Efficacy of repeated doses of diminazene aceturate (Dinazene®) in the treatment of experimental *Trypanosoma brucei* infection of Albino rats

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Summary

The efficacy of repeated doses of Dinazene® in Albino rats experimentally infected with *Trypanosoma brucei* (Gboko strain) was investigated. A total of 30 adult female Albino rats weighing 130-190 g were used for the study. They were assigned to six groups (groups A-F) of five rats each. Groups A-D were infected intraperitoneally with 1.0×10^6 trypanosomes in 400 µL of PBS diluted blood while groups E (uninfected treated) and F (uninfected untreated) served as controls. The rats in the groups A-D as well as those in group E were treated with 7.0 mg/kg body weight at day 11 post infection. Groups B, C and D however received two, three and four repeated doses of the drug at weekly intervals following initial treatment. There was complete clearance of the parasite within 120 h post treatment. Parasitaemia, packed cell volume (PCV), total red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration (Hb), rectal temperature, and body weight were used to assay the efficacy of treatment. Following treatment and parasite clearance from the blood, there was improvement ($P<0.05$) in the values of parameters measured when compared to the uninfected controls. However, relapse infection was observed in the rats of group A, B and C, with a resultant decline in clinical condition and values of parameters used to assess efficacy. We concluded that four consecutive treatments using same dose at weekly intervals proved efficacious in the experimental management of *T. brucei* infection in rats.

Key words: Dinazene®, Haematology, Rat, Repeat treatment, *Trypanosoma brucei*

Introduction

Trypanosomosis is a disease complex caused by several *Trypanosoma* species, transmitted cyclically by the *Glossina* (tsetse flies), and mechanically by biting flies (*Tabanids* and *Stomoxys*) (Luckins and Dwinger, 2004). Trypanosomes are haemo-flagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their hosts. These parasites cause diseases that affect man and animals, including cattle (Ikede and Losos, 1972), horses (Neitz and McCully, 1971), dogs (Adewunmi and Uzoukwu, 1979; Omamegbe *et al*., 1984; Ezeokonkwo *et al*., 2010) and laboratory animals (Anosa, 1983). The disease is characterized by pyrexia, fluctuating parasitaemia, anaemia and immuno-suppression (Ikede and Losos, 1972; Anosa, 1983), and has been described as a complex debilitating and often fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted haemoflagellate protozoan parasites of the genus *Trypanosoma* (Anene *et al*., 2001).

There is no effective vaccine against trypanosomes because of the phenomenon of antigenic variation exhibited by the parasites. The available means of control involves tsetse control, chemoprophylaxis, chemotherapy and use of trypanotolerant livestock. At present, control of trypanosomosis is chiefly done by chemotherapy and chemoprophylaxis using the salts of three compounds: Diminazine, Homidium, and Isometa-midium (Leach and Roberts, 1981; ILRAD Reports, 1990).

Diminazene aceturate is probably the most commonly used therapeutic agent for trypanosomosis in livestock in Sub-Saharan Africa (Geerts and Holmes, 1998), even in Nigeria. However, complete dependence on drugs in many situations of trypanosomosis has been hampered in many areas by their toxic effects, high cost and frequent development of resistance to these drugs by the parasites. This is considered a very serious problem in trypanosomosis control in Africa. This results from the fact that the drugs effectively eliminate the parasites from the blood stream and the animal appears recovered, and then undergoes relapse infection which may be characterized by severe neurological infection leading to

the death of the affected animal. In Nigeria, the occurrence of drug resistance to available trypanocides has been attributed to the presence of fake drugs, abuse of the existing drugs and inadequate dosing of the drugs in trypanosomosis therapy (Ezeokonkwo *et al*., 2007).

Therefore, the current challenge to the majority of African pastoralists is to optimize the use of the relatively old existing drugs (Ezeokonkwo *et al*., 2007). In view of this, the use of drug combinations, new therapeutic regimens and the use of Slow Release Devices (SRD) of existing trypanocides have been suggested (De dekens *et al*., 1989; Atonguia and Costa, 1999). Evaluation of blood indices and parameters helps to determine the health status of animals, establish the degree of damage to hosts tissues and assess the severity of the infection (Coles, 1986).

