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Preliminary Report on the Effects of Varied Diminazene Diaceturate Dosages and Treatment Regimens on *Trypanosoma brucei* Clearance and Relapse in Experimentally Infected Wistar Rats

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ABSTRACT

Comparison of the diminazene diaceturate (DD) varied treatment regimen in male Wistar rats experimentally infected with Trypanosoma brucei was done. Forty-nine rats were randomly assigned to seven groups of seven rats each (A - G). Group A was the uninfected untreated control while groups B – G were infected with 1.4×10^6. Groups C and D were treated with 3.5 mg/kg DD once and for five consecutive days while group B was left untreated. Groups E and F were treated with 7 mg/kg DD once and for five consecutive days while group G was treated with 7 mg/kg DD and repeated after 14 days. The effect of varied doses and treatment regimen was evaluated using parasites clearance and relapse of infection. Parasitaemia was established 4 - 9 days post infection in all groups except for one rat in Group G. Trypanosomes were cleared 3 - 5 days post treatment (PT) in all the treated groups. Trypanosomes relapsed in groups C, D, E, F, and group G on days 14, 42, 25, 56, and 39 post treatment, respectively. Relapse also occurred in about 67% of the rats in Group D and 14% of the rats in Group F within 80 days post treatment (PT). Increasing the dosage and or extending the duration of treatment with DD, marginally delayed the clearance of parasite in the infected rats but delayed the onset of relapse and reduced its occurrence in the infected rats. The curative efficacy of DD in this study appeared to be dose dependent as increasing the dosage and duration of treatment elicited a better therapeutic outcome. However, further experimental trials are imperative in rats and other animal species.

Keywords: Diminazene diaceturate; Parasitaemia; Treatment regimen; Trypanosomosis; Relapse; Wistar rats

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INTRODUCTION

African trypanosomiasis is an infectious disease of humans and animals with similar aetiology and epidemiology [1]. It is caused by protozoan parasites of the genus *Trypanosome*, which is transmitted cyclically by the bite of an infected tsetse fly (*Glossina* spp) or mechanically by other haematophagus flies of the genera *Haemaptera*, *Tabanus* and *Stomoxys* [2]. Trypanosomes live and multiply extracellularly in blood and tissue fluids of the mammalian host [3]. Due to antigenic variation of trypanosomes, the prevention of trypanosomiasis through vaccination is difficult [4]. Thus, the eradication or control of trypanosomiasis is largely based on chemotherapy and chemoprophylaxis [5].

However, there are limited numbers of chemotherapeutic agents available for the treatment of animal and human trypanosomiasis [6]. Most drugs administered as per standard regimen appeared to be highly ineffective in the treatment of human or animal trypanosomiasis [7]. Therefore, a possible option for the achievement of adequate treatment and prevention of trypanosomiasis is to make the optimal use of existing drugs. Some of these options included the use of slow-release devices (SRD) of existing drugs [8], using repeated standard doses [9], varied doses [10] and combination therapy using Diminazene and other drugs such as Secinidazole [11] including other different drugs combinations, especially those with different modes of action.

Diminazene diaceturate (DD), a diamidine in the same family with diminazene aceturate, is a widely used trypanocide, which binds irreversibly to DNA or nucleotides and interferes with DNA formation and parasite replication [12]. The recommended therapeutic dosages for Diminazene are 3.5 – 7mg/kg body weight by deep intramuscular route [15], however, relapses have been recorded in dogs when 3.5mg/kg bodyweight and 7mg/kg body weight were used respectively [19]. It also interferes with aerobic glycolysis and DNA synthesis [13]. In addition, studies have shown that DD partly modulates host inflammatory/immune responses in a manner that dampens the excessive immune activation and the production of pathology-producing pro-inflammatory cytokines in experimental rodents [14]. Diminazene diaceturate is rapidly absorbed after intramuscular administration and appear to enter the cerebrospinal fluid (CSF) but at a much lower concentration in infected dogs than is found in the plasma of uninfected dogs [15, 16]. The CSF levels are higher in infected dogs with African trypanosomiasis than what is found in uninfected healthy dogs, probably due to meningeal inflammation [15]. Varied elimination half-lives of diminazene reported range from 10-30 hours in dogs, goats and sheep to over 200 hours in cattle [16]. Treatment of experimental *T. brucei* infection in animals with drugs becomes less effective as the interval between infection and treatment increases [17]. Besides, there are reports of treatment failures due to drug resistance [18] as well as the reported relapse of infection after treatment with the standard regimen of DD at 7.0 mg/kg body weight at two weeks interval in dogs [19]. Treatment failure or the return of parasitemia was attributed to the failure of the drug to cross the blood-blood barrier or due to sub-therapeutic doses [20]. However, in another study involving *Trypanosoma evansi* in cats and rats, it was reported that extending DD treatment dose and duration gave a better outcome [21, 22]. Trypanosomosis is one of the major problems confronting animal health and production in the humid tropic and sub-

Saharan Africa. However Diamidines (diminazene diaceturate and diminazene aceturate) appear to be the most preferable chemotherapeutic agents for the control of this disease [5]. Therefore, it has become imperative to investigate variable treatment options involving dosage variation and duration of therapy with the known DD to mitigate the age-long devastating disease condition among animals in these parts of the world. This study aims at evaluating the effects of varied diminazene diaceturate dosages and treatment regimens on *Trypanosoma brucei* clearance and relapse in experimentally infected Wistar rats

MATERIALS AND METHODS

Drug

Diminazene diaceturate granules (1.05 g in 2.36 g sachet) were procured from Sequent Scientific Limited (A-68, Additional Ambemath, MIDCIndl. Area, Ambemath (E), India).

