African Journal of Biomedical Research, Vol. 8 (2005); 197 - 201
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group
Available online at http://www.bioline.org.br/md

Full Length Research Article

Pathogenicity of *Trypanosoma congolense* Infection following Oral Calcium Chloride Administration in Rats

T.N. Egbe-Nwiyi[*], I. O. Igbokwe and P. A. Onyeyili[2]

Departments of Veterinary Pathology, Physiology and Pharmacology[2], University Of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria

Received: August, 2005
Accepted: September, 2005

Abstract
Eighty healthy adult albino rats of both sexes weighing 180-200g were used in two experiments to study the effects of oral calcium chloride treatment on the pathogenicity of *Trypanosoma congolense* infection. Experiment 1 was terminated at the peak of parasitaemia while experiment II was allowed to run a full course. In each experiment, forty rats were divided into four groups of 10 rats each, namely: A, uninfected untreated control; B, treated uninfected control; C, infected untreated and D, treated infected. Aqueous solution (10%) of $CaCl_2$ was administered daily using stomach tube to each rat at 100mg/kg in groups B and D from 7 days before infection to the end of the study. Each rat in groups C and D was infected by intraperitoneal injection of $1x10^6$ trypanosomes in phosphate buffered saline solution (pH 7.4) diluted donor rat blood. The infected untreated group exhibited higher ($P< 0.05$) parasitaemia, more severe anaemia and hepatic and renal damage than the infected treated group. In conclusion, oral calcium chloride treatment in rats seemed to reduce the pathogenicity of *T. congolense* infection by delaying the onset of parasitaemia and reducing the levels of parasitaemia, accompanied anaemia as well as organ damage.

Keywords: Rats, *Trypanosoma congolense*, calcium chloride treatment, pathogenicity.

INTRODUCTION

Trypanosomosis is a disease of man and animals (Losos and Ikede, 1972). The disease in animals is produced by *Trypanosoma vivax, T. congolense, T. Simiae, T. b. brucei, T. evansi* and *T. equiperdum*. The human trypanosomes are *T. b. rhodesiense, T. b. gambiense* and *T. cruzi* (Radostits *et al*, 1994). The pathology and pathogenesis of the disease have been described previously (Losos and Ikede, 1970; Losos, 1986; Radostits *et al*, 1994). *Trypanosoma congolense* has affinity for the microvasculature (Losos, 1986). Nutrition is known to modulate the severity of trypanosomal infection in animals (Otesile *et al*, 1991; Igbokwe, 1995; Egbe-Nwiyi, 2003). Calcium is an important cation in the animal's body and membrane function of both the trypanosomes and the hosts (Kaneko, 1989). Calcium plays a role in blood coagulation, cell membrane and capillary permeability (Guyton and Hall, 1996). Calcium is present in variable amount in almost all feed stuff (National Research Council, 1971). Milk and milk products are the best food sources of calcium in man and animals. Several non feed supplemented sources of calcium are used in animal diet, with the most common being ground limestone. Other common sources include Oyster shell, Calcium Sulphate, Calcium Chloride, Phosphate and bone meal (NRC, 1971). Many sources of calcium are

inexpensive and animals have a wide tolerance level for calcium.

This study was designed to determine the effect of oral calcium chloride treatment on the pathogenicity of *Trypanosoma congolense* infection in rats.

MATERIALS AND METHODS

Experimental animals: Eighty healthy adult albino rats of both sexes weighing 180-200g were obtained from the laboratory animals unit of the Department of Pharmacology, University of Jos, Nigeria. They were housed in clean cages at room temperature (30-35^{0}C), fed standard commercial diet (ECWA Feeds Ltd) and provided clean water *ad libitum*. Before the commencement of the experiments, the animals were screened for the presence of blood parasites using standard techniques (Schalm *et al*, 1975). Forty rats were used in each of the two experiments (I and II) each with four groups (A,B,C and D) of 10 rats. The groups were treated as follows; A, uninfected untreated control; B, treated uninfected control; C, infected untreated and D, treated infected. Experiment I was terminated at the peak of parasitaemia while experiment II was allowed to run a full course.

