THE CHEMOPROPHYLACTIC EFFICACY OF KELAMIDIUM® IN ALBINO RATS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI*

T. A. Ezeudu[1*], I. O. Ezeh[1], R. C. Ezeokonkwo[1], I. K. Idika[1], C. N. Iheagwam[1], N. C. Nzeakor[2]
[1] *Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.*
[2] *Department of Veterinary Services, Ministry of Agriculture, Anambra State.*
**Corresponding author: E-mail – terry.ezeudu@unn.edu.ng Phone - 08038043211*

Abstract
The chemoprophylactic efficacy of Kelamidium® (a brand of Isometamidium chloride) was investigated in Trypanosoma brucei infected albino rats. A total of 25 female albino rats divided into five groups (I – V) of five rats each were used for the study. Group I served as uninfected control, group II as the infected control and groups III, IV and V pre-treated with Kelamidium® at day zero and infected at 8, 10 and 12 weeks respectively with 10^6 trypanosomes suspended in 0.2ml of normal saline. The packed cell volume (PCV), parasitaemia, and total leucocyte counts were used to assess the efficacy of Kelamidium®. The infection established parasitaemia in groups IV and V which were pre-treated at day zero with Kelamidium®, whereas no parasitaemia was recorded in group III throughout the experimental period. The infection was chronic in the untreated control, however only one rat showed the infection till the end of the experiment. The failure to establish infection in rats of group III shows that Kelamidium® was protective up to 8 weeks. It was concluded that the chemoprophylactic efficacy of Kelamidium® at 1.0mg/kg is protective in albino rats for 8 weeks only.

Key words: Trypanosoma brucei, Kelamidium, isometamidium chloride, chemoprophylaxis

Introduction

Trypanosomosis is a debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse-transmitted haemoflagellate protozoan parasites of the genus *Trypanosoma* (Anene *et al.*, 2001). The disease affects animals and man, and is widespread in tsetse infested areas of Africa (PAAT, 2001; Shaw, 2004). The impact of the disease in sub-Saharan Africa extends over some 10 million square kilometres (a third) of the continent (Finelle, 1983), and it has been estimated to cause great losses amounting to US $5 billion dollars annually in meat production, milk yield and tractive power (ILRAD, 1994). Infection is associated with irregular fever, anaemia, emaciation or weight loss, impairment of immune function, reproductive disorders, and death if the animals are not treated (Anosa 1988; Horst, 1996; Taylor and Authie, 2004).

Chemotherapy with trypanocides is the most widely applied method of control (PAAT, 2001; Holmes *et al.*, 2004). Over the years, treatment of animal trypanosomosis relied on three drugs – Diminazene aceturate, Homidium bromide (or chloride) and Isometamidium (Leach and Roberts, 1981; Holmes *et al.*, 2004). However, the commonly used drug for chemotherapy is Diminazene aceturate at 3.5-7 mg/kg body weight (Kuttler, 1988) whereas the most widely used chemoprophylactic agent is Isometamidium chloride at 0.5-1 mg/kg (Sone *et al.*, 1988; Toure S.M., 1970; Kaggwa E., et al 1988). The frequently and widely reported decreasing efficiency of available trypanocides, difficulties of sustaining tsetse control and little hope that a conventional anti-trypanosome vaccine will be produced in the near future have increased the need for new drugs for the control of trypanosomosis (**Anene, *et al.*, 2000**).

Isometamidium chloride is widely used in tropical countries for the control of animal trypanosomiasis principally in cattle but also in sheep, goats, buffalos, donkeys, horses, camels and dogs. It is given at doses of 0.5 or 1.0 mg/kg body weight by deep intramuscular injection. Kelamidium® is a new brand of Isometamidium chloride, and its chemotherapeutic and chemoprophylactic efficacy has been evaluated in dairy cows (Nonga and Kambarage 2008) and reported to have a prophylactic efficacy of 99.4% comparable to Samorin and was thus advocated as an alternative choice to Samorin. The objective of this present study was to evaluate the chemoprophylactic efficacy Kelamidium® in animal trypanosomosis, using albino rats as the experimental model.

Materials and Methods

2.

