THE EFFICACY OF INCREASING DOSES OF SAMORENIL® IN THE TREATMENT OF *TRYPANOSOMA BRUCEI* INFECTED ALBINO RATS.

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SUMMARY

The efficacy of increasing doses (3.5, 7.0, 14, and 21 mg/kg) of Samorenil®; a diminazene based trypanocide was investigated in albino rats experimentally infected with *Trypanosoma brucei*. Thirty albino rats were used for the study. The rats were divided into six groups of five rats each. Groups III, IV, V, VI were infected with 1.0 x 10^6 trypanosomes intraperitoneally and were later treated at day 9 post infection with 3.5, 7.0, 14 and 21 mg/kg of samorenil® respectively. Group I served as negative control (uninfected untreated) while Group II served as positive control (infected untreated). The parameters monitored for the therapeutic assessment of the increasing doses of Samorenil® were parasitaemia, rectal temperature, packed cell volume (PCV), and body weight. Following treatment, there was complete aparasitaemia at 96 hours post treatment in all the infected treated rats. There was also an improvement in the clinical condition of the experimental rats. Four rats in the positive control (group II) (infected untreated) died by day 35 post infection and the remaining one died at day 70 post infection. Relapse infection however occurred at days 35, 49, and 63-post treatment in groups treated with 3.5, 7.0, and 14 mg/kg respectively. There was no relapse infection in the group treated with 21 mg/kg. It was thus concluded that there may be merit in using higher doses of Samorenil® in the treatment of trypanosomosis. The possible toxic effect of these higher doses however needs to be further investigated to ascertain the safety or otherwise of the higher doses used.

KEY WORDS: Efficacy, Samorenil®, *Trypanosoma brucei*, Rats.

INTRODUCTION

African trypanosomosis is a disease caused by haemoprotozoan parasites normally transmitted by *Glossina* and affects man and economically important domestic animals throughout 10 million km^2 (about 1/3) of the African continent (Finelle, 1983).

Animal trypanosomosis is most important in cattle, but can cause serious losses in pigs, camels, goats, and sheep and pet animals like dogs. Infection of cattle by one or more of the *Trypanosoma* species results in acute, subacute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea, rapid loss of condition, and often terminates in death. (Mulligan, 1970).

FAO (1987) put the direct losses in meat production and milk yield, and the cost of programmes that attempt to control trypanosomosis at between US $ 600 million and US $1.26 billion each year. The report also stated that over US $20 million are spent per year on trypanocides accounting for over 44% of the total expenditure on Veterinary drugs. At present, control of typanosomosis is chiefly by chemotherapy and chemoprophylaxis using the salts of three compounds, diminazene, an aromatic diamidine, homidium, a phenathridine and isometamidium, a phenathridine aromatic amidine, (leach and Roberts, 1981 and ILRAD, 1990). In addition, quinapyramine, suramin and recently cymelarsan have been used for the treatment and prevention of *Trypanosoma evansi* (Leach and Robert, 1981, Zhang *et al.*, 1991and Ndoutamia *et al.*, 1993).

Diminazene aceturate is the most commonly used therapeutic agent while isometamidium chloride is most commonly used prophylactic agent. There is yet no prospect of the development and use of vaccines because of

the well-known phenomenon of antigenic variation. Most of these trypanocides have been in use for over five decades with development of resistance of trypanosomes to the drugs. (Stevenson *et al.*,1995; Schrevel *et al.*, 1996; Anene *et al.*, 2000; Tabel *et al*, 2000; Roderick *et al.*,2000 and Geerts *et al.*,2001.). Furthermore, relapse infections occur following treatment of trypanosomosis and characterized by severe neurological syndrome and death (Uchendu and Chukwu, 1988). This has been a serious hindrance in pet survival in the Eastern parts of Nigeria and beyond.

