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Research article

Effects of Oral Supplementation with Manganese chloride on the severity of *Trypanosoma brucei* and *Trypanasoma congolonse* infections in rats

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ABSTRACT: Eighty healthy adult albino rats of both sexes were used in two experiments to study the effect of Manganese chloride supplementation on the severity of *Trypanosoma brucei brucei* and *Trypanosoma congolense* infections. In each experiment, forty rats were divided into four groups of 10 rats each, namely; A, unsupplemented control; B, supplemented control; C, infected supplemented and D, infected unsupplemented. Aqueous solution (10%) of $MnCl_2$ was administered daily using stomach tube to each rat at 100mg/kg in groups B and C from 10 days before infection and during the course of the infection. Each rat in groups C and D was infected by intraperitoneal injection of 1 x 10^6 trypanosomes (*T. b. brucei* or *T. congolense*) in phosphate buffered saline diluted donor blood. The prepatent periods were shorter ($P < 0.05$) in *T. brucei* than *T. congolense* infections and shorter ($P < 0.05$) in infected unsupplemented than infected supplemented rats. The infected unsupplemented groups had higher ($P<0.05$) parasitaemia, more severe anaemia ($P<0.05$) and hepatic and renal damage than infected supplemented groups. Therefore, oral Manganese chloride supplementation in rats appeared to reduce the severity of trypanosome infections by delaying the onset of parasitaemia, reducing the levels of parasitaemia and accompanying anaemia and organ damage.

Key Word: *Trypanosoma brucei*, *Trypanosoma congolense*, manganese chloride supplementation

INTRODUCTION

Trypanosomosis accounts for poor animal production in most parts of Africa (Losos, 1986). The major pathogenic species of trypanosomes are *T. vivax*, *T. brucei*, *T. congolense*, *T. evansi* and *T. simiae* (Nantulya, 1990). The disease causes anaemia, poor milk production, orchitis in males, infertility in females and death in some cases if untreated (Radostits *et al*, 1994). Nutritional status plays a significant role in the pathogenesis of African trypanosomosis (Murray and Dexter, 1988). Better nutrition can boost trypanotolerance or at least improve the recovery rate of cured animals (Otesile *et al* 1991; Igbokwe, 1995). Poor nutrition can increase the severity of the disease (Losos 1986; Igbokwe, 1995). Egbe-Nwiyi *et al.* (2003, 2004) reported that rats infected with *T. brucei* or *T. congolense* and supplemented with oral Magnesium chloride and Zinc chloride withstood the effects of the infection better than infected unsupplemented animals. Manganese is one of the least toxic elements to mammals and birds (McDonald *et al*, 1995). Dietary Manganese intake as high as 1000 – 2000ppm has been reported not to affect the growth rate of rats (McDonald

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et al., 1995). Infertility associated with low dietary manganese intake has also been observed(McDonald *et al*., 1995). Manganese chloride oral supplementation (50mg/Kg) has been observed not to affect the PCV but reduced the severity (levels of parasitaemia and anaemia) of *T. brucei brucei* and *T. congolense* infections in rats (Egbe-Nwiyi *et al*., 2005). This study was designed to determine the severity of $MnCl_2$ oral supplementation(100mg/Kg) on the organ damage of *T. b. brucei* and *T. congolense* infections in rats.

MATERIALS AND METHODS

Experimental Animals

Eighty healthy adult albino rats of both sexes weighing 180-200g were obtained from the Laboratory animal unit of the Department of Veterinary Pathology, University of Maiduguri. They were housed in clean cages at 30 – 35^0C, fed standard commercial diet (ECWA feed Jos, Nigeria) and provided clean water *ad libitum*. Forty rats were used in each of the two experiments (I and II) in which there were four groups (A, B, C and D) of 10 rats. The groups were A, unsupplemented control; B, supplemented control, C, supplemented infected and D, unsupplemented infected.

Trypanosome Infection

Trypanosoma congolense (Gboko Strain) and *Trypanosoma brucei* (Lafia Strain) were obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Vom, Nigeria. The trypanosomes were maintained in rats by serial passages. Phosphate buffered saline (pH 7.4) diluted infected blood from donor rats containing 1 x 10^6 trypanosomes was intraperitoneally injected into each rat in groups C and D in experiments I and II using *T. brucei* and *T. congolense*, respectively.

Oral Manganese Chloride Supplementation

Manganese Chloride ($MnCl_2$) (BDH Chemicals Ltd, Poole, England) Solution (10% aqueous) was administered daily by stomach tube to each rat at a dose rate of 100mg/kg in groups B and C in both experiments. The solution was administered from 10 days before infection and during the course of the infection.