Trypanosome Infection: *Trypanosoma congolense* (Gboko strain) obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Nigeria were maintained in rats by serial passages. Phosphate buffered saline solution (pH 7.4) diluted infected blood from donor rats containing 1x10^6 trypanosomes was intraperitoneally injected into each rat in groups C and D in experiments I and II.

Oral calcium chloride ($CaCl_2$) treatment: Calcium chloride (May and Baker Laboratory Chemicals Ltd, Dagenham, England) solution (10% aqueous) was administered daily by stomach tube to each rat at 100mg/kg in groups B and D in both experiments. The solution was administered from 7 days before trypanosome infection to the end of the study.

Haematological and biochemical analysis:

Tail blood samples were collected from rats at intervals pre and post parasite inoculation and evaluated for parasitaemia and haematological changes. Parasitaemia was measured by haemocytometry (Schalm *et al*, 1975) while packed cell volume (PCV) was used to assess the haematological response and determined by microhaematocrit method (Schalm *et al*, 1975).

The rats in experiment I were decapitated under ether anaesthesia at 28 days post-infection (pi) and blood from the neck vessels collected, allowed to clot and serum harvested after centrifuging at 1000g for 5 minutes. Serum alanine (ALT) and aspartate (AST) aminotransferases activities, serum cholesterol and calcium levels were determined using commercial reagents kits (Randox, Laboratory LTD, UK). Serum urea and creatinine concentrations were estimated by methods of Tietz (1986).

Statistical analysis: The data obtained were summarized as means ± standard deviations and means were compared by analysis of variance (ANOVA) and students's t-test (Chatfield, 1983).

RESULTS

Parasitological findings: In groups C and D, the mean prepatent periods in experiment I (Ex 1) were 10.1 ± 1.4 and 14.5 ± 2.5 days respectively while in experiment II (Ex II), they were 10.2 ± 1.3 and 13.3 ± 2.3 days respectively. The prepatent periods were significantly shorter ($P < 0.05$) in group C than group D in Ex 1 and Ex. II. The level of parasitaemia was progressive in both infected groups however group C exhibited higher ($P< 0.05$) level of parasitaemia than group D (Table 1).

Haematological findings: The packed cell volume (PCV) decreased significantly ($P< 0.05$) in all the infected rats in groups C and D when compared with the values in the uninfected untreated or uninfected treated control rats in groups A and B respectively in both Ex1 and Ex II. The level of decrease was more severe ($P < 0.05$) in group C than group D in both Ex 1 and Ex II.

Biochemical findings: The serum ALT, AST, Cholesterol, calcium, urea and creatinine values are presented in Table 3. The ALT, AST, urea and creatinine levels increased significantly ($P < 0.05$) in groups C and D with the level being higher ($P<0.05$) in group C than group D. The serum cholesterol and calcium levels on the other hand, decreased significantly ($P<0.05$) in both infected groups C and D with the level of decrease observed to be more ($P < 0.05$) in group C than group D.

Table 1: Mean (± SD), parasitaemia (x 10^3/µl) of rats orally treated or untreated with $CaCl_2$ and infected with *T. congolense* when experiment terminated at the peak of parasitaemia (PP) or allowed to run a full course (FC)

