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Original Research Article

Comparative evaluation of the chemotherapeutic efficacies of two salts of diminazene aceturate in *Trypanosoma brucei brucei* infected dogs

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Abstract

Purpose: *To compare the anti-trypanosomal efficacies of 4,4-(diazoaminedibenzamidinetrihydrate) diacetate (4,4-DDBT) and 4,4-(diazoamino) benzamidine (4,4-DB) in experimental canine trypanosomosis.*

Methods: *The efficacies of 4,4-DDBT and 4,4-DB were evaluated in 4 groups of dogs (n = 3) designated A-D. Group A was normal control without infection or drug treatment, group B did not receive any drug treatment but was infected with Trypanosoma brucei brucei, while groups C and D were infected with T. b. brucei and treated with 4,4-DDBT(3.5 mg/kg) and 4,4-DB (3.5 mg/kg), respectively.*

Results: *The incubation period of the infection was 6 - 9 days post-infection. Treatment of the dogs with 4,4-DDBT led to zero parasitaemia 48 h post-treatment, while there was only a decrease in parasitemia to log 6 in 4,4-DB-treated dogs. Resurgence of parasite into the blood stream occurred in 4,4-DDBT-treated dogs 6 days after initial parasite clearance. Blood analyses post-treatment revealed elevated leucocytes and lymphocytes in 4,4-DB-treated dogs ($p < 0.05$). Packed cell volume was also observed to be higher in 4,4-DDBT-treated group when compared to 4,4-DB group ($p < 0.05$).*

Conclusion: *These findings suggest that 4,4-DDBT is more efficacious in the clinical management of canine trypanosomosis caused by T. b. brucei. However, it does not prevent relapse of infection. Based on these findings, therefore, 4,4-DDBT should be the diminazene salt of choice when indicated in the clinical management of T. b. brucei infection in dogs.*

Keyword: *Dogs, Trypanosomes, 4,4-(Diazoamino) benzamidine, 4,4-(diazoaminedibenzamidinetri-hydrate) diacetate*

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INTRODUCTION

Trypanosomes are one of the endemic heamoprotozoan parasites that cause severe mortality in domestic dogs in Nigeria. This is due to the ubiquitous nature of *Glossina spps* in the country [1,2]. Human and animal African trypanomosis are usually fatal without

chemotherapy [3]. In veterinary medicine, diminazeneaceturate (DA) and isomethamidium chloride are the trypanocides commonly employed for curative and prophylactic regimens respectively [4]. DA is the most common and available trypanocide used in the treatment of trypanosomosis in dogs. Unfortunately, the drug is outmoded and no new drug has been produced against the organism [5]. It has been reported that the recommended dose of DA; 3.5 – 7mg/kg for canine trypanosomosis caused by *Trypanosoma b. brucei* does not clear the parasite from the animal's system [6] and this has been linked to resistance by trypanososmes [7].

The total dependence on drug in the treatment of trypanosomosis has led to the emergence of resistant trypanosomes [8]. Rapid development of resistance by trypanosomes to DA has been reported in different species of animal [1,9,10]. This problem of resistance is of high public health importance, because dogs live in close proximity to humans, and, therefore, can easily transmit the resistant trypanosomes to man [11]. Resistance to trypanocide is usually manifested as relapse of infection. Variability in the quality of DA in the market has been reported with majority of the brands containing less than the active compound (DA) as indicated on the label [1]. Substandard preparations at lower price have led to the development of therapeutic failure and possible emergence of DA resistant trypanosomes [12]. However, apart from drug quality, other factors such as under-dosing and rapid re-exposure after treatment with therapeutic agents with short duration of action may also lead to development of resistance [9].

In Nigeria, trypanosomosis is one of the commonest endemic diseases severely affecting domestic dogs. The issue of relapse of infection is now commonplace and has resulted in the infected dogs being treated severally with the same drug because there is limited range of trypanocides. This study compared the efficacies of two salts of DA; 4,4-DDBT and 4,4-DB in the chemotherapy of canine trypanosomosis caused by *Trypanosoma b. brucei*, with a view of achieving complete cure without relapse infection.

EXPERIMENTAL

Animals

Twelve (12) clinically healthy male dogs between the ages of 6 to 12 months were used. The dogs were housed in the Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria dog kennel protected from flies. The dogs were fed with pelletized dried dog food twice daily and water was provided *ad libtum*. The dogs were allowed 3 weeks for acclimatization. The dogs were dewormed using Prasizam© (combination of Febentel, Pyrantelpalmoate and praziquantel). They were vaccinated against rabies and then DHLPP (distemper, parvoviral enteritis, parainfluenza, canine hepatis and leptospirosis polyvalent vaccine). They were then screened for blood parasites before the commencement of the experiment. The protocol for the experiment is in compliance with National Institute of Health (NIH) guidelines [13] and received approval from the Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (approval no: UNFVM/08/18/7).

