

Full Length Research Paper

The role of intraperitoneally administered vitamin C during *Trypanosoma congolense* infection of rabbits

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Accepted 5 April, 2010

The effects of daily intraperitoneally administered doses of 100 mg/kg bd. wt. vitamin C on levels of some endogenous antioxidants as well as hepatic and renal function were investigated in a group of rabbits infected with a strain of *Trypanosoma congolense* (strain number: BS2/TC/SP28/P4). Values of parameters estimated in this group during and after 5 weeks of infection were compared with those from a group of similarly infected, but vitamin-free rabbits as well as two groups of healthy rabbits, one group of which was similarly treated with vitamin C. *T. congolense* infection caused significant ($P < 0.01$) decrease in packed cell volume (PCV), blood and organ glutathione, plasma and liver ascorbic acid as well as serum creatinine. Treatment of infected animals with vitamin C kept the parasitaemia significantly ($P < 0.01$) lower than in the vitamin-free infected animals after the 3rd week of infection. The vitamin treatment also prevented, to a significant ($P < 0.01$) degree (and in some cases completely), the disease-induced decreases in blood and organ glutathione (GSH) as well as plasma ascorbic acid. The trypanosomal anaemia was partially, but significantly ($P < 0.01$) ameliorated by vitamin treatment. Infection without vitamin therapy also caused significant ($P < 0.01$) increase in the levels of serum total bilirubin and proteins as well as aspartate and alanine aminotransferases. Vitamin C completely, or to a significant ($P < 0.01$) degree, prevented the disease-induced increases in all these parameters. It was concluded that vitamin C at the dose and route used prevented the disease-induced depletion of endogenous antioxidants, hepatic dysfunction and to a significant degree, anemia.

Key words: *Trypanosoma congolense*, antioxidant, oxidative damage.

INTRODUCTION

African trypanosomosis is a major health concern because of its devastating effect on man and animals. The most important pathogenic trypanosomes in this regard include: *Trypanosoma brucei gambiense*, *Trypanosoma brucei Rhodesiense*, *Trypanosoma evansi*, *Trypanosoma simiae*, *Trypanosoma brucei brucei*, *Trypanosoma vivax* and *Trypanosoma congolense*. The last three species are the most important species associated with bovine trypanosomosis. The major patho-

logical effects of these parasites are a varying degree of anemia and degenerative changes in organs and tissues. Oxidative stress has been associated with the pathogenesis of the disease. The oxidative stress is thought to be imposed by increased production of superoxides and hydrogen peroxide (H_2O_2) by macrophages of the activated and expanded mononuclear phagocytic system (Askonas, 1985), production of H_2O_2 by *T. brucei* (Meshnick et al., 1977) and depletion of blood and organ antioxidant reserves during trypanosome infections. Depletion of blood and tissue glutathione (GSH) has been reported in *T. gambiense* (Ameh, 1984) and *T. brucei* infected rats (Igbokwe et al., 1998; Umar et al., 2000, 2001) as well as in *T. brucei* infected mice (Igbokwe et al., 1994) and *T. vivax* infected cattle (Igbokwe et al., 1996). Plasma and tissue ascorbic acid has also been reported to be depleted in *T. evansi* (Nyden,

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Abbreviations: GSH, Glutathione; PCV, packed cell volume; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GSH, glutathione.

1948), *T. brucei* infected rats (Umar et al., 2000, 2001) and guinea pigs (Roskin and Nastiukova, 1941).

The levels of retinol and carotenoids in the liver of *T. brucei*-infected rats were also reported to be depleted by infection (Ihedioha and Anwa, 2002). The oxidative stress thus imposed causes oxidative damage to plasma membranes thereby contributing to the cellular degenerative changes observed in trypanosomosis. Administration of exogenous antioxidants to *T. brucei* infected rabbits (Umar et al., 1999a) and rats (Umar et al., 1999b, 2000, 2001) ameliorated the anaemia, hepatocellular and renal damage caused by the disease.

