International Journal of Animal and Veterinary Advances 4(2): 93-98, 2012
ISSN: 2041-2908

Submitted: December 30, 2011 Accepted: February 17, 2012 Published: April 20, 2012

Effects of DMSO on Diminazene Efficacy in Experimental Murine *T. brucei* Infection

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Abstract: This study evaluated the influence of dimethyl sulfoxide (DMSO) daily supplementation on diminazene treatment of trypanosomosis. Four groups of *Trypanosoma brucei brucei* infected rats received 7.0 mg/kg diminazene aceturate on day 7 post infection. Three of the four groups received different doses of DMSO (0.5, 1.0 and 2.0 g/kg, respectively) in addition to diminazene treatment. The changes in hematological parameters and the weights of liver, spleen and heart caused by *T. brucei* infection were monitored and used to assess the efficacy of treatment. The prepatent period of infection was four days. Infection caused significant increases in WBC count, spleen and liver weights but it caused decreases in PCV, HB and RBC by day 7 post infection (PI_7). By PI_{14}, spleen weight and WBC counts were reduced from the PI_7 level without treatment. Diminazene/DMSO combination did not reduce liver weight or increased hematological parameters more significantly than diminazene treatment alone. Increase in the dose of DMSO caused increases in liver weight. Diminazene/DMSO combination reduced spleen weight more significantly than diminazene treatment alone. Diminazene/DMSO combination delayed re-emergence of parasites beyond PT_{21} at which time parasites were detected in the blood of rats treated only with diminazene. The limited advantages of diminazene/DMSO combination over diminazene alone as well as the possible liver toxicity of DMSO at high doses would not make DMSO supplementation a viable addition to trypanosomosis chemotherapy.

Key words: Diminazene, DMSO, liver weight, parasitemia, rat, spleen weight, trypanosome

INTRODUCTION

Despite many decades of research and actions at vector and chemotherapeutic control of trypanosomosis, effective control strategies have yet to materialize. The two traditional methods of using chemotherapeutic drugs for treatment and prophylaxis as well as insecticides for vector control have met with unsatisfactory outcomes. Drug resistance and toxicity are two important complicating factors in the chemotherapy of trypanosomosis and the widespread use of insecticides is not environmentally friendly. The failure of these traditional methods has been complicated by the lack of vaccine and new drugs. New drug development for the control of trypanosomosis is unappealing for commercial reasons (Trouiller and Olliaro, 1999; Murray *et al*., 2000) hence; it is pertinent to find how the old drugs can do their therapeutic best.

Dimethyl Sulfoxide (DMSO) has antioxidant properties being a free radical scavenger with high degree of specificity for the damaging hydroxyl radical (Panganamala *et al*., 1976; Santos *et al*., 2003). The cardiovascular protective effect of DMSO in copper-deficient rats is thought to occur by an antioxidant mechanism (Yokoi *et al*., 1990). With the allusion that oxidant/antioxidant imbalance contributes to the pathogenesis of trypanosomosis, ascorbic acid, an antioxidant has been combined with diminazene in the treatment of *T. brucei* infection. Treatment with diminazene/ascorbic acid combination led to better remission of the disease than diminazene alone (Eghianruwa *et al*., 2009). The result with ascorbic acid indicated that trypanocide/antioxidant combination may enhance efficacy of trypanocides. Hence, this study was undertaken to elucidate the influence of DMSO (a compound with antioxidant properties) on diminazene efficacy in experimental *T. brucei* infection.

MATERIALS AND METHODS

Eighty Wistar albino rats (90-110 g) of mixed sexes procured from the Nigerian Institute for Trypanosomiasis Research, Vom, Nigeria were housed in standard rat cages in a fly proof house. The animals were humanely cared

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for in compliance with the principles stipulated in the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 2010). The study was conducted at the University of Ibadan, Nigeria in 2010 and the institution's ethics committee approved and monitored the protocol. Animals were fed ad libitum with commercially formulated 8 mm pelletized mouse cubes (Ladokun Feeds, Ibadan, Nigeria). Water was provided *ad libitum* using plastic bottles equipped with sipper tubes. Excess feed and water were removed and replaced with fresh ones daily.