The objective of the study was to assess the efficacy of repeated doses of Dinazene® (diminazene aceturate) in the treatment of Albino rats experimentally infected with *Trypanosoma brucei*.

Materials and Methods

Experimental animals

Thirty adult female Albino rats weighing between 130-190 g were used for this study. They were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were randomly assigned into six groups of five rats each and kept in their respective rat cages in the Laboratory Animal Unit of the Department of Veterinary Parasitology and Entomology. The rats were acclimatized for two weeks before the commencement of the experiment. Within this period, they were confirmed free of blood parasites and were dewormed with albendazole (Zolat®) for gastrointestinal parasites. They were fed standard rat feed and given water *ad libitum*.

Trypanosomes

The *T. brucei* used in this experiment was originally obtained from goats during a survey in Gboko Benue State and designated "Gboko strain". They were subsequently maintained in mice in the Laboratory Animal Unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

Infection of the experimental rats

The donor rats were bled from the retro orbital plexus through the median canthus of the eye. The infected blood was diluted using phosphate buffered saline (PBS). Each rat in groups A-D was then infected with a total of 1.0×10^6 trypanosomes suspended in 0.4 ml of PBS.

Drugs

The drug used for the experiment is Diminazene aceturate (Dinazene®) (Vetindia Pharmaceuticals Ltd., India) and was administered to the rats according to the study design.

Duration of the experiment

The rats were acclimatized for about 2 weeks after which they were infected with 1.0×10^6 trypanosomes per ml intraperitoneally. Treatment of the rats began 11 days post infection after parasitaemia had been confirmed in all the infected rats. The experiment lasted for 10 weeks after initial treatment during which parameters such as parasitaemia, PCV, RBC count, WBC count, Haemoglobin concentration, differential leucocyte counts, weight, temperature, blood glucose were monitored weekly.

Experimental design

A total of thirty rats were used for the experiment, which were assigned into six groups of five rats each. The groups include:

Group A: Infected and treated at day 11.
Group B: Infected and treated at day 11, then repeated 7 days later.
Group C: Infected and treated at day 11, then repeated 7 and 14 days later.
Group D: Infected and treated at day 11, then repeated 7, 14 and 21 days later.
Group E: Treated at day 11 only and not infected.
Group F: Not infected and not treated (control).

Pre-infection parameters were obtained one week before the day of infection of the rats. These parameters were taken weekly thereafter until 10 weeks post treatment.

Parasitaemia

The level of parasitaemia was estimated using the rapid matching method as described earlier (Herbert and Lumsden, 1976). This was done daily from 3 days post infection, up to treatment until total parasite clearance from the blood. Subsequently, it was then taken weekly till the end of the experiment.

Clinical signs

The clinical manifestations observed in the rats include anaemia, emaciation or anorexia, pale mucous membrane and dullness.

Rectal temperature

Was determined using a digital clinical thermometer.

Determination of weight

The weekly weights of the rats were measured in grams using an electronic kitchen scale (Laica®), Italy.

PCV, RBC count and total WBC counts

The standard procedures for Veterinary Haematology and Practical Haematology as described by Schalm *et al*. (1975) and Coles (1986) were used to determine the PCV, RBC count, total and differential WBC counts.

Haemoglobin concentration

As described by Schalm *et al*. (1975) using the cyanomethaemoglobin method was used to determine the haemoglobin concentration.

Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA). The least significant difference was used to separate the means at post hoc. Probability values of <0.05 were considered significant.