Sample Collection and Analysis:

Tail blood from the infected rats was examined daily until parasitaemia established, and the level of parasitaemia was estimated using haemocytometry method (Coles, 1980).The packed cell volume (PCV) of the tail blood of the rats was determined every four days by the microhaematocrit method (Coles, 1980). The *T. brucei* and *T. conglolense* infected rats and their corresponding controls on days 12 and 22 post infection respectively, were decapitated under ether anesthesia and the blood from the neck vessels was collected, allowed to clot and serum harvested after centrifugation at 1,000g for 5 min. Serum alanine (ALT) and aspartate (ALT) aminotransferases activities were determined using commercial reagent kits (Randox Laboratories, Ireland). Serum urea and creatinine concentrations were estimated by standard methods. (Tietz, 1986).

Statistics

The data obtained were summarized as means ± standard deviations and means were compared by analysis of variance (ANOVA) and Students' t-test (Chatfield, 1983).

RESULTS

The prepatent periods (PP) were 3.8±0.9 and 6.1±0.7 days in *T.. brucei* infected unsupplemented and supplemented rats, respectively; 5.4±0.5 and 8.3 ±0.7 days respectively, in *T. congolense* infected unsupplemented and supplemented rats. The prepatent periods were shorter ($P < 0.05$) in *T. brucei* than *T. congolense* infections, and shorter ($P < 0.05$) in both infected unsupplemented than infected supplemented groups.

The mean parasitaemia in both *T. brucei* and *T. congolense* infected supplemented and unsupplemented rats are presented in Table 1. The *T. brucei* and *T. congolense* infected unsupplemented rats had higher ($P < 0.05$) levels of parasitaemia than the infected supplemented groups. On day 12 post-infection (pi), when the *T.* brucei experiment was terminated; the level of parasitaemia was higher ($P < 0.05$) in *T. brucei* infected than *T. congolense* infected rats. On day 22pi, when the *T. congolense* experiment was terminated, the level of parasitaemia was higher ($P < 0.05$) in *T. congolense* infected than *T. brucei* infected on day 12 pi.

The mean PCV decreased in all the infected groups (Table 2). The mean PCV values in both *T. brucei* and *T. congolense* unsupplemented groups were significantly ($P < 0.05$) lower than those of the infected supplemented groups from day 8 pi with *T. brucei* infection and from day 12 pi with *T. congolense* infection.

Table 1:
Parasitaemia of rats* supplemented(S) or unsupplemented (U) with 10% aqueous $MnCl_2$ and infected with *T. b. brucei* (Tb) or *T. congolense*(Tc).

Days post infection	Trypanosome count (x10^3/per ml)			
	Tb + S	Tb + U	Tc+ S	Tc + U
2	*0.0± 0.0*[a]	0.2± *0.6*[b]	0.0± *0.0*[a]	0.0± *0.0*[b]
4	*0.0± 0.0*[a]	2.4± *0.5*[b]	0.0± *0.0*[a]	0.0± *0.0*[b]
6	1.6 ± *1.4*[a]	5.2± *0.8*[b]	0.2± *0.6*[a]	2.2± *0.9*[b]
8	3.1 ± *0.9*[a]	6.3± *0.5*[b]	1.0± *1.4*[a]	4.5± *0.5*[b]
10	6.1± *1.0*[a]	10.4± *1.7*[b]	2.8± *1.0*[a]	7.3± *1.3*[b]
12	8.7± *0.9*[a]	13.1± *0.7*[b]	4.4± *0.7*[a]	9.8± *1.5*[b]
14	ND	ND	5.7± *0.7*[a]	11.0± *1.1*[b]
16	ND	ND	8.0± *0.9*[a]	12.6± *0.7*[b]
18	ND	ND	10.7± *1.5*[a]	14.6± *1.1*[b]
20	ND	ND	12.6± *1.5*[a]	17.6± *0.8*[b]
22	ND	ND	14.1± *2.1*[a]	21.5± *1.5*[b]

ND: No Data
[a,b]Values *in rows for Tb or Tc* with different superscripts differ significantly (P < 0.05); * n = 10

Table 2:
Mean packed cell volume(%) of control and *T. brucei* infected rats* orally supplemented (S) or unsupplemented (U) with 10% aqueous $MnCl_2$

Days pi	Control		*T. congolense* infected	
	U	S	U	S
0	50.2±*0.4*[a]	50.4±*0.8*[a]	50.2±*0.5*[a]	50.2±*0.4*[a]
4	50.6±*0.8*[a]	50.2±*0.9*[a]	50.5±*0.7*[a]	50.3±*0.7*[a]
8	50.8±*0.3*[a]	50.6±*0.2*[a]	48.9±*0.7*[a]	50.0±*0.8*[a]
12	50.9±*0.4*[a]	50.7±*0.4*[a]	45.3±*0.5*[b]	48.9±*0.3*[a]
16	50.6±*0.6*[a]	50.6±*0.4*[a]	23.5±*8.7*[b]	37.4±*2.5*[b]
20	50.4±*0.5*[a]	50.8±*0.5*[a]	21.4±*1.3*[b]	30.9±*1.9*[c]