Animals were divided into six groups (I-VI) and allowed to acclimatize for 2 weeks before commencement of experiments. Group I (n = 5) served as uninfected control. Groups II to VI which consisted of 15 rats each were infected with the Federe strain of *T. brucei brucei* obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Vom, Nigeria where it was preserved in liquid nitrogen. The parasites were maintained in rats by serial passage after removal from liquid nitrogen. Infection of experimental animals was by intraperitoneal injection of $1x10^6$ trypanosome cells obtained from the tail blood of a rat showing heavy parasitemia. The blood was diluted in normal saline to obtain trypanosome concentration of 1×10^5 per mL. Each animal received 0.1 mL of the diluted blood.

Seven days post infection (PI_7), the infected rats in groups III, IV, V, and VI were treated once with 7.0 mg/kg diminazenediaceturate (Trypadim®, Merial, France) injected intramuscularly. The rats in group II were untreated (infected control). Beginning from the day of diminazene treatment (PI_7; DOT), rats in groups IV, V and VI received daily oral supplement of dimethyl sulfoxide (DMSO) (BDH Laboratory Reagents, England) at 0.5, 1.0 and 2.0 g/kg, respectively in addition to the single diminazene treatment. The DMSO required by each group per day was dispensed in small volumes of water. Fresh water was given after medicated water was exhausted.

Five rats in group II were sacrificed on PI_7 to generate data for the day of diminazene treatment (DOT). The three surviving rats in the group were sacrificed on PI_{14}. Five rats were sacrificed from each of groups III-VI on days 7, 14 and 21 post diminazene treatment. The sacrificed animals were euthanized with chloroform (BDH Laboratory Reagents, England) inhalation. Following euthanasia, blood was withdrawn from the hearts into sample bottles containing Na_2EDTA. The liver, heart and spleen were dissected out and immediately weighed.The organs were then processed routinely for histopathology and sections stained with hematoxylin and eosins were examined with the light microscope.

Changes in parasitemia; Packed Cell Volume (PCV); Haemoglobinemia (Hb); Erythrocyte (RBC) count; leukocyte (WBC) count; weights of the liver, spleen and heart were measured to determine the effect of DMSO supplementation on diminazene efficacy. The PCV, Hb and RBC, WBC were determined using established methods as described by (Walters *et al*., 1986). The rapid matching method described by (Herbert and Lumsden, 1976) was used to estimate parasitaemia in blood samples.

ANALYSIS OF RESULTS

The differences in the means of parameters were analyzed statistically with Instat® software (GraphPad Inc., USA) using one-way analysis of variance (ANOVA) (for $\geq$3 means) and two-tailed p-value (for 2 means). Probability values less or equal to 0.05 ($p\leq0.05$) were considered significant.

Infection effects: Parasites appeared in the blood of infected rats from PI_4. The first deaths were recorded on PI_9. By PI_{14}, only three of the fifteen infected animals in group II (infected control) survived; five were sacrificed on PI_7; while seven died between PI_9 and PI_{12}. *Trypanosoma brucei brucei* caused significant ($p<0.05$) increases in liver and spleen weights by PI_7. There were no significant ($p>0.05$) increases in heart weight. Pack cell volume, haemoglobin content and RBC counts were significantly ($p<0.05$) reduced by the infection by PI_7. The significant ($p<0.05$) increase in WBC count on PI_7 was significantly reversed by PI_{14} (Fig. 1).