Results

Parasitaemia

This became detectable in all infected rats 5 days post infection and progressively increased until a peak on day 8. Treatment was then administered to all infected rats of groups A, B, C, and D on day 11 post infection, with the groups being treated once, twice, three and four times, respectively (Fig. 1). In group A (treated at day 11 only), the parasites cleared from three of the five rats 48 h post treatment, and in the other remaining rats 72 h and 96 h post treatment, respectively. However, relapse infection occurred in four of the animals at day 35 post infection while the fifth rat remained aparasitaemic till the group was sacrificed. In group B (treated at days 11 and 18) four rats were observed to be aparasitaemic 48 h post treatment, while parasite clearance occurred in the 5th rat 96 h post treatment. Relapse was also noticed in two rats at day 49 while the others remained aparasitaemic till the experiment was terminated on day 70. In group C (treated at days 11, 18 and 25), three rats showed clearance of the parasite 48 h post treatment while the other two became free of the parasite 5 days after treatment. Relapse occurred in the first rat only at day 49 post treatment others remained aparasitaemic till the experiment was terminated. In group D (treated at days 11, 18, 25 and 32), one rat became aparasitaemic 48 h post treatment, while three others were aparasitaemic at 72 h post treatment. The last rat became aparasitaemic at 96 h post treatment. No relapse was recorded in this group.

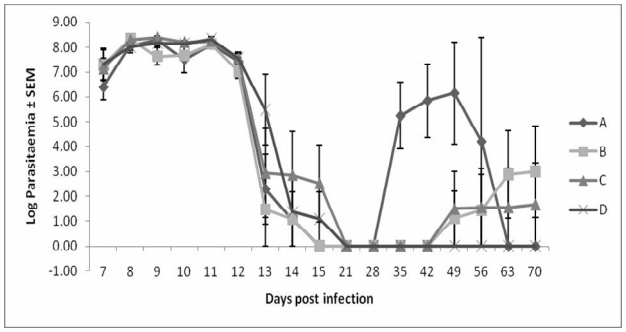

Fig. 1: Mean log parasitaemia of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

Packed cell volume (PCV %)

Figure 2 shows the mean PCV. There was significantly lower (P<0.05) PCV of the rats in groups A-D two weeks post infection when compared to the control. Although the rats were treated on day 11 post infection, the effect of the infection on PCV was still evident 4 days after. However following parasite clearance from the blood, the treated rats in groups A-D had increased PCV which was not significantly different (P>0.05) from the uninfected controls. Following relapse in group A, the PCV of rats decreased significantly (P<0.05) than other treated groups and the controls at weeks 6 and 7. Although groups B, C and D had no significant difference (P≥0.05) in their PCV following treatment, the relapse infection in groups B and C at week 7 caused a slight reduction in their mean PCV. This reduction became significantly lower (P≤0.05) than group D and the controls (E and F) on weeks 9 and 10.

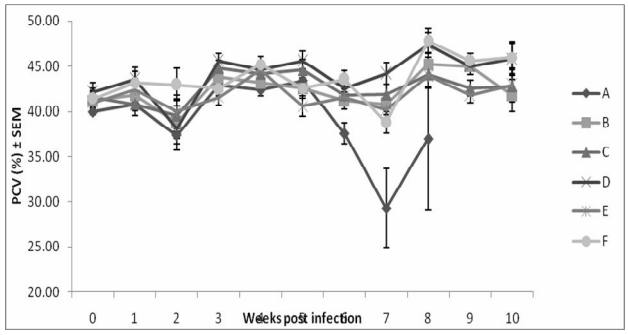

Fig. 2: Mean PCV values of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

Total red blood cell count/haemoglobin concentration

Figures 3 and 4 show the mean total RBC count and mean haemoglobin concentration, respectively, which showed a significantly lower (P<0.05) value in the rats of groups A-D 2 weeks post infection when compared to the control groups. As with the mean PCV, the effects on the RBC counts and haemoglobin concentration of the infection were still evident as at 4 days post treatment. However, following clearance of the parasites from the blood of infected rats, there was increased total RBC counts and haemoglobin concentration when compared (P<0.05) with the controls. By weeks 6 and 7, there was