Experimental Animals

Forty-nine apparently healthy Wistar rats aged between 5 - 6 months and weighing between 100 – 232 g were used in the study. They were conditioned to the laboratory for 28 days before the commencement of the study. The rats were fed with pelleted proprietary poultry/rat feed and provided with clean water *ad-libitum* throughout the period of the study.

Trypanosoma brucei Parasite

The *Trypanosoma brucei* (Field strain) was obtained from a natural clinically infected dog at the Veterinary Teaching Hospital, University of Nigeria, Nsukka, Nigeria, and identified at the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria. However, the isolate was maintained in mice prior to the experimentation.

Experimental Design

Forty-nine rats were randomly assigned to seven groups of seven rats each: Groups A - G. Group A was the uninfected untreated control while groups B – G were infected with 1.4×10^6 trypanosomes. Group B was left untreated, Groups C and D were treated with 3.5 mg/kg DD once, and for five consecutive days respectively. Groups E and F were treated with 7 mg/kg DD once, and for five consecutive days respectively while group G was treated with 7 mg/kg DD and repeated after 14 days.

The effect of varied doses and treatment regimen was evaluated using parasite clearance time and relapse of infection. Clearance time was determined as the interval between the time of treatment and the time when no trypanosome was observed in the peripheral blood sample.

Data Analysis

The results of the study were analysed in percentages and presented in tables.

RESULTS

The rats became parasitaemic between 4-and 9-days post infections as shown in (Table 1). However, one rat in group G was aparasitemic up to day 15 post-infection. The rats were treated on day 15 post infection. Tables 2 and 3 showed the parasitaemia clearance and relapse respectively following *Trypanosoma brucei* infection and varied DD treatment regimes. Parasitic clearance and relapse following the varied DD treatment regimens was observed in all the treated groups However, the clearance and relapse duration varied between the treatment groups.

Table 1: Percentage parasitaemia among Wistar rats experimentally infected with *T. brucei*

Days of exposure	Groups						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Day 0	0/7 (0%)	0/7(0%)	0/7 (0%)	0/7(0%)	0/7(0%)	0/7(0%)	0/7(0%)
Day 4	0/7 (0%)	2/7(28%)	0/7 (0%)	0/7(0%)	0/7(0%)	0/7(0%)	2/7(28%)
Day 5	0/7 (0%)	3/7(43%)	0/7 (0%)	1/7(14%)	0/7(0%)	0/7(0%)	2/7(28%)
Day 6	0/7 (0%)	3/7(43%)	2/7 (28%)	2/7(28%)	2/7(28%)	0/7(0%)	2/7(28%)
Day 7	0/7 (0%)	5/7(71%)	6/7(86%)	7/7(100%)	4/7(57%)	3/7(43%)	6/7(86%)
Day 8	0/7 (0%)	7/7(100)	6/7(86%)	7/7(100%)	7/7(1000%)	5/7(71%)	6/7(86%)
Day 9	0/7 (0%)	7/7(100)	7/7(100%)	7/7(100%)	7/7(100%)	7/7(100%)	6/7(86%)
Day 10	0/7 (0%)	7/7(100)	7/7(100%)	7/7(100%)	7/7(100%)	7/7(100%)	6/7(86%)
Day 15	0/7 (0%)	7/7(100)	7/7(100%)	7/7(100%)	7/7(100%)	7/7(100%)	6/7(86%)

Group A: Uninfected and untreated; Group B: Infected and untreated; Group C: infected and treated (DD at 3.5 mg/kg single dose); Group D; infected and treated (DD at 3.5 mg/kg for 5 days); Group E: infected and treated (DD at 7.0 mg/kg single dose); Group F; infected and treated (DD at 7.0 mg/kg daily for 5 days); Group G: infected and treated (DD at 7.0 mg/kg stat, repeated 14 days later)

Numerator = Number of rats with established parasitaemia
Denominator = Total number of rats in the group
Day 0 = indicates Day of infection
DD = Diminazene diaceturate

Table 2: Percentage clearance of *Trypanosoma brucei* parasitaemia following varied treatment regimen of Diminazene diaceturate

Days of exposure	**Groups**				
	C	**D**	**E**	**F**	**G**
Day 0	6/6(0%) DT	7/7 (0%) DT	7/7 (0%) DT	7/7 (0%) DT	6/6 (0%) DT
Day 1	6/6(0%)	7/7(0%) DT	7/7(0%)	6/7 (14%) DT	5/6(17%)
Day 2	2/6(67%)	6/7 (14%) DT	2/7(71%)	4/7 43%) DT	5/6(17%)
Day 3	0/6(100%)	1/7 (86%) DT	1/7(86%)	2/7(71%) DT	2/6(67%)
Day 4	0/6(100%)	1/7 (86%) DT	0/7(100%)	0/7 (100%) DT	0/6(100%)
Day 5	0/6(100%)	0/7(100%)	0/7(100%)	0/7(100%)	0/6(100%)

