Trypanocidal efficacy of diminazene in diabetic rats

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Abstract

The experiment was conducted to investigate the impact of hyperglycaemia on the trypanocidal efficacy of diminazene aceturate. Groups of alloxan-induced diabetic rats infected with *T. brucei* and *T. congolense* were treated with diminazene aceturate, and trypanocidal effects compared with normal non-diabetic controls. Results showed that the prepatent period was shorter in the diabetic (11.25±1.65 days) than non-diabetic-*T. congolense* (15.0±1.73 days), and also variations in responses to the trypanocidal therapy between the diabetic and non-diabetic groups were detected. Parasite clearance time did not differ significantly between the diabetic and non-diabetic (43.2±8.89 versus 52.8±8.89 hours in *T. brucei* and 33.6±5.9 versus 36.0±6.93 hours in *T. congolense*, respectively). The relapse intervals were shorter in the diabetic than non-diabetic (16 days versus 23 days in *T. brucei*, and 7 days versus 14 days in *T. congolense*, respectively). Proportion of relapses was greater in the diabetic- (100%) than non-diabetic-*T. congolense* (66.7%). We also find parasite species-related differences in susceptibility to the trypanocide, with a higher apparent cure rate in the *T. brucei* than *T. congolense* group. We conclude from the results of this study that the chemotherapeutic effectiveness of diminazene aceturate may be diminished in patients with diabetes mellitus. Further study is needed to validate this hypothesis.

Keywords: *Trypanosoma brucei*, *T. congolense*, trypanocide, diabetes, rat.
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تأثير الدايمينازين المميت للتريبانوسوما في الجرذان التي تعاني من السكري

يو إس تشكيوزي، أي بي مادوكا و جاي جي أيفيني

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير السكري على التأثير المميت للدايمينازين على التريبانوسوما في الجرذان. تم إحداث السكري بالألوكسان في الجرذان التي خمجت بالتريبانوسوما *T. brucei* and *T. congolense* وعولجت بالدايمينازين. ولوحظ إنخفاض تأثير الدايمينازين ضد التريبانوسوما في الجرذان التي تعاني من داء السكري.

Introduction

Diabetes mellitus is a serious lifelong metabolic disorder with a rapidly increasing incidence worldwide (1-3). It has been predicted that about 360 million of the world's population will be affected by the year 2025 (4). Depressed immunological mechanisms consequent upon persistent hyperglycaemia has been reported as one of the complications of the disease (5-7). Several workers have in fact demonstrated increased susceptibility of diabetics to infections (8-10) including trypanosomosis (11-13) but

Chigozie and Anene (14) did not observe enhanced susceptibility to trypanosome infection in diabetic rats.

Trypanosomosis is endemic in vast areas of African continent infested by the *Glossina* vector (15). It is a very important disease with considerable health and economic impact on animals and humans in these areas (16). There may be need to administer trypanocidal drug therapy to diabetics who are concurrently infected with trypanosomosis. Cognizant of the involvement of the host immunity in optimizing medical therapies (17,18) we have thus examined the chemotherapeutic effectiveness of diminazene aceturate - a commonly used trypanocide in Africa, in alloxan-induced diabetic rats artificially infected with *Trypanosoma brucei* and *T. congolense*.

Materials and methods

Animals

We used albino rats weighing between 83.5 and 159.5 grams (116.9±3.9) bred in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. Rats 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 had free access to food and water throughout the experiment.

Parasites

The *T. congolense* used in this study was obtained from National Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. It was a primary isolate from a cow in Gwarzo area of Kaduna State, Nigeria in 2009. *Trypanosoma brucei* used was a primary isolate from a pig slaughtered at the Nsukka Municipal abattoir. The trypanosomes were passaged into donor rats before infection of experimental rats with 1.5 x 10^6 trypanosomes i.p. Trypanosome counts were estimated by the rapid matching method of Hebert and Lumsden (19). Parasitaemia was monitored using buffy coat method (20).