Because of the relatively limited market in Africa and the high cost of developing and licensing new drugs, International Pharmaceutical companies have shown little interest in the development of new trypanocides for use in either animals or humans. The current challenge to majority of African Pastoralist therefore is to achieve optimal use of the relatively old existing drugs. In this vein, some workers have suggested the use of drug combinations, new therapeutic regimens and use of slow release devices (SRD) of the existing trypanocides (Dedeken *et al.*, 1989; Atonguia and Costa, 1999 and Geerts and Holmes, 1999). Even human trypanocide, Pentamidine Isethionate has been experimentally used in the management of animal trypanosomosis (Anene *et al.*, 2006). The aim of the present study is to investigate the efficacy of varying doses of Samorenil® in the treatment of albino rats experimentally infected with field isolates of *Trypanosoma brucei*.

MATERIALS AND METHODS

Experimental animals

Thirty adult albino rats (10 males and 20 females) weighing 125-285g bred within the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used. They were kept in a fly proof house and were fed and watered ad libitum. The rats were acclimatized in the laboratory animal unit of the Dept. of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka for two weeks before commencement of the experiment.

Drug

The drug used for this study is Samorenil®, a brand of diminazene diacaeturate (Alfasan International BV Woerden, Holland). The drug was reconstituted in distilled water to make 7% aqueous solution prior to use.

Trypanosomes

Trypanosoma brucei used in this study was obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The parasite was originally isolated from a slaughtered pig in Nsukka main abattoir early in 2006 and was maintained and multiplied in mice prior to use.

Infection of the Experimental rats

The donor mice were bled by puncture of the vein at the median canthus of the eye. The infected blood so obtained was serially diluted with Phosphate buffered saline (PBS). The experimental rats were each inoculated intraperitoneally with 1 x 106 trypanosomes. The rapid matching method of Herbert and Lumsden (1976) was used for quantifying the parasites used.

Experimental Design

The rats were randomly divided into six groups of five rats each (groups I - VI). Group I served as the uninfected untreated control while group II served as infected untreated control. Groups III - VI were infected and treated with 3.5, 7.0, 14, and 21 mg/kg of Samorenil® respectively. The infected rats each received 1 x 106 trypanosomes in PBS intraperitoneally (i/p). After infection, the rats were screened for parasitaemia from the 4th day post infection using wet mount and haematocrit concentration technique with a view to establishing the onset of parasitaemia (Soulsby, 1982). The infected rats were treated nine days post infection. Thereafter, the rats were screened every seven days for the presence of the parasites in the blood. Other parameters monitored for the assessment of therapeutic efficacy of the increasing doses of Samorenil®, were clinical signs, mortality/survivability, packed cell volume (PCV), rectal temperature, and body weight.

Statistical Analysis

The results of this study were statistically analysed using one way analysis of variance (ANOVA) and Student's test.

RESULTS

Parasitaemia/mortality/survivability

The parasites were detectable in the blood of all the infected rats five to nine (5-9) days post infection. The level of parasitaemia persistently increased and reached anti log 8.1 (1.25x108 trypanosomes/ml) by day 9 post infection when treatment was instituted in the treated groups (III VI). Four rats in the infected untreated control (group II) died due to the infection by 35 days post infection. The remaining one in that group died at 70 days post infection. All the rats in the uninfected, untreated control (group I) survived till the end of the experiment.

There was complete clearance of parasites from the blood of all the infected treated rats at 96 hours post treatment following treatment with the varying doses (3.5, 7.0, 14, and 21 mg/kg) of the drug. One rat each was aparasitaemic at 24 hours post treatment in groups III, IV, V, while two rats were aparasitaemic in Group VI within the same period. Relapse infection however occurred in one of the rats in groups III, IV, V, on days 35, 49, and 63 respectively. The other rats in these groups were parasite free by the end of the study. There was no relapse infection in rats in Group VI (21 mg/kg) Table I.