Trypanosome parasite

Trypanosoma brucei brucei used was the *federe* strain obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The dogs were infected with 1 million trypanosomes suspended in 1 ml of phosphate buffered saline (PBS).

Drugs

The brand containing 4,4-DB is a commonly used trypanocide which costs about $0.69per sachet. Each sachet contained 2.36g and administered as a 7% solution (3.5 mg/kg) intramuscularly stat. The brand containing 4,4-DDBT is a new trypanocide recently introduced into the Nigerian market. It is sold at $8.33 per vial. Each vial contains 1.0 g powder which is administered as a 5% solution (3.5 mg/kg) intramuscularly stat.

Experimental design

The dogs were assigned to 4 groups (A-D) (n = 3). Group A (uninfected untreated), each dog in groups B-D was infected with 1 million trypanosomes intraperitoneum. After infection, group B was left untreated, group C and D dogs were each treated with 4,4-DDBT and 4,4-DB respectively following onset of parasitemia 10 days post-infection. Parameters such as survivability/clinical signs, PCV, hemoglobin concentration (Hb), parasitaemia, temperature, heart rate, weight, erythrocyte count, total and differential leucocyte count were used to evaluate the efficacy of the drugs. The clinical signs, parasitaemia, heart rate and temperature were monitored daily, while PCV, Hb, erythrocyte count and total and differential leucocyte counts were obtained 0, 7, 14 and 21 days post-infection.

Collection of blood samples

Blood samples were obtained through the cephalic vein using 21 gauge needles. Blood samples for haematology (1 ml) was collected into EDTA sample bottles, while 0.1 ml for evaluation of parasitaemia was collected directly into a microscope slide and covered with cover slip.

Parasitaemia was quantified using standard technique [14]. Hemoglobin concentration, PCV, total and differential leucocyte counts, and erythrocyte counts were determined using standard techniques [15,16].

Data analysis

Data generated were presented in figures and tables as mean with standard errors of means. Analysis of variance was used to analyze the data. Duncan multiple range tests were used to separate variant means, and $p < 0.05$) was considered significant.

RESULTS

Clinical signs and parasitological finding

The incubation period observed in this study was 6 - 9 days post infection. Clinical signs such as anorexia, pale mucous membrane, depression, reluctance to move and pyrexia were observed in the infected dogs. These observed clinical signs disappeared following treatment in the treated groups after parasitaemia disappeared in group treated with 4,4-DDBT (group C) or declined in those treated with 4,4-DB (group D). The clinical signs gradually returned 6 days post-treatment when the infection relapsed. However, in the infected untreated group (group B), the signs gradually developed into more serious signs such as severe emaciation, ocular and nasal discharges, edema, enlarged abdomen and death.

Parasites cleared from the blood of dogs treated with 4,4-DDBT 48 h post-treatment and the dogs remained aparasitaemic until day 6 post-treatment when relapse occurred and parasitaemia gradually returned. There was a decline but not total clearance of parasitaemia (log 6.9) in dogs in group D and level of parasitaemia continued to fluctuate until the experiment was terminated on day 21. Parasitaemia was high, fluctuating and very evident in infected untreated dogs and eventually led to their death between days 17 - 19 post-infection (Figure 1).

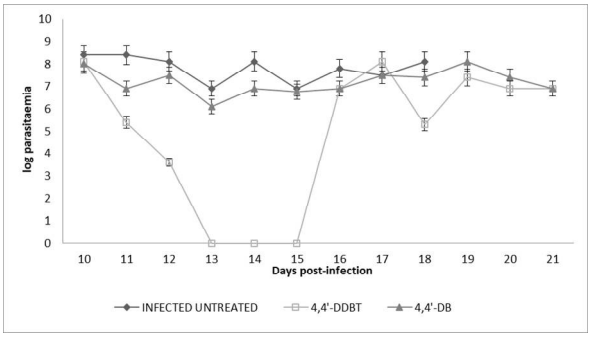

Figure 1: Effect of 4,4-DDBT and 4,4-DB on parasitaemia in *T. b. brucei* infected dogs

Effect of 4,4-DDBT and 4,4-DB on rectal temperature in *T. b. brucei* infected dogs

Twenty-four hours post-treatment, rectal temperatures of dogs in groups C and D declined significantly ($p < 0.05$) in relation to dogs in group B and this was maintained until day 16 post-infection when relapse occurred. However, dogs treated with 4,4-DB had lower temperatures when compared with dogs treated with 4,4-DDBT (Figure 2).