By PI_{14}, liver weight, PCV, Hb and RBC in group II had increased by 8.15, 6.35, 3.93, and 13.68%, respectively from their values on PI_7 (Table 1). On the contrary, on PI_{14}, spleen weight and WBC count were respectively 21.81 and 47.20% less than the values recorded on PI_7. The changes in WBC count between PI_7 and PI_{14} were significant ($p<0.05$). The histopathological lesions observed on PT_{21} include moderate hyperplasia of Kupffer cells with haemosiderin pigments, diffused vacuolation of hepatocytes and moderate lymphocytic and mild neutrophilic infiltrates in the portal triad of the liver (Fig. 2). Germinal center hyperplasia, proliferation of the

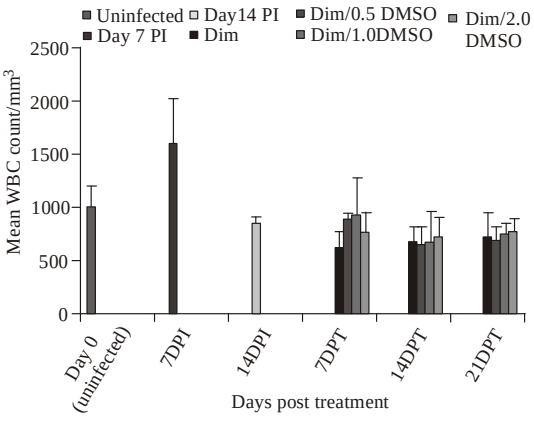

Fig. 1: Effects of DMSO supplementation on WBC count in *T. b. brucei* infected and diminazene treated rats

Table 1: Effect of *T. b. brucei* infection on organ weights and haematology in infected and untreated rats. Values are expressed as Means±S.D. Values in a row with different superscripts differ significantly (p<0.05)

		Infected; untreated	
Parameter	Uninfected (n = 5)	PI_7 (n = 5)	PI_{14} (n = 3)
Liver weight (% BW)	3.58±0.33*	5.07±0.35♣	5.52±0.75♣
Spleen weight (% BW)	0.36±0.08*	2.98±0.61♣	2.33±0.46♣
Heart weight (%BW)	0.35±0.06*	0.31±0.06*	0.37±0.06*
PCV (%)	37.5±2.63*	25.75±4.1♣	27.5±2.12♣
Hb (g/dL)	11.8±0.73*	8.55±1.21♣	8.9±0.57♣
$RBCx10^6/mm^3$	7.07 ± 0.53*	4.48±0.47♣	5.19±0.03♣
WBC/mm^3	10,025.00±2,080.11*	16,100.00±4,177.72♣	8,500.00±707.11§

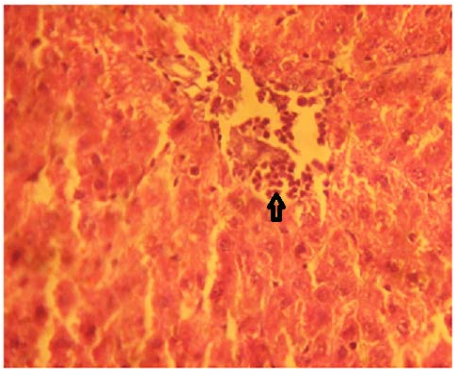

Fig. 2: Photomicrograph of rat liver showing periportal cellular infiltration on day 21 following infection with *T. b. brucei* (H&E) × 25

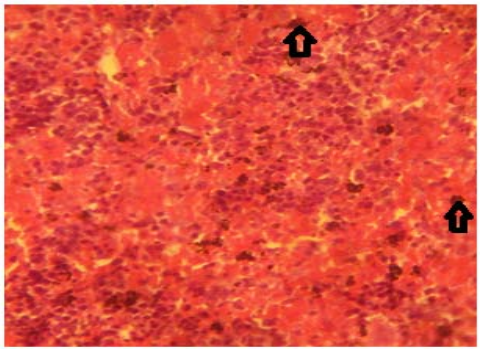

Fig. 3: Photomicrograph of rat spleen showing haemosiderosis (arrow) on day 21 following infection with *T. b. brucei*. (H&E) × 25

reticular tissue, macrophages and plasma cells and marked deposits of haemosiderin pigments were observed in the spleen (Fig. 3). No histopathological lesions were seen in the heart.