infection Stage	Days post - infection	*T. congolense* infected untreated	*T. congolense* infected treated
PP	8	4.0±12.6 [a]	0.0 [b]
	10	26.0±23.1 [a]	4.0±12.6 [b]
	12	55.0±12.6 [a]	14.0±23.1 [b]
	14	75.0±12.6 [a]	24.0±32.2 [b]
	16	97.0±11.5 [a]	48.0±32.9 [b]
	18	120.0±16.3 [a]	72.0±26.9 [b]
	20	202.0±28.9 [a]	94.0±26.7 [b]
	22	258.0±40.4 [a]	121.0±28.4 [b]
	24	358.0±44.6 [a]	144.0±33.7 [b]
	26	732.0±10.2 [a]	162.0±33.2 [b]
	28	848.0±10.4 [a]	248.0±25.2 [b]
FC	8	4.0±12.6 [a]	0.0 [b]
	10	30.0±27.0 [a]	4.0±12.6 [b]
	12	59.0±19.1 [a]	18.0±23.9 [b]
	14	75.0±15.0 [a]	38.0±25.9 [b]
	16	96.0±15.0 [a]	61.0±29.1 [b]
	18	122.0±19.8 [a]	76.0±21.1 [b]
	20	163.0±23.1 [a]	87.0±19.4 [b]
	22	185.0±25.4 [a]	99.0±21.8 [b]
	24	224.0±42.9 [a]	115.0±23.6 [b]
	26	282.0±56.1 [a]	134.0±25.0 [b]
	28	593.0±14.0 [a]	156.0±24.5 [b]
	30	613.0±48.1 [a]	340.0±82.7 [b]
	32	-	382.0±51.8

a, b values in rows with different superscript differ significantly (P < 0.05); - = death

The serum calcium level in group B ($CaCl_2$ treated and uninfected control) was slightly higher (P > 0.05) than that in group A (uninfected and untreated control) while the level was significantly lower (P < 0.05) in group C (infected and untreated rats) when compared with group D (infected and treated rats). The mean survival times in groups C and D rats were 26.5 ± 0.5 and 32.0 ± 1.6 days respectively, with the value being shorter (P < 0.05) in group C than group D.

DISCUSSION

In the present study, calcium chloride treatment appeared to have ameliorated the pathogenicity of *Trypanosoma congolense* infection in rats. This is based on the premise that the observed prepatent period and survival time were shorter, level of parasitaemia higher and anaemia more severe in *T. congolense* infected and calcium chloride untreated (group C) than in the infected and treated (group D) rats. Calcium chloride ($CaCl_2$) treatment can be considered as the only exogenous factor that could have accounted for the differences in observations of the parameters assessed. Infective dose of trypanosome species has been reported to influence variations in some parameters in trypanosomosis (Losos, 1986; Murray and Dexter, 1988) but such phenomenon is not applicable in this study, since all the infected rats got similar dose.

Table 2: Packed cell volume (%) of control rats and rats infected with *T. congolense* (TC) with or without oral $CaCl_2$ treatment when the experiment was terminated at the peak of parasitaemia (PP) or allowed to run a full course (FC).

Stage of infection	Days post – infection	Uninfected untreated control	Treated uninfected control	TC infected untreated	TC infected treated
PP	0	50.1 ± 0.8 [a]	50.6± 0.8 [a]	50.8±0.5 [a]	50.1±0.7 [a]
	4	50±0.7 [a]	50.6±0.9 [a]	50.7±0.5 [a]	50.1±0.7 [a]
	8	50.2±1.0 [a]	50.5±0.9 [a]	50.6±0.5 [a]	50.1±0.7 [a]
	12	50.4±0.7 [a]	50.7±0.8 [a]	47.2±1.2 [b]	49.6±0.2 [a]
	16	50.2±0.7 [a]	50.6±0.7 [a]	44.3±0.8 [b]	48.0±2.2 [c]
	20	50.2±0.7 [a]	50.6±0.7 [a]	39.6±0.5 [b]	46.0±1.6 [c]
	24	50.2±0.7 [a]	50.6±0.8 [a]	30.4±2.7 [b]	41.4±3.6 [c]
	28	50.3±0.7 [a]	50.6±0.8 [a]	23.1±1.7 [b]	36.0±1.2 [c]
FC	0	50.3±0.5 [a]	50.6±0.0 [a]	50.0±0.0 [a]	50.0±0.0 [a]
	4	50.3±0.5 [a]	50.4±0.0 [a]	50.0±0.0 [a]	50.0±0.0 [a]
	8	50.3±0.5 [a]	50.0±0.0 [a]	49.8±0.1 [a]	50.0±0.0 [a]
	12	50.3±0.5 [a]	50.0±0.0 [a]	43.6±2.7 [b]	49.1±1.1 [a]
	16	50.3±0.5 [a]	50.0±0.0 [a]	39.1±1.1 [b]	47.1±1.1 [c]
	20	50.3±0.5 [a]	50.0±0.0 [a]	28.8±2.5 [b]	45.2±1.0 [c]
	24	50.3±0.5 [a]	50.0±0.0 [a]	24.0±0.7 [b]	43.4±1.1 [c]
	28	50.3±0.5 [a]	50.0±0.0 [a]	19.6±1.0 [b]	39.6±1.0 [c]
	32	50.4±0.6 [a]	50.2±0.7 [a]		32.4±2.1 [b]

