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Full Length Research Paper

Effect of intraperitoneal administration of vitamin c (ascorbic acid) on anemia in experimental *Trypanosoma congolense* infected rabbits

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The effect of Vitamin C supplementation on anemia in experimental *Trypanosoma congolense* infected rabbits was investigated. Locally bred rabbits were infected with 6×10^6 trypanosomes per rabbit and infection was monitored for 5 weeks. Packed cell volume (PCV), Total leucocytes count (TLC) and parasite load were determined weekly. Vitamin C supplementation did not significantly affect parasitaemia in the first two weeks of infection but parasitaemia was significantly decreased ($p < 0.01$) in the last three weeks of infection. Anemia developed in the *T. congolense* infected rabbits as evidenced by reduced PCV during the course of the experiment. Treatment of infection with Vitamin C had slightly, though not to a significant extent ameliorated the *T. congolense* induced anemia. Leucocytosis was lower in the infected treated rabbits than in the infected untreated rabbits. It was concluded that Vitamin C did not prevent the anemia or the leucocytosis caused by *T. congolense*, but it slightly though not to a significant level ameliorated the condition.

Key words: *Tryponosoma congolense*, vitamin C (Ascorbic acid), anemia.

INTRODUCTION

World Health Organization (WHO) estimates that sleeping sickness affects between 300,000 and 5000,000 people in Africa's so-called "tsetse belt" covering approximately ten million square kilometres and stretching from Senegal in West Africa through all central Africa to Uganda in East Africa and several other tropical African countries south of the Equator. A related challenge is Nagana, or African animal trypanosomiasis which has several impact on the region's agriculture, causing annual losses of cattle production of more than US$ 1 billion (WHO, 2005).

Trypanosomiasis still remains a constraint to livestock production in Nigeria (Abenga et al., 2002; Opasima and Ekwurek, 1988; Anene et al., 1991a; Anena et al., 1991b) and other parts of Africa (Doko et al., 1991; Gaturaga et al., 1991).

Trypanosome infections are generally characterized by anemia, leucopenia, thrombocytopenia as well as biochemical aberrations such as hypoglycemia, hypoalbuminemia and hypergammaglobulinemia due to elevated IgM levels (Anosa, 1988). The severity of the haematological and biochemical changes associated with various host-parasite combinations is determined by the levels of parasitaemia, which develops during the early phase of infection.

The hematological and biochemical abnormalities induced by trypanosomes arose from their direct effect via their products on host cells such as Red blood cell (RBC), White blood cell (WBC), Platelets and tissues such as liver, kidney, bone marrow and lymphoid organs, resulting in cell destruction and organ malfunction as well as extractions from and additions to host chemistry associated with parasite Metabolism (Anosa, 1988).

T. congolense exerts its effect mainly by causing severe anemia and mild to moderate organ change. The cause of anemia in trypanosomiasis is multifactoral, which includes haemadilution, erythrophagocytosis, haematopoietic response, haemolytic factor and bone marrow dyserythropoiesis. More recently, oxidative stress has been considered as one of the factors that causes anemia in trypanosomiasis (Umar et al., 1999). The

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Table 1. Mean weekly parasitaemia in *T. congolense* infected rabbits supplemented/unsupplemented with vitamin C.

Post infection time	GROUP 3	GROUP 4
(days)	$\times 10^6$	$\times 10^6$
Day 8	0.793 ± 0.280^a	0.531 ± 0.128^a
Day 15	1.314 ± 0.441^a	0.891 ± 0.169^a
Day 22	1.497 ± 0.463^a	0.788 ± 0.168^b
Day 29	3.483 ± 0.911^a	1.350 ± 0.490^b
Day 36	1.900 ± 0.160^a	0.900 ± 0.230^b

All values represent mean ± standard error of mean (SEM). Comparison was done between the groups and values with different superscript are statistically different ($p < 0.01$).

