 means). Statistical estimates were made at confidence interval of 95%. Probability values less or equal to 0.05 (P≤0.05) were considered significant.

**Results**

*Infection and treatment effects on PCV. Trypanosoma brucei* caused significant (P<0.01) reductions in the Pack Cell Volumes of infected rats by day 7 post infection (PI). Treatment with diminazene alone caused significant increases (P<0.05) in the mean PCV by day 7 post treatment (PT) compared to the value on the day of treatment (DOT). The PCV value in the diminazene treated group was not significantly (P>0.05) different from the value in the uninfected control group by day 7 PT. The diminazene/selenium and diminazene/tocopherol groups also recorded significant increases in mean PCV by day 7 PT (Fig. 1). The mean PCV in the diminazene/tocopherol and diminazene/selenium groups were significantly higher than the PCV recorded in the diminazene group (P<0.05) and in the uninfected control group (P<0.001) on day 7 PT. Variations in the doses of tocopherol and selenium did not result in significant variations in mean PCV; hence response of PCV to antioxidant supplementation was not dose-dependent.

The mean PCV increased further in both the diminazene and diminazene/selenium groups by days 14 and 21 PT but declined in the diminazene/tocopherol group. By day 21 PT, the

![Fig. 1. Efficacy of diminazene alone and in the presence of selenium or tocopherol on normalization of reduced PCV in *T. brucei* infected rats](image-url)
mean PCV value in both the diminazene and diminazene/200 mcg/kg selenium groups were significantly (P<0.05) higher than the values on day 7 PT. There were no significant (P>0.05) differences in the means of PCV recorded in the diminazene and diminazene/selenium groups on days 14 and 21 post treatment. By day 21 PT, the mean PCV in the diminazene and diminazene/selenium groups were significantly (P<0.01) higher than the values recorded on DOT and in the uninfected control group. However, the opposite was the case in the diminazene/tocopherol groups. The mean PCV in these groups declined from the level on day 7 PT. By day 21 PT, the mean PCV values were significantly (P<0.01) lower than the values recorded on day 7 PT and in the uninfected control group but were still significantly (P<0.01) higher than the DOT value.

Infection and treatment effects on hemoglobin (Hb) concentration. On day 7 post infection with *T. brucei*, the hemoglobin contents of infected rats were significantly (P<0.01) reduced compared to those of uninfected control. Treatment with diminazene alone and diminazene plus daily supplementation with tocopherol or selenium reversed the reduction in Hb concentration by day 7 post treatment (Fig. 2). There were no significant (P>0.05) differences in the means of Hb in the diminazene and diminazene/selenium groups by day 7 PT but the means of Hb concentration in the diminazene/tocopherol groups were significantly (P<0.05) higher than both the diminazene and diminazene/selenium groups.

The Hb concentration in the diminazene and diminazene/selenium groups increased consistently and by 21 days PT the means of Hb were significantly (P<0.05) higher than the DOT day 7 PT values. The means of Hb were statistically the same in both treatment groups on days 14 and 21 post treatment. On the contrary, the Hb concentration in the

![Fig. 2. Efficacy of diminazene alone and in the presence of selenium or tocopherol on normalization of depressed hemoglobin concentration in *T. brucei* infected rats](image-url)
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tocopherol supplemented groups declined significantly from the values on day 7 PT and by day 21 PT, the mean Hb values in the diminazene/tocopherol groups were significantly (P<0.05) lower than in the diminazene group but were still significantly (P<0.001) higher than the DOT value.

*Infection and treatment effects on erythrocyte (RBC) count.* Seven days after infection the mean RBC count of infected rats were significant (P<0.01) lower than control values. The decline in erythrocyte count in *T. brucei* infected rats responded with increase towards normal values following treatment with diminazene alone and also with diminazene plus daily supplementation with selenium or tocopherol (Fig. 3). The means of RBC count in the selenium and tocopherol supplemented groups were not significantly (P>0.05) higher than the means of erythrocyte count recorded in the diminazene group on day 7 PT. The means of RBC count in all treated groups were significantly (P<0.05) higher than the values recorded on DOT.

On days 14 and 21 PT, RBC counts were further elevated beyond the day 7 PT values both in the diminazene and diminazene/selenium groups. However, there were no significant (P>0.05) differences in the means of RBC count recorded in the diminazene treated group compared to the diminazene/selenium groups on days 14 and 21 post treatment. By day 21 PT, the means of erythrocyte count in the diminazene and diminazene/selenium groups were significantly higher than the values recorded on DOT. The RBC counts in the tocopherol supplemented groups declined from the level on day 7 PT. By day 21 PT, the means of erythrocyte count in the groups were significantly lower than the values recorded in the diminazene group (P<0.05) and on day 7 PT (P<0.001) but were still significantly higher than the DOT value (P<0.05).