- = death; [a,b,c] Values in rows with different superscripts differ significantly (p<0.05)

Table 3:
Serum activities of alanine (ALT) and aspartate (AST) aminotransferases, serum urea and creatinine concentrations, and serum Cholesterol and calcium levels in rats orally treated or untreated with $CaCl_2$ and infected with *T.* congolense.

Parameters	Uninfected untreated control	Treated uninfected control	*T. congolense* infected untreated	*T. congolense* infected treated
ALT (i.u/L)	9.2±1.0 [a]	9.4±1.1 [a]	60.2±3.5 [b]	30.2±1.9 [c]
AST (i.u/L)	10.3±1.8 [a]	10.1±1.8 [a]	60.1±3.4 [b]	30.1±1.8 [c]
Cholesterol (mg/dl)	140.6±2.9 [a]	140.7±2.1 [a]	50.5±3.9 [b]	93.0±3.2 [c]
Calcium (mg/dl)	9.1±0.4 [a]	9.9±0.5 [a]	2.7±0.4 [b]	5.0±0.4 [c]
Urea (mmol/dl)	4.8±0.1 [a]	4.8±0.1 [a]	13.2±0.3 [b]	8.4±0.2 [c]
Creatinine (µmol/l)	63.3±1.7 [a]	63.5±1.7 [a]	125.7±3.9 [b]	90.5±4.3 [c]

[a,b,c] Values in rows with different superscripts differ significantly ($p<0.05$)

Anaemia which was more severe in the infected and untreated rats generally determines the severity of trypanosome infection in animals (Lisos, 1986; Murray and Dexter, 1988). The anaemia coincided with the onset of parasitaemia and progressed with the height of the latter.

The biochemical parameters indicated liver and kidney damage. The increase in serum ALT and AST and reduced cholesterol levels may be associated with liver damage (Whitelaw *et al*, 1980; Baron, 1982; Kaggwa *et al*, 1984), while the elevated serum urea and creatinine levels may probably be due to renal damage (Anosa, 1988). The increase in the serum ALT, AST, urea and creatinine levels was less in the *T. congolense* infected and $CaCl_2$ treated rats and this also buttresses the positive effect of the treatment on trypanosome infections. Furthermore, the serum calcium levels were low in *T. congolense* infected untreated and treated groups of rats with the level being much lower in the *T. congolense* infected untreated rats. The hypocalcaemia reported in the present study did not differ from earlier reports (Fiennes *et al*, 1946; Zia-Ur-Rahman *et al*, 1996) and may be associated with hypoproteinaemia (Losos, 1986' Anosa, 1988). It is also important to note that in the control groups, the calcium level was slightly higher but not significant in the $CaCl_2$ treated uninfected rats when compared with the values of the uninfected untreated rats. The slight increase in the treated uninfected rats may be associated with high intestinal absorption potential of calcium chloride. Calcium chloride treatment did not manifest any negative effect on the PCV value of the treated uninfected control rats. Calcium compounds ingested orally usually are not considered toxic. But high intake of calcium in animals can have a depressing effect on utilization of other nutrients like phosphorous, zinc, magnesium and iron (Guthrie *et al*, 1975).