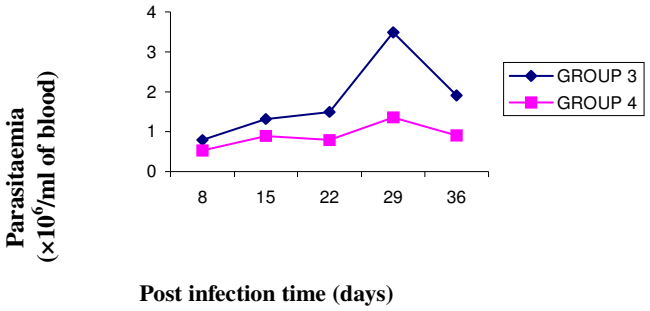

Figure 1. Profile of parasitaemia in *T. congolense* infected rabbits supplemented/unsupplemented with Vitamin C.

oxidative stress is as a result of systematic ascorbic acid depletion due to increased ascorbic acid consumption in infected animals. This oxidative stress leads to per oxidative tissue damage, which increases erythrocyte per oxidation, oxidative haemolysis and depletion of erythrocyte and liver glutathione by free radicals generated by the trypanosome. As a result membrane Phospholipids and Proteins are attacked leading to alteration in membrane structure, which also affects the membrane fluidity.

Vitamin C (Ascorbic acid) is a water-soluble antioxidant, which is capable of protecting against oxidative injuries in the aqueous compartments. The aim of this work was to investigate whether exogenous supplementation of Vitamin C could alleviate or prevent anemia caused by *T. congolense* in rabbits

MATERIALS AND METHODS

The *T. congolense* (Basa Strain was obtained form the Nigeria Institute for Trypanosomiasis Research (NITR) Vom, Nigeria. It was isolated from a goat and has been passaged into a mice nine (9) times. Ascorbic Acid Standard used was purchased from Shangai Siful Pharmaceutical Co;Ltd, Shangai, China.

ANIMALS

Twenty-eight male (Mixed-breed) rabbits were obtained from local breeders within Maiduguri metropolis weighing between 300-1600 g. In the Laboratory, they were all treated with Neo-terramycin and Amprolium for coccidiasis for 5-days. They were fed on groundnut haulm, cornhusk, fresh vegetables and water for three weeks before the onset of the experiment. The rabbits were randomly distributed into 4 groups of 7 animals each and treated as follows:
Group 1 (Normal control) received normal diet and water *ad libitum* only. Group 2 (Vitamin C only): this group was given the normal diet daily intraperitoneal injection of Vitamin C 100 mg/kg body weight throughout the periods of the experiment. Group 3 (Infected Control). The rabbits were each infected by intraperitoneal injection of *T. congolense* (6×10^6 trypanosomes in diluted mouse blood-which serves as the donor). No further treatment was given to them.
Group 4 (Infected + Vitamin C supplement) rabbits apart from normal diet were infected with *T. congolense* (6×10^6) trypanosomes by intraperitoneal injection and Vitamin C supplement was then given to each of them after infection (100 mg/kg body weight) daily by intraperitoneal injection.

Preparation and analysis of samples

One week post infection, blood was collected from the ear vein of each rabbit in groups 3 and 4 (infected control and infected treated) for determination of parasitaemia using wet mount (Herbert and Lumsden, 1976). Similarly, blood was collected into ethylene diamine tetra acetic acid (EDTA) tubes from the ear vein of all the rabbits weekly for determination of Packed cell volume (PCV) using microhaematocrit method and Total leukocyte count (TLC) was counted using the haemocytometer as described by (Schalm et al., 1975).

Analysis of data

All results were expressed as mean ± Standard error of mean (SEM) and difference between two means was determined by using student's t-test.

RESULT AND DISCUSSION

The profiles of Parasitaemia for group 3 (Infected control) and group 4 (Infected + Vitamin C) are shown in Table 1 and Figure 1. The parasitaemia was detected on Wet mount day 8 post- infections.