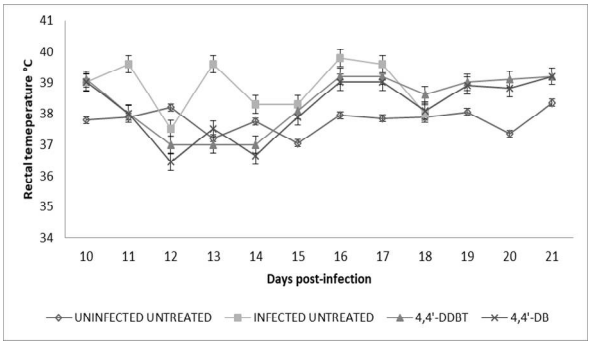

Figure 2: Effect of 4,4'-DDBT and 4,4'-DB on rectal temperature of *T. b. brucei* infected dogs

Effect of 4,4'-DDBT and 4,4'-DB on Heart rate of dogs in experimental *Trypanosoma. b. brucei* infection

Heart rate of dogs in group B was significantly ($p < 0.05$) increased from day 12 to 14 post-infection. However, lower heart rate ($p < 0.05$) was recorded in dogs in group C 11 days post-infection compared to those in groups B and D (Figure 3).

Hematological findings

Red blood cell counts, PCV and hemoglobin (Hb) concentration

Following infection, the RBC number, PCV and

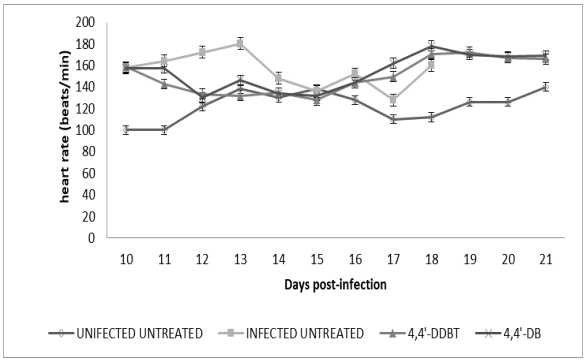

Figure 3: Effect of 4,4-DDBT and 4,4-DB on the heart rate of dogs in experimental *Trypanosoma b. brucei* infection

Hb concentration declined ($p < 0.05$) in all the infected dogs on day 7 post-infection.

Nevertheless following institution of treatment, there was an increase ($p < 0.05$) in the level of these erythrocytic indices in groups C and D dogs on day 14, though the levels of RBC in groups C and D were still lower ($p < 0.05$) than those of dogs in group A on day 21. Meanwhile, the PCV of dogs in group C was higher ($p < 0.05$) than those of group D on day 21; however, there was no difference ($p > 0.05$) in their RBC number and Hb concentrations (Table 1).

White blood cells

By day 7 post-infection, the total WBC count of all the infected dogs had significantly decreased in relation to those of dogs in group A. Although, treatment with either 4,4-DDBT or 4,4-DB lead to significant increase of the WBC of the treated dog, those of the untreated remained low on day 14 post-infection. Nevertheless, by day 21, the total WBC of dogs treated with 4,4-DB was significantly higher than those of groups A and C (Table 2).

Differential white blood cell count

Infection of dogs with *Trypanosoma b. brucei* lead to increase ($p < 0.05$) in the lymphocyte

Table 1: Effect of treatment with either 4,4-DDBT or 4,4-DB on the RBC, PCV and Hb concentration of dogs infected with *Trypanosoma b. brucei*

Parameter	Days post-infection			
	0	7	14	21
RBC (x 10^6 /µL)				
A	4.6±10.4	4.8±13.6 [a]	4.8±80.0[a]	4.8±13.9 [a]
B	4.8±[34].1	3.0±30.0 [b]	2.6±22.6 [b]	NA
C	4.6±24.9	3.1±37.2 [b]	4.1±17.7[a]	3.6±90.2 [c]
D	4.7±63.7	3.2±62.6 [b]	3.9±14.0[a]	3.3±53.9 [c]
PCV (%)				
A	41.5± 0.50	41.0± 0.6[a]	42.5± 6.5 [a]	42.5±0.3 [a]
B	40.6±6.76	34.5±0.5[b]	20.0±0.2[b]	NA
C	41.0± 0.57	33.0±1.2[b]	30.3± 1.5 [c]	28.6±1.5 [c]
D	40.6 ±4.05	34.6±2.2[b]	28.5±3.5 [c]	18.3±2.3 [d]
Hb conc. (g/dL)				
A	13.2±2.6	13.5±3.3[a]	13.7±2.7 [a]	13.9±1.5 [a]
B	13.1±0.5	9.5±1.3 [b]	6.9±0.3 [b]	NA
C	13.2±0.6	9.0±1.7[b]	11.0±0.2 [a]	9.2±0.3 [c]
D	13.3±0.2	9.6±2.7[b]	10.6±1.0 [a]	8.1±0.2 [c]

