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The effect of oral magnesium chloride supplementation on the pathogenicity of *T. brucei brucei* and *T. congolense* infections in rats

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ABSTRACT: Eighty healthy adult albino rats of either sex weighing between 190 – 200 grams were used to assess the effect of oral magnesium chloride ($MgCl_2$) (at 50mg/kg body weight) supplementation on the severity of *T. b. brucei* (lafia strain) or *T. congolense* (Karu strain) infections. Two studies were carried out and the rats were divided into 4 groups of 10 rats each (per study) namely: Group A (uninfected, unsupplemented control), Group B (supplemented with magnesium chloride), Group C (*T.b. brucei or T. congolense* infected, 1 x 10^6 trypanosomes and supplemented) and Group D (*T.b. brucei or T. congolense*, infected, 1 x 10^6 trypanosomes and unsupplemented). Each rat in groups B and c received approximately 0.01ml of $MgCl_2$ intragastrically daily for 8 days before and during the course of the infection. The prepatent periods, survival times and levels of anaemia in the *T.b. brucei or T. congolense* infected supplemented rats were comparable ($P > 0.05$) with those of the *T.b. brucei or T. congolense* infected unsupplemented. However, parasitaemia, hepatomegaly and splenomegaly were significantly higher ($P < 0.05$) in the rats infected with *T.b. brucei or T. congolense* and unsupplemented. The $MgCl_2$ at 50 mg/kg body weight has not exhibited any significant effects on the severity of *T.b. brucei* (lafia strain) or *T. congolense* (karu strain) infections in rats. There is the need to try higher doses of the agent and possibly determine bioavailability of the $MgCl_2$ in subsequent studies.

Key words: Magnesium chloride; Supplementation; Trypanosomes; Pathogenicity; Rats.

Introduction

Man and animals have been affected by disease-causing trypanosomes in Africa over the years (7). The disease causes sleeping sickness in man while in animals, it causes anaemia, infertility in females, orchitis in males, low productivity and sometimes death if untreated (11). *Trypanosoma brucei* affects extra and intravasculature while *T. congolense* and *T. vivax* affect intravasculature mainly (2, 8). As the trypanosome causes serious disease in both man and animal, nutrition plays vital role in the regulation of the disease (10, 5). Otesile *et al* (10) observed that pigs placed on high plane of nutrition were able to withstand the effect of *T. brucei* infection better than pigs on low plane of nutrition. Egbe-Nwiyi (3) reported that rats infected

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with *T. brucei or T. congolense* and supplemented with oral alcium chloride withstood the effect of the infection better than the *T. brucei or T. congolense* infected, unsupplemented rats.

Magnesium acts directly on cells and tissues as part of their environment (14). The magnesium is closely associated with calcium and phosphorus and it is essential for the efficient metabolism of carbohydrates and lipids (14). The principal sites of absorption appeared to be the duodenum and small intestine. The purpose of the present study was to ascertain whether magnesium chloride supplementation enhances or reduces the severity of trypanosome infections [*T. brucei* and *T. congolense*] in rats.

Materials and Methods

Experimental animals:

Eighty healthy adult albino rats of either sex, weighing 190-200 grams were obtained from the National Institute for Trypanosomiasis Research (NITR), Vom, Nigeria and fed a commercial diet (ECWA Feeds, Jos). Water was given *ad libitum*. The rats were screened for the presence of blood parasites before the commencement of the experiment using standard techniques (12) study, namely:

Group A: Uninfected unsupplemented control

Group B: Supplemented with magnesium chloride

Group C: Infected with *T.b. brucei* (1×10^6 trypanosomes) or *T. congolense* (1×10^6 trypanosomes) and magnesium chloride supplemented.

Group D: Infected with *T.b. brucei* (1×10^6 trypanosomes or *T. congolense* (1×10^6 trypanosomes) and unsupplemented.

Trypanosomes:

Trypanosoma brucei brucei (Lafia strain) and *T. congolense* (Karu strain) used were obtained from NITR, Vom and the parasites were each passaged serially in donor rats.