T. congolense infected rabbits became parasitaemic by 8th day post infection (PI). From the profiles, it is clearly indicated that the disease ran a chronic course during the experiment. There was a gradual increase in parasitaemia in both the groups with the highest parasitaemia recorded on the 4th Week (day 29) in both the groups, which later dropped on the 5th week (36) with termination of the experiment. This means that there was a fluctuating parasitaemia during the course of the experiment, which is a typical characteristic of a chronic infection in trypanosomal infection as reported by Rurangirwa et al. (1978). In the 3rd, 4th and 5th week of infection, the parasitaemia in the Vitamin treated infected animals (groups 4) was significant ($P<0.01$) lower than in the untreated infected rabbits of group 3. Supplementing infection with Vitamin C did not significantly affect parasi-taemia in the first two weeks of infection, but there was a significant decrease ($P<0.01$) in the parasitaemia in the last three weeks of infection (days 22, 29 and 36, respectively) indi-

Table 2. Mean weekly Packed Cell Volume (PCV) of all the groups.

Time (weeks)	Group I	Group II	Group III	Group IV
	Normal control	Normal +	infected control	Infected +
		Vitamin C.		Vitamin C.
0	36.75 ± 2.72	37.57 ± 3.21	36.50 ± 5.29	36.50 ± 1.41
1	35.26 ± 3.06	36.57 ± 4.43	27.38 ± 2.56	29.63 ± 3.81
2	36.00 ± 3.59	37.14 ± 4.56	27.14 ± 3.24	25.13 ± 3.44
3	36.71 ± 2.14	37.43 ± 2.81	27.57 ± 2.44	26.25 ± 1.98
4	36.00 ± 3.37	37.40 ± 5.92	24.67 ± 4.51	26.75 ± 0.96
5	36.50 ± 1.29	37.50 ± 4.09	23.67 ± 3.06	25.67 ± 1.53

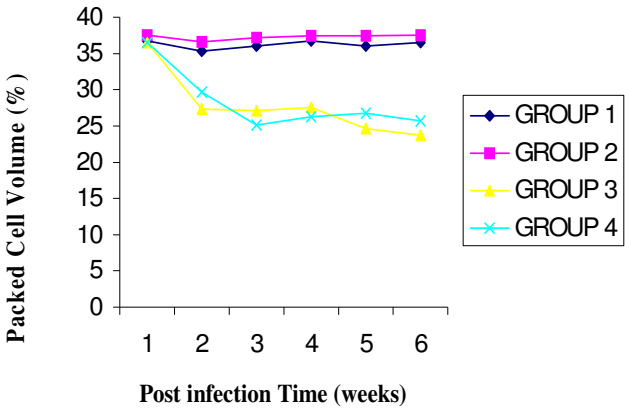

Figure 2. Mean weekly PCV Profiles of Vitamin C supplemented/unsupplemented rabbits infected/uninfected with *T. Congolense.*

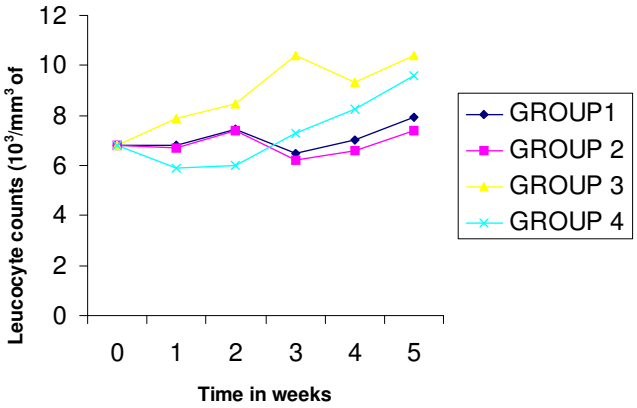

Figure 3. Profiles of Leucocyte Count in *T. Congolense* infected/uninfected rabbits supplemented / unsupplemented with Vitamin C.

cating that the vitamin has interfered with the metabolism or cellular division of the parasite.