A total of 25 female albino rats were used for this study. They were randomly assigned into five groups I, II, III, IV, V . Where Group I = Uninfected control (Negative control), Group II = Infected on week 4 and not treated (Positive control), Group III = Pre-treated with Kelamidium® at day 0 and subsequently infected

with *T. brucei* after 8 weeks; Group IV = Pre-treated at day 0 and infected after 10 weeks; Group V = Pre-treated at day 0 and infected at week 12 post treatment. The *Trypanosoma brucei* used for this study was first isolated from a natural infection in pigs presented for slaughter at the Nsukka Municipal abattoir. The test drug was Kelamidium® a new brand of Isometamidium chloride, manufactured by KELA Belgium. These rats were acclimatized for about 3 weeks after which groups III, IV, V were pre-treated with Kelamidium® at 1.0mg/kg IM. Groups II, III, IV and V were inoculated with approximately 10^6 trypanosomes suspended in 0.2ml of normal saline intra-peritoneally at weeks 4, 8, 10 and 12 respectively.

The packed cell volume (PCV), parasitaemia and total leucocyte count (TLC) of the experimental rats were used to assess the efficacy of Kelamidium®. These parameters were determined weekly. The PCV was determined by the microhaematocrit method, while the TLC was done by the haemocytometer method (Coles, 1986). The level of parasitaemia (LOP) was estimated using the rapid matching method (Herbert & Lumsden, 1976).

Results and Discussion

Following the infection of the rats in group II the parasites became detectable in blood from the 4th day post infection with an average prepatent period of 5 days. The LOP increased and consequently led to anaemia and death of four out of the five rats in the untreated group (groups II). Also, the LOP in the infected untreated rats was significantly higher ($p < 0.05$) than the LOP of the pre-treated infected rats; this suggested that though Kelamidium® might not be able to proffer chemoprophylaxis beyond 8 weeks, it inhibited to an extent the multiplication of the parasites (trypanosomes) and thus a resulting LOP.

The clinical signs of trypanosomosis observed in the infected rat groups included pale mucous membrane, anaemia, rough hair coat and dullness. Deaths were recorded in the infected untreated control group (4 out of 5 rats). The clinical signs observed in the rats such as irregular fever, anaemia, pale mucous membrane, impairment of immune functions and death of untreated rats was consistent with earlier reports by Losos & Ikede (1970), Anosa (1988), Ilorst (1996), Onah *et al.* (2000), and Taylor & Authie (2004).

There was a significant decrease of the PCV (%) ($p < 0.05$) of the rats in groups II (infected untreated) at week 5 when compared with the uninfected control (group I) (Table 1); this was probably as a result of the *T. brucei* infection. At weeks 3, 7, and 8 the PCV of the various groups did not differ significantly ($p > 0.05$). The PCV (%) of groups II, III, IV and V further dropped by week 9, with a sharp decrease at week 10 and these were significantly lower than that of the uninfected control ($p < 0.05$). The anaemia and the resultant progressive fall in PCV is characteristic of trypanosomosis (Uzokwe, 1990). The results are consistent with the findings of Anene *et al.* (2006). Many factors have been reported in literature to be responsible for the reduction in the PCV in animal trypanosomosis; these include depression of erythropoiesis, immunolytic factors, disorders of coagulation, increased plasma volume and haemodilution (Murray & Dexter, 1988; Authie & Pobel, 1990; Taylor & Authie).

The uninfected control rats had significantly higher TLC ($p < 0.05$) when compared with groups II, III, IV and V at weeks 8 and 13 (Table 2). However, rats in group V which were pre-treated with Kelamidium and infected at week 12 had a significantly lower ($p < 0.05$) TLC at week 13 than those in groups II, III, and IV (Table 2). The significant reduction in the TLC of the infected group in this experiment agrees with the findings of Omamegbe & Uche (1985), Anika *et al.* (1987) and Akpa *et al.* (2008), and supports the long held view that trypanosomosis is associated with immuno-suppression and or immuno-depression.

Conclusion

Based on the result of this study, it was concluded that Kelamidium® has chemoprophylactic properties up to 8 weeks in rats when administered at the dose of 1.0 mg/kg body weight.

References

Anene BM, Onah DN, Nawa Y (2001). Drug resistance in pathogenic African trypanosomes, what hopes for the future? *Vet. Parasitol.*, 96:83-100.

