

EFFICACY OF MAGNESIUM CHLORIDE SUPPLEMENTATION AND DIMINAZENE ACETURATE IN RATS INOCULATED WITH *TRYPANOSOMA BRUCEI*

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ABSTRACT

The effect of MgCl₂ and Diminazene aceturate in experimental *Trypanosoma brucei* infected rats was investigated. Infection with the parasite caused a progressive increase in parasitaemia which resulted in a significant (P<0.05) decrease in packed cell volume, Red blood cell count and white blood cell count in the infected not treated (group F), the positive control and other groups C,D,E, and G respectively. The treated groups C,D,E and G improved remarkably, but were not clear of the parasites, most especially group D, which was given subtherapeutic dose of diminazene aceturate and supplemented MgCl₂ orally at 100 mg/kg per os. This prolonged the life of the rats to up to 27 days. The combination of MgCl₂ and diminazene reduced the severity of trypanosome infection and hence alleviated the anaemia and leukopenia.

Key words: MgCl₂, Diminazene aceturate, Trypanosoma, blood, Parasitaemia

INTRODUCTION

African trypanosomosis is a serious threat to human and animal health in sub-Saharan Africa. It is estimated that 60 million people and over 3 million herds of cattle are at risk (WHO, 2005). The disease is characterized by anaemia, weight loss, weakness, anorexia, abortion, low milk production, jaundice, geophagia and

decrease in reproductive capability (Radostitis *et al.*, 2003). Human African Trypanosomosis now poses as an emerging zoonosis in sub-Saharan African. (Kabayo, 2002; Waisawa *et al.*, 2003). Trypanosomosis is described as “one of the most neglected disease” (Truc, 2003) especially in terms of drug development (WHO, 1998). Chemotherapy and chemoprophylaxis are the main methods of controlling trypano-

somes (Onyiah, 1997). Combination therapy was advocated by some workers (Ajagbonna and Olaniyi, 1999; Ajagbonna *et al.*, 2005). Egbe-Nwiyi *et al.* (2003), suggested the need for more careful study on a trial combining Magnesium chloride with a trypanocide. The objective of the study, therefore, is to determine the effect of combining a synthetic Magnesium salt with diminazene aceturate in *T. brucei* infection.

MATERIALS AND METHODS

Experimental Animals

Thirty five healthy albino-rats of both sexes with weights ranging between 180-220 grams were purchased from Abeokuta, Ogun State, Nigeria. *The animals were fed ad libitum* and were stabilized for 2 weeks. They were confined in cages with wire mesh and were screened for haemoparasites using standard methods as adopted by Ajagbonna *et al.* (2005).

Experimental Design

The rats were categorized into 7 groups of 5 each

GROUP A: Uninfected unsupplemented control

GROUP B: Uninfected Supplemented with magnesium chloride for 10 days

GROUP C: Infected Supplemented with magnesium chloride

GROUP D: Infected supplemented with MgCl₂ and treated with subtherapeutic dose of diminazene aceturate.

GROUP E: Infected unsupplemented treated with half dose diminazene aceturate. (1.75mg/kg)

GROUP F: Infected unsupplemented not treated control

GROUP G: Infected treated with full dose

of diminazene aceturate (3.5mg/kg)

Trypanosome Stock

Trypanosoma brucei used in the study was obtained from Nigerian Institute for Trypanosomiasis and onchocerciasis Research, Vom, Nigeria. The Parasites were each passaged serially in donor rats. The rats were inoculated using a method adopted by Egbe-Nwiyi *et al.* (2003).

Parameters For Assessing Therapeutic Activity of MgCl₂-diminazene combination
Wet mount method was used as adopted by (Ajagbonna and Onyelili, 2003; Ajagbonna *et al.*, 2005).

Magnesium Chloride Administration

Analar® brand magnesium chloride salt prepared by BDH chemical Limited Poole England was administered using a method adopted by Egbe-Nwiyi *et al.* (2003).

Trypanocide

The Trypanocide used was diminazene aceturate made in India Bio-Product, PVT India Products. The drug was administered Intramuscularly as adopted by Ajagbonna *et al.* (2005).

Blood Sample Collection

A 21" gauge syringe needle was used to carry out cardiac puncture and 2-4ml of blood was extracted by aspiration. This was collected into bottles containing Ethylene diamine tetra acetic acid 20mg/ml of blood as anticoagulant. The packed cell volume (PCV), Red blood cell count (RBC) and white blood cell count (WBC) were determined using standard method (Schalm *et al.* (1975).