The profile of the healthy untreated rabbits indicated that the PCV of this group fluctuated between narrow insignificant changes, while the profile for vitamin C treated rabbits remained constant throughout the duration of the experiment (Figure 2). The profiles of the two infected groups indicated that the PCVs in these groups constantly dropped during the course of the experiment with little or no attempt at recovery.

The results indicated that infection without treatment caused a significantly higher drop in PCV than was observed in the two healthy groups. Anemia was developed in the *T. Congolense* infected animals and this is indicated by reduced PCV from 36.50 ± 5.29% to 23. 67 ± 3.06% in the infected control group and 36.50 ± 1.41% to 25.67 ± 1. 53% during the course of the experiment (Table 2).

Anemia in trypanosmal infection had been reported (Bengaly et al., 2002; Murray, 1979) and has been attributed to several factors which include immunological factors, erythrophagocytosis, erythropoietic response and oxidative damage, all of which to haemolysis of red blood cells. Oxidative haemolysis is presume to occur due to increased production of free radicals in the body of infected animals (Slater,1984; Baltz et al.,1985) and depletion of endogenous antioxidant reserves of the body (Zwart et al., 1991).

Vitamin C is an antioxidant for membrane compounds against free radicals generated during Trypanosomiasis. In this present work, supplementing infection with vitamin C slightly, though not to a significant level ameliorated the anemia in the infected animals. The fact that the anemia caused by *T. congolense* was not completely prevented by supplementation with vitamin C indicates the role of other factors in the etiology of trypanosomal anemia. Groups 1 and 2 animals had similar profiles of total leucocytes count throughout the experiment. In the group 3 animals infected with *T. congolense*, this was a gradual and steady rise in the total leucocytes count with a drop recorded only in the 4th week of infection (Figure 3). In the Vitamin C treated infected animals of group 4, there was little change in the total leucocyte count in the first to second weeks of infection but the total leucocytes count steadily increased for the remaining duration of the experiment.

Infection of the rabbits with *T. congolense* stimulated their immune system (defense mechanism) to level that is adequate to completely abort the infection (as shown in Table 3). This means that the disease ran a chronic

Table 3. Total leucocyte count profiles of vitamin C supplemented/ unsupplemented rabbits Infected/uninfected with *T. Congolense.*

Time (weeks)	Group I	Group II	Group III	Group IV
	Normal control	Normal +	infected control	Infected +
	$10^3/mm^3$	Vitamin C.		Vitamin C.
		$10^3/mm^3$	$10^3/mm^3$	$10^3/mm^3$
0	6.81 ± 0.85^a	6.81 ± 0.85^a	6.81 ± 0.85^a	6.81 ± 0.85^a
1	6.81 ± 0.85^a	6.70 ± 0.50^a	7.89 ± 0.86^b	5.88 ± 0.30^c
2	7.43 ± 0.54^a	7.41 ± 0.47^a	8.44 ± 0.39^b	601 ± 0.43^c
3	6.47 ± 0.24^a	6.21 ± 0.22^b	10.37 ± 0.41^c	7.29 ± 0.34^d
4	7.00 ± 0.84^a	6.60 ± 0.40^a	9.30 ± 0.32^b	8.25 ± 0.46^c
5	7.93 ± 084^a	7.40 ± 060^b	10.40 ± 0.51^b	9.57 ± 0.65^c

All values represent mean ± standard error of mean (SEM). Comparison was done between the groups and values with different superscript are statistically different ($p < 0.05$).

course during the experiment. There was therefore a pronounced leucocytosis in the unsupplement *T. congolense* infected rabbits being observed in this work.

Conclusion

Vitamin C did not prevent the anemia caused by *T. congolense* but it was slightly, though not to a statistically significant level, ameliorated. Similarly, vitamin C did not have any effect on the leucocytosis caused by this parasite.

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