Treatment effects on liver weight and histopathology: Treatment with diminazene alone and in combination with DMSO resulted in marginal, insignificant (p>0.05) reduction in liver weight from the DOT value of 5.07±0.35% BW by PT_7 (Fig. 4). The variations in the means of liver weight between the treated groups on

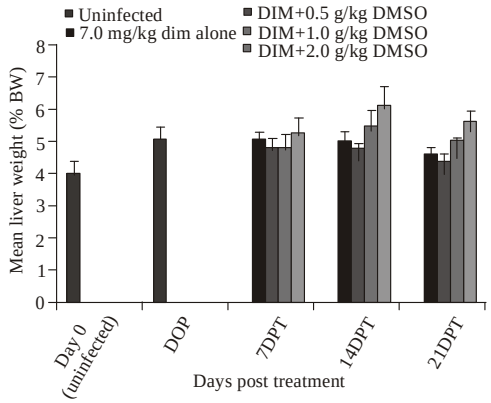

Fig. 4: Effects of DMSO daily supplementation on liver weight in *T. b. brucei* infected and diminazene treated rats

PT_7were also not significant (p>0.05). There were no significant differences (p>0.05) in the mean values of liver weight on $PT_{14}$and PT_{21}in both the diminazene alone and diminazene/DMSO groups. The mean liver weight values of 4.57±0.41 and 4.33±0.55% BW on PT_{21} in the diminazene and diminazene/0.5 g/kg DMSO groups respectively were significantly (p<0.05) lower than the value on DOT. Increase in the dose of DMSO from 0.5 to 1.0 and 2.0 g/kg gave generally higher mean liver weights. The mean liver weight values of 6.08±0.20 and 5.67±0.35% BWon PT_{14} and PT_{21}, respectively in the 2.0 g/kg DMSO supplemented group was significantly (p<0.05) higher than the values recorded in the diminazene, diminazene/0.5g/kg DMSO groups and on DOT. Histopathological lesions were observed by PT_{21} in both the diminazene and diminazene/DMSO groups.

Diminazene/DMSO effects on spleen weight and histopathology: The prominent increase in spleen size and weight caused by the infection with *T. brucei* was significantly (p<0.05) reduced from the DOT value on PT_7 by diminazene and diminazene/DMSO combinations without any significant (p>0.05) differences in the reduction caused by the two treatment options (Fig. 5).

The combinations of diminazene with 1.0 and 2.0 g/kg DMSO caused marginally lower but statistically

Table 2: Effects of diminazene alone and diminazene/DMSO combinations on hematological parameters in *T. b. brucei* infected rats. Values are expressed as Means±S.D. PI = post infection; PT = post treatment while superscript indicate the day; Values in a column with different superscript differ significantly ($p<0.05$)

		Parameter		
Treatment group	Test day	PCV (%)	Hb (g/dL)	RBC ($x10^6/mm^3$)
Uninfected		39.75±2.22 *	13.45±0.76 *	6.87±0.48 *
DOT (PI_7)	PI_7	25.75±4.10 ♣	8.55±1.21 ♣	4.48±0.47 ♣
Diminazene (7 mg/kg)	PT_7	39.50±3.42 *	12.18±1.21*	6.96±0.75*
	PT_{14}	41.75±4.19 *	13.45±1.43*	7.36±0.54*
	PT_{21}	47.25±0.96 §	15.18±0.56§	7.92±0.38§
Diminazene + 0.5 g/kg DMSO	PT_7	37.00±2.16 *	12.65±0.53*	6.35±0.52*
	PT_{14}	45.00±2.45 §	14.7±0.68§	7.88±0.44§
	PT_{21}	45.00±2.45 §	14.7±0.68§	7.88±0.44§
Diminazene + 1.0 g/kg DMSO	PT_7	38.75±2.99 *	12.95±1.02*	6.75±0.46*
	PT_{14}	41.25±3.95 *	13.22±1.18*	7.70±0.50§
	PT_{21}	46.25±4.27 §	15.00±1.42§	7.84±0.71§
Diminazene + 2.0 g/kg DMSO	PT_7	34.75±2.99 ¤	11.73±1.11 ¤	6.16±0.40*
	PT_{14}	42.33±4.04 ¤	13.33±1.68*	7.16±0.39*
	PT_{21}	44.50±1.50 §	15.05±0.35§	7.47±0.16§