Drug administration

Diminazene diaceturate (Dimivet®, SKM Pharma PVT. Ltd, Nashik-422 010, Bangalore-560 001, India) was use as recommended by the manufacturers. A stock solution was made by dissolving 2.3 g granules containing 1.05 g of the drug in 12.5ml of sterile water, which was appropriately diluted to achieve the dosage administered to each rat.

Induction of Diabetes

Diabetes was induced in the rats essentially as described by Venugopal *et al*. (21). Alloxan monohydrate was freshly dissolved in normal saline to make 100 mg/ml stock solution, and administered to the rats intraperitoneally (i.p.) after 24 hours fasting at a dose of 150 mg/kg body weight. Control groups of rats received injections of equivalent volumes of normal saline. Blood glucose concentrations were determined after 18 hours fasting on a tail blood using Glucometer (ACCU-CHEK active serial no. GN: 10023338). All the rats including alloxan-treated rats were infected seven days after induction.

Haematology

Packed cell volume (PCV) was determined by microhaematocrit method, haemoglobin by cyanomethaemoglobin method, red blood cell (RBC) and total white blood cell (WBC) counts using improved Neubuauer counting chamber technique (22). Differential WBC counts were determined on Giemsa-stained blood films. Blood for the test were obtained by the orbital bleeding technique.

Statistical analysis

Data obtained were expressed as arithmetic mean ± S.E. 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

Induction of diabetes in rats

Significant hyperglycaemia was detected in the rats by day seven after alloxan treatment (Table 1). Control rats had less than 102 mg/dl compared with at least 221 mg/dl fasting blood glucose in the diabetic. Rats were considered diabetic if their blood glucose was greater than115 mg/dl. Blood glucose was not significantly ($P>0.05$) influenced by trypanosome infection in both diabetics and non-diabetics. The levels remained stable in both the normal and infected groups throughout the experiment.

Trypanosome susceptibility of diabetic rats

The prepatent period (PP) of infection for the *T. brucei* group was 4-7 days in both the diabetic and non-diabetic, with a mean prepatent period (MPP) of 6.2±0.58 and 5.2±0.54 days, respectively, and was not significantly different ($P>0.05$). For *T. congolense* group, the PP in the diabetic was 7-15 days (MPP, 11.25±1.65 days) and non-diabetic 12-18 days (MPP, 15.0±1.73 days) differing significantly ($P<0.05$). The parasites cleared within 24 – 72 hours post-treatment (P.T.) in the diabetic- and non-diabetic-*T. brucei* in mean times of 43.2±8.98 and 52.8±8.98 hours, respectively, and within 24 – 48 hours, mean times of 33.6±5.9 and 36.0±6.93 hours, respectively in the *T. congolense* showing no significant differences ($P>0.05$). Relapsed infections occurred by 16 and 23 days

P.T., respectively, in the diabetic and non-diabetic *T. brucei*, and by 7 and 14 days P.T., respectively, in the *T. congolense* groups (Table 2). The proportions of *T. brucei*-infected rats in each category that relapsed was similar, however, only two of the non-diabetic-*T. congolense* relapsed, whereas all the diabetic-*T. congolense* infected rats relapsed including two rats that showed apparent refractoriness (Table 2).

Table 1: Mean ± S.E. blood glucose concentration (mg/dl) of *T. brucei* and *T. congolense* infected diabetic and non-diabetic rats treated diminazine diaceturate.