Statistical Analysis

The data obtained were summarized as means and standard deviation and means were compared by Analysis of variance (ANOVA), Probability level at ($P < 0.05$) was considered.

RESULTS

The rats exhibited progressive parasitaemia that resulted in earlier death of rats in group F as shown in tables "1" and "2". The administration of the $MgCl_2$ in combination with diminazene aceturate, in group D Improved the survival time to groups time to 27 days. the results of other test groups are summarized in Table 2. while group G, the group infected treated with full dose (3.5 mg/kg) showed a relapse of infection and various resurgences as summarized in Table 2.

Anaemia occurred in all the infected groups (C,D, E, F, and G). There was significant decrease in PCV, RBC and WBC respectively ($P < 0.05$). This is summarized in Table 3. There was however marked improvement towards the normal uninfected untreated group (A) in group D E and G after various treatments but not in group F. The untreated infected control. The rats in the group did not improve towards the normal group A.

DISCUSSION

Results of the present study suggest that *T. brucei* has lethal effect that causes tissue damage and death of animals. This is in agreement with the observations of (Mikail *et al.*, 2002; Egbe Nwiyi *et al.*, 2003; 2004; Ajagbonna *et al.*, 2005). The study shows that magnesium chloride a source of Magnesium must have acted (due to its antioxidative effect) by scav-

enging and mopping up free radicals peroxides and other stress factors produce by antigenic factors from the parasite as adduced by Egbe-Nwiyi *et al.* (2003).

The study shows that WBC count also improved due to the use of the combination. This is in agreement with the work of Delbet (1992), who observed a rejuvenating effect and an increase of WBC count by 300%. magnesium chloride as adduced by Igbokwe (1994) must have dissociated to produce magnesium ion Mg^{2+} , so this cation in the extra cellular fluid would have spared the red blood cell integrity due to its action on the AT Pase. This would enable the red blood cells to withstand stress caused by the parasite and this would stabilize the red cell membrane and prevent it from osmotic lysis following parasitic infection. (Kaneko, 1989). This, with the trypanocide effect would have treated the experimental groups inoculated and this most likely is the reason for the prolongation of the average time of survival of rats. The resurgences in the infected and treated with full dose (3.5mg/kg, therapeutic) of the trypanocide diminazene aceturate is as a result of the relapse of infection, other workers have advocated that *T. brucei* is a tissue invasive parasite. In this study, this assertion might have prevented the trypanocide from been accessible to the parasite to elicit its trypanocidal effect. This will eventually cause the reappearance of the parasite in the peripheral circulation over time which will consequently, result to parasitaemia again. This assumption is in line with the studies of Ezeokonkwo *et al.* (2007) and Onyeili and Egwu (1995). In conclusion Magnesium chloride and diminazene combination is a potential therapy in management of African trypano-

somiasis. Further research, therefore, should be carried out of investigate supplementation of various minerals with various trypanocides combinations.

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Table 2: Summary of Total Number of Deaths, Cured Animals and Average Survival period in Treated and Untreated Groups in Magnesium Chloride Supplemented Study

	A	B	C	D	E	F	G
Indices determination	UNINFECTED supplemented for 10 days and throughout experiment	UNINFECTED supplemented for 10 days and throughout experiment	INFECTED supplemented mgcl ₂	INFECTED supplemented and give ½ dose of Diminazine aceturate	INFECTED Treated with ½ dose of Diminazine aceturate	INFECTED Not treated control	INFECTED Treated with full dose of Diminazine aceturate
Total number of deaths	0	0	4	4	5	5	0
Numbers cleared of parasites	5	5	1	1	0	0	5
Average survival days	30	30	30	27	20	14	30

Table 3: Effects of Magnesium Chloride, Berenil® and its Combination on Haematological Values 11 days post infection and after treatment. Haematological values mean ±SD

Haematological indices	A	B	C	D	E	F	G
Pcv (0/0)	45.4±1.82	42.5±3.54	37±5.5	42±1.41	46.3±1.53Q	22.8±3.96*	44.4±2.19
Rbc (106/μl)	5.62±0.26	6.5±1.7	5.9±0.2	6.9±0.7*	5.7±0.6	2.90±0.38*	5.90±0.38
Wbc (103/μl)	4.4±0.2	4.4±0.1	4.6±0.1	5.7±1.2*	4.2±0.3	2.5±0.3*	6.4±1.1*

*P<0.05 compared to control untreated group