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Full Length Research Paper

Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamins A and C to *Trypanosoma brucei brucei*-infected rats

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The effect of combined administration of 1000 i.u/100g body weight (bd.wt.) vitamin A and 100 mg/kg bd wt. vitamin C to *Trypanosoma brucei brucei*-infected rats daily for twenty-one days was investigated. The anaemia caused by *T. brucei* infection in rats not administered the vitamins was significantly (P<0.05) more severe than that recorded in the group of infected rats given the daily doses of the vitamins. Indices of hepatic function such as serum alanine- and aspartate transaminases, and serum alkaline phosphatase activities as well as indices of renal function such as serum urea and creatinine levels were all significantly (P<0.05) elevated above levels in uninfected rats by *T. brucei* infection. However, combined administration of vitamins A and C to infected animals prevented the disease-induced increases in these parameters. *T. brucei* infection also caused significant hepatomegaly and splenomegaly in vitamin-free rats; however administration of the vitamins to infected rats completely prevented the hepatomegaly but only partially prevented the splenomegaly caused by infection. It was concluded that intraperitoneally administered vitamins A and C alleviated *T. brucei* – induced anaemia and organ damage.

Key words: *T. brucei*, anaemia, vitamins A and C, free radicals, organ pathology.

INTRODUCTION

Trypanosomes are protozoan parasites, and the causative agents of “nagana” in animals, sleeping sickness and chagas’ disease in humans (Mhlanga, 1996). The mechanism of pathogenesis is in part due to the generation of free radicals and superoxides during trypanosomal infec-tion which causes degenerative changes in vital tissues and organs of infected animals due to cellular injury (Anosa and Kaneko, 1984; Igbokwe, 1994). These oxidative species attack both the membrane polyunsaturated fatty acids and proteins (Slater, 1984) of red blood cells (RBC) leading to heamolysis, and consequently anaemia; and deplete endogenous antioxidants reserves in the blood and some organs of trypanosome infected animals (Ameh, 1984; Igbokwe et al., 1998), presumably because they scavenge the trypanosome-generated free radicals.

Vitamins A and C are lipid soluble and water-soluble antioxidants, respectively, which protect against oxidative injuries in the aqueous compartment and lipid bilayer of cell membranes (Halliwell and Gutteridge, 1985). The ability of vitamin C to reduce organ damage (Umar et al., 2000) and vitamins C and E to reduce the severity of anaemia (Umar et al., 1999) in *Trypanosoma brucei*-infected animals have all been reported. It was also reported that *T. brucei* infection of rats caused depletion of liver stores of retinol and carotenoids (Ihedioha and Anwa, 2002). To our knowledge, the ability of vitamin A alone or in combination with any other antioxidant vitamin to ameliorate the trypanosome-induced anaemia and/or organ damage has not been documented.

Therefore, in this study, the effects of combined intraperitoneal administration of vitamins A and C on heamatological parameters as well as indices of hepatic and

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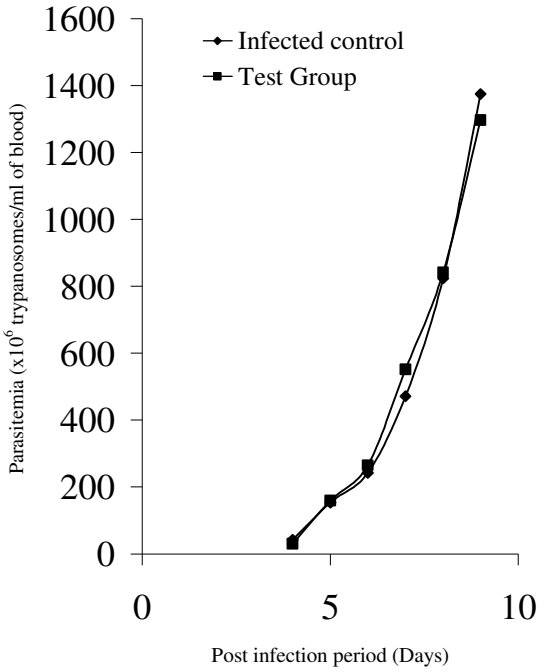

Figure 1. Profile of parasitemia in *T. brucei* infected rats untreated (infected control) and treated (test group) with vitamins A and C.

renal functions in *T. brucei brucei*-infected rats were evaluated.