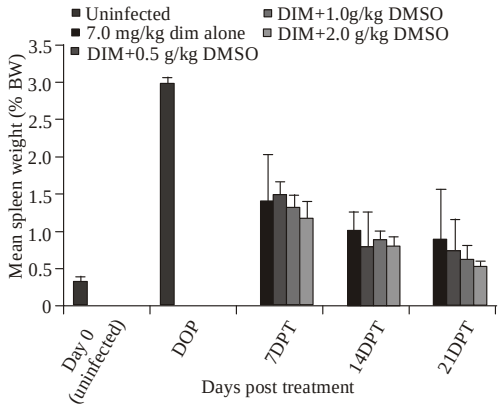

Fig. 5: Effects of DMSO daily supplementation on spleen weight in *T. b. brucei* infected and diminazene treated rats

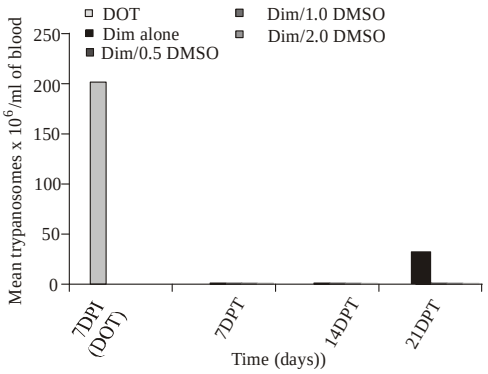

Fig. 6: Effects of DMSO daily supplementation on parasitemia in *T. b. brucei* infected and diminazene treated rats

insignificant ($p>0.05$) reduction in spleen weight compared to that of diminazene treatment alone. The mean values of spleen weight on PT_7 in all treated groups were significantly ($p<0.01$) higher than the values obtained on PT_{14}. However, there were no significant ($p>0.05$) differences in the mean values of spleen weight between the groups treated with diminazene alone and those with diminazene/DMSO combinations. On PT_{21}, diminazene plus 2.0 g/kg DMSO produced significant ($p<0.05$) reduction of mean spleen weight from PT_{14} value. The mean spleen weight of 0.52±0.01% BW in the diminazene/2.0 g/kg DMSO group on PT_{21} was the lowest recorded and the value was significantly ($p<0.05$) lower than that recorded in the group which received diminazene treatment alone.

Diminazene/DMSO effects on hematology and parasitemia: Treatment of *T. b. brucei* infection with diminazene and diminazene/DMSO combinations significantly ($p<0.01$) reversed the depressed PCV, Hb and RBC by PT_7. There were no significant ($p>0.05$) differences in the mean values of PCV, Hb and RBC in both the diminazene and diminazene/DMSO groups on 7 PT_7. These hematological parameters fully returned to normal by PT7 in all treated groups as there was no significant ($p>0.05$) difference between the values in the treated groups and uninfected rats (Table 2). The parameters also rose steadily from PT_7 to PT_{21}. The PCV and Hb concentration in all treatment groups on PT_{21} were significantly ($p<0.001$) higher than the values recorded in normal rats.

The increase in mean WBC count was reversed within seven days in untreated rats. Treatment with diminazene with and without DMSO also caused significant reduction in WBC count by PT_7. The mean WBC count was significantly ($p<0.05$) lower in the group treated with diminazene alone than in the diminazene/DMSO groups. There were no statistical differences in the mean WBC count in all treated groups by PT_{14} and PT_{21} (Fig. 1).

Diminazene alone and in combinations with DMSO were effective in clearing parasites from the blood by PT_7.

Infection relapsed only in the diminazene treated group by PT_{21} (Fig. 6).

