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Full Length Research Paper

The effects of diabetes mellitus on the pathogenicity of trypanosomosis in rats

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Alloxan monohydrate was used to induce diabetes in rats, and then followed post infection with *Trypanosoma congolense* and *Trypanosoma brucei* to determine the impact of diabetes on the course of trypanosome infection. Significant (P<0.05) hyperglycaemia occurred in the alloxan treated rats starting on day seven to the end of the experiment. Trypanosome infection did not affect the glycaemic status of either the normal or the hyperglycaemic rats. The mean prepatent period (MPP) was significantly (P<0.05) longer in the diabetic *T. brucei*- infected rats (5.6±0.23 days) than the non-diabetics (3.8±0.34 days) whereas in the *T. congolense* it was similar in both the diabetic (13.1±0.73days) and the non -diabetic (13.3±0.67 days). The levels of parasitaemia which were comparable in the diabetic and non-diabetic groups increased progressively until death of the rats. The mean survival time (MST) for the diabetic *T. brucei*- infected rats (17.2±0.86 days) was not significantly (P=0.49) different from that of the non-diabetics (19.5±1.15 days) while that of the diabetic *T. congolense* (34.8±4.15 days) was significantly (P<0.001) longer than that of the non-diabetics (25.7±2.1 days). *T. brucei* infection in the diabetics was significantly (P<0.001) more acute than *T. congolense* (MST, 17.2±0.86 versus 34.8±4.15 days) unlike in the non-diabetic (MST, 19.5±1.15 versus 25.67±2.11 days, P=0.08). The red blood cell parameters (PCV, HB and RBC) were significantly (P<0.05) decreased by trypanosome infections, unaffected by the diabetic status but was trypanosome species dependent, being significantly lower in the *T. brucei* than *T. congolense*-infected rats. A significant (P<0.05) decrease in the percentage body weight gains between the diabetic and the non-diabetic controls occurred as a result of the weight decreasing effect of diabetes mellitus. It was thus concluded that the course of *T. brucei* and *T. congolense* infections was not significantly altered in alloxan-induced diabetic rats, and trypanosome infection may not confound the results of blood glucose monitoring for the diagnosis of diabetes.

Key words: *Trypanosoma brucei*, *Trypanosoma congolense*, rat, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is traditionally described as hyper-glycaemia due to absolute or relative deficiency of insulin secretion (Alberti et al., 1998; WHO, 2003; Mbaya and Ramiaya, 2006). Diabetes remains the most common endocrine disease encountered in both human (Hassan, 1985; King et al., 1998) and small animals (dogs and cats) (Ettinger and Feldman, 2005; Kim et al., 2006) although it has also been reported in cattle, sheep, pigs and horses (Gould, 1981; Tajima et al., 1999; Clark, 2003). Diabetes is considered to increase susceptibility to infections (Ponce-De-Leon et al., 2004; Joshi et al., 1999; Martens et al., 2007) depress immunity (Bagdade et al., 1974) and impair phagocytosis (Abrass and Hori, 1984, 1987).

However, a contrary report showed that diabetic mice were less anaemic, controlled parasitaemia better and showed enhanced phagocytic activity compared with normal mice (Elased et al., 1995).

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Coinfections of diabetes and trypanosomosis, a debilitating haemoparasitic disease of animals and humans (Ekejindu et al., 1989; Hill et al., 2005) may occur in areas of tropical Africa endemic for the disease. It would be useful therefore, to investigate the impact of hyperglycaemia on the course of trypanosome infection. In the present study we have infected alloxan-treated rats with *Trypanosoma brucei* and *Trypanosoma congolense* and measured parasiteamia, blood glucose, temperature, weight changes, survival and development of anaemia – a cardinal symptom of trypanosomosis during the course of infection.

MATERIALS AND METHODS

Animals

Thirty three (33) male albino rats weighing between 83.5 and 159.5 g (116.9±3.9) were used in the study. They were kept in clean rat cages and housed in well ventilated fly proof experimental animal house. Animals were humanely cared for in compliance with the principles of laboratory Animal care. They were fed with commercial broiler ration and water was given *ad libitum*.

Induction of diabetes

Diabetes was induced in the rats using the method described by Venugopal et al. (1998). Alloxan monohydrate used in the induction was freshly dissolved in a normal saline to make 100 mg/ml stock solution and then kept at room temperature prior to use. The rats were intraperitoneally (i.p.) injected with alloxan monohydrate after 24 h fasting at a dose of 150 mg/kg body weight.

Trypanosome infections

T. congolense used in this study was obtained from National Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. It was isolated from a cow in Gwarzo Area of Kaduna State, Nigeria in 2009. The trypanosomes were passaged in donor rats before infection of the experimental animals, i.p. with 0.1 ml of saline diluted blood containing 1.5×10^6 trypanosomes. The number of infective trypanosomes was determined using the rapid matching method of Herbert and Lumsden (1976).