TABLE 1: Parasitaemia of rats infected with T. brucei and treated with varying doses of Samorenil®

Days PI	TREATMENT GROUPS					
	I (Uninfected and untreated control)	II (Infected and untreated control)	III (Infected and treated with 3.5mg/kg)	IV (Infected and treated with 7.0 mg/kg)	V (Infected and treated with 14 mg/kg)	VI (Infected and treated with 21 mg/kg)
O*	0/5	5/5	5/5	5/5	5/5	5/5
7**	0/5	5/5	0/5	0/5	0/5	0/5
14	0/5	5/5	0/5	0/5	0/5	0/5
21	0/5	5/5	0/5	0/5	0/5	0/5
28	0/5	5/5	0/5	0/5	0/5	0/5
35	0/5	5/5	1/5	0/5	0/5	0/5
42	0/5	1/1	1/5	0/5	0/5	0/5
49	0/5	1/1	1/5	1/5	0/5	0/5
56	0/5	1/1	1/5	1/5	0/5	0/5
63	0/5	1/1	1/5	1/5	1/5	0/5
70	0/5	0/0	1/5	1/5	1/5	0/5

Days PI: No of days post treatment.
O* = day of treatment;
7 = day seven PI.**
Numerator = Number of dogs positive for Trypanosoma brucei.
Denominator = Number of dogs surviving in the group.

Clinical signs

Clinical signs observed in most of the infected rats were fever, dullness, depression, sleepy syndrome, emaciation, anorexia, pale mucous membrane and ascitis. These signs gradually disappeared following treatment and reappeared on emergence of relapse infection. All the rats in Group II (infected untreated) however died between day 35 - 70 post infection.

Rectal Temperature

Fig. 1 shows the effect of increasing doses of Samorenil® (3.5, 7.0, 14, 21 mg/kg) on the rectal temperature of the experimental rats. The mean rectal temperature was elevated in Group II (infected untreated). This elevation continued until all the rats in that group died by day 70 post infection. The mean rectal temperature of rats in Group I (uninfected untreated) was stable at about 34°C until day 70 when it decreased significantly ($P<0.01$). A close look at figure 1 shows that the mean rectal temperature in groups III, IV, V, and VI treatment groups declined by day seven post infection and afterwards significantly increased ($P<0.01$) by day 28 through day 70 of the experiment in group III. There was rather an unsteady fluctuation in the rectal temperature in the three remaining treatment groups (IV, V, and VI), the temperature declining significantly ($P<0.01$) by day 70 of the experiment.

Packed Cell Volume (PCV)

The Packed cell volume of the rats in group I (uninfected untreated) was unaffected whereas infection caused significant decline ($P<0.05$) in the packed cell volume (PCV) of the rats in group II (Infected untreated) with all the rats in that group dead by day 70 post infection. Fig. 2 Comparative study of the PCV in the four drug treated groups (3.5, 7.0, 14, and 21 mg/kg) revealed that there was a sharp drop in the PCV in all the four groups by day seven of infection. The PCV latter rose gradually from day 14 (5 days post treatment) reaching the peak by day 35 and latter declined significantly ($P<0.05$) until day 63, however it slightly increased on day 70 in all the treated groups.

Body weight

Figure 3 shows the effect of increasing doses of Samorenil® (3.5, 7.0, 14, and 21 mg/kg) on the body weight of experimental rats. The body weights of the experimental rats were unaffected in the uninfected untreated control (group I). However, *Trypanosoma* infection caused significant reduction in the body weight of the experimental rats ($P<0.05$) in group II (infected untreated) with all the rats dead in that group by day 70 of infection. A Comparative assessment of mean body weights of the four treated groups (3.5, 7.0, 14.0, and 21 mg/kg) revealed that the mean body weights of the rats were unaffected up to day 42 of the experiment but increased slightly by day 49 through day 70 of the experiment.

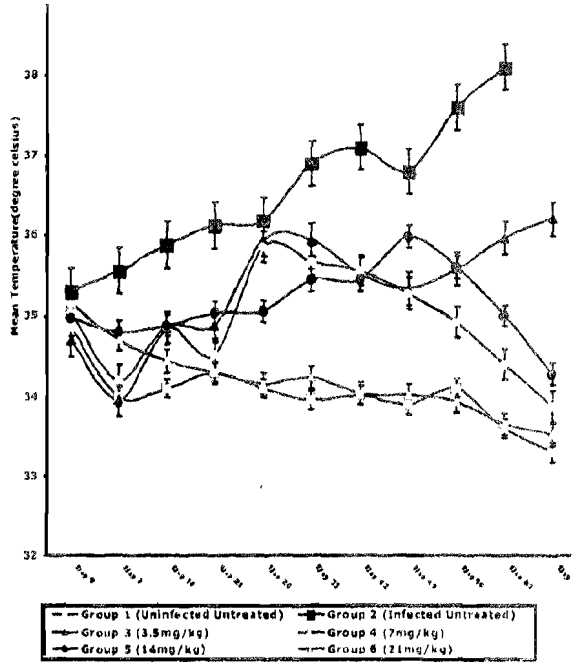

Fig. 1: Mean Temperature values [°C] of rats infected with *T. brucei* and treated with varying doses of Samorenil®.

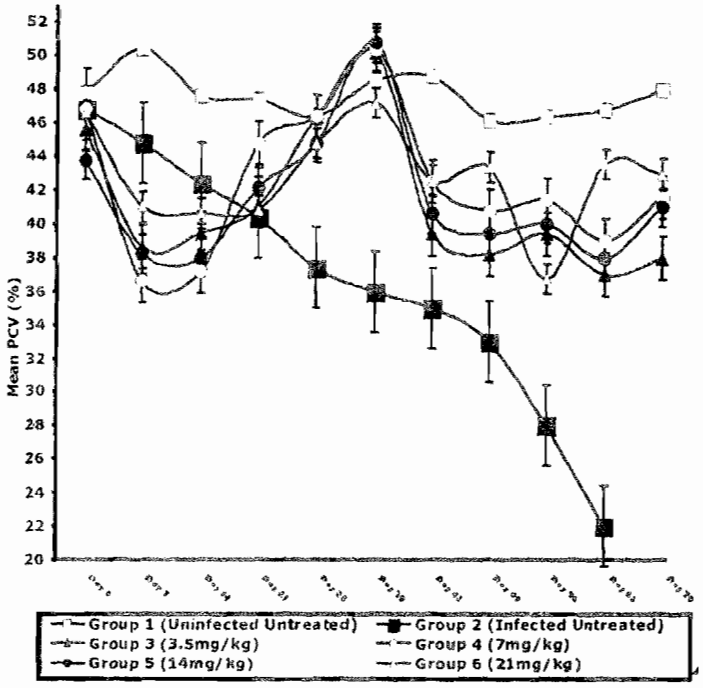

Fig. 2: Mean Packed Cell Volume [%] of rats infected with *T. brucei* and treated with varying doses of doses of Samorenil®

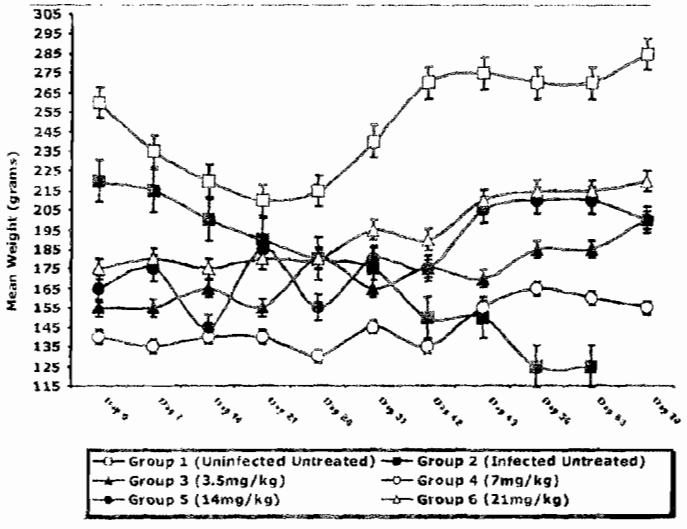

Fig. 3: Mean Weight Values (gm) of rats infected with T. brucei and treated with varying doses of Samorenil®

DISCUSSION

The parasites were detectable in the blood of all the infected rats 5-9 days post infection. Parasitaemia increased exponentially culminating in the death of all the rats in the infected untreated rats (group II) by day 70 of the infection. The short pre-patent period (5-9 days) recorded in this experiment is in conformity with the findings of Onyeyili and Anika (1991) in dogs, Anene *et al* (1999) in dogs and Anene *et al* (2006) in rats.