Group A: Uninfected and untreated; Group B: Infected and untreated; Group C: infected and treated (DD at 3.5 mg/kg single dose); Group D; infected and treated (DD at 3.5 mg/kg for 5 days); Group E: infected and treated (DD at 7.0 mg/kg single dose); Group F; infected and treated (DD at 7.0 mg/kg daily for 5 days); Group G: infected and treated (DD at 7.0 mg/kg stat, repeated 14 days later)

Numerator: Number of rats cleared of parasite.
Denominator: Total number of rats in the group post treatment.
DT = days of treatment.
DD = Diminazene diaceturate

Table 3: Percentage r elapse of *Trypanosoma brucei* in Wistar rat following varied treatment regimen with Diminazene diaceturate

Days of exposure	**Groups**				
	C	**D**	**E**	**F**	**G**
Day 7	0/7(0%)	0/7(0%)	0/7(0%)	0/7(0%)	0/6(0%)
Day 14	2/7(29%)	0/7(0%)	0/7(0%)	0/7(0%)	0/6(0%)
Day 21	4/7(57%)	0/7(0%)	1/7(14%)	0/7(0%)	0/6(0%)
Day 25	7/7(100%)	0/7(0%)	2/7(29%)	0/7(0%)	0/6(0%)
Day 28	ND	0/7(0%)	ND	0/7(0%)	0/6(0%)
Day 39	ND	0/7(0%)	ND	0/7(0%)	4/6(67%)
Day 42	ND	1/6(17%)	ND	0/7(0%)	ND
Day 49	ND	1/6(17%)	ND	0/7(0%)	ND
Day 56	ND	2/6(33%)	ND	1/7(14%)	ND
Day 80	ND	4/6(67%)	ND	1/7(14%)	ND

Group A: Uninfected and untreated; Group B: Infected and untreated; Group C: infected and treated (Diminazene diaceturate at 3.5 mg/kg single dose); Group D; infected and treated (Diminazene diaceturate at 3.5 mg/kgdaily for 5 days); Group E: infected andtreated (Diminazene diaceturate at 7.0 mg/kg single dose); Group F; infected and treated (Diminazene diaceturate at 7.0 mg/kg daily for 5 days); Group G: infected and treated (Diminazene diaceturate at 7.0 mg/kg stat, repeated 14 days later)

Numerator: Number of rats with relapse
Denominator: Total number of rats in the group post treatment
ND: Not done

DISCUSSION

The mean prepatent period of 7 days recorded in this study was at variance with the five days reported by [23]. This might be due to strain variation and individual infectivity or host susceptibility. The clearance of the parasite following treatment with DD within 3-5 days in all the treated rats showed that the parasite was sensitive to the trypanocide used. There were no attributable signs of DD toxicity in all the groups, except one rat in group F that manifested sign of neurological disorder in the form of cycling when held on the tail. This study suggested that increased dosage or concentration of DD may not reduce the time of clearance of parasitemia or rather may marginally delay the time of parasitemia clearance from the rats treated with DD as seen in Table 2 (C = 3, D = 5, E = 4, F = 4, and G = 4). A possible explanation for this delay could be pro-inflammatory cytokines suppression [14] which may be directly proportional to the dosage concentration of DD administered to the rats. However, the onset of relapse was inversely proportional to the duration and concentration of DD administered to the rats as seen in Table 3 (C=14, D=42, E=25, F=56, G=39).

Some unknown intrinsic factors could make some animals have permanent cure irrespective of delayed treatment or time of treatment as observed in this study and other studies [9, 10]. [13] have demonstrated dose and treatment time - dependent efficacy of diminazene aceturate against *Trypanosoma cruzi*. It seems that the curative efficacy of DD is dose dependent as shown in table 3.

In this study, all the treatment groups were associated with relapse in the treated rats. However, the rats in the group F that received higher dosage and extended treatment of DD had a lower relapse rate. It was observed that this group F with higher dose of 7.0 mg/kg body weight for five consecutive days had a relapse rate of 14% within 80 days observation period post first treatment (PFT) with a much longer clearance period of 56 days post first treatment. Group D treated with 3.5 mg/kg body weight for five consecutive days had a relapse rate of 69% and shorter period post clearance before relapse (42 days). The dosage of 7.0 mg/kg body weight for 5 days appeared to be the best regimen out of those investigated for the treatment of the late-stage trypanosomiasis as show in table 3. It has also been reported that when high concentration of Berenil is used on trypanosomes, it promoted reduction in parasite growth without affecting the viability of the parasite cell [12]. This explains why despite prolong period of clearance in group F (56days) the relapse was later observed within 80days post treatment period. However, further experimental trials are required to determine if this finding is applicable to the treatment of trypanosomiasis in other animals.

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