T. brucei organism used was a primary local isolate from a slaughtered pig in the Nsukka Municipal abattoir. Infection of the rats was as described for *T. congolense* above.

Experimental procedure

All the rats were allowed to acclimatize for one week before the study began. They were randomly divided into six groups (1 to 6). Groups 1, 2 and 3 contained six rats each while groups 4, 5 and 6 had five rats each. Group 1 was non-diabetic control rats that received only 0.1 ml of normal saline. Groups 2 and 3 were non-diabetic rats infected with *T. congolense* and *T. brucei,* respectively. Group 4 was diabetic control rats while Groups 5 and 6 were infected with *T. congolense* and *T. brucei*, respectively.

The following parameters were monitored at weekly intervals; parasitaemia, body weight, temperature, haematology (PCV, HB conc., RBC count). Deaths in any group were recorded. Blood glucose levels were determined using Glucometer. The pre- patent period (PP) of infection was determined by daily examination of the tail blood of each infected rat beginning from day 2 post infection (p.i.) using wet smear and buffy coat methods of Murray et al. (1977). Estimation of the levels of parasitaemia was done by the method of Herbert and Lumsden (1976). Blood samples for haematology were collected from the rats via orbital bleeding technique.

Differences between groups were analyzed using one way analysis of variance (ANOVA) and Duncan`s multiple range test. A probability value of less than 0.05 ($P<0.05$) was considered statistically significant. In addition, the Kaplan-Meier survival method (Bland and Altman, 1998) was applied to estimate the proportion of rats surviving at each time point post infection. Subsequently, the log rank statistical test was applied to compare the survival curves produced (Bland and Altman, 2004). Statistical analysis was carried out using SPSS version 16 software.

RESULTS

Seven days after alloxan treatment, significant mean fasting blood glucose levels were recorded with values of 287.6±77.4, 259.6±66.1 and 255±59.24 mg/dl, respectively, in the uninfected control, *T. brucei*- and *T. congolense* groups, against 105.3±1.1, 103±1.81 and 97.5±3.4 mg/dl in the respective untreated group. Significant hyperglycaemia persisted in the alloxan treated rats throughout the period of the study. The mean blood glucose concentrations did not differ significantly between the controls and infected diabetic and non-diabetic rats.

The mean prepatent period (MPP) in diabetic and non-diabetic *T. brucei* infected rats were 5.6±0.23 and 3.8±0.34 days, respectively, and in *T congolense* infected rats 13.1±0.73 and 13.3±0.67 days, respectively.

The mean prepatent period (MPP) in diabetic and non-diabetic *T. congolense* infected rats were similar with values of 13.1±0.73 and 13.3±0.67 days, respectively, while that of *T. brucei* infected rats were 5.6±0.23 and 3.8±0.34 days, respectively, and differed significantly ($P<0.05$). The levels of parasitaemia which were comparable in the both diabetic and non- diabetic groups increased progressively in all the infected rats until death (Table 1).

The proportion of rats surviving post infection in each group at any given time over the study period is presented in the Kaplan-Meier survival curves (Figure 1). Vertical steps downward correspond to day's p.i. when death of each individual was observed. There was a significant difference ($P=0.009$) between the survival curves. The diabetic *T. congolense* infected rats had a mean survival time (MST) of 34.8±4.15 while the non-diabetic *T. congolense* had MST of 25.7±2.1 days, differing significantly. The MST for diabetic *T. brucei*

Table 1. Mean (±SE) parasitaemia (log_{10} trypanosomes/ml) of *T b brucei* and *T congolense* infected diabetic and non-diabetic rats.

Days of post infection	Diabetics		Non-diabetics	
	T. brucei	***T. congolese***	***T. brucei***	***T. congolense***
7	6.96±0.06		7.0±0.06	
14	8.2±0.36	7.2±0.12	8.3±0.1	7.1±0.18
21		7.7±0.24	8.7±0.0	7.35±0.15
28		7.8±0.17		8.25±0.15
35		8.2±0.1		