Anene BM, Ezeokonkwo RC, Mmesirionye TI, Tettey JNA, Brock JM, Barret MP, Dekoning HP (2006). A diminazene-resistant strain of *Trypanosoma brucei* isolated from a dog in cross resistant to pentamidium in experimentally infected albino rats. *Parasitol.*, 132:127-133.

Anosa VO (1988) Haematological and biochemical changes in human and animal trypanosomosis. Parts 1. *Revue Elev Med Vet Pays Trops.*, 41:65-78

Authie E, Pobel T (1990). Serum haemolytic complement activity and C3 levels in bovine trypanosomosis under natural

conditions of challenge early indications of susceptibility to disease. *Vet. Parasitol.*, 35:43-59.

Coles EH (1986). Veterinary Clinical Pathology, 3rd ed. W.B. Saunders Company, Philadelphia, pp, 145-151.

Finelle P (1983). African Trypanosomosis. In FAO *Animal Production and Health* Paper. 37, 19–22.

Holmes PH, Eisler MC, Geerts S (2004). Current chemotherapy of animal trypanosomosis. In: Maudlin I, Holmes P.H, Miles M.A. (eds) Trypanosomosis. CAB International, Wallingford, pp 431-444.

Horst SHS (1996). Trypanosomosis. In: Horst SHS (Ed.) Tropical Animal Health. Kluwer Academic, Dordrecht, pp 152-169.

ILRAD (1994). Trypanosomosis. International Laboratory for Research on *Animal Diseases Reports*, Nairobi, pp: 21-29.

Kaggwa E, Munya WK, Mugera GM (1988). Relapse in dogs experimentally infected with *Trypanosoma brucei* and treated with diminazene aceturate or isometamidium chloride. *Vet Parasitol.*, 27:199-208.

Kuttler KL (1988). Chemotherapy of babesiosis. In: Ristic M (Ed.), Babesiosis of Domestic Animals and Man, CRC Press Inc., Boca Raton, Florida, pp. 227-243.

Leach TM, Roberts CJ (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomosis in the eastern hemisphere. *Pharmacol Ther.*, 13:91-147.

Losos GJ, Ikede BO (1972). Review of the pathology of diseases in domestic and laboratory animal caused by *T. congolense, T. vivax, T. brucei, T. rhodesiense* and *T. gambiense*. *Vet. Pathol.*, 9:1-72.

Lumsden WHR, Herbert WJ (1976). *Trypanosoma brucei*. A rapid matching method for estimating the host parasitaemia. *Exp. Parasitol.*, 40:427-431..

Murray M, Trail JCM, Davis CE, Black SJ (1984). Genetic resistance to African trypanosomosis. *J. Infect. Dis.*, 149: 311–319.

Nonga HE, Kambarage DM (2008). Evaluation of the chemotherapeutic and prophylactic efficacy of Kelamidium® in bovine trypanosomosis. *Bull. Anim. Hlth. Prod. Afri.*, 56:4

Omamegbe JO, Orajaka LJE, Emchelu CO (1984) The incidence and clinical forms of naturally occurring canine trypanosomosis in two veterinary clinics in Anambra state of Nigeria. *Bull. Anim. Health Prod. Afr., 32; 23-29.*

Onah DN, Hopkins J, Luckins AG (1999). Changes in the peripheral blood lymphocyte sub-populations and parasite specific antibody response in *Trypanosoma evansi* infection of sheep. *Parasitol. Res.*, 85:263–269.

PAAT (2001). Facts and figures on African trypanosomosis. *Programme Against African Trypanosomosis (PAAT) Newsletter*, 10, 5–7.

Sones KR, Njogu AR, Holmes PH (1988). Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride: a comparison of tests using cattle and mice. *Acta Trop.*, 45:153-164.

Taylor K, Authie ML (2004). Pathogenesis of animal trypanosomosis. In: Maudlin I, Holmes P.H, Miles M.A (eds) Trypanosomosis. CAB International, Wallingford, UK, pp 331-353

Toure SM (1970). Le prothidium et l'isometamidium dans le traitment de la trypanosomiase du chien à *Trypanosoma brucei. Rev. Elev. Med. Vet. Pays Trops.*, 42:55-61

Table 1: The mean packed cell volume (%) ± SEM of rats pre-treated with Kelamidium® and subsequently infected with *Trypanosoma brucei*

Time (weeks)	Group I	Group II	Group III	Group IV	Group V
1	...	45.8 ± 1.16	47.6 ± 0.81	47.4 ± 0.81	48 ± 1.58
3	48.2 ± 1.07	44.75 ± 2.17	45.4 ± 2.66	46.4 ± 1.44	49.2 ± 1.24
5	46.6 ± 1.72^{ab}	42.75 ± 0.75^{b}	43.8 ± 0.97^{ab}	47.4 ± 0.68^{a}	45.8 ± 1.39^{ab}
7	46 ± 1.92	43.5 ± 1.55	46.2 ± 1.98	48.8 ± 1.43	47.8 ± 2.40
8	46 ± 1.48	44.25 ± 1.31	43.4 ± 2.89	47.4 ± 1.75	45.2 ± 1.69
9	47 ± 1.79^{ab}	45 ± 3.06^{b}	48.2 ± 0.92^{ab}	46.4 ± 1.03^{ab}	50.4 ± 0.81^{a}
10	43.6 ± 1.12^{a}	31 ± 1.00^{c}	35.8 ± 1.28^{b}	34.6 ± 0.81^{b}	36 ± 1.05^{b}
11	44.8 ± 0.80^{a}	38.5 ± 1.50^{b}	47 ± 2.10^{a}	42.4 ± 1.21^{ab}	46.6 ± 1.17^{a}
12	49.8 ± 1.66^{a}	40 ± 0.00^{b}	46 ± 1.95^{ab}	44.6 ± 3.61^{ab}	50.4 ± 0.75^{a}
13	45.6 ± 1.29^{ab}	49 ± 0.00^{a}	44.4 ± 1.36^{ab}	42.8 ± 1.36^{b}	42.4 ± 1.50^{b}

$^{a, b, c}$ Different superscripts in a row indicate significant differences at $p < 0.05$

Table 2. The mean total leucocyte count (10^3 cells/mm^3) ± SEM of rats pre-treated with Kelamidium® and subsequently infected with Trypanosoma brucei

Time (weeks)	Group I	Group II	Group III	Group IV	Group V
1	14.59 ± 2.10	13.41 ± 2.10	12.35 ± 2.11	11.84 ± 1.51	14.59 ± 2.02
3	12.17 ± 1.61	10.21 ± 1.03	13.11 ± 1.24	13.71 ± 2.91	11.44 ± 1.72
5	15.92 ± 2.24	14.21 ± 1.50	17.59 ± 4.36	13.03 ± 1.29	13.59 ± 2.33
7	16.04 ± 1.54	12.23 ± 2.30	19.59 ± 2.23	14.99 ± 2.31	18.87 ± 3.47
8	20.16 ± 2.98^{a}	10.55 ± 1.02^{bc}	14.20 ± 2.23^{abc}	15.47 ± 1.74^{ab}	8.66 ± 0.51^{c}
9	16.96 ± 2.34	12.62 ± 1.16	12.35 ± 1.82	14.31 ± 1.93	13.68 ± 2.31
10	14.93 ± 1.51	8.85 ± 1.89	13.55 ± 1.97	9.38 ± 3.18	15.83 ± 2.10
11	14.25 ± 2.65	8.58 ± 3.83	9.76 ± 3.95	13.67 ± 2.34	14.38 ± 1.86
12	19.38 ± 5.96	8.20 ± 0.00	10.08 ± 1.59	11.05 ± 1.39	13.13 ± 2.54
13	15.04 ± 3.74^{a}	14.30 ± 0.00^{ab}	9.71 ± 0.86^{ab}	8.94 ± 1.67^{ab}	6.99 ± 1.13^{b}

$^{a, b, c}$ Different superscripts in a row indicate significant differences at $p < 0.05$

[Group I = Uninfected control (Negative control), Group II = Infected on week 5 a nd not treated (Positive control), Group III = Pre -treated with Kelamidium® at day 0 and subsequently infected with T. bruceiafter 8 weeks; Group IV = Pre -treated at day 0 and infected after 10 weeks; Group V = Pre -treated at day 0 and infected at week 12 post treatment].