Trypanosome infection:

The tail-blood from a donor rat earlier inoculated with *T.b. brucei* (lafia strain) or *T. congolense* (Karu strain) was collected and diluted in cold normal saline. Forty rats were infected by intraperitoneal injection of the diluted blood containing 1×10^6 trypanosomes (*T.b. brucei or T. congolense*). The tail blood from the infected animals was examined daily and the level of parasitaemia was determined using haemocytometer method (12). The remaining forty rats served as uninfected, unsupplemented or supplemented controls.

Magnesium Chloride:

Five grams of magnesium chloride ($MgCl_2$) was weighed and dissolved in 100ml of distilled water. The $MgCl_2$ was administered to the rats intragastrically (13) and each rat in groups B and C received approximately 0.01ml of the $MgCl_2$ daily for 8 days before and during the course of the infection.

Determination of Packed Cell Volume (PCV):

Tail-blood was used to determine the PCV using microhaematocrit method (12).

Post-morten examination of carcasses:

When the infected rats died and the uninfected sacrificed at the end of the experiment, post-morten was performed on each rat using standard method (4). The liver and spleen were removed and weighed and lesions were recorded. The survival time of each rat was calculated.

Statistics:

The data obtained in the study were summarised as Means ± Standard deviations. Analysis of Variance (ANOVA) and students' t-test were used to analyse the data (using statistix, computer software, version 3.1, 1992).

Results

The prepatent periods and surivival times of the *T.b. brucei* and *T. congolense* infected supplemented and unsupplemented rats are presented in Table 1. The levels of parasiyaemia are presented in Tables 2 and 3 while the levels of anaemia are shown in Tables 4 and 5.

The prepatent periods in both *T.b. brucei* infected supplemented and unsupplemented rats did not differ significantly ($P > 0.05$). In *T. congolense* infected supplemented and unsupplemented rats, the prepatent periods were also comparable ($P > 0.05$). The survival times of *T.b. brucei or T. congolense* infected, supplemented rats did not differ ($P > 0.05$) from those infected but unsupplemented. However, the levels of parasitaemia were significantly higher ($P < 0.05$) in *T.b. brucei* (especially days 8 – 10 post-infection) or *T. congolense* (especially days 14-20 post-infection) infected, unsupplemented animals when compared with the levels in the rats infected with *T.b. brucei or T. congolense* and supplemented. The levels of anaemia in the *T.b. brucei or T. congolense* infected supplemented were comparable ($P > 0.05$) with those rats infected with *T.b. brucei or T. congolense* and unsupplemented. There were severe hepatomegaly and splenomegaly in the *T.b. brucei or T. congolense* infected, unsupplemented rats when compared with the moderate level recorded in the *T.b. brucei or T. congolense* infected supplemented rats (Table 6).

Table 1: Mean (± Sd) prepatent periods and survival times of rats infected with *T. brucei* (lafia strain) or *T. congolense* (Karu strain) with or without oral magnesium chloride (50mg/kg body weight) supplementation.

Parameters	**Treatment groups**	
	T.b. brucei or *T. congolense* infected and supplemented (n = 10)	*T.b. brucei* or *T. congolense* infected and unsupplemented (n = 10)
Prepatent period (days)	4.6 ± 0.5^a	4.3 ± 0.7^a
	(11.3 ± 0.9^a)	$(10.5 \pm 0.7^a$
Survival time (days)	12.0 ± 0.9^a	10.5 ± 0.8^a
	(20.3 ± 1.2^a)	(19.7 ± 1.3^a)

( ) = value of *T. congolense* rats per group

n = number of rats per group

Values in rows with the same superscripts are not significant ($P > 0.05$).

Table 2: Mean (± SD) parasitaemia (x 10^3/µl) level of rats infected with *T.b. brucei* with or without oral magnesium chloride (50 mg/kg body weight) supplementation.