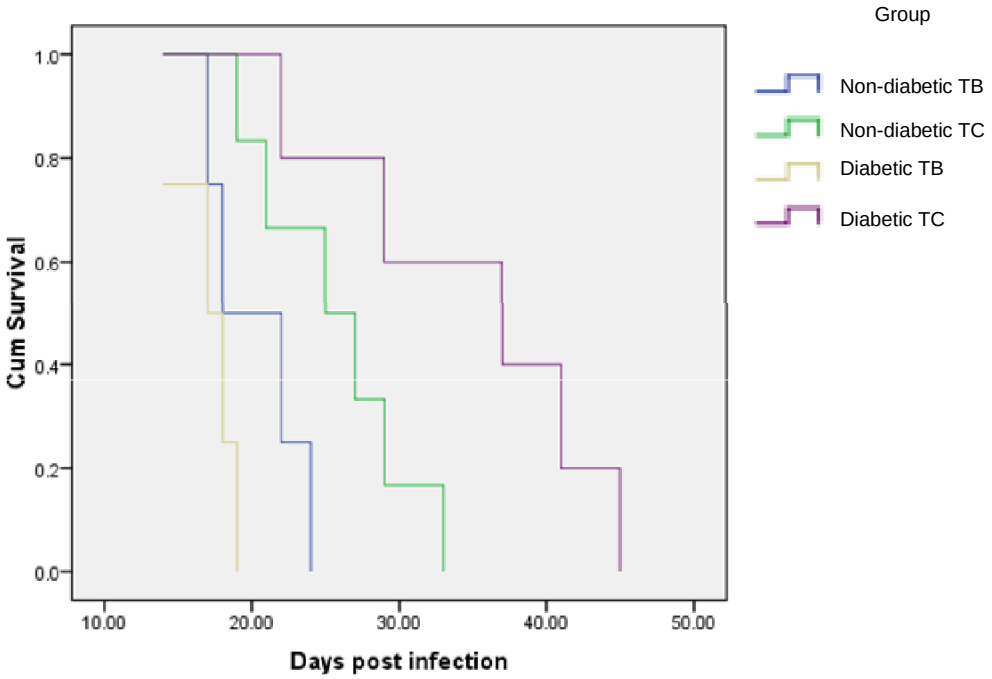

Figure 1. Kaplan-Meier survival curves for non-diabetic *T. brucei* (TB) and *T. congolense* (TC), and diabetic *T. brucei* (TB) and T. *congolense* (TC) infected rats.

(17.2±0.86 days) and non-diabetic *T. brucei* (19.5±1.15 days) did not differ significantly (P=0.49). In the diabetics, the species of trypanosome influenced the survival time (*T. congolense*, 34.8±4.15 versus *T. brucei* 17.2±0.86, P<0.001) unlike in the non-diabetics (*T. congolense* 25.67±2 versus 19.5±1.15, P<0.077). The trypanosome infections caused significant temperature increases in both parasites which fluctuated in the course of the disease especially in the diabetics.

The mean red blood cell parameters (PCV, HB concentration and RBC count) of the trypanosome infected rats significantly decreased progressively from day 14 p.i. The values for the diabetic and non-diabetic infected rats were similar, but significantly lower in the *T. brucei* than *T. congolense* group.

The mean percentage weight gains were significantly higher in the non-diabetic control rats than in all the other groups including the diabetic control rats.

DISCUSSION

Alloxan monohydrate has been used previously to induce diabetes in laboratory animals (Igbokwe et al., 1990, 1998; Ahmed and Tarannum, 2009; Gwarzo et al., 2010). It induces Type-2 diabetes in animals by causing

selective necrosis of the beta cells of the islets (Dunn and McLetchie, 1943; Lenzen, 2008). In the present study, rats were rendered diabetic by day 7 of alloxan administration with fasting blood glucose levels above 220 mg/dl considered diabetic for rats (Ahmed and Tarannum, 2009) even though Iwalewa et al. (2008) also considered levels of 115mg/dl significant. The diabetic state produced in the rats was stable throughout the experiment and unaltered by trypanosome infections, thus corroborating the results of (Igbokwe et al., 1990, 1998) . It is to be noted, however, that Elased et al. (1995) using murine malaria parasites showed that infection induced hypoglycaemia in normal mice and normalized the hyperglycaemia of moderately diabetics.

The mean prepatent period (MPP) was shorter in non-diabetic *T. brucei* infected rats than in the diabetics whereas in *T. congolense* MPP was similar in both the diabetics and non-diabetics. Also the levels of parasitaemia were comparable in the non- diabetics and diabetics (Table 1) . These results showed that diabetes did not aggravate parasitaemia in the rats. This is in agreement with the findings of Seed and Sechelski (1989) who did not observe any alterations in parasitaemia in diabetic *T. rhodesiense*-infected mice but contradicts Amole et al. (1985) and Tanowitz et al. (1988) who showed that hyperglycaemia significantly increased parasitaemia and mortality in mice infected with *T. brucei* and *T. cruzi*, respectively . Also, unlike in the present study, Igbokwe et al. (1998) reported that parasitaemia appeared earlier in infected diabetics than non-diabetics.

The concurrence of significantly increased temperature in all the infected rats with the period of onset of parasitaemia and appearance of anaemia accorded with earlier reports that the appearance of parasites in the blood is associated with fever and anaemia (ILRAD, 1990; Taylor, 2004). Anaemia recorded in the infected rats was independent of their glycaemic status and contradicted the results of Igbokwe et al. (1998) who reported more severe anaemia in the diabetics than the non-diabetics.

Significant body weight gains occurred in the non-diabetic control rats compared with diabetic control and was attributed to the weight decreasing effects of diabetes (Clark, 2003).

The survival time for diabetic *T. congolense*- infected rats was longer than for the non- diabetics whereas it was unaffected by diabetic state in *T. brucei*-infected rats. However, Igbokwe et al. (1998) and Amole et al. (1985) