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ARTICLE

Comparative Trypanocidal Efficacy of a New Commercial Brand of Diminazene in *Trypanosoma congolense* Infected Rats.

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Summary

The trypanocidal efficacy of ProZant Vet®, a new ready to use generic brand of diminazene aceturate was compared with two standard trypanocides; Trypamidium Samorin® (isometamidium chloride) and Dimivet® (diminazene diaceturate) in rats artificially infected with *Trypanosoma congolense.* Thirty albino rats (fifteen males and fifteen females) randomly divided into five groups (A, B, C, D and E) of six rats each were used for the study. Each group comprised three males and three females but each sex category was caged separately to avoid mating and pregnancy. Group C was the infected untreated control while group E was the uninfected untreated control. Groups A, B and D were infected with *T. congolense* and treated on day 14 post infection with isometamidium chloride (Trypamidium Samorin®) at a dose of 2 mg/kg body weight, diminazene diaceturate (Dimivet®) and diminazene aceturate (ProZant Vet®) at doses of 7 mg/kg body weight, respectively. Parameters used for assessing the efficacy of treatments including Packed Cell volume (PCV) Hemoglobin concentration (Hb) and parasitaemia showed some variations between the drugs. Dimivet®–treated group PCV and HB responses to trypanocidal administration were the best. Cases of relapse parasitaemia were recorded in all the three treated groups, which occurred earlier in the Trypamidium–Samorin® and ProZant® treated groups (14 days, respectively, PI) compared with Dimivet® treated group (21 days PI). All rats in the Trypamidium-Samorin® and Dimivet® groups died, whereas one rat in the ProZant group survived beyond the experimental period. A sex-related mortality pattern occurred which indicated greater susceptibility of the male than female rats. After a careful consideration of our results, we concluded that there was no appreciable difference between the efficacies of Dimivet® and ProZant®, but were apparently superior to Trypamidium – Samorin®. However, none of the drugs cured the infection to prevent incidences of relapse parasitaemia thus indicating that the *T. congolense* strain used in this study resisted the normal therapeutic dose of the drugs.

KEY WORDS: *T. congolense*, diminazene, new brand, rat.

INTRODUCTION

The African Animal Trypanosomosis (AAT) has been described as a major obstacle to sustainable livestock production and food security, and according to Swallow (2000) is an important factor of underdevelopment in sub-Saharan Africa. The disease has an economic importance which is related to high morbidity and mortality of susceptible animals, and the high cost of treatment of such animals as well as a decreased production of meat and milk. It is the most single devastating disease in Africa in terms of poverty and loss of agricultural production amounting to 3 billion pounds annually (Hursey, 2000). The disease is usually characterized by anemia (seen as pale mucous membrane, reduced packed cell volume and haemoglobin concentration) weakness, emaciation, fever, lethargy, weight loss and a heavy mortality rate (Urquhart *et al*., 1996). Transmission is either mechanically by biting flies such as *Tabanids, Stomoxys, Liperosa,* and *Haematopota* or cyclically by tsetse fly belonging to the genus *Glossina* (Soulsby, 1986).

Chemotherapy and chemoprophylaxis, coupled with vector control programmes, have been the main stay of trypanosomosis control in Africa in the absence of effective vaccine against the disease. Drug control of animal trypanosomosis in Africa is mainly dependent on the use of diminazene and isometamidium chloride (Aliu *et al*., 1984; Aliu and Odegaard, 1985; Anika *et al.,* 1987). Diminazene is a curative drug while isometamidium chloride, which has a long lasting action, is both curative and preventive trypanocide. Their continued use and effectiveness as a tool for the treatment and/or chemoprophylaxis of trypanosomosis has been threatened by the availability in the market of poor quality, substandard and adulterated veterinary products. Drugs used in animal trypanosomosis are generally subject to lower

standards of quality control than those used in human African trypanosomosis (Maudlin *et al*., 2004). Multiple generic variants of trypanocidal drugs, including isometamidium and diminazene are available and a recent analysis of commonly used diminazene preparations in the country has revealed some discrepancies in their drug quality (Anene *et al*., 2006). The release of preparations that contain low qualities of active drug creates ideal conditions for the selection of drug resistance as well as leading to therapeutic failures. It is thus important to evaluate any new trypanocidal formulation for its chemo-therapeutic effectiveness before presentation and adoption for field use in any given area.

In this study therefore, we have undertaken to evaluate the comparative trypanocidal efficacy of ProZant®, a new ready to use diminazene formulation against standard formulations of isometamidium chloride and diminazene aceturate in *Trypanosoma congolense* infected rats.