*[a,b c] Values in rows with different superscripts differ significantly (P < 0.05); *n= in each = 10*

Table 3:
Serum activities of alamine (ALT) and aspartate (AST) aminotransferases and serum urea and creatinine concentrations in control rats and *T. b. brucei* infected (Tb) (12 days post infection) or *T. congolense* (Tc) infected (22 days post infection) rats* unsupplemented (U) or supplemented with 10% aquous $MnCl_2$

Parameters	Infection (Tb or Tc)	Control		*T. congolense* infected	
		U	S	U	S
ALT(i.u./L)	Tb	10.6±*0.8*[a]	10.0±*0.9*[a]	60.2±*1.4*[b]	27.3±*2.9*[c]
	Tc	10.0±*0.8*[a]	10.4±*0.9*[a]	58.9±*2.8*[b]	32.6±*2.c*[c]
AST (i.u/L)	Tb	*10.0*±*0.7*[a]	10.0±*0.8*[a]	58.8±*3.1*[b]	28.6±*61.3*[c]
	Tc	*12.7*±*0.3*[a]	12.8±*0.2*[a]	65.3±*3.1*[b]	35.1±*0.6*[c]
Urea (mmol/L)	Tb	4.4±*0.3*[a]	4.4±*0.3*[a]	12.2±*0.8*[b]	8.1±*0.4*[ca]
	Tc	4.8±*0.1*[a]	4.7±*0.2*[a]	11.1±*0.6*[b]	7.0±*0.5*[c]
Creatinine (µ mol/L)	Tb	63.4±*1.3*[a]	63.6±*1.1*[a]	128.9±*6.8*[b]	91.7±*4.5*[c]
	Tc	65.7±*0.6*[a]	65.9±*0.7*	112.6±*5.0*[b]	83.8±*0.9*[c]

*[a,,b,c] Values in rows with different superscripts differ significantly (P < 0.05); *n= in each = 10*

There were no significant ($P < 0.05$) variations in the PCV of the unsupplemented and supplemented control groups, both within and between the groups during the period of the experiments.

The serum ALT and AST activities, urea and creatinine concentrations increased significantly ($P < 0.05$) in the infected groups when compared with the uninfected groups (Table 3). The mean values in both *T. brucei* and *T.* congolense infected supplemented were lower ($P < 0.05$) than those of the infected unsupplemented groups.

DISCUSSION

All the *T. brucei* and *T. congolense* infected rats received the same infective dose of the trypanosomes and the prepatent periods were shorter in both *T. brucei* and *T. congolense* infected unsupplemented than infected supplemented rats. The onset of parasitaemia was also earlier in both *T. brucei* and *T. congolense* infected unsupplemented than infected supplemented groups. The level of parasitaemia was equally higher in all the infected unsupplemented animals when compared with the infected supplemented groups. Murray and Dexter (1988) reported that the prepatent period and level of parasitaemia in animal trypanosomosis could be influenced by the infective dose of trypanosomes. Since there was no difference in the infective dose of trypanosomes administered to all the infected rats, it was likely that manganese chloride supplementation could have influenced on the course of the infection in the rats. Similar observations using different mineral supplements in trypanosome infected rats were earlier reported (Egbe-Nwiyi *et al*., 2004; 2005a and b). There was anaemia in all the infected rats and the level of anaemia appeared more severe in all the trypanosome infected unsupplemented rats. Higher level of parasitaemia usually relates to more severe anaemia (Murray and Dexter, 1988).

The *T. brucei* has been reported to be more tissue invasive than *T. congolense* and trypanosome invasion of tissues may cause organ damage (Losos, 1986; Anosa, 1988). Both *T. brucei* and *T. congolense* infections caused hepatocellular damage which was less severe in the infected supplemented rats judging from the activities of ALT and AST. The renal damage caused by the trypanosome infections was indicated by increases in serum concentrations of urea and creatinine and the degree of the organ damage was less severe in infected supplemented than infected unsupplemented groups. The reduced less severity of organ damage by *T. b. brucei* and *T. congolense* infections in the supplemented rats at days 12 and 22 pi respectively could be attributed to the $MnCl_2$ supplementation.

McDonald *et al*. (1995) reported high intake of $MnCl_2$ in many animal species without adverse effects. Therefore, the dosage of $MnCl_2$ administered to the rats in the present study was safe as data obtained showed no effects on the PCV and other parameters in the control groups. In conclusion, oral $MnCl_2$ supplementation in rats appeared to delay the onset of parasitaemia and reduce parasitaemia, anaemia and organ damage in *T. brucei* and *T. congolense* infections. Therefore, MnCl supplementation may boost trypanotolerance in domestic anilans.

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