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Full Length Research Paper

Effects of intraperitoneal administration of vitamins C and E or A and E combinations on the severity of *Trypanosoma brucei brucei* infection in rats

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The effects of intraperitoneal administration of combinations of vitamins C and E (100mg/Kg b.w each) or vitamins A and E (10,000i.u/Kg, 100mg/Kg b. w., respectively) on *Trypanosoma brucei brucei* infection in rats were compared. The rats were infected intraperitoneally with the same parasite load and the experiment lasted for 17 days. The two vitamin combinations significantly (P<0.05) and persistently kept the parasitaemia lower than was recorded in the untreated infected rats. Terminal parasitaemia of the infected group given the vitamins A and E combination (AE) was significantly (P<0.05) lower than that of the group given the vitamins C and E combination (CE) . Although all infected animals developed anaemia, its severity in the untreated infected animals was significantly (P<0.05) higher than observed in the two infected groups treated with the vitamin combinations; with the group given AE developing a significantly (P<0.05) less severe anaemia than those given CE. *Trypanosoma brucei* infection, without vitamin treatment caused general increases in serum alanine- and aspartate aminotransferases, urea and creatinine. Although CE had no significant effect on the infection levels of serum ALT and AST; AE significantly (P<0.05) prevented the disease-induced increases in these parameters. The two vitamin combinations prevented, to a significant degree, the disease-induced elevation of serum urea and creatinine; however, AE was more effective at this than CE. It was concluded that intraperitoneal administration of either vitamins C and E or vitamins A and E combinations alleviates the degenerative changes in tissues and organs associated with *Trypanosoma brucei* infection of rats; with the vitamins A and E combination being more effective.

Key words: *Trypanosoma brucei brucei*, antioxidant vitamins, anaemia, tissue damage.

INTRODUCTION

African trypanosomosis caused by the *Trypanosoma brucei* sub-group is associated with hepatocellular degeneration, glomerulonephritis and anaemia (Anosa and Kaneko, 1984; Bruijin et al., 1987; Suliman and Feldman, 1989). The role of oxidative stress in the pathogenesis of the disease is becoming increasingly relevant. Blood stream forms of *T. b. brucei* were reported to produce enormous amounts of H_2O_2 (Meshnick et al., 1977) and the systemic oxidative stress thus imposed was corroborated by decreases in hepatic reduced glutathione levels in *Trypanosoma brucei gambiense*-infected- (Ameh, 1984), *T. b. brucei*-infected rats (Igbokwe et al., 1998) and guinea pigs (Roskin and Nastuikova, 1941), decreases in liver retinol and ß-carotenes in *T. brucei*-infected rats (Ihedioha and Anwa, 2002) as well as increased susceptibility of erythrocytes to oxidative haemolysis in *T. gambiense*-infected rats and *T. brucei*-infected mice (Igbokwe et al., 1994). Supplementation of infected animals with antioxidant vitamins tended to reduce the oxidative stress and the associated degeneration of tissues and organs. The administration of vitamin C or E to *T. brucei*-infected rats and rabbits (Umar et al., 1999a, 2000, 2001) or *Trypanosoma congolense*-infected rabbits (Umar et al., 2007a) and the combinations of vitamins C and E or C and A in trypano-some infections (Umar et al., 1999b; 2007b) boosted the reserves of endogenous antioxidants and reduced the tissue damages caused by the disease. Thus antioxidant

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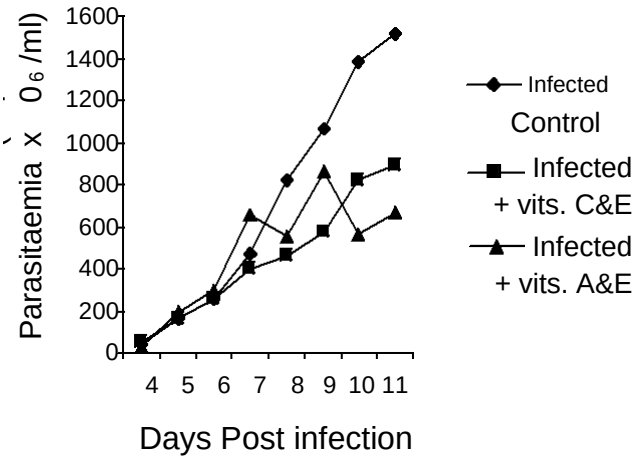

Figure 1. Parasitaemia of *T. brucei* infected rats with or without vitamins A and E or C and E.