[a,b,c]*p < 0.05; NA: not available because the dogs died. A: uninfected, untreated, B: infected untreated, C: 4,4-DDBT (3.5 mg/kg), D: 4,4-DB (3.5 mg/kg)*

Table 2: Total WBC (x 10^3 /µL) count of dogs infected with *T. b. brucei* and treated with either 4,4-DDBT or 4,4-DB

	Days post-infection			
	0	***7***	***14***	***21***
A	18.2±0.6	18.2±2.4[a]	18.2±2.5[a]	18.1±0.7 [a]
B	17.9±3.2	15.9±2.2[b]	12.0± 0.2[c]	NA
C	18.6±4.3	16.8±4.5[b]	21.7±7.6[b]	21.9±0.3[c]
D	17.6±1.9	16.0±7.1[b]	20.7±4.2 [b]	24.6±3.1 [b]

[a,b,c]*p* < 0.05; .NA: not available because the dogs died. A: uninfected untreated, B: infected untreated, C: 4,4-DDBT (3.5 mg/kg), D: 4,4-DB (3.5 mg/kg)

Table 3: Lymphocyte and neutrophil counts of *T. b. brucei* infected dogs treated with 4,4-DDBT and 4,4-DB

Variable	Days (post-infection)			
	0	***7***	***14***	***21***
Lymphocytes(x10^3 /µL)				
A	13.7±0.1	13.1±2.3[a]	13.1±4.3[a]	13.4±0.5[a]
B	13.7±2.5	17.0±2.0[b]	23.2±2.1[b]	NA
C	13.5±2.4	16.8±3.4[b]	21.7±0.1[b]	14.0±0.4[a]
D	12.9±1.8	16.9±1.1[b]	20.0±2.6[b]	19.2±1.2[b]
Neutrophil (x 10^3/µL)				
A	4.9±1.2	5.0±0.3[a]	5.0±0.8[a]	5.1±0.5[a]
B	5.3±0.6	3.3±2.2[b]	1.9±2.0[b]	NA
C	5.9±2.1	4.8±01.1[a]	3.5±0.4[c]	2.8±0.0[c]
D	5.2±0.3	4.9±1.8[a]	2.8±0.2[c]	2.1±1.3[c]

[a,b,c]*p* < 0.05;.NA: not available because the dogs died. A: uninfected untreated, B: infected untreated, C: 4,4-DDBT (3.5 mg/kg), D: 4,4-DB (3.5 mg/kg)

Table 4: Body weights (kg) of dogs infected with *T. b. brucei* and treated with 4,4-DDBT or 4,4-DB

	Days (post-infection)			
	0	***7***	***14***	***21***
A	5.7±0.3	5.9±0.3[a]	6.3±0.3[a]	6.2±0.8[a]
B	5.8±0.3	4.7±0.3[b]	3.6±0.0[c]	NA
C	5.8±0.8	4.6±0.5[b]	4.3±0.3[b]	4.1±0.2[b]
D	5.7±0.8	4.3±0.7[b]	4.0±0.8[b]	4.2±0.5[b]

[a,b]*p* p < 0.05;.NA: not available because the dogs died. A: uninfected untreated, B: infected untreated, C: 4,4-DDBT (3.5 mg/kg), D: 4,4-DB (3.5 mg/kg)

counts of all the infected dogs from day 7 post-infection as against those of the uninfected dogs. However, by day 21, Lymphocyte counts of the dogs treated with 4,4-DB were significantly higher than those of groups A and C (Table 3).

Effect of 4,4-DDBT and 4,4-DB on body weight of dogs experimentally infected with *Trypanosoma brucei brucei*

There was a significant (p <0.05) decrease in the mean body weight of dogs in group B compared to groups A, C and D dogs on day 14. There was no significant difference in weight between Dogs in groups C and D, but dogs in group A had significantly higher body weight than those in groups C and D on days 14 and 21.