![Fig. 3. Efficacy of diminazene alone and in the presence of selenium or tocopherol on normalization of reduced RBC count in *T. brucei* infected rats](image)
Discussion

Anemia was prominent in *T. brucei* infections as indicated by reduced PCV, Hb and RBC in infected rats. Anemia in trypanosome infection has also been reported by several workers (LOSOS and IKEDE, 1972; MURRAY and DEXTER, 1988; KAIKABO and SALAKO, 2006; TOMA et al., 2008). Hemoglobin concentration attained the highest level in the diminazene/tocopherol group by day 7 PT compared to the diminazene/selenium and diminazene groups. The mean PCV in the diminazene/tocopherol and diminazene/selenium groups were significantly higher than the PCV recorded in the diminazene group on day 7 PT. Thus, recovery rate of Hb concentration was fastest in the tocopherol supplemented group while PCV recovered faster in the tocopherol and selenium groups than in the diminazene group. Hematological parameters (PCV, Hb and RBC) maintained upward trends in the diminazene and diminazene/selenium groups with the values of PCV, Hb and RBC becoming higher in the diminazene/selenium groups but values declined in the diminazene/tocopherol groups by day 14 and 21 PT.

The higher levels of hematological parameters in the antioxidant supplemented groups within the first week showed that antioxidant supplementation lead to more rapid return of these parameters to normal. The relatively higher values in the diminazene/selenium groups by day 21 PT indicate that diminazene treatment followed by selenium supplementation was superior to diminazene treatment alone. There is lack of data in this aspect of trypanosomosis therapy to enable adequate comparison of these results. However, EZE (2004) reported survival rates of 50% and 66.67% respectively in diminazene alone and diminazene/selenium combination in *Trypanosoma brucei* infected rats.

The superiority of diminazene/selenium combination over diminazene alone may be associated with the observations that selenium supplementation enhance the immune system and increase the natural resistant of animals by increasing response of the organism to antigenic stimuli (CALNAGO et al., 1984; MADRON and VRZGULOVA, 1988; ARSHAD et al., 2005). Profound immunosuppression has been observed as a significant and complicating factor in the pathogenesis of trypanosomosis (LOSOS and IKEDE, 1972). Selenium superiority may also be associated with its antioxidant property. Selenium is known to eliminate reactive oxygen species and modulate redox-sensitive enzyme (SAXENA and JAISWAL, 2007). Oxidative stress has been cited as a cause of anemia and depletion of some endogenous antioxidants such as vitamin C, Vitamin E, glutathione and carotenoids in trypanosomosis (IGBOKWE, 1994; UMAR et al., 2000).

The decline in the values of hematological parameters in the diminazene/tocopherol groups by day 14 PT cannot the explained from the results of this study. The beneficial effects of vitamin E supplementation in some disease states have been reported. OGNJANOVIĆ et al. (2003) observed that cadmium causes anemia characterized by decrease in RBC count, PCV value and Hb concentration similar to the observations in this study.
These workers further observed that Vitamin E pretreatment reversed the effects of cadmium on hematological parameters and concluded that vitamin E has a protective role in anemia caused by cadmium. KAIKABO and SALAKO (2006) reported better hematologic status in vitamin E supplemented animals following *T. brucei* infection. Also, EZE and OCHIKE (2007) observed enhanced PCV, reduced parasitemia and prolonged survival interval when vitamin E was supplemented at low doses in *T. brucei* infected mice. These authors also observed that Vitamin E supplementation at higher doses reversed the effects on the parameters. The short-lived efficacy of diminazene/tocopherol combination in this study may therefore be attributed to high dose or the continuous daily supplementation causing accumulation of tocopherol. It has been observed that certain antioxidants become prooxidants at high doses. This condition has been documented in the case of vitamins E and C. In humans, the high supplementation of vitamin E has been shown to induce a pro-oxidant activity making them react directly with other free radicals or induce lipid oxidation under mild oxidative stress but not under severe situations (KONTUSH et al., 1996).

Results from this study showed that the superiority of diminazene/tocopherol over diminazene alone was short lived but that of diminazene/selenium was long lasting. These results indicate that trypanocide/antioxidant combination may have significant therapeutic application in trypanosomosis.

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SAŽETAK

Ključne riječi: *Trypanosoma brucei*, liječenje, diminazen, tokoferol, selen