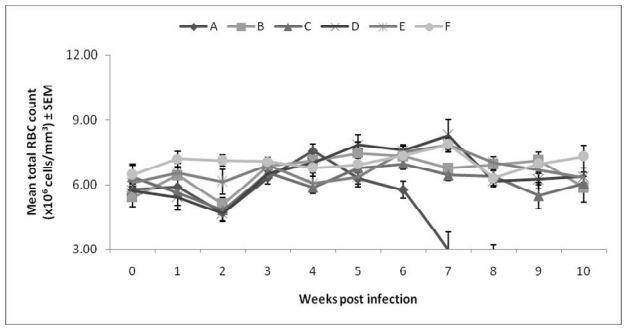

Fig. 3: Mean total RBC count of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and: F: Uninfected and untreated

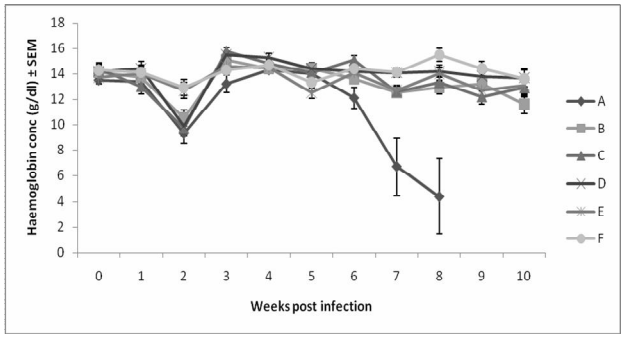

Fig. 4: Mean haemoglobin concentration of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

a significant decrease (P<0.05) in the levels of both parameters in group A when compared with other treated groups and the control. Groups B and C showed no significant difference (P>0.05) in their total RBC counts and haemoglobin concentration following treatment. However, relapse infection in these groups at week 7 caused a slight decrease in these values, consequently showing significantly reduced values (P<0.05) when compared with group D and the controls (E and F).

Total white blood cell count

Infection with the parasite led to a decrease in the total WBC counts of the rats. After treatment, there was a gradual increase in the WBC counts, which was not statistically significant (P>0.05) in all the groups. However, there was a significantly lower (P<0.05) WBC count in group A at week 7. Groups E and F showed no significant difference in their values (P>0.05) throughout the experiment (Fig. 5).

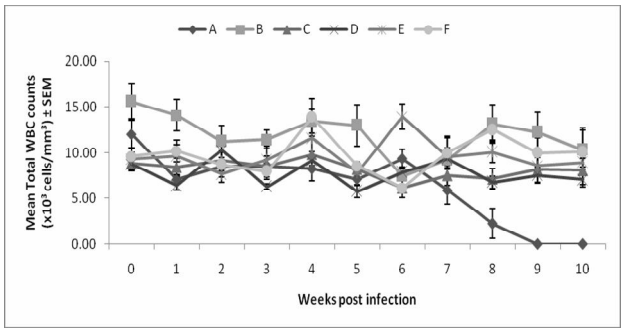

Fig. 5: Mean total WBC count of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

Temperature

Figure 6 shows the mean temperature. There was no significant difference (P>0.05) in both infected and control groups throughout the course of the experiment.

Weight

Figure 7 shows the mean body weight of rats. There was no significant difference (P>0.05) in both infected and control groups from infection to the end of the experiment.