DISCUSSION

In this study, 4,4-DDBT showed a better therapeutic effect than 4,4-DB in the clinical management of *T. b. brucei* infection at the dose of 3.5mg/kg in dogs. However, 4,4-DDBT could not prevent relapse of the infection in the treated dogs indicating resistance of the trypanosomes to the drug.

The establishment of parasitaemia in this study was observed between day 6-9 post-infection, which continued to fluctuate especially in infected untreated group. This fluctuating pattern has been reported by several authors as one of the characteristics of trypanosomosis [1,17]. This is as a result of the ability of the trypanosomes to continuously change its surface antigen. This observation agrees with other works done on *T. b. brucei* infection in animals [18]. There was a total clearance of parasitaemia in group treated with 4,4-DDBT within 48 h post-treatment. However, 4,4-DB could not clear the parasitemia, rather, it caused a decrease to log 6.9. This finding is consistent with the reports of previous researchers who observed that DA does not clear trypanosomes from the animal's system [6].

The efficacy of 4,4-DDBT is, therefore, superior to that of 4,4-DB. Relapse of infection occurred in the 4,4-DDBT treated dogs, indicating that *T. b. brucei* is resistant to this salt of DA. There have been reports on the relapse of infection by *T. b. brucei* after treatment with DA [1, 7, 18]. Reports of relapse of *T. b. brucei* infection have been attributed to the presence of *T. b. brucei* in drug-inaccessible sites such as the brain; and this often occurs when there is a prolonged period between infection and treatment [18] such that the parasite migrates to brain and after treatment recede from the brain to the blood stream [19].

However, in the present study, the treatment was started on day 10; a day after parasitaemia was established, suggesting that the parasites may not have invaded the brain as treatment was started immediately. Therefore, the relapse may

not be as a result of the parasites resurfacing into the blood stream from the brain but may be attributed to other factors such as mutation, amplification or deletion, altered drug uptake, drug metabolism, drug-target interaction or efflux [3].

The undulating pyrexia observed in the infected groups could have been responsible for the observed anaemia because erythrocytes were exposed to temperatures higher than normal body temperature which led to increased RBC destruction [20]. High body temperature can equally lead to increased rate of immunochemical reactions with subsequent increase in lipid peroxidation of RBCs.[20].

The 4,4–DB was better at controlling the observed high temperature when compared to 4,4-DDBT because of its antipyrine content. The increased heart rate observed in the infected groups was better controlled by 4,4-DDBT than 4,4-DB. This could be because 4,4'-DDBT was better at ameliorating anaemia than 4,4-DB and this could be related to the more efficient control of parasitaemia by 4,4-DDBT.

Increased heart rate during anaemia is mainly due to compensatory mechanisms of the heart to tissue hypoperfusion [21]. Anaemia observed in this study has been reported by many authors as a constant finding in trypanosomosis [1]. In this study, anaemia was observed in all the infected groups as evidenced by decreases in PCV, Hb concentration and RBC count by day 7 post-infection. However, these haematological parameters were higher in the group treated with 4,4-DDBT than in infected untreated and 4,4-DB treated group. This finding also indicated that 4,4-DDBT was more effective than 4,4-DB in the clinical management of *T. b. brucei* infection in dogs. The anemia in this study could be attributed to the overwhelming hemolytic activities of the trypanosomes [22].

Following infection with *T. b. brucei*, there was elevation of the total white blood cell count of the entire infected groups. Leucocytosis in trypanosomosis is usually as a result of lymphocytosis. This condition is usually an immunological response by the animal to trypanosome infection [22]. A decline in neutrophil count was observed in this study following infection with trypanosomes. This observation agrees with the findings of Allam *et al,* [23] and this may be as a result of overwhelming immunosuppression in the infected groups.

In this study, the clinical signs such as pale mucus membranes, rough hair coats, emaciation, depression, ocular and nasal discharges, edema, dullness and anorexia were observed. These observations were in agreement with the reports of Ezeokonkwo and Agu, who reported similar clinical signs in *T. b. brucei* infected rabbits [24]. These signs gradually disappeared after treatment in the treated groups. However, the disappearance of these signs were faster in dogs treated with 4,4-DDBT than in dogs treated with 4,4-DB. The decrease in body weight observed in the infected dogs may have direct relationship with anorexia experienced by the dogs during infection.

CONCLUSION

The findings show that 4,4-DDBT has greater efficacy than 4,4-DB in the clinical management of *T. b. brucei* infection in dogs. However, the effect of 4,4-DDBT was short-lived as there was relapse of infection in the treated dogs, indicating drug resistance. Therefore, further search for alternative trypanocide (other than DA) should be encouraged to curb the increasing resistance.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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