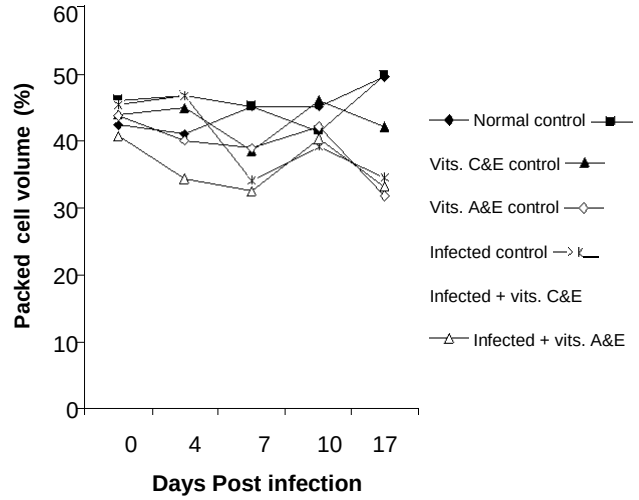

Figure 2. Packed cell volume of *T. brucei* infected rats with or without vitamins A and E or C and E.

vitamin supplementations may reduce the severity of trypanosome infections by offering protection against possible oxidative injuries associated with the disease.

Control of trypanosomes presently relies mainly on use of trypanocidal drugs, but has not been satisfactory largely due to the slow discovery of new drugs and high cost of existing ones as well as toxicity to the hosts and acquired resistance to the drugs by the parasite (Fairlamb, 1982). It is postulated that combining trypanocidal drugs with antioxidant therapy may improve the efficacy and efficiency of treatment of trypanosome infections. However, the interactions of the easily available antioxidant vitamins must first be understood.

The present research, compared the effects of intraperitoneal administration vitamins C-E and A-E combinations on the severity of *T. brucei* infection in rats.

MATERIALS AND METHODS

Experimental animals and design: Forty- eight adult albino rats of both sexes, weighing 200 – 300 g, were used for the experiment. The rats were fed, *ad libitum*, with a standard feed (Vital Feeds, Jos, Nigeria) and drinking water was continuously provided. The rats were randomly divided into six groups of eight rats each. Rats in three of the groups were each infected, while rats in the other three groups were uninfected. A pair of infected and uninfected groups was treated intraperitoneally with a combination of vitamins (Jinling PI, china) C (100 mg/Kg) and E (100 mg/Kg) or A (10,000i.u/Kg) and E (100 mg/Kg) from day 1 post infection to the end of the experiment on day 17 p.i. The remaining pair was not treated with vitamins.

Trypanosome infection

The *Trypanosoma b. brucei* (Basa strain) was obtained from the Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The infected blood from a donor rat (at peak parasitaemia) was collected and diluted with cold physiological saline. The number of parasites in the diluted blood was determined (Herbert and Lumsden, 1976) and a volume containing approximately 10^6 trypanosomes was intraperitoneally injected into each rat in the infected groups.

Samples collection and analyses

Tail-blood was collected daily for the estimation of parasitaemia (Herbert and Lumsden, 1976) and packed cell volume by the microhaematocrit method. The rats were sacrificed by humane decapitation, blood collected in plain containers, serum harvested and used for the estimation of alanine, aminotransferase and aspartate aminotransferase activities by methods described by Bergmeyer et al. (1978) using commercial reagent kits (Gasellchaft fur Biochemica und Diagnostica, Wiesbgden, Germany). The serum samples were also used for estimation of urea and creatinine by the diacetylmonoxime and Jaffe's reactions, respectively, as described by Kaplan et al. (1988).

Statistical analyses: All data are presented as mean ± SEM and analyzed by the one-way ANOVA. Paired means were analyzed by the Student's t-test.

RESULTS

The parasitaemias of the vitamin-treated infected groups (Figure 1) were significantly ($P<0.05$) lower than in the untreated group (Table I); with vitamins A and E causing significantly ($P<0.05$) lower parasitaemia than vitamins C and E.

The *Trypanosoma brucei* infected rats developed anaemia, as indicated by the downwards displacement of their PCV profiles (Figure 2) in comparison to those of uninfected groups. The infected groups of rats recorded significant decreases in their PCV values at the end of the experiment (Table 1). The percentage decreases in PCV were similar for the untreated and vitamins C and E treated infected groups, but significantly ($P<0.05$) lower in the vitamins A and E treated infected group.

Table 2 presents the results of the serum biochemical parameters estimated in this experiment. Infection, without treatment, caused increases in the levels of

Table 1. Mean ± SEM (n = 8) of terminal parasitaemia and percentage change in packed cell volume of all groups of rats.