Much of the work done on the role of oxidative stress in trypanosomosis has been with the tissue invading trypanosome species, that is, the *T. brucei* subgroup (Ameh, 1984; Igbokwe et al., 1994, 1998; Umar et al., 1999a, 1999b, 2000, 2001; Ihedioha and Anwa, 2002). Very little information exists on the extent to which oxidative damage contributes to infections by haematitic species such as *T. congolense*. The present investigation was, therefore, designed to assess the antioxidant status of *T. congolense* infected rabbits and the effect of administration of vitamin C on degree of anaemia and organ damage during the infection.

MATERIALS AND METHODS

Experimental animals and treatment

Twenty-eight mixed-breed, male rabbits weighing 1.30 to 1.60 kg were treated with neo-tetramycin and amprolium (Pfizer, Nigeria) for coccidiosis and randomly divided into four equal groups. All animals were allowed unrestricted access to drinking water and a diet consisting of unmeasured quantities of groundnut haulm, cereal husks and fresh leafy vegetables. The rabbits were acclimatized for 3 weeks before commencement of experiment. Animals in two of the four groups were each infected with 2×10^6 trypanosomes in 0.5 ml of cold saline-diluted infected blood from a donor mouse by intraperitoneal injection. Intraperitoneal injections of daily doses of 100 mg/kg body weight vitamin C (Shanghai sifu pharmaceutical Co. Ltd., Shanghai China), were started two hours after infection in one of the infected groups and continued till termination of experiment at the end of the 5th week of infection. The other infected group was kept vitamin-free. One of the other two non-infected groups was also kept vitamin-free while the other was treated with vitamin C as described above. The experiment was terminated five weeks after infection.

Trypanosome

The *T. congolense* (strain number BS₂/TC/SP28/P4) used in this experiment was obtained from Nigerian Institute for Trypanosomosis Research, (NITR), Vom, Nigeria. The parasite was isolated from a goat and had been serially passaged through mice nine times before we received it.

Sampling and analyses

Venous blood was collected weekly from the ear lobe for determination of parasitaemia (Herbert and Lumsden, 1976) and

packed cell volume (PCV) by the microhaematocrit method. At the end of the fifth week of infection, all rabbits were sacrificed by humane jugular decapitation and blood collected into two separate containers, one of which contained ethylene diamine tetra acetic acid (EDTA) as anticoagulant while the other was plain. Plasma and serum were harvested by centrifuging whole or coagulated blood at 3000xg. Whole blood was used for estimation of blood glutathione (Beutler et al., 1963), plasma for determination of ascorbic acid (Roe, 1973), serum for estimation of total bilirubin (Varley, 1976), total proteins (Reinhold, 1953), as well as urea and creatinine (Tietz, 1982). Serum alanine (ALT) and aspartate (AST) aminotransferase activities were determined by the method of Reitman and Frankel (1957) using wet reagent kits (Randox Laboratories, 35, Diamond Road, Antrim, U.K. BT294QY).

Livers and kidneys of the rabbits were carefully extracted, blotted, weighed and homogenized in ice-cold physiological saline solution using a tissue homogenizer. The homogenate was centrifuged at 3000xg and the supernatant harvested for estimation of tissue GSH and ascorbic acid as described for blood and plasma above.

Analysis of data

All data are presented as mean \pm SEM. Data were analysed by the one-way analysis of variance (ANOVA) and Student's t-test.

RESULTS

Parasitaemia and packed cell volume

The parasitaemia profiles (Figure 1) of both infected groups showed that there was a gradual but small increase in parasitaemia in the first 3 weeks of post infection (pi), which was followed by a sharp rise to a peak value in the 4th week pi; parasitaemia dropped equally sharply in the 5th week pi. In the first 2 weeks of infection, the parasitaemias of the 2 groups were statistically similar; but by the 3rd week, through to the 5th week, the parasitaemia in the vitamin-free group was consistently significantly ($P < 0.01$) higher than in the vitamin-treated group.

The profiles of the mean weekly PCVs of the 2 infected groups of rabbits (Figure 2) indicated an essentially similar pattern of changes in PCV during the course of the disease, though the terminal percentage decrease in PCV from pre-infection value (Table 1) was significantly ($P < 0.01$) higher in the vitamin-free infected rabbits than in the vitamin-treated infected ones.