In conclusion, the oral $CaCl_2$ treatment in rats appeared to delay the onset of parasitaemia and reduce the levels of parasitaemia, anaemia, liver and kidney damages in *Trypanosoma congolense* infection.

Acknowledgement:
The authors appreciate the technical assistance of J. Jubril, E. Ibrahim, Mallam Adamu, N.D. Nwagbara and Yusuf Abubakar.

REFERENCES

Anosa, V. O. (1988): Haematological and biochemical changes in human and animal trypanosomosis. Parts I and II. Rev. Elev. Med. Vet. Pays Trop. 41 65-78, 151-164.

Baron, D. N. (1982): Textbook of Chemical Pathology, 4th edn., Hodder and Stoughton Educational Ltd., Kent,

Chatfield, C. (1983): Statistics for Technology. A course in Applied Statistics, 3rd edn. Chapman and Hall, London,

Egbe-Nwiyi-, T. N., Nwaosu, S.C. Tsuya, R.D. (2003): The effects of high oral magnesium chloride supplementation on the pathogenicity of *Trypanosoma brucei brucei* and *T. congolense* infections in rats. Trop. Vet. 21 152-159.

Fiennes, R. N. T.W., Jones, E.R. Laws, S.G. (1946): The course and pathology of *T. congolense*(Broden) disease of cattle. J. Comp. Path., 56 1-27.

Guyton, A.C. Hall, J.E. (1996): Textbook of medical physiology, 9th edn., W.B. Saunders Company, Philadelphia,

Guthrie H. A (1975): Introductory nutrition, 3rd edn., Saint Lous,

Igbokwe, I. O. (1995), Nutrition in the pathogenesis of Africa trypanosomiasis Protozoological Abstracts, CAB International, 19 797-807.

Kaggwa, E. Munyua, W.K. Mugera, G. M (1984): The pathogenicity of *Trypanosoma brucei* in dogs. Bull. Anim. Hlth. Prod. Afri. 32 360-368.

Kaneko, J.J. (1989): Clinical Biochemistry of Domestic Animals, 4th edn., Academic Press, Philadelphia,

Losos, G. J. Ikede, B. O., (1970): Pathology of experimental trypanosomiasis in albino rats, rabbits, goat and sheep. A preliminary report. Canad. J. Comp. Med., 32 209-212.

Losos, G. J. Ikede, B. O . (1972), Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense* and *T*. gambiense. Vet. Pathol. 9 1-17.

Losos, G. J. (1986), Infectious Tropical diseases of domestic animals, Churchill Livingstone Inc., New York,

Murray, M. Dexter, T.M. (1988): Anaemia in bovine Africa trypanosomiasis: a review. *Acta trop*., 45 389-432.

National Research Council (NRC) (1971). Atlas of nutrition data. United States and Canadian Feeds, National Academic of Science, Washington D.C.

Otesile, E. B., Fagbemi, B. O. Adeyemo, O. (1991), The effect of *Trypanosoma brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. Veterinary parasitology, 40 207-216.

Radostits, O. M (1994): Blood, D. C. Gay, C.C., Veterinary Medicine, 8th edn., Bailliere Tindall, London,

Schalm, O. W., Jain, N. C. Carroll, E. J. (1975)., Veterinary Haematology. 3rd edn. Lea and Febiger, Philadephia,

Tietz, N. W (1986): Clinical chemistry, W. B. Saunders Company, Philadelphia.

Whitelaw, D.D., Macaskill, J. A., Holes, P. H., **Jennings, F.W. Urquhart, G.M (1980):** Genetic resistance of *T. congolense* infections in mice. Infect. Immun., 27 (1980) 707-713.

Zia-Ur-Rahman, Butt, A.A., Asif, M.M., Haq, I. U., Ahmad, A., Ashab, M. Shaukat, S.A.J. (1996): Concentration of serum micro and macro-elements in the sera of control and trypanosome-infected camel. Trop. Vet., 14 (1996) 133-136

* *Author for correspondence: e-mail: tncegbe@hotmail.com Phone No.: 08035642698*