Research Journal of Veterinary Sciences

ISSN 1819-1908

Academic Journals Inc.

www.academicjournals.com

Research Journal of Veterinary Sciences 8 (2): 36-41, 2015
ISSN 1819-1908 / DOI: 10.3923/rjvs.2015.36.41

Comparative Haematological Changes in Rats Experimentally Infected with *Trypanosoma brucei brucei* and Treated with Imidocarb Dipropionate and Diminazene Aceturate

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ABSTRACT

Trypanosomosis is a disease that causes great haematological alterations in the infected. Despite the importance of the disease in animals, there is yet scarce trypanocides available for treatment of infected mammals. The present limitation in chemotherapy in trypanosomosis lead to assessment of the usefulness of imidocarb di propionate as an alternative trypanocide. Twenty one pathogen free albino rats were used in the study. They were randomly grouped into 3 of 7 members each. The GPA was uninfected control, GPB was infected with *T. brucei brucei* and treated with diminazene aceturate and GPC was infected with *T. brucei brucei* and treated with imidazole dipropionate. By 5 to 6 days post infection, there was a significant decrease in the PCV and Hb concentration values of the infected groups (GPB and GPC) and up to day 7 in GPC. There were haematological improvement in the infected groups by day 8 post infection (day 3 post treatment) on treatment with imidocarb dipropionate and diminazene in GPB and GPC, respectively. The rapid haematological improvements in the groups were attributed to prompt treatment and acuteness of the disease in the rats. It was concluded that *T. brucei brucei* alters both the PCV and Hb values of infected rats and treatment with imidocarb dipropionate significantly ($p<0.05$) improved altered haematological values and therefore could serve as an alternative trypanocide.

Key words: *Trypanosoma brucei brucei,* pack cell volume, hemoglobin conc, imidocarb dipropionate, diminazene aceturate

INTRODUCTION

African trypanosomosis is caused by a flagellate protozoan, *Trypanosoma brucei brucei* especially coined after David Bruce a Scottish parasitologist (Hoffman *et al*., 2013). *Trypanosoma brucei brucei* inhabits the blood plasma, intercellular tissues and body cavity fluid of an infected animal precipitating anaemia and tissue damage (Sharma *et al*., 2000). Blood cells are useful indicator of ill health and are invaluable in diagnosis, treatment and prognosis of vast number of disease condition. Trypanosomosis causes significant decrease in Pack Cell Volume (PCV) and hemoglobin concentration (Ekanem and Yusuf, 2008; Akanji *et al*., 2009). Their presence in the blood induces increased red blood cell destruction and changes in biochemical constituents of blood (Igbokwe and Mohammed, 1992; Taiwo *et al*., 2003; Ekanem and Yusuf, 2008; Akanji *et al*., 2009). The mechanism of anaemia is not clearly understood however, in goats, anaemia is characterized

by macrocytosis, reticulocytosis, hyperplasia of bone marrow and spleen and increased hemosiderin deposits (Sharma *et al*., 2000). It may also result from haemolysis as indicated by a shortened half life of Cr labeled RBCs (Jennings, 1976). Imidocarb dipropionate is a derivative of imidazole that commonly comes as salt of dipropionate or hydrochloride primarily used in treatment of babesiosis in dogs (EMEA., 2001). The present study accesses the potency of imidocarb di propionate and diminazene aceturate in reversal of the reduction in pack cell volume and hemoglobin concentration in *T. brucei brucei* infection in rats.

MATERIALS AND METHODS

Twenty-one nine weeks old pathogen free albino rats of both sexes that weighed between 150-300 g were used in the study. The rats were breed in the laboratory animal house of Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The rats were fed and watered *ad libitum* prior to commencement of the study. Each rat was identified with picric acid stain.

Imidocarb dipropionate (12%) (Imizole®. Intervet/Merck Animal Health NADA 141-071, Approved by FDA Germany) was administered to the GPC rats at the dose of 24 mg kg^{-1} subcutaneously for 2 consecutive days.

Diminazene aceturate (Veribin® CEVA Sante Animale- La Ballasteiére 33501 Libourne Cedex, France) a generic brand of trypanocide was also administered to GPB at the dose of 3.5 mg kg^{-1} given intramuscularly stat.

The *T. brucei brucei* parasite used in this study was a "Federe" strain obtained from the National Institute of Trypanosomosis and Onchocerciasis Research (NITOR) Vom, Plateaue State, Nigeria. The parasites were cryopreserved in liquid nitrogen from where donor rats were initially infected. The parasites were maintained by serial passage in rats at the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

An estimated 2.5×10^6 trypanosomes suspended in 1 mL of normal saline was used to infect each experimental rat through the intraperitoneal route using 1 mL tuberculin syringes. The quantity of parasite was estimated using the rapid matching method of Herbert and Lumsden (1976).