Treatment groups	**Days post -infection**			
	4	**6**	**8**	**10**
T.b. brucei infected and supplemented (n= 10).	15.0 ± 19.5[a]	51.0 ± 9.9[a]	67.0 ± 6.7[a]	81.0 ± 3.1[a]
T.b. brucei infected and unsupplemented (n = 10).	31.0 ± 27.6[b]	73.0 ± 4.8[b]	95.0 ± 5.2[b]	140.0 ± 13.3[b]

( ) = value of *T. congolense* infected rats per group
n = number of rats per group
Values in rows with the same superscripts are not significant (P > 0.05).

Table 3: Mean (± SD) parasitaemia (x 10^3/µl) level of rats infected with *T. congolense (*karu strain) with or without oral chloride (50mg/kg) body weight) supplementation.

Treatment groups	**Days post-infection**					
	10	**12**	**14**	**16**	**18**	**20**
T. congolense infected and supplemented (n=10).	9.0 ± 14.4[a]	40.0 ± 15.6[a]	59.0 ± 7.3[a]	76.0 ± 6.9[a]	100.0 ± 4.7[a]	124.2 ± 7.8[a]
T. congolense infected and unsupplemented (n=10).	23.0 ± 20.0[b]	66.0 ± 9.6[b]	104.0 ± 15.7[b]	130.0 ± 17.6[b]	173.0 ± 14.9[b]	236.7 ± 23.3[b]

n = number of rats per group
Values in columns with different superscripts are significantly different (P < 0.05).

Table 4: Mean (± SD) packed cell volume (PCV %) of uninfected supplemented and unsupplemented control rats and rats infected with *T.b. brucei* with or without oral magnesium chloride (50 mg/kg body weight) supplementation.

Treatment groups	**Days post-infection**		
	4	**6**	**8**
Uninfected control (n=10).	50.4 ± 0.5[a]	50.4 ± 0.5[a]	50.4 ± 0.7[a]
Uninfected supplemented (n=10).	50.4 ± 0.7[a]	50.4 ± 0.7[a]	50.4 ± 0.5[a]
T.b. brucei infected and supplemented (n=10).	50.4 ± 0.5[a]	50.4 ± 0.5[a]	36.4 ± 4.2[b]
T.b. brucei infected and unsupplemented (n=10).	50.4 ± 0.7[a]	50.4 ± 0.7[a]	35.1 ± 0.7[a]

n = number of rats per group

Values in columns with different superscripts are significantly different (P < 0.05).

Table 5: Mean (± SD) packed cell volume (PCV %) of uninfected supplemented and unsupplemented control rats and rats infected with *T. congolense* with or without oral magnesium chloride (50mg/kg body weight) supplementation.

Treatment groups	**Days post-infection**					
	0	**4**	**8**	**12**	**16**	**20**
Uninfected control (n=10)	50.5 ± 0.3^a	50.5 ± 0.3^a	50.5 ± 0.3^a	50.4 ± 0.5^a	50.4 ± 0.5^a	50.4 ± 0.7^a
Uninfected supplemented (n=10)	50.4 ± 0.7^a	50.4 ± 0.7^a	50.4 ± 0.5^a	50.5 ± 0.3^a	50.4 ± 0.7^a	50.5 ± 0.5^a
T. congolense infected and supplemented(n=10)	50.5 ± 0.7^a	50.5 ± 0.7^a	50.5 ± 0.3^a	50.4 ± 0.7^a	50.1 ± 0.2^a	35.7 ± 4.4^b
T. congolense infected and unsupplemented (n=10)	50.1 ± 0.3^a	51.0 ± 0.4^a	51.0 ± 0.4^a	50.5 ± 0.7^a	50.1 ± 1.3^a	34.9 ± 4.4^b

n = number of rats per group

Values in columns with different superscripts are significantly different ($P < 0.05$).

Table 6: Mean (± SD) liver and spleen weights (g/100g body weight) of uninfected rats and rats infected with *T.b. brucei* or *T. congolense* with or without oral magnesium chloride (50 mg/kg body weight) supplementation.