Days post infection	Groups					
	Non-diabetic			Diabetic		
	uninfected control	*T. brucei* infected non diabetic	*T. congolense* infected non diabetic	Diabetic uninfected	*T. brucei* infected diabetic	*T. congolense* infected diabetic
-5	ND	ND	ND	94.00±4.56^{a}	92.40±4.50^{a}	90.20±4.26^{a}
0	88.40±5.70^{a}	86.60±5.34^{a}	84.00±49 ^{a}	276.25±6.38^{b}	293.80±46.4 ^{b}	224.20±25.00^{b}
7	102.20±4.05^{a}	100.20±5.14^{a}	98.60±6.24^{a}	360.00±79.11^{c}	235.60±43.44^{b}	225.80±43.05^{b}
14	97.00±2.77^{a}	109.80±3.61^{ab}	93.60±3.61^{a}	279.25±80.26^{c}	220.20±60.68^{bc}	201.80 ± 8.39^{ab}
21	89.20±7.22^{a}	92.20±5.90^{a}	91.60±5.31^{a}	286.50±87.21^{b}	234.80±65.05^{b}	199.00±16.50^{ab}
28	93.20±6.24^{a}	95.80±3.62^{a}	97.75±3.38^{a}	261.00±76.99^{b}	233.00±49.98 ^{b}	200.25±21.5^{ab}
35	92.80±4.22^{a}	96.20±3.25^{a}	94.25±6.81^{a}	221.75±49.27^{b}	220.00±52.53^{b}	196.00±19.21^{b}
42	92.20±4.77^{a}	94.00±5.38^{a}	96.25±4.01^{a}	237.00±62.43^{b}	228.00±56.54^{b}	160.75±12.15^{ab}

Different superscripts $^{a, b, c}$ in a row indicates significant difference between the means at the level of probability: $P \leq 0.05$, ND= Not done.

Table 2: Parasitaemia of *T. brucei* and *T.congolense* infected diabetic and non- diabetic rats treated with diminazene diaceturate.

Days post Infection	Groups					
	Non diabetics			Diabetics		
	Uninfected Control	*T. brucei* infected	*T. congolense* Infected	Diabetic uninfected control	*T. brucei* infected	*T. congolense* infected
0	0/6	0/6	0/6	0/5	0/5	0/5
7	0/6	6/6	0/6	0/5	5/5	1/5
14	0/6	2/5 *	2/6	0/5	1/5 *	3/5
21	0/6	0/5	5/5 **	0/5	0/5	5/5* *
28	0/6	0/5	0/4	0/4	1/5	2/4
35	0/6	1/5	2/4	0/4	1/5	2/4
42	0/6	1/5	2/4	0/4	0/4	4/4
49	0/6	1/5	0/2	0/4	0/4	1/1
56	0/6	0/4	0/2	0/4	0/4	1/1
63	0/6	0/4	0/2	0/4	0/4	1/1
70	0/6	0/4	0/2	0/4	0/4	1/1
77	0/6	0/4	0/2	0/4	0/4	1/1

**T. brucei* infected groups were treated with diminazene aceturate on day 12 post infection. ***T. congolense* infected groups were treated with diminazene aceturate on day 21 post infection. + Numerator = the number of rats parasitaemic. Denominator = the number of rats infected (variations due to mortality). 0 = Day of infection.

Haematology

Infection caused significant drop in the red cell parameters (PCV, HB and RBC count) in both the diabetic and non-diabetic *T. brucei* and *T. congolense* by day 14 and 28 p.i., respectively, but subsequently improved with treatment and was significantly better in the non-diabetic *T. congolense* than *T. brucei* groups (Fig. 1, 2 and 3).

Diabetic state in this study was associated with leucocytosis due to neutrophilia, monocytosis, but also eosinopaenia and lymphopaenia (Fig. 4). Infection with trypanosomes induced leucopaenia in the diabetics *T.*

congolense, due to lymphopenia, neutropenia, monocytopaenia and eosinopenia, but not in the non-diabetics or *T. brucei* group.

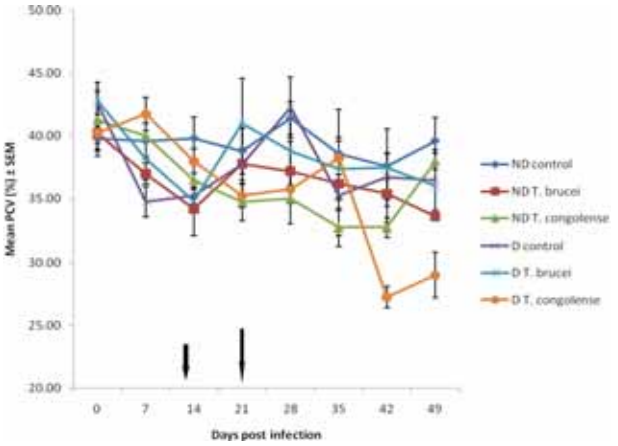

Fig. 1: Mean packed cell volume (%) of diabetic (D) and non diabetic (ND) *T. brucei* and *T. congolense* infected rats treated with diminazene aceturate. *Arrows indicate days of diminazene aceturate administration (*T. brucei,* day 12; *T. congolense,* day 21).