The clinical signs of pale mucous membrane, anorexia, listlessness, dullness, emaciation, depression, sleepy syndrome, ascites, and central nervous system signs as recorded in this experiment are characteristic and typical of trypanosomosis in animals. These findings are in agreement with that of Ezeokonkwo and Agu (2003; 2004) and Anene *et al* (2006). Fluctuating temperature as recorded in this work is characteristic of trypanosomosis in animals (Stephen, 1986 and Anene *et al.*, 1999). It is speculated that pyrexia is precipitated in the infected animals by the metabolism of tryptophan to tryptophol by trypanosome infections. The accumulation of trypatophol in pharmacological doses in animals have been reported by Seed and Hall (1977) to be responsible for rectal temperature changes in humoral antibody response to heterologous antigens. Treatment with increasing doses of Samorenil® (3.5, 7.0, 14, 21 mg/kg) was able to reverse the increasing temperature by day 70 post infection.

Relapse infection of one rat each occurred on day 35 in Group III (3.5 mg/kg); day 49 in group IV (7.0 mg/kg), and on day 63 in group V (14 mg/kg). However, no relapse infection was recorded in group VI (21 mg/kg). It appears from the result that the higher the dose of Samorenil® administered; the longer it took for the relapse to occur. The 21 mg/kg dose appears to be the most efficacious since no relapse infection occurred in the course of the study.

One however has to be careful in using this high dose in livestock since diminazene aceturate is known to be very toxic to dogs and horses (personal observations). Relapse infection has been reported by so many other workers (Ezeokonkwo and Agu, 2004; Anene *et al.*, 2006 and Whitelaw *et al.*, 1985). Relapses after treatment, have been attributed to sequestration of parasites in the brain tissues where the parasites are unaffected by the trypanocide (Samorenil®) probably because of their inability to cross the blood brain barrier (Jennings, 1976). These parasites then return to the blood when the effect of the drug had waned, they latter multiply leading to further detection of parasites in the blood (Jennings *et al.*, 1979).

The mean PCV (%) fell sharply in the infected untreated control (group II) leading to the death of four rats in that group by 35 days post infection and the remaining one by the 70th day of infection. This progressive fall in PCV following infection implies anaemic condition in the experimental rats which is characteristic of *T. brucei* infection in animals. This observation is in conformity with the finding of previous workers (Ezeokonkwo and Agu, 2003; Obidike, *et al*, 2005 and Anene *et al.*, 2006). It has been reported that anaemia in trypanosomosis is caused by a combination of factors which include haemolysis of erythrocytes by trypanosomes (Fiennes, 1953), haemodilution (Clarkson, 1968), and erythrophagocytosis (Mackenzie *et al.*, 1975). Samorenil® at all the doses used (3.5, 7.0, 14, and 21 mg/kg) effectively reversed the anaemic conditions hence the appreciation in the PCV values in the four treated groups (groups III, IV, V, and VI) from day 14 through day 63 of the infection as observed in the study.

The mean body weight of the experimental rats in Group II (Infected untreated) significantly reduced ($P<0.05$). However, the mean body weights of the rats in the other four treated groups (III, IV, V, & VI) were unaffected up to day 42 of the experiment but increased slightly by day 49 through day 70 of the experiment. The reduction in body weight of the rats is probably due to anorexia induced by trypanosomosis in animals. This finding is in conformity with the findings of Anosa and Isoun (1976), Fiennes (1970) and Anene *et al* (1991). Treatment with Samorenil® at various doses in this experiment appear to have reversed the anorexia which may have lead to the return to normal mean body weight in the four treated groups.

CONCLUSION

The result of this study has shown that 21.0mg/kg of Samorenil® is efficacious in the treatment of *T. brucei* infection of rats since no relapse infection occurred. However, more work is needed in this direction to ascertain whether this high dose is toxic to the animals.

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