Blood biochemical parameters

The concentrations of all biochemical parameters estimated in blood are summarized in Table 1. Serum total bilirubin was significantly ($P < 0.01$) elevated above normal levels by *T. congolense* infection, but intraperitoneal administration of vitamin C to infected rabbits significantly ($P < 0.01$) prevented this disease-induced increase. The concentrations of endogenous antioxidants, GSH and ascorbic acid, in the blood were significantly ($P < 0.01$)

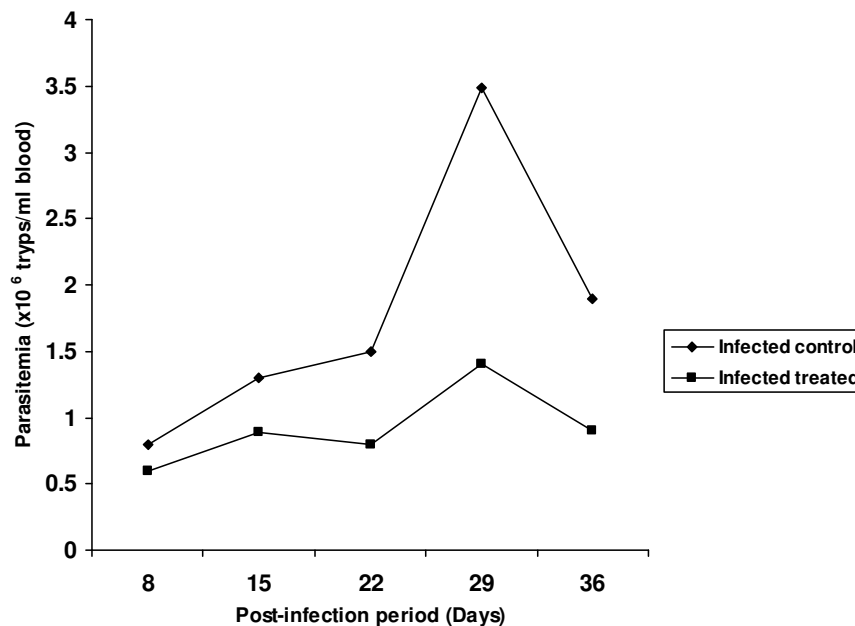


Figure 1. Parasitaemia profiles of infected rats treated/untreated with vitamin C.

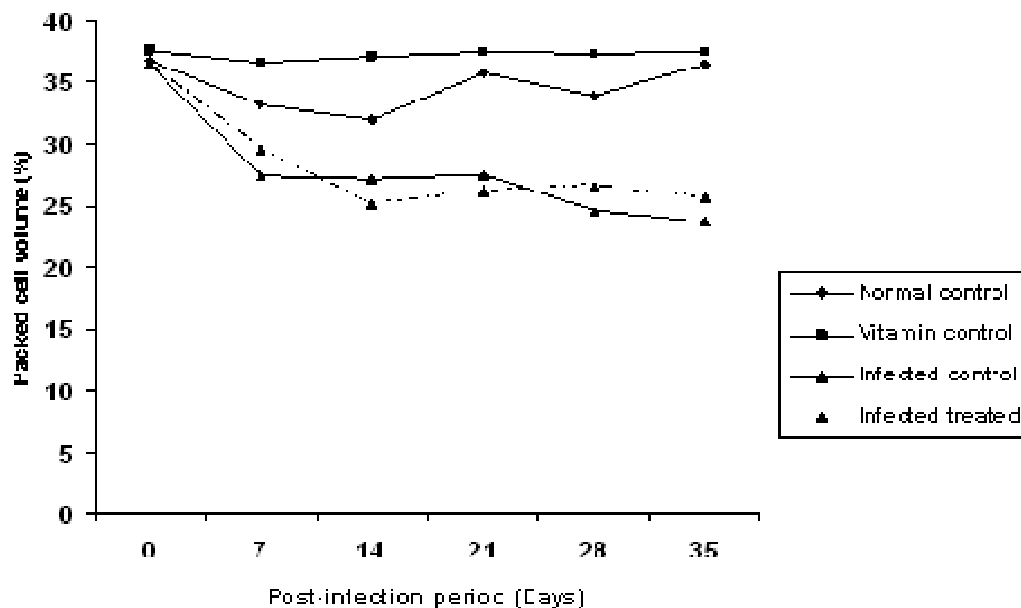


Figure 2. Profiles of PCV of infected/healthy rats treated/untreated with vitamin C.

lowered from the normal levels in healthy animals, by the infection. Vitamin C administration significantly ($P < 0.01$) augmented the levels of these 2 antioxidants in both healthy and diseased animals.