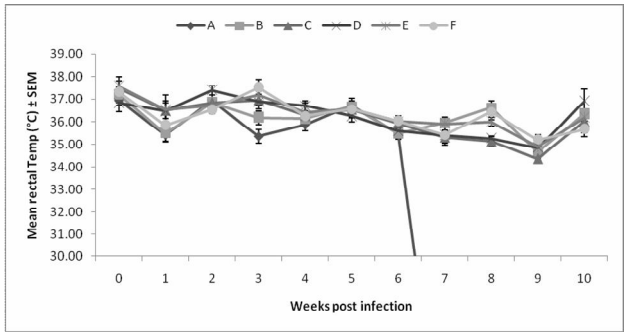

Fig. 6: Mean temperature of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

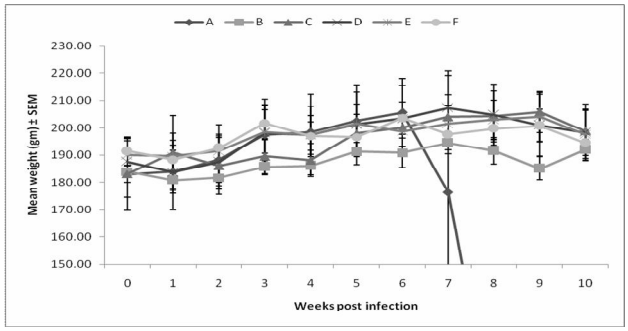

Fig. 7: Mean weight of rats infected with *T. brucei* and treated with repeated doses of Dinazene®. A: Infected and treated once, B: Infected and treated twice, C: Infected and treated thrice, D: Infected and treated four times, E: Uninfected and treated, and F: Uninfected and untreated

Discussion

The short pre-patent period (7 days) obtained from this study is in conformity with the findings of Nyindo (1992) who reported onset of parasitaemia in *T. brucei* infected animals to be 6-10 days. The high levels of parasitaemia and anaemia in the infected group was typical of trypanosome infections in rodents (Nyindo, 1992; Horst, 1996; Taylor and Authie, 2004). The clinical manifestations observed in the rats such as anaemia, emaciation, pale mucous membrane, agree with the observations (Ikede and Losos, 1972; Anene *et al*., 1999; Onah *et al*., 2000). Relapse infection was also recorded and this agrees with the findings of Ezeh *et al*. (2009). The reduction in parasitaemia following treatment which went on over a period of 5 days showed that the trypanosomes may be resistant to the trypanocide. Diminazene aceturate achieves therapeutic concentration in 24-48 h post treatment within which there is clearance of the parasite from the blood. Failure to achieve this may then suggest drug inefficiency or resistance. It was therefore not surprising that the relapse infection occurred after 24 days of single treatment and

31 days of double treatment in groups A and B, respectively.

The significant reduction ($P<0.05$) in the total WBC counts (leucopenia) in the infected group in this experiment which is as a result of immunosuppression, is an important attribute of trypanosomosis and has been reported in infected animals (Ngure *et al*., 2008). Similarly, anaemia and the resultant progressive fall in PCV, RBC count and haemoglobin concentration have been reported by various researchers (Samaddar *et al*., 1962; Anosa and Isoun, 1976; Dargie *et al*., 1979; Griffin and Allonby, 1979; Ngeranwa *et al*., 1993). These parameters however returned to pre-infection values following the initial and subsequent treatments. Infection and treatment with Dinazene® did not significantly affect the changes in body weight and temperature throughout the period of the experiment.

Relapse infection occurs when parasites invade tissues which are not accessible to the trypanocidal drugs (Ezeh *et al*., 2009). Although the parasites cleared from the blood following treatment, this lasted for about 24 days after the initial treatment (day 11 pi). The relapse which occurred in group A from day 35 and in groups B and C from day 49 suggests that the repeat treatments were only able to reduce relapse but not eliminate it permanently. This was, however, not the case with group D where all animals treated remained aparasitaemic till the end of the experiment.

Relapse infection may be attributed to drug resistance by the parasite following under dosing and low concentration of the active principle in the drugs among other factors. It has been reported that the preponderance of fake and adulterated drugs can also lead to emergence of drug resistant trypanosomes (Ezeokonkwo *et al*., 2007). Relapse can also result from sequestration of the parasites in the brain. This is because molecules of diminazene aceturate are too large to cross the blood-brain barrier and parasites which occur at these areas reappear in the blood when the effect of the drug may have waned (Jennings *et al*., 1979).

The results obtained from this study showed that repeated doses of Dinazene® (diminazene aceturate) was effective in the clearance of the parasite but prevented relapse only after the fourth consecutive treatment. Also, repeated treatments with Dinazene® did not produce adverse effect on the haematology values of the animals. This was reinforced by the fact that the haematological parameters remained relatively normal throughout the experiment. This treatment regimen is recommended in cases of relapse infection in trypanosomosis, bearing in mind its safety.

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