RESULTS

Twenty one pathogen free albino rats were randomly grouped into 3 groups of 7 members each. The GPA was the un-infected control; GPB was infected with *T. brucei brucei* and treated with diminazene aceturate at the dose of 3.5 mg kg^{-1}. The GPC was infected with *T. brucei brucei* and treated with Imidocarb di propionate at the dose of 24 mg kg^{-1} for 3 consecutive days.

Parasitaemia was determined using two methods; the wet blood mounts and the haematocrit buffy coat methods as described by Woo (1970).

Hematology: Two milliliters of blood was collected through the media canthus of the eyes of each of the experimental rats into labeled ethylenediamine tetra-acetic acid (EDTA) containing bottle for the determination of both PCV and hemoglobin (Hb) concentrations.

Capillary tubes were filled with labeled blood samples from the rats and centrifuged at 11000 rev for 5 min, the PCV values were read using the PCV reader (Coles, 1986).

Hemoglobin concentration was determined using the cyanomethaemoglobin method (Van Kampen and Zijlstra, 1961).

Data obtained was presented as Mean±Standard Error of Mean (SEM). Statistical significance was determined using one way analysis of variance (ANOVA) and Duncan's multiple range tests of SPSS version 16 soft ware packages. The level of Significance was accepted at $p<0.05$ (Scenedor and Cocharm, 1973).

In Table 1, there was no significant difference ($p<0.05$) in the PCV of the infected groups (GPB and GPC) until by day 5 and 6 post infection when there was a significant ($p<0.05$) decrease in both GPB and GPC. The decrease continued up to day 7 in GPC. The commencement of treatment on day 5 in GPB with diminazene aceturate at 3.5 mg kg^{-1} once achieved haematological improvement by day 8 post treatment. There was equally haematological improvement by day 8 post treatment in GPC on treatment with imidocarb di propionate at 24 mg kg^{-1} for 3 consecutive days. Subsequently, there was no recorded of haematological alterations in both GPB and GPC till the end of experiment.

In Table 2, *Trypanosoma brucei brucei* infection of the experimental groups (GPB and GPC) done on day 1 was established by day 4 post infection. By day 5 and 6, there was a significant decrease in hemoglobin concentration in both GPB and GPC and up to day 7 in GPC. Treatment with diminazene aceturate in GPB and Imidocarb dipropionate in GPC on day 5 gave haematological improvements in both groups by day 8 till the end of the experiment.

Table 1: Mean±SE PCV (%) of rats with experimental *T. brucei brucei* and treated with diminazene aceturate and imidocarb dipropionate

Experimental period (days)	GPA (control)	GPB	GPC
0	35.71±2.59^{a}	32.43±2.64^{a}	35.14±3.33^{a}
1#	36.31±1.50^{a}	34.71±1.34^{a}	36.001±0.21^{a}
2	36.71±2.09^{a}	35.71±2.19^{a}	35.01±0.59^{a}
3	37.51±1.56^{a}	36.01±0.50^{a}	35.01±2.09^{a}
4	37.71±2.59^{a}	35.01±0.19^{a}	33.71±1.00^{a}
5*+	38.43±0.65^{a}	24.71±0.94^{b}	32.29±2.37^{b}
6*	35.71±2.59^{a}	26.31±2.59^{b}	28.71±2.50^{b}
7*	34.29±2.75^{a}	29.00±1.57^{ab}	27.57±1.39^{b}
8	35.71±2.59^{a}	31.71±0.59^{a}	30.71±2.39^{a}
9	35.71±2.59^{a}	32.76±0.50^{a}	31.61±0.51^{a}
10	35.71±2.59^{a}	33.01±0.19^{a}	34.01±0.49^{a}
11	37.70±0.50^{a}	34.71±2.00^{a}	33.01±0.53^{a}
12	38.14±1.12^{a}	35.00±1.66^{a}	34.00±1.15^{a}

a, b Homogeneity between the experimental groups at probability $p<0.05$, #: Day of *Trypanosoma brucei* infection, +: Day of treatment with diminazene aceturate, *: Day of treatment with imidocarb dipropionate

Table 2: Mean±SE Hemoglobin concentration of rats with experimental *T. brucei brucei* and treated with diminazene aceturate and imidocarb di propionate