MATERIALS AND METHODS

Experiment animals

A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the World Health Organization (WHO document, 1998). Apparently healthy white albino rats (Wistar strain) of both sexes weighing 98.0 – 198.0 g were obtained from Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in well ventilated laboratory cages with 12 h day/night cycles. They were maintained on a ration containing commercial poultry feed (Vital feeds, Jos) made up of 54% carbohydrate, 20% protein, 2% minerals, 10% fibre, 1% vitamin and 13% fat. Water was also supplied *ad libitum*.

Trypanosome parasites

T. brucei brucei (Basa strain) were obtained from an experimentally infected rat previously inoculated with the parasite from Department of parasitology, Faculty of veterinary medicine, Ahmadu Bello University, Zaria.

Treatment of the experimental animals

Thirty two rats were divided into four groups and treated as follows:

Uninfected controls: The eight rats in this group were neither infected nor treated with the vitamins.

Vitamin controls: This group consisted of eight uninfected rats that received daily intraperitoneal injection of 1000 i.u/100g bd.wt. of vitamin A (Jinling pharmaceutical industries, China) and 100 mg/kg bd wt. of vitamin C (Jinling pharmaceutical industries, China).

Infected controls: Eight rats were each intraperitoneally inoculated with about 10^6 *T. brucei brucei* (Basa strain) per 100 g bd.wt. in infected blood from a donor rat previously inoculated with the parasites. No further treatment was administered to these animals.

Test group: The eight rats in this group were similarly infected with *T. brucei brucei* and given daily injections of vitamins A and C as earlier described.

The rats were sacrificed humanely by jugular decapitation at the end of twenty one days, post infection (p.i), and organs, blood and serum samples collected.

Assays

The level of parasitemia was monitored daily as described by Herbert and Lumsden (1976) while the packed cell volume was monitored weekly by the microhaematocrit method. Alanine and aspartate transaminase activities were assayed by the method of Bergmeyer et al. (1978) using a commercial reagent kit (Human, Gasellchaft fur Biochemica und Diagnostica, Max-Planck-Ring 21, Wiesbgden, Germany) while alkaline phosphatase activity was determined by the method of McComb and Bowers (1972) also using a commercial reagent kit (Human, Gasellchaft fur Biochemica und Diagnostica, Max-Planck-Ring 21, Wiesbgden, Germany). Serum urea and creatinine concentrations were determined by the diacetylmonoxime and Jaffe's reactions as described by Kaplan et al. (1988).

Statistical analysis

The results are presented as mean ± standard error of means (SEM) and student t-test was used to analyze the results.

RESULTS AND DISCUSSION

The parasitemia of both the infected controls and the test group are presented in Figure 1. *T. brucei* were first detected on day 4, p.i, and progressively increased until day 18 p.i. There was no statistically significant difference ($P<0.05$) in the parasitemias of the two groups throughout the duration of the experiment. There were no significant ($P>0.05$) differences in the pre-infection PCV of all groups of rats. However, the two infected group developed anaemia as the infection progressed, as indicated by significant ($P<0.05$) drops in PCV. The anaemia observed in the infected controls was significantly ($P<0.05$) more severe than that of the test group which was given vitamin therapy (Table 1).

Table 2 presents the results of the indices of hepatic and renal function analysed in the experiment. Infection with *T. brucei* caused significant increases ($P<0.05$) in the activities of serum alanine- and aspartate transaminases which were significantly prevented ($P<0.05$) by the vitamin administration. However, the vitamin administration had no significant effect ($P>0.05$) on the disease-

Table 1. Means ± SEM (n = 8) of the initial and final packed cell volumes of all groups of rats.

Post infection days	Packed cell volume (%)			
	uninfected control	Vitamin control	Infected control	Test group
0	42.25±2.87 ^{a,b}	41.00±1.83^{b}	43.75±2.63^{a,b}	44.50±1.29^{a}
18	49.33±1.25^{a}	45.33±2.05^{b}	31.75±3.86^{c}	40.50±0.70^{d}
% change	17.10±7.06^{a}	10.56±0.09^{a}	-27.70±6.33^{b}	-8.96±1.51^{c}

Values with different superscript within a row are statistically different (P>0.05).

Table 2. Means ± SEM (n = 8) indices of hepatic and renal function of all groups of rats.

Biochemical parameters	uninfected control	Vitamin control	Infected control	Test group
Alanine ransaminase (i.u/l)	20.47±1.11^{a}	21.32±0.92^{a}	30.92±8.49^{b}	11.19±1.08^{c}
Aspartate transaminase (i.u/l)	30.64±4.69^{a}	34.25±7.67^{a}	43.15±1.05^{b}	17.87±0.99^{c}
Alkaline phosphatase (i.u/l)	79.04±4.44^{a}	94.06±9.96^{a}	212.33±28.95^{b}	230.87±16.89^{b}
Urea (mmol/l)	172.67±3.34^{a}	187.28±2.97^{b}	316.80±29.12^{c}	148.21±0.76^{d}
Creatinine (mmol/l)	60.27±1.39^{a}	52.81±2.08^{b}	103.41±3.18^{c}	62.81±0.95^{d}

Values with different superscripts within a row are statistically different (P<0.05).

Table 3. Means ± SEM (n = 8) of organ : body weight ratios of all groups of rats.

Groups	Organ : body weight ratios		
	Liver ($x10^{-2}$)	Spleen ($x10^{-2}$)	Kidney ($x10^{-2}$)
uninfected control	3.13±0.62^{a}	0.47±0.50^{a}	0.72±0.09^{a,b}
Vitamin control	3.40±0.42^{a}	0.60±0.31^{a}	0.66±0.07^{a}
Infected control	4.80±0.41^{b}	1.95±0.67^{b}	0.83±0.16^{b}
Test group	3.61±1.32^{a}	1.38±0.73^{b}	0.64±0.18^{a}

Values with different superscripts within a column are statistically different (P<0.05).

induced increases in the activity of alkaline phosphatase. Both the disease-induced increases in serum urea and creatinine concentrations were significantly prevented (P<0.05) by the vitamin administration.

The *T. brucei* infection caused a significant (P<0.05) hepatomegaly seen as an increase in liver : body weight ratio but was completely prevented in the test group by the administration of the vitamins. However, the disease-induced splenomegaly (in the infected controls) was only slightly ameliorated by the vitamins. The kidney : body weight ratio was not affected in all the groups (Table 3).

The parasitemia observed in this experiment rose progressively without any period of drop, which indicates an acute phase of the disease. Similar observation has been previously reported with this strain of the parasite (Umar et al., 1999). The inability of the combined vitamins A and C administration to affect the parasitemia suggests that the vitamins neither interfered with the normal metabolism of the parasite nor enhance the host's ability to destroy the parasite.

The acute anaemia recorded in the *T. brucei* infected rats has been a consistent feature of trypanosomosis (Murray, 1974; Ameh, 1984; Igbokwe et al., 1996). Several factors contribute to the development of anaemia among which is the oxidative damage of RBC membranes by free radicals and peroxides generated during the course of the infection (Igbokwe, 1994). The administration of the vitamins significantly (P<0.05) ameliorated the disease induced anaemia and may be attributable to the antioxidant activity of these vitamins; by scavenging the trypanosome-generated free radicals; thus reducing the free radical load. However, the inability of the vitamins to completely prevent the disease-induced anaemia indicated that other aetiological factors were involved in the development of anaemia in the infected rats (Umar et al., 1999).

The hepatomegaly and splenomegaly consistently reported (Morrison et al., 1978) in trypanosomosis was also observed in the present investigation. The enlargment of liver and spleen is caused by the activation and expansion of the reticuloendothelial system during trypanosome infection. The severity of hepatomegaly and splenomegaly was often associated with the onset and degree of parasitaemia. The vitamin therapy significantly

(P<0.05) prevented the disease induced hepatomegaly but only partially affected the associated splenomegaly. The *T. brucei* infection of the rats caused significant (P<0.05) increase in serum levels of alanine and aspartate transaminases, which confirms earlier report (Moon et al., 1968; Singh and Gaur, 1983; Adah et al., 1992). Increases in the serum levels of these enzymes are indications of damage to liver, brain and cardiac muscle (Kaplan et al., 1988). The combined administration of vitamins A and C completely prevented the disease-induced increases in alanine- and aspartate transaminases which suggest that the levels of free radicals and peroxides in the infected animals might be kept at relatively lower levels by the vitamins and thus reducing the oxidative challenge to which cellular membranes of hepatocytes are exposed. Also, the damage to renal structures reflected as increases in serum urea and creatinine concentrations were significantly (P<0.05) prevented by the vitamins which may therefore indicate the protection of renal structures by the vitamins against the oxidative damage by the trypanosome-generated free radicals.

We concluded that the combined administration of vitamins A and C ameliorates anaemia and organ damage during *T. brucei* infection of rats. The findings further support the significant roles of trypanosome-generated free radicals in the pathogenesis of African trypanosomosis.

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