DISCUSSION

The reduced PCV, Hb and RBC levels which are indicative of anemia have been reported consistently by other workers (Losos and Ikede, 1972; Murray, 1979; Jenkins and Facer, 1985; Bengaly *et al*., 2002; Kaikabo and Salako, 2006; Toma *et al*., 2008; Eghianruwa *et al*., 2009). The observed increases in the weights of liver and spleen are in agreement with those of Morrison *et al*. (2005) and Kaikabo and Salako (2006). The histopathological lesions observed in this study have also been described earlier (Eghianruwa *et al*., 2009). The absence of lesion in the heart which is reported here is contrary to the observation of (Kaikabo and Salako, 2006) but consistent with earlier report (Eghianruwa *et al*., 2009). The infection with *T. brucei* in this study produced leukocytosis followed within seven days by leucopenia. This pattern of response of WBC has been reported in *T. evansi* infection in dogs in which leucocytosis was detected on day 6 and leucopenia on day 12 post infection (De La Rue *et al*., 2000). Toma *et al*. (2008) reported leucocytois in *T. congolense* infected rabbits. Leucopenia has been reported by several authors (Losos and Ikede, 1972; Anosa, 1988). The discrepancies in these reports may be attributed, in part, to the time of observation. Early response to trypanosome infection has been shown to involve cellular proliferation especially of lymphocytes (De La Rue *et al*., 2000). The level to which this response proceeds is thought to be due to the level of tolerance. It has been postulated that tolerant animals develop leukocytosis and non-tolerant ones develop leucopenia (Paling *et al*., 1991).

Diminazene alone or in combination with DMSO supplementation was ineffective in reducing liver weight but both treatment protocols caused consistent reduction in spleen weight. Treatment with diminazene plus daily supplementation with 2 g/kg DMSO was more effective on the long run in reducing spleen weight since the mean spleen weight on day 21 PT was significantly lower than the corresponding value in the group treated with diminazene alone. This result is contradictory to that obtained with the liver in which increase in the dose of DMSO exacerbated the increased liver weight caused by the infection. There is no data in the literature on the combination of DMSO and a trypanocide but this observation have been made in *T. congolense* infection and its cause attributed, at least in part, to hepatotoxicity by DMSO (Eghianruwa and Anika, unpublished data). This observation has also been made when diminazene was combined with high doses of ascorbic acid in *T. brucei* infection (Eghianruwa *et al*., 2009). Apart from hepatotoxicity induced by high doses of DMSO, these observations may be due to the fact that certain antioxidants become prooxidants at high doses. This condition has been documented in the case of vitamins E and C (Kontush *et al*., 1996; Kraus *et al*., 2004; Eze and Ochike, 2007).

Diminazene alone or in combination with DMSO was ineffective in healing the histopathological lesions observed in the liver and spleen. These results are contradictory to those obtained with ascorbic acid in which diminazene treatment followed by supplementation with 200 mg/kg ascorbic acid was effective in healing the hepatic and splenic histopathological lesions (Eghianruwa *et al*., 2009).

Treatments with diminazene alone and in combination with DMSO were equally effective in completely reversing the depressed PCV, Hb and RBC levels due to the infection. Drug treatment did not appear to have significant effect on WBC count since the leucocytosis recorded on PI_7 spontaneously reverted to leucopenia by PI_{14} without drug treatment. The levels of WBC in the untreated group by PI_{14} and in the treated groups by PT_7 (14 days after infection) were not significantly different.

Treatment with diminazene followed by DMSO supplementation appears to have superiority over treatment with diminazene alone in reducing spleen weight and delaying relapse. Both treatments were equally effective in restoring hematological values. The ineffectiveness in restoring the liver at low doses and possible hepatotoxicity at high doses makes diminazene/DMSO combination an unlikely candidate in the therapy of trypanosomosis. This is further reinforced by the knowledge that DMSO in combination with diminazene had no outstanding effect over diminazene treatment alone in *T. congolense* infection (Eghianruwa and Obidike, 2011).

ACKNOWLEDGMENT

The authors acknowledge the technical support of Mrs. J. Ademakinwa and Mrs. A. F.Adeyemi, staff of Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Ibadan. The technical contribution of staff of the histopathology laboratory, Faculty of Veterinary Medicine, University of Ibadan, Messrs J. U. Augustin, A. O Nwagbara and I. C. Mpama is highly appreciated.

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