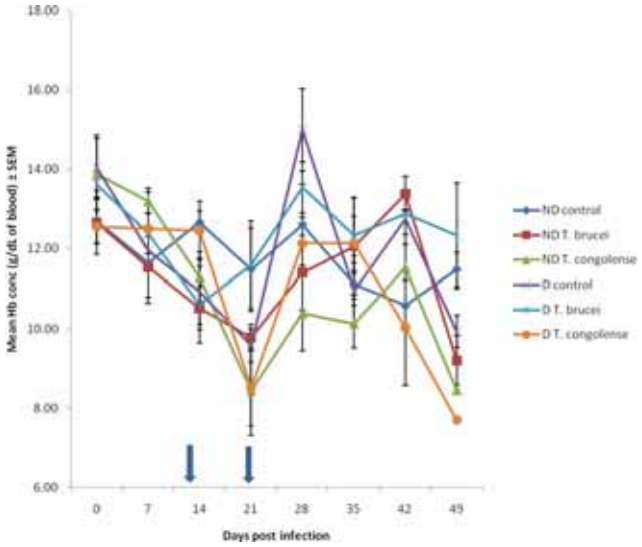

Fig. 2: Mean haemoglobin concentration (g/dL) of diabetic (D) and non diabetic (ND) *T. brucei* and *T. congolense* infected rats treated with diminazene aceturate. *Arrows indicate days of diminazene aceturate administration (*T. brucei* day 12; *T. congolense* day 21).

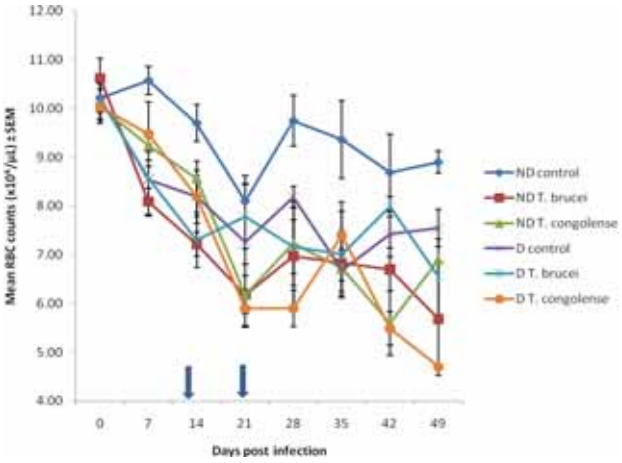

Fig. 3: Mean red blood cell counts (x10^6 /µL) of diabetic (D) and non diabetic (ND) *T. brucei* and *T. congolense* infected rats treated with diminazene aceturate. *Arrows indicate days of diminazene aceturate administration (*T. brucei* day 12; *T. congolense* day 21).