Groups	Normal Controls	Vits. C and E Controls	Vits. A and E Controls	Infected Controls	Infected + Vits. C and E	Infected + Vits. A and E
Terminal Parasitaemia ($x 10^6$ Tryps./ml)	-	-	-	1520.00 ± 34.99^a	823.75 ± 50.19^b	564.50 ± 35.66^c
Initial PCV (%)	42.25 ± 1.02	46.00 ± 1.04	44.00 ± 1.04	43.75 ±0.93	45.25 ± 0.97	40.75 ± 0.88
Final PCV (%)	49.33 ± 0.44	49.75 ± 0.34	42.00 ± 0.76	31.75 ±1.37	34.50 ± 1.02	33.00 ±0.87
% change in PCV*	$+17.03 \pm 2.49^a$	$+8.47 \pm 2.42^b$	-4.45 ± 0.74^c	-27.70 ± 2.24^d	-23.87 ± 0.86^e	-19.06 ± 0.28^f

Values with different superscript along a row are statistically different (P<0.05). * Positive signs (+) indicate increases and negative signs (-) indicate decreases.

Table 2. Mean ± SEM (n = 8) of some serum biochemical indices in all groups of rats.

Groups	Normal Controls	Vits. C and E Controls	Vits. A and E Controls	Infected Controls	Infected + Vits. C and E	Infected + Vits. A and E
ALT (i.u./L)	20.47 ± 1.11^a	20.60 ± 1.85^a	17.77 ± 1.15^b	30.92 ± 1.49^c	33.26 ± 6.79^c	28.25 ± 0.26^d
AST (i.u./L)	30.64 ± 4.69^a	31.53 ± 2.18^a	38.22 ± 1.20^b	43.15 ± 1.05^c	42.69 ± 1.07^c	40.37 ± 1.46^d
Urea (mM)	172.67 ± 3.34^a	191.00 ± 5.36^b	141.75 ± 5.56^c	316.80 ± 29.12^d	163.50 ± 5.64^e	136.75 ± 5.56^c
Creatinine (µM)	60.27 ± 1.39^a	56.01 ± 1.34^b	59.38 ± 1.53^a	103.41 ± 3.18^c	64.35 ± 0.99^d	38.50 ± 2.60^e

Values with different superscripts along a row are statistically different (P<0.05).
ALT- Alanine aminotransferase
AST- Aspartate aminotransferase

serum alanine- and aspartate aminotransferases, as well as urea and creatinine significantly (P<0.05) above baseline levels. Both vitamins combination significantly (P<0.05) prevented the disease- induce increases in serum urea and creatinine, but only the vitamins A and E combination was able to prevent the disease-induced increases in serum alanine- and aspartate aminotransferases.

DISCUSSION

The decrease in parasitaemia caused by the administration of antioxidant vitamins has been reported earlier (Umar et al., 1999a) and was attributed to the vitamins' ability to boost immune response to disease (Passmoore and Eastwood, 1986). The combination of vitamins A and E seemed to suppress parasitaemia than vitamins C and E. Anaemia is a consistent feature of trypanosome infections caused by, amongst other factors, oxidative damage to erythrocyte membrane components. Administration of antioxidant vitamins has had the effect of reducing the severity of anaemia in experimental infections with different species of trypanosomes (Umar et al., 1999a, 2000, 2001, 2007a,b). This effect was attributed to the protection of membrane and cellular components against oxidative species by the vitamins. In the present investigation, vitamins A and E ameliorated anaemia to a greater extent than vitamins C and E.

Increases in levels of serum alanine- and aspartate aminotransferases, urea and creatinine have all been reported in experimental trypanosomosis (Hudson, 1944; Kalu et al., 1989; Adah et al., 1992) and are indications of damage to hepatic and renal tissues (Kaplan et al., 1988). Administrations of some antioxidant vitamins have been shown to reduce the damage to organs and tissues caused by trypanosome infections (Umar et al., 1999b, 2000, 2001, 2007a,b) as corroborated by the findings in this report. Again in this instance, the vitamins A and E combination was better at reducing organ damage in the infected rats than the combination of vitamins C and E.

In the present investigation, the combination of vitamins A and E seemed better at keeping parasite-load low as well as ameliorating anaemia and organ damage than the vitamins C and E combination. Vitamin C, being water-soluble, is an active antioxidant in the aqueous phase of the cytoplasm. Furthermore, vitamin C is easily excreted in the urine and so is not stored to any appreciable extent in the body. On the other hand, Vitamin A is lipid-soluble and stored in tissues and organs (Robert et al., 2000). It is also involved in maintaining a number of immune cell-types and haematopoiesis. The pro- vitamin A (ß-Carotene) is a powerful antioxidant which scavenges free radicals from the lipid bilayer of membranes; the administered vitamin A would keep the cellular stores of this powerful lipid-phase antioxidant high. Replenishment of vitamin A in deficient animals has been shown to restore lymphoid organ development, circulating immune cell population and function; as well as resistance to infection by pathogens (Calder and Jackson, 2000). These factors, perhaps amongst others, may be respon-

sible for the greater potency of the vitamins A and E combination.

In conclusion, parenteral administration of antioxidant vitamins reduced the parasitaemia, severity of the anaemia, and the damage to the liver and kidney of *T. brucei* infected rats, with the vitamins A and E, seemingly, having a greater effect than vitamins C and E.

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