Increased severity of acute Trypanosoma brucei brucei infection in rats with alloxan-induced diabetes

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Short note

Increased severity of acute *Trypanosoma brucei brucei* infection in rats with alloxan-induced diabetes

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Abstract – Twenty rats were made diabetic by treatment with alloxan monohydrate (10 % solution, 100 mg/kg body weight). Ten diabetic and ten non-diabetic rats were intraperitoneally infected with the same infective doses of *Trypanosoma brucei brucei* (Lafia strain). The uninfected controls were ten diabetic and ten non-diabetic rats. The prepatent period was shorter in the diabetics (3.5 ± 0.5 days) than the non-diabetics (4.2 ± 0.4 days). Although the infected diabetic and non-diabetic rats had comparable levels of peak parasitaemia, the diabetics had significantly ($P < 0.05$) higher parasitaemia before this peak. The survival time was shorter ($P < 0.05$) for the infected diabetics (12.1 ± 3.2 days) than for the infected non-diabetics (14.8 ± 1.7 days). The infection did not affect the level of diabetic hyperglycaemia, but caused a more severe anaemia in the diabetics than the non-diabetics, with the percentage decreases in packed cell volume in the diabetics being higher ($P < 0.05$) from days 3 to 12 post-infection. In conclusion, the pathogenic effects of trypanosome infection may be more severe in rats having alloxan-induced diabetes. © Inra/Elsevier, Paris.

***Trypanosoma brucei brucei* / rat / diabetes**

Résumé – Infection par *Trypanosoma brucei brucei* aggravée chez des rats présentant du diabète induit par de l'alloxan. Vingt rats ont été rendus diabétiques par un traitement avec de l'alloxan monohydrate (solution à 10 %, 100 mg/kg de poids corporel). Dix rats diabétiques et dix rats non diabétiques ont été infectés par voie intrapéritonéale avec la même dose de *Trypanosoma brucei brucei* (Lafia strain). Les témoins non infectés étaient répartis en dix rats diabétiques et dix rats non diabétiques. La période prépatente a été plus courte chez les diabétiques (3,5 ± 0,5 j) que chez les non diabétiques (4,2 ± 0,4 j). Bien que les diabétiques aient présenté des pics de parasitémie de niveau comparable, les diabétiques ont eu une parasitémie significativement ($p < 0,05$) plus importante avant ce pic. Le temps de survie a été plus court ($p < 0,05$) pour les diabétiques infectés (12,1 ± 3,2 j) que pour les diabétiques non infectés (14,8 ± 1,7 j). L'infection n'a pas affecté le niveau d'hyperglycémie

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diabétique, mais a provoqué une anémie plus sévère chez les diabétiques que chez les non diabétiques, avec des pourcentages de diminution de l'hématocrite chez les diabétiques plus élevés ($p < 0,05$) du jour 3 au jour 12 après l'infection. En conclusion, les effets pathogènes de l'infection par le trypanosome semblent plus importants chez les rats ayant un diabète induit par l'alloxan. © Inra/Elsevier, Paris.

***Trypanosoma brucei brucei* / rat / diabète**

1. INTRODUCTION

The pathogenesis of African trypanosomosis is associated with an anaemia predominatly caused by extravascular haemolysis [19]. Recently, low dietary energy nutrition in animals was reported to increase the severity of the anaemia caused by the disease [5, 12]. Jennings [10] earlier suggested that inadequate energy supply in erythrocytes might alter the erythrocyte membrane surface during infection. Therefore, cellular energy deficit may probably predispose to enhanced trypanosome-mediated erythrocyte destruction.

Human trypanosomosis (sleeping sickness) is endemic in parts of Africa [4] where some people may be diabetic, and therefore, concurrence of both disease conditions could exist. Transmembrane glucose transport [11] and intraerythrocytic glycolysis [13] normally activated by insulin, may be impaired in diabetes characterized by insulin deficiency; thereby causing decreased cellular glucose utilization. Parenteral administration of alloxan monohydrate induces diabetes in animals by destruction of beta-cells of pancreatic islets of Langerhans [17]. Infection of such diabetic animals with *Trypanosoma brucei brucei,* a model for human trypanosome species, might provide insight into whether trypanosomosis could be aggravated in diabetics.

In our pilot experiment, *T. b. brucei*-infected rabbits with the same infective parasite dose died 2.7 days earlier with a more severe anaemia if pretreated intravenously with alloxan. In the present study, rats made diabetic by alloxan treatment and non-diabetic rats were infected with *T. b. brucei* and both groups compared with respect to parasitaemia, packed cell volume (PCV) and survival time.

2. MATERIALS AND METHODS

2.1. Animals

Forty male albino rats, weighing 110 ± 10 g were obtained from the National Institute for Trypanosomiasis Research (NITR, Vom, Nigeria) and fed a commercial diet (ECWA feeds, Jos).

2.2. Inducing diabetes

Twenty rats were injected intramuscularly (at the base of the tails) with 10 % alloxan monohydrate (Sigma, USA) at 100 mg/kg body weight after an overnight fasting. The fasting tail-blood glucose concentrations were determined, before and at intervals after alloxan treatment, using a method by Asatoor and King [2]. The rat was considered diabetic with a fasting tail-blood glucose concentration of more than 8.0 mmol/L [21]. Infection was carried out 4 days after alloxan treatment.

2.3. Trypanosome infection

Tail-blood from a donor rat previously inoculated with *Trypanosoma brucei brucei* (Lafia strain) at NITR, Vom, was dropped in 10 mL of cold normal saline and mixed. Ten diabetic and ten non-diabetic rats were infected by intraperitoneal injection of 0.5 mL of diluted blood containing 1.5×10^7 trypanosomes. Tail-blood from these animals was examined and parasitaemia was estimated from wet mounts by the method of Herbert and Lumsden [6]. Ten diabetic and ten non-diabetic rats served as uninfected controls.

Table I. Mean (± SD) parasitaemia (log_{10}·mL $^{-1}$) in *T. b. brucei*-infected non-diabetic and diabetic rats ($n = 10$).

Days post-infection	Non-diabetics	Diabetics
3	0	3.4 ± 3.6
6	7.8 ± 0.3[a]	8.6 ± 0.2[b]
9	8.7 ± 0.1[a]	8.7 ± 0.3[a]
12	8.6 ± 0.3[a]	8.9 ± 0.3[a]

Values with different superscripts (a, b) differ significantly ($P < 0.05$).

2.4. PCV Determination

PCV was measured by a microhaematocrit method.

2.5. Statistics

The data were summarized as means ± standards deviations (SD). One-way ANOVA was performed within groups and the comparison of means between groups was by two-tailed Student's *t*-test [3].

3. RESULTS

The prepatent period of 3.5 ± 0.5 days in infected diabetics was shorter ($P < 0.05$) than 4.2 ± 0.4 days in the infected non-diabetics. All the infected rats were parasitaemic on day 6 p.i. and mean parasitaemia score was significantly ($P < 0.05$) higher in the diabetic than non-diabetic rats *(table I)*. However, there was no difference in parasitaemia between the groups on days 9 and 12 p.i. The survival time of the infected diabetics (12.1 ± 3.2 days) was shorter ($P < 0.05$) than that of infected non-diabetics (14.8 ± 1.7 days).

The mean fasting blood glucose (FBG) concentrations in the non-diabetic and diabetic rats are shown in *table II*. The FBG in the infected non-diabetics was significantly ($P < 0.05$) higher on day 6 p.i. and lower on day 12 p.i. than the control values. Hyperglycaemia was maintained in the diabetics on days 0–12 p.i. and no significant differences occurred between the FBG values of the infected and uninfected diabetics.

The mean PCV of the uninfected (control) non-diabetic and diabetic rats did not vary significantly during the experiment,

Table II. Mean (± SD) blood glucose concentrations (mmol·L $^{-1}$) of control (uninfected) and *T. b. brucei*-infected diabetic and non-diabetic rats.

Days post-infection	Non-diabetic rats		Diabetic rats	
	Control*	Infected*	Control*	Infected*
0	4.5 ± 0.4[a]	4.4 ± 0.3[a]	10.1 ± 0.6[a]	9.6 ± 0.7[a]
9	4.5 ± 0.3[a]	5.1 ± 0.2[b]	10.6 ± 0.6[a]	10.5 ± 2.0[a]
12	4.4 ± 0.3[a]	3.9 ± 0.4[b, †]	9.8 ± 0.6[a]	9.5 ± 2.5[a, ††]

Values with different superscripts (a, b) differ significantly ($P < 0.05$).
* $n = 10$; † $n = 8$; †† $n = 6$ after mortality.

Table III. Mean (± SD) packed cell volume (PCV) of control (uninfected) and *T. b. brucei*-infected non-diabetic (ND) and diabetic (D) rats.