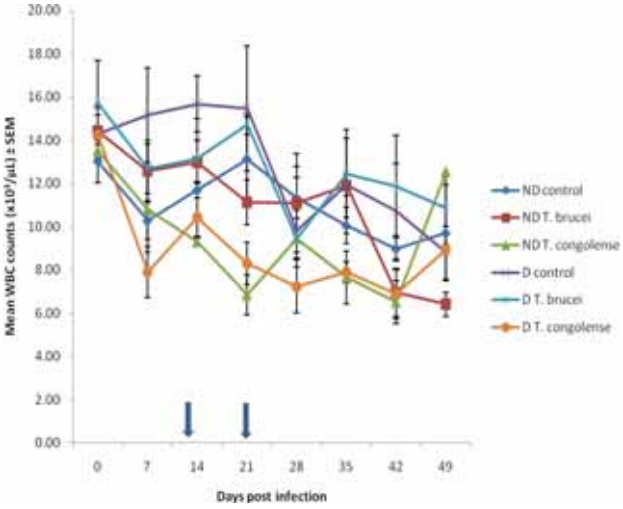

Fig. 4: Mean white blood cell counts (x10^3 /µL) of diabetic (D) and non diabetic (ND) *T. brucei* and *T. congolense* infected rats treated with diminazene aceturate. *Arrows indicate days of diminazene aceturate administration (*T. brucei* day 12; *T. congolense* day 21).

Discussion

Alloxan has been reported to induce inhibition of pancreatic glucokinase function and there is selective beta-cell loss, leading to insulin deficient diabetes (23-25). The resultant hyperglycaemia is a common feature of type-1 and -2 diabetes (23-28). Alloxan injection resulted in stable hyperglycaemia throughout the period of this experiment, and it has commonly been used by other workers to render

rats diabetic (13,23-25,29). Blood glucose was unaffected by *Trypanosoma* infection as it neither did not make normal rats hypoglycaemic nor normalize blood glucose concentrations in hyperglycaemic rats unlike in the results of Elased *et al*. (30) in murine malaria parasites.

The PP was significantly shorter ($P<0.05$) in the *T. brucei* (4-7 days) than *T. congolense* (7-18 days) group in agreement with the findings of other workers (31-33). Whereas PP in the diabetic- and non-diabetic was similar for *T. brucei*, it was significantly ($P<0.05$) shorter in the diabetic- than non-diabetic-*T. congolense* group. Results of the relapse interval and relapse proportion of infection indicated superior trypanocidal effects in the non diabetic compared with the diabetic, particularly the *T. congolense* group. It is to be noted, however, that the small number of samples per group, as well as the relatively short duration of this experiment may have limited the power of this study to demonstrate any significant differences. Martens *et al.* (10) reported that impaired immunity is a feature of chronic diabetes of three months duration, but not acute diabetes, although Yamashiro *et al*. (7) was able to demonstrate impaired tuberculosis defenses as early as 14 days after infection in mice.

The results of the blood parameters (PCV and Hb) showed that the rate of infection with trypanosomes caused anaemia consistent with the findings of other workers (34-36). This decrease, however, was reversed by treatment before the recrudescence of parasitaemia and associated anaemia. Diabetes per se in this study caused leucocytosis due to neutrophilia and monocytosis in association with eosinopenia and lymphopenia. There is partial disagreement between our results and that of Warley et al. (37) who reported a decrease in WBC counts due to lymphopenia, although the neutrophils and monocytes were unaffected. The decreased WBC in the peripheral circulation was associated with thymic atrophy in diabetic mice and rats (38,39). There is however, a report of increase in neutrophils in the blood of diabetic animals (40,41) which is in agreement with our result. We found no alterations in the leucocyte counts between diabetic and non-diabetic *T. brucei* infected groups similar to the results of Martens *et al*. (10) who found no noticeable differences in the proportions of lung T-cells, monocytes/macrophages or granulocytes between diabetic and non-diabetic mice infected with tuberculosis. However, diabetic *T. congolense group* had significant leucopenia starting from day seven after infection.

Host immune response in trypanosomosis requires an effective humoral immune response and monocyte/macrophage functions to restrict trypanosome replication (17,42-45). Hypothetically, defects in these immune mechanisms triggered by diabetic condition (5-7) could result in diminished effectiveness of trypanocides (5,18). The immunological basis for immunosuppression in diabetes is poorly understood. However, depressed neutrophil and macrophage functions have been associated with diabetes (46,47).

It was concluded from the results of the study that the chemotherapeutic effectiveness of trypanocides may be compromised in diabetic state. However, further research using larger numbers of animals may be required to confirm the hypothesis.

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