Experimental period (days)	GPA (control)	GPB	GPC
0	10.96±0.46^{a}	9.14±0.28^{a}	9.97±0.25^{a}
1#	12.51±0.28^{a}	10.21±0.48^{a}	10.21±0.18^{a}
2	13.32±0.40^{a}	11.71±0.20^{a}	11.51±0.40^{a}
3	14.51±0.28^{a}	12.01±0.40^{a}	12.30±0.23^{a}
4	13.20±0.03^{a}	12.51±0.18^{a}	13.01±0.18^{a}
5*+	13.01±0.43^{a}	8.27±0.46^{b}	9.16±0.79^{b}
6*	14.51±0.28^{a}	8.01±0.20^{b}	7.51±0.48^{b}
7*	13.60±0.20^{a}	10.91±0.65^{a}	9.86±0.35^{b}
8	13.51±0.48^{a}	11.51±0.23^{a}	10.01±0.42^{a}
9	12.01±0.78^{a}	12.02±0.48^{a}	11.01±0.20^{a}
10	13.51±0.20^{a}	12.51±0.21^{a}	12.01±0.88^{a}
11	12.01±0.08^{a}	11.90±0.40^{a}	12.00±0.08^{a}
12	13.01±0.42^{a}	12.51±0.28^{a}	12.01±0.19^{a}

a, b Homogeneity between the experimental groups at probability $p<0.05$, #: Day of *Trypanosoma brucei brucei* infection, +: Day of treatment with diminazene aceturate, *: Day of treatment with imidocarb dipropionate

DISCUSSION

The period of establishment of *T. brucei brucei* infection in both GPB and GPC were in line with previous works done in *T. brucei brucei* infection in animals (Akpa *et al*., 2008; Gerasimos and Kent, 2014). The significant decreases in Pack Cell Volume (PCV) and hemoglobin concentration (Hb) signified some degree of blood loss in the infected groups. This corroborates previous work done in trypanosomosis in animals (Eloy and Lucheis, 2009; Ezeokonkwo *et al*., 2010; Nwoha and Anene, 2011a). A sufficient blood loss could result to anaemia which is one of the cardinal signs of trypanosomosis in animals (Sadun *et al*., 1973; Finelle, 1973; Ohaeri and Eluwa, 2011) and in humans (Woodruff *et al*., 1973) and clinically anaemia is presented by paleness of the mucous membrane (Nwoha and Anene, 2011b). In trypanosomosis, matrix of factors may be contributory to the significant deficits in blood parameters observed in infected animals and these may include haemolysis as previously recorded by Jennings (1976). It may also result from hemorrhage due to mechanical damage to the parasitized erythrocytes (Eloy and Lucheis, 2009). Such damage is usually severe during peak of parasitaemia (Holmes, 1976). Other possible cause of anaemia which before now has been disregarded is malnutrition which often arise sequel to anorexia. Anorexia is a common clinical finding in infected animals and over time compromises the availability of essential elements necessary for haematopoesis which are obtainable from food. Anorexia plays a significant role in anaemia especially in chronic conditions which enhances depletion of nutrient body reserves. Several workers have already recorded significant decrease in PCV and Hb in *T. brucei brucei* infection in dogs (Nwoha and Anene, 2011a), in *T. evansi* infection in Sheep (Onah *et al*., 1996), in *T. brucei rhodesiensi* infection in Vevert monkeys (Kagira *et al*., 2006) and in *T. evansi* infection in goats (Sharma *et al*., 2000). Treatments with diminazene aceturate and imidocarb dipropionate were curative and caused haematological improvement in both the PCV and Hb values of the infected rats. This apparently occurred post elimination of trypanosome parasites from the rats. The result of this investigation shows that imidocarb dipropionate is as potent as diminazene aceturate in the elimination of trypanosomes parasites from an infected blood. The comparative efficacy of imidocarb dipropionate to diminazene aceturate could be utilized as an alternative trypanocide especially in zones or areas currently faced with challenges of drug resistance to the latter. Until now resistant strains of trypanosomes are major limitation to effective use of diminazene aceturate in the treatment of trypanosomosis in animals (Chigozie *et al*., 2012). The rapid haematological improvement in both the PCV and Hb on treatment could be associated with the acuteness of the disease and also prompt administration of therapy on the infected rats. Both factors enhanced quick and rapid restoration of reduced hematology. The study therefore concludes that *T. brucei brucei* infection resulted in a decrease in both PCV and Hb in infected rats and also exposes the potency of imidocarb dipropionate as an alternative trypanocide.

REFERENCES

Akanji, M.A., O.S. Adeyemi, S.O. Oguntoye and F. Sulyman, 2009. *Psidium guajava* extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. EXCLI J., 8: 148-154.

Akpa, P.O., R.C. Ezeokonkwo, C.A. Eze and B.M. Anene, 2008. Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei* brucei infection in dogs. Vet. Parasitol., 151: 139-149.

Chigozie, U.S., A.B. Maduka and J.G. Ifeanyi, 2012. Trypanocidal efficacy of diminazene in diabetic rats. Iraqi J. Vet. Sci., 26: 33-38.