The activities of the marker enzymes, AST and ALT were significantly ($P < 0.01$) increased above normal levels in the sera of the vitamin-free infected animals. Intra-peritoneally administered vitamin C, however, completely prevented the disease-induced increases in the activities

of these enzymes in the vitamin-treated infected rabbits. The levels of serum total proteins were also significantly ($P < 0.01$) elevated by disease, but treatment with vitamin C partially, but significantly ($P < 0.01$) prevented this rise in serum total proteins. *T. congolense* infection had no effect on serum level of urea but significantly ($P < 0.01$) lowered the level of serum creatinine. While administered vitamin C had no significant effect on serum urea, it caused considerably significant ($P < 0.01$) decreases in

Table 1. Some blood biochemical parameters and percentage decreases in packed cell volume of rabbits after five weeks of infection with *T. congolense* and/or treatment with vitamin C.

Parameters	Groups (n = 7)			
	Uninfected control	Vitamin control	Infected Control	Infected treated
# Percentage decrease in PCV. (%)	0.40 ± 0.84	0.39 ± 0.38	35.00 ± 1.04*	29.73 ± 1.48**
Serum total bilirubin (µmole/L)	20.00 ± 2.04	22.50 ± 1.44	36.67 ± 2.36*	26.25 ± 3.15**
Blood glutathione (mmoles/100ml RBC)	0.68 ± 0.04	1.56 ± 0.36*	0.52 ± 0.10**	1.57 ± 0.29*
Plasma ascorbic acid (mM)	0.27 ± 0.04	0.34 ± 0.02*	0.21 ± 0.02**	0.27 ± 0.04*
Serum AST (i.u./L)	56.50 ± 14.37	50.80 ± 10.50	77.00 ± 5.57*	62.00 ± 7.75
Serum ALT (i.u./L)	37.50 ± 1.02	30.50 ± 2.56*	49.88 ± 1.94**	30.13 ± 3.48*
Serum total proteins (g/L)	52.55 ± 0.86	56.75 ± 2.06*	67.70 ± 3.31**	61.75 ± 2.63*
Serum urea (mM)	6.73 ± 0.29	5.84 ± 0.38	7.20 ± 0.39	6.32 ± 0.23
Serum creatinine (µmoles/L)	254.8 ± 13.61	176.17 ± 27.18*	231.92 ± 7.27**	184.56 ± 8.99*

All values are means ± SEM. (***)Values along a row are statistically different (P<0.01).

These values represent the % differences between pre-infection PCV and terminal values.

Table 2. Organ glutathione and ascorbic acid levels of rabbits after five-weeks of infection with *T. congolense* and/or treatment with vitamin C.

Parameters	Groups (n = 7)			
	Uninfected Controls	Vitamin control	Infected Controls	Infected treated
Glutathione (mmoles/100 g) × 10 ⁻³	a. 3.60 ± 0.40	3.30 ± 0.50	1.60 ± 0.30*	4.50 ± 1.70
	b. 2.30 ± 0.20	4.80 ± 0.60*	1.30 ± 0.30**	1.40 ± 0.30**
Ascorbic acid (mmoles/100 g) × 10 ⁻¹	a. 4.15 ± 0.64	3.89 ± 0.41	2.52 ± 0.36*	2.89 ± 0.28*
	b. 1.54 ± 0.25	2.04 ± 0.45	1.63 ± 0.18	2.77 ± 0.51*

All values for liver (a) and kidney (b) are presented as means ± SEM. (***) values along a row are statistically different (P<0.01).

serum creatinine in both healthy and diseased rabbits.