MATERIALS AND METHOD

Experimental Animal

Thirty albino rats (fifteen males and fifteen females) bred in the animal house of the Faculty of Veterinary Medicine, University of Nigeria Nsukka were used for the experiment. They were kept in clean cages inside a well ventilated fly-proof experimental animal house. They were humanely cared for in compliance with the principle of laboratory animal care. They were given food and clean water *ad libitum* throughout the experiment.

Trypanosoma congolense

The *Trypanosoma congolense* was obtained from National Institute for Trypanosomiasis Research (NITR) Vom, Plateau State. It was isolated from a cow in Gwarzo area of Kaduna State in 2009. It was maintained by intra-peritoneal (p.i.) passage in white albino rats.

Drugs

- ProZant Vet® (Neospark Drug and Chemicals Private Limited, 241, B.L. Bagh, Panjagutta, Hyderabad-500 082, A.P. , India). It was a ready to use injectable solution, containing diminazene aceturate and phenazone.
- Trypamidium-Samorin® (Merial, 29, Avenue Tony Gamier-69007-France). Trypamidium-Samorin® is a brand of isometamidium chloride. It was presented in 125 mg sachet as a sterile red powder and was reconstituted with 6.25ml of sterile water to make 2% solution.
- Dimivet® (SKM Pharma PVT. Ltd, Nashik-422 010, Bangalore-560 001, India). Dimivet is a 2.3g granules containing 1.05g of Diminazene diaceturate and reconstituted with 12.5ml of sterile water.

Experimental Design

The rats were randomly divided into five groups (A, B, C, D and E) of six rats each. Each group was made up of three males and three females but each sex in each group was kept in a separate cage to avoid mating and pregnancy. Each of the rats in groups A, B, C and D was inoculated i.p. with *Trypanosoma congolense* infected rat blood containing $2.6x10^6$ trypanosomes per milliliter. Group E was used as the uninfected and untreated control. Group C was the infected but untreated control whereas on day 14 post infection, groups A, B and D rats were administered trypanocides i.p. as follows:

Group A: Isometamidium chloride (Trypamidium-Samorin®) at a dose of 2 mg/kg body weight.

Group B: Diminazene diaceturate (Dimivet®) at a dose of 7 mg/kg body weight.

Group D: Diminazene aceturate (ProZant Vet®) at a dose of 7 mg/kg body weight.

Parasitaemia by buffy coat method (Murray *et al*., 1977) and haematological parameters (PCV, Hb; Schalm *et al*., 1975) were measured weekly, except for daily tail blood examinations to determine parasite clearance time post treatment. Deaths were recorded daily. Blood samples for haematology were obtained by the orbital bleeding technique. The parameters were monitored for 63 days with the exception of parasitaemia which was monitored for 77 days to accommodate the sixty days recommended for the evaluation of drug sensitivity in trypanosome chemotherapy (Geerts and Holmes, 1998).

Statistical analysis

Data obtained were expressed as arithmetic mean ± s.e.m. Statistical significance was assessed using one-way analysis of variance (ANOVA) and Duncan's multiple range test with SPSS version 16 soft ware package. P values <

0.05 were considered significant.

RESULTS

Parasitaemia

The results of the parasitaemia are presented in table I. The infection was patent by day 14 post infection (PI). The mean parasite clearance time post treatment with Samorin®, Dimivet®, and ProZant® was 72 ± 0.0, 48 ± 0.0 and 66 ± 15.6 hours, respectively (not shown in the table). Relapse parasitaemia occurred with all of the three trypanocides used, and following re-treatment of the relapse, parasite clearance time was 72 ± 19.6, 48 ± 0.0 and 48 ± 0.0 hours, respectively, for the drugs. The relapse interval was 14 days post treatment for Samorin® and ProZant®, and 21 days post treatment for Dimivet®.

Mortality

All the infected untreated rats died by day 35 PI (Table I) with mean time to death (MTD) of 23.3 ± 3.6 days. Of the 12 infected male rats, seven (58%) died on day14 PI while none of the female infected rats died within this period. All the Samorin® treated- and Dimivet® treated-rats (100%) died by day 49 (MTD, 42.5 ± 3.7) and day 77 (MTD, 68.3 ± 2.3) PI, respectively, whereas (83.3%) of ProZant® treated rats died by day 70 (MTD, 51.7 ± 9.7) PI, with one survival beyond the experimental period.