Organ weights	Treatment groups			
	Uninfected control (n=10)	Uninfected supplemented (n=10)	*T.b. brucei* or *T. congolense* infected and suplemeneted (n=10)	*T.b. brucei* or *T. congolense* infected and unsupplemented (n=10)
Liver (g/100 g body weight)	2.7 ± 0.4^a	2.5 ± 0.4^a	7.7 ± 1.0^b	13.2 ± 1.4^c
	(2.2 ± 0.3^a)	(2.3 ± 0.3^a)	(8.3 ± 2.1^b)	(13.6 ± 1.8^c)
Spleen (g/100g body weight)	1.7 ± 0.3^a	1.7 ± 0.2^a	3.4 ± 0.7^b	6.9 ± 0.3^c
	(1.6 ± 0.3^a)	(1.7 ± 0.3^a)	(3.3 ± 0.7^b)	(0.6 ± 0.4^c)

n = number of rats per group
( )= value of *T. congolense* infected rats per group
Values in rows with different superscripts are significantly different ($P < 0.005$).

Discussion

Trypanosoma brucei brucei is tissue invasive while *T. congolense* affects the blood mainly and the supplementation of magnesium chloride ($MgCl_2$) to the *T.b. brucei* (lafia strain) and *T. congolense* (Karu strain) infected rats has not significantly influenced the severity of the infections. This is based on the fact

that the level of anaemia in the *T.b. bruce* or *T. congolense* infected and supplemented animals was comparable with level observed in the unsupplemented *T.b. brucei* or *T. congolense* infected rats. However, the height of parasitaemia was progressively higher in the rats infected with *T.b. brucei* or *T. congolense* and unsupplemented. The height of parasitaemia in the present study was never commensurate with the level of anaemia generated in those groups of rats. The unsupplemented *T.b. brucei* or *T. congolense* infected rats were expected to be more anaemic for being more parasitaemic than the supplemented *T.b. brucei* or *T. congolense* infected animals. In some reports (8), animals with higher level of parasitaemia exhibited more severe infection. The severity of trypanosome infections is shown mainly by the level of anaemia (2). Therefore, the higher level of parasitaemia in the infected unsupplemented groups but comparable level of anaemia in the supplemented and unsupplemented rats contradicts earlier reports (7,8). It is tempting then to speculate that the parasites were not too virulent to have generated severe anaemia commensurate with the level of parasitaemia exhibited.

The hepatomegaly and splenomegaly observed in the rats infected with *T.b. brucei* or *T. congolense* supplemented and unsupplemented agree with the observations of previous workers (9,6,3). But the unsupplemented *T.b. brucei* or *T. congolense* infected rats exhibited more severe hepatomegaly and splenomegaly. Such severe parameters were not commensurate with the level of anaemia recorded in those groups of rats. This may among other things, indicate that hepatomegaly or splenomegaly does not contribute significantly to the level of anaemia in trypanosome infections. This agrees with the reports of Igbokwe and Nwosu (6) where splenomegaly or hapetomegaly did not contribute much to the severity of anaemia, but disagrees with the observations of Anosa and Kaneko (1) who reported that the parameters (hepatomegaly and splenomegaly) were directly related to the severity of anaemia.

Mortality was first noticed in the usupplemented *T.b. brucei* or *T. congolense* infected animals but the survival time was comparable in the supplemented and unsupplemented *T.b. brucei* or *T. congolense* infected rats. However, the survival time was slightly longer in the supplemented *T.b. brucei* or *T. congolense* infected rats and these groups of rats were also less anaemic. This may call for further studies. It is possible that the magnesium chloride may have significant influence but the dose was not enough or the duration of supplementation was short and or there was poor bio-availability of the agent in the small intestine. Magnesium chloride is easily absorbed from the small intestine and duodenum (14).

In conclusion, rats infected with *T. brucei* or *T. congolense* and supplemented exhibited higher level of parasitaemia, hepatomegaly and splenomegaly but comparable prepatent periods, survival times and anaemia with the *T.b. brucei* or *T. congolense* infected and unsupplemented rats. Magnesium chloride at 50mg/kg body weight had no significant influence on the severity of trypanosome infections in rats and there is need to try higher doses of such agent and possibly determine bio-availability of the agent in subsequent studies.

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