Organ antioxidants

The levels of GSH and ascorbic acid in the livers and kidneys of all groups of rabbits are presented in Table 2. *T. congolense* infection, without vitamin treatment, caused significant (P<0.01) depletion of GSH in the two organs. While Vitamin C did not prevent the disease-induced drop in kidney GSH, it elevated the level of liver GSH in the vitamin-treated infected rabbits significantly (P< 0.01) above even the levels recorded in the healthy control rabbits. Liver ascorbic acid was also significantly (P<0.01) depleted by infection and treatment with vitamin C did not change the situation. Kidney ascorbic acid levels were not affected by infection.

DISCUSSION

Administration of vitamin C to infected animals had a significant effect on level of parasitaemia only by the 3rd week of treatment. Observations in the first 2 weeks of treatment confirmed an earlier report, which indicated that administration of the same dose of vitamin C (along

with vitamin E.) to *T. brucei* infected rabbits (Umar et al., 1999a) for 2 weeks had no significant effect on the onset and level of parasitaemia. The significant reduction in parasitaemia caused by vitamin C administration after the 3rd week of treatment may be attributed to the effect of the vitamin on cell mediated immunity. The vitamin has been reported to enhance cell-mediated immunity in supplemented humans by increasing serum levels of some immunoglobulins, such as IgA, IgM and C-3 complement (Passmore and Eastwood, 1989). The anaemia in the vitamin treated infected rabbits was less severe than the one in the vitamin-free infected ones. This may be partly attributed to the lower parasitaemia in the latter animals since the onset and level of parasitaemia has been positively correlated with the degree of anaemia (Dargie et al., 1979; Maxie et al., 1979) in the disease. Another possible contributory factor may be the antioxidant action of Vitamin C, which would have kept the level of free radicals in the blood low, thereby preventing oxidative damage to erythrocyte membrane.

The levels of GSH and ascorbic acid in the blood, liver and kidney were depleted by *T. congolense* infection, confirming several earlier reports in which *T. brucei* (Umar et al., 1999a, b, 2000, 2001), *T. evansi* (Nyden, 1948) and *T. gambiense* (Ameh, 1984) have been used for experimental infection of various species of animals.

This indicates that depletion of endogenous antioxidant may be a significant factor in the pathogenesis of *T. congolense* infection as in the other species mentioned earlier. Administration of exogenous vitamin C to infected animals prevented these disease-induced decreases in GSH and ascorbic acid. This suggests that the administered vitamin C had a sparing effect on endogenous antioxidants as earlier reported (Umar et al., 1999b, 2000, 2001). The increase in serum AST and ALT strongly indicated liver damage (Kaplan et al., 1988) in the vitamin-free rabbits, which confirms results of earlier experiments with *T. congolense* (Whitelaw et al., 1980; Adah et al., 1992). Vitamin C administration prevented, to a significant extent, the disease-induced increases in serum AST and ALT, agreeing with results of earlier work with *T. brucei* (Umar et al., 1999a, 2000). This suggests that vitamin C protected the liver against disease-generated oxidative species during the disease probably by virtue of its antioxidant property that provided greater protection to plasma membrane and other susceptible cellular structures against oxidative agents.

The level of serum urea remained at normal levels while that of serum creatinine fell significantly ($P < 0.01$) below normal levels in vitamin C-free infected rabbits. Serum urea has been reported (Hudson, 1944) to be at normal levels during periods of low parasitaemia while serum creatinine falls to sub-normal levels (Welde et al., 1974) in cattle chronically infected with *T. congolense*. Vitamin C administration to infected rabbits lowered the level of serum urea significantly ($P < 0.01$) below infection level and caused the serum creatinine levels to drop even below the low levels recorded in the vitamin-free infected rabbits. These observations indicated that vitamin C gave some degree of protection to the kidneys during the course of the disease. It was concluded that *T. congolense* infection caused depletion of GSH and ascorbic acid in the body as well as cause hepatic and renal damage, and that administration of vitamin C to infected animals annulled these effects suggesting that oxidative stress probably plays a significant role in the pathogenesis of *T. congolense* infection as in *T. brucei* infections.

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