Packed cell volume (PCV)

Infection caused a significant decrease ($P < 0.05$) in the PCV of the rats by day 14 PI (Table II). Trypanocidal administration reversed the haematocrit to normal values in all the treated groups by day 21 PI. It subsequently decreased significantly ($P< 0.05$) in the Samorin®-treated group by day 28 PI, and in the Dimivet® and ProZant®-treated group by day 42 PI, being significantly ($P < 0.05$) lower in the Dimivet® group compared with ProZant® group.

Haemoglobin (Hb) Concentration

The Hb concentration of all the infected groups significantly decreased ($P < 0.05$) by day 14 PI (Table III). Trypanocidal treatment caused significant improvement ($P < 0.05$) in the Hb concentration by day 21 PI in the Dimivet® and ProZant® groups but not Samorin®.

TABLE I: Parasitaemia of *T. congolense* infected rats treated with either Samorin® Dimivet® or ProZant®.

Days post infection	A (treated with Samorin ®)	B (treated with Dimivet ®)	C (infected untreated)	D (treated with ProZant ®)	E (uninfected control)
0	*0/6	0/6	0/6	0/6	0/6
7	0/6	0/6	0/6	0/6	0/6
14+	5/5	4/4	5/5	4/4	0/6
21	0/4	0/4	1/1	0/4	0/6
28++	4/4 ++	0/4	1/1	3/3 ++	0/6
35	1/3	1/4	1/1	2/3	0/6
42+++	3/3	4/4+++	---	2/3	0/6
49	---	¼	---	2/3	0/6
56	---	2/4	---	2/3	0/6
63	---	1/3	---	½	0/6
70	---	2/2	---	0/1	0/6
77	---	---	---	0/1	0/6

\+ Day of treatment (trypanocide administration)
++ Day of re-treatment of relapsed infection in groups A and D.
+++ Day of re-treatment of relapsed infection in group B
* Number of rats parasitaemic; Denominator = the number of rats per group.

TABLE II: Mean PCV (%) ± SEM of *T. congolense* infected rats treated with either Samorin®, Dimivet® or ProZant®

Days post infection	A (treated with Samorin®)	B (treated with Dimivet®)	C (infected but untreated)	D (treated with ProZant®)	E (uninfected control)
0	38.17 ± 0.91 ^{a}	37.8 ± 0.95 ^{a}	37.17 ± 0.87 ^{a}	37.3 ± 0.84 ^{a}	39.17 ± 0.4 ^{a}
7	37.67 ± 2.14 ^{a}	37.67 ± 1.59 ^{a}	37.5 ± 2.19 ^{a}	40.17 ± 1.58 ^{a}	41.67 ± 1.022 ^{a}
14+	32 ± 0.82 ^{b}	32 ± 1.9 ^{b}	29.3 ± 1.56 ^{b}	30.8 ± 1.54 ^{b}	41.17 ± 0.79 ^{a}
21	38.25 ± 1.03 ^{a}	41.75 ± 0.85 ^{a}	-	39.75 ± 2.1 ^{a}	38.5 ± 1.09 ^{a}
28++	31.25 ± 2.06 ^{a}	40.75 ± 2.14 ^{b}	-	39.67 ± 0.33 ^{b}	43.3 ± 0.715 ^{b}
35	38.3 ± 4.41 ^{a}	38.8 ± 3.77 ^{a}	-	44 ± 1.0 ^{a}	41.17 ± 0.40 ^{a}
42+++	27.7 ± 0.9 ^{a}	27.7 ± 2.6 ^{a}	-	33 ± 0.6 ^{b}	39 ± 0.3 ^{c}
49	-	36 ± 1.9 ^{a}	-	40 ± 2.1 $^{a b}$	40.8 ± 0.31 ^{b}
56	-	34 ± 2.8 $^{a b}$	-	28 ± 4.0 ^{a}	36.5 ± 0.5 ^{b}
63	-	35 ± 1.5 ^{a}	-	37.5 ± 3.5 ^{a}	38 ± 0.5 ^{a}

\+ Day of treatment (trypanocide administration)
\+ Day of re-treatment of relapsed infection in groups A and D.
+++ Day of re-treatment of relapsed infection in group B
Different superscripts in a row indicate significantly difference ($p< 0.05$).

TABLE III: Mean haemoglobin concentration (gm/dl) ± SEM of *T. congolense* infected rats treated with Samorin®, Dimivet® or ProZant®.