Coles, E.H., 1986. Veterinary Clinical Pathology. 4th Edn., WB Saunders Co., Philadelphia PA., ISBN-13: 978-0721618289, pp: 145-151.

EMEA., 2001. Committee for veterinary medicinal products Imidocarb. EMEA/MRL/785/01-FINAL, May 2001, The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines and Inspections.

Ekanem, J.T. and O.K. Yusuf, 2008. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. Afr. J. Biomed. Res., 11: 79-85.

Eloy, L.J. and S.B. Lucheis, 2009. Canine trypanosomiasis: Etiology of infection and implications for public health. J. Venomous Anim. Toxins Incl. Trop. Dis., 15: 589-611.

Ezeokonkwo, R.C., I.O. Ezeh, J.I. Onunkwo, P.O. Obi, I.W. Onyenwe and W.E. Agu, 2010. Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Vet. Parasitol., 173: 48-54.

Finelle, P., 1973. African animal trypanosomiasis. World Anim. Rev., 8: 24-27.

Gerasimos, L. and L.H. Kent, 2014. Motility and more: The flagellum of *Trypanosoma brucei*. Nat. Rev. Microbiol., 12: 505-518.

Herbert, W.J. and W.H.R. Lumsden, 1976. *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitemia. Exp. Parasitol., 40: 427-431.

Hoffman, R., E.J. Benz Jr., L.E. Silberstein, H. Heslop, J. Weitz and J. Anastasi, 2013. Hematology: Basic Principles and Practice. Elsevier Health Sciences, London, UK., ISBN-13: 9781437729283, Pages: 2343.

Holmes, P.H., 1976. The Use of Radioisotopic Tracer Techniques in the Study of the Pathogenesis of Trypanosomiasis. In: Nuclear Techniques in Animal Production and Health, IAEA (Ed.). International Atomic Energy Agency, Vienna, ISBN-13: 9789200102769, pp: 436-474.

Igbokwe, I.O. and A. Mohammed, 1992. Some plasma biochemical changes in experimental *Trypanosoma brucei* infection of Sokoto red goats. Rev. Elev. Med. Vet. Pays Trop., 45: 287-290.

Jennings, F.W., 1976. The Anaemia of Parasitic Infections. In: Pathophysiology of Parasitic Infection, Soulsby, E.J.L. (Ed.). Academic Press, New York, USA., pp: 41-67.

Kagira, J.M., J.K. Thuita, M. Ngotho, R. Madachi, D.M. Mwangangi, J.M. Ndung'u, 2006. Haematology of experimental *Trypanosoma brucei rhodesiense* infection in vervet monkeys. Afr. J. Health Sci., 13: 59-65.

Nwoha, R.I.O. and B.M. Anene, 2011a. Changes in packed cell volume and hemoglobin concentration in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and *Ancylostoma caninum*. Philippian J. Vet. Anim. Sci., 37: 151-158.

Nwoha, R.I.O. and B.M. Anene, 2011b. Clinical signs and pathological changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei brucei* and *Ancylostoma caninum*. J. Vet. Parasitol., 25: 97-102.

Ohaeri, C.C. and M.C. Eluwa, 2011. Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. Vet. Parasitol., 177: 199-202.

Onah, D.N., J. Hopkins and A.G. Luckins, 1996. Haematological changes in sheep experimentally infected with *Trypanosoma evansi*. Parasitol. Res., 82: 659-663.

Sadun, E.J., A.J. Johnson, R.B. Nague and R.C. Duxbury, 1973. Experimental infection with African trypanosomes: Veterinary preliminary parasitological clinical haematological serological and pathological observations in rhesus monkeys infected with trypanosome rhodesiense. Am. J. Trop. Med. Hyg., 22: 323-330.

Scenedor, G.W. and W.G. Cocharm, 1973. Statistical Method. 6th Edn., Iowa State University Press, Amess, Iowa, USA.

Sharma, D.K., P.P.S. Chauhan, V.K. Saxena and R.D. Agrawal, 2000. Haematological changes in experimental trypanosomiasis in Barbari goats. Small Rumin. Res., 38: 145-149.

Taiwo, V.O., M.O. Olaniyi and A.O. Ogunsanmi, 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental *Trypanosoma congolense* and *T. brucei* infections in sheep. Israel J. Vet. Med., 58: 112-117.

Van Kampen, E.J. and W.G. Zijlstra, 1961. Test-combination haemoglobin. Clin. Chem. Acta, 6: 538-538.

Woo, P.T., 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. Acta Trop., 27: 384-386.

Woodruff, A.W., J.L. Ziegler, A. Hathaway and T. Gwata, 1973. Anaemia in African trypanosomiasis and big spleen disease in Uganda. Trans Res. Sci. Trop. Med. Hyg., 67: 329-337.