Days post-infection	PCV (%)				% Decreased in PCV in infected rats	
	Control		Infected rats			
	ND*	D*	ND*	D*	ND	D
0	42.6 ± 3.1	46.3 ± 5.1	43.9 ± 4.0[a]	43.2 ± 5.1[a]	–	–
9	42.5 ± 2.6	46.7 ± 4.9	43.0 ± 4.2[a]	40.2 ± 5.2[a]	3.1 ± 3.1[a]	7.2 ± 2.1[b]
9	42.6 ± 2.8	47.6 ± 5.0	41.0 ± 4.9[a]	36.1 ± 5.3[b]	6.5 ± 4.5[a]	16.6 ± 4.5[b]
9	42.3 ± 2.7	47.8 ± 5.0	38.8 ± 4.1[a]	33.3 ± 4.8[b, ††]	11.6 ± 4.6[a]	24.0 ± 4.4[b]
12	42.6 ± 2.4	48.1 ± 5.2	37.0 ± 5.1[a, †]	31.5 ± 5.0[b, †††]	14.5 ± 5.8[a]	28.8 ± 3.6[b]

Values with different superscripts (a, b) differ significantly ($P < 0.05$).
* $n = 10$; † $n = 8$; †† $n = 9$; ††† $n = 6$ after mortality.

but decreased ($P < 0.05$) in the infected rats. The infected non-diabetics and diabetics had comparable mean PCV on day 0, but on days 6, 9 and 12 p.i., the PCV values were significantly ($P < 0.05$) lower in the infected diabetics than non-diabetics *(table III)*. The mean percentage decreases in PCV from values on day 0 were significantly ($P < 0.05$) higher in the infected diabetics than non-diabetics on days 3, 6, 9 and 12 p.i. *(table III)*.

4. DISCUSSION

Parasitaemia appeared earlier in infected diabetics than in non-diabetics and the parasitaemia was higher in the diabetics before the parasitaemic peak. However, the level of peak parasitaemia was comparable in the infected diabetics and non-diabetics. The persistent hyperglycaemia in the diabetics might have hastened the initial multiplication of the parasites without affecting the intensity of peak parasitaemia. Seed and Sechelski [18] reported that continuous high plasma glucose concentrations in *T. rhodesiense*-infected mice made diabetic by treatment with streptozotocin did not alter the parasite growth curve and peak parasitaemia during the plateau phase. Also, injection of large quantities of glucose into *T. rhodesiense*-infected mice prior to peak parasitaemia did not produce any alteration in parasitaemia [18].

Furthermore, increasing the number of parasites in the inoculum was reported to shorten the prepatent period without affecting the height of parasitaemia and time to death after peak parasitaemia in *T. b. brucei*-infected mice [15]. Therefore, a shorter prepatent period in the diabetics was not likely owing to a higher infective dose, since each rat in both the infected diabetic and non-diabetic groups received the same volume in inoculum and similar number of parasites.

The infected diabetics had a shorter survival time and more severe anaemia than the infected non-diabetics. Since the height of parasitaemia was similar in the infected diabetics and non-diabetics, the greater severity of the disease in the diabetics could not be attributed only to any difference in parasitaemia. The pathophysiological changes associated with diabetes may have aggravated the pathogenic processes in trypanosomosis. Igbokwe et al. [9] reported that acute *T. brucei* infection of rats caused impaired oral glucose tolerance. Therefore, the infected diabetics may have more severe

cellular energy deprivation since diabetes decreases glucose uptake and utilization in tissues [21] including erythrocytes [13]. Reduced glucose metabolism in erythrocytes and subsequent reduction in NADH and NADPH supplies may quicken erythrocyte ageing by lipid peroxidation [7] followed by extravascular haemolysis in the expanded mononuclear phagocytic system associated with trypanosomosis [1]. With reports of increased susceptibility of erythrocytes to in vitro peroxidation in trypanosomosis [8] and increased lipid peroxidation in diabetes [16], the concurrence of the infection with diabetes could possibly exacerbate the oxidative injury to erythrocytes by hydrogen peroxide produced by trypanosomes [14] and activated macrophages [20]. Lack of mortality and no change in the PCV of the alloxan-treated uninfected rats precluded any apparent toxicity of alloxan as a confounding factor in the experiment. It was reported that alloxan caused only renal tubular necrosis at toxic levels [17].

Trypanosome infection in the non-diabetics caused an initial increase and terminal decrease in blood glucose concentrations in agreement with our earlier report [9], but the infection did not affect the level of diabetic hyperglycaemia, indicating that when blood glucose concentrations are monitored for diagnosis of diabetes in patients with trypanosomosis, the values obtained may not be spurious.

In conclusion, acute *T. b. brucei* infection was more severe in diabetic than non-diabetic rats, judging from the shorter survival time and greater decrease in PCV in the infected diabetics.

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