Days post infection	A (treated with Samorin®)	B (treated with Dimivet ®)	C (infected but untreated)	D (treated with ProZant ®)	E (uninfected control)
0	10.9 ± 0.5 ^{a}	11.17 ± 0.74 ^{a}	12.3 ± 0.61 ^{a}	10.9 ± 0.6 ^{a}	11.9± 0.42 ^{a}
7	10.9 ± 0.41 ^{a}	11.6 ± 0.7 ^{ab}	13.1 ± 0.77 ^{b}	11.6 ± 0.89 ^{ab}	10.8 ± 0.27 ^{a}
14+	8.7 ± 0.28 ^{bc}	8.9 ± 0.48 ^{c}	7.9 ± 0.37 ^{b}	6.9 ± 0.28 ^{b}	14.1 ± 0.59 ^{a}
21	11.93 ± 0.32 ^{c}	13.78 ± 0.48 ^{ab}	-	15.15 ± 0.4 ^{bc}	14.8 ± 0.5 ^{a}
28++	9.3 ± 1.14 ^{a}	9.1 ± 0.93 ^{a}	-	10.6 ± 1.23 ^{a}	11.7 ± 0.47 ^{a}
35	14.7 ± 0.77 ^{ab}	12.4 ± 1.45 ^{a}	-	15.6 ± 0.32 ^{b}	14.8 ± 0.52 ^{b}
42+++	10.8 ± 1.3 ^{ab}	8.1 ± 1.5 ^{a}	-	12.3 ± 1.1 ^{b}	13.9 ± 0.64 ^{b}
49	-	13.3 ± 1.0 ^{a}	-	13.1 ± 0.5 ^{a}	14.4 ± 0.3 ^{a}
56	-	13.2 ± 1.2 ^{a}	-	11.4 ± 1.5 ^{a}	14.2 ± 0.2 ^{a}
63	-	10.8 ± 1.5 ^{a}	-	12.2 ± 2.6 ^{a}	13.9 ± 0.35 ^{a}

\+ Day of treatment (trypanocide administration)
++ Day of re-treatment of relapsed infection in groups A and D.
+++ Day of re-treatment of relapsed infection in group B.
Different superscripts in a row indicate significantly difference ($p< 0.05$).

DISCUSSION

The results of this study showed that *T. congolense* infection of rats produced patent parasitaemia within 14 days PI. This result is in

agreement with the findings of Abenga *et al*. (2005) and Ezeokonkwo (2009) but contrasted with the findings of Onyeyili and Anika (1991) and Anene *et al.* (1989) in *T. congolense* infected animals. The parasitaemia increased progressively leading to the death of all the rats by day 35 PI, with greater susceptibility of the male rats than females similar to the finding of Turay *et al*. (2005). The course of *T. congolense* infection in this study was acutely fatal, similar to the finding of Ezeokonkwo (2009) but differs from that of Anene *et al*. (1989) in *T. congolense* infected dogs. Treatment with the three drugs cleared the parasites from the blood of the infected rats within 3 days of treatment but with varying levels of efficacy.

Relapse parasitaemia occurred at similar time interval (2 weeks post treatment) in the Trypamidium-Samorin® and ProZant-treated groups but was longer (3 weeks) in the Dimivet®-treated group. Relapse after trypanocidal treatment has been reported by other workers (Jennings *et al*., 1977; Peregrine *et al*., 1991; Sutherland *et al*., 1991). Relapse is usually considered to indicate resistance to the drug under test at the dose rate employed (Williamson and Stephen 1960; Ainanshe *et al*., 1972). Relapse of infection can also occur due to the ability of the trypanosome to penetrate the brain and other intracellular tissue beyond the action of trypanocides which subsequently replicate and re-establish a circulating parasitaemia (Jennings and Gray 1983).

It is noteworthy, that one rat in the ProZant® – treated group survived beyond the period of this experiment, whereas survival was zero in the Trypamidium – Samorin® – treated group by day 49 PI and in the Dimivet® - treated group by day 77 PI. The results of the red blood cell parameters (PCV and Hb) showed that the infection of the rats with *T. congolense* caused anaemia consistent with the findings of other workers (Anosa, 1977; Ikede *et al.,*1977; Onyeyili and Anika, 1989; Anene *et al*., 1989). This decrease however, improved progressively following treatment to the level comparable with the uninfected control group before subsequent decreases initiated by recrudescence of parasites in the blood.

CONCLUSION

It was concluded from the results of this study that Dimivet® and ProZant® appeared to have comparable efficacies which were superior to Trypamidium - Samorin® in the treatment of *T. congolense* infection, although none of the drugs was able to cure the infection and prevent relapses of infection. The far longer stability of ProZant in solution compared with the other trypanocides, which has financial benefit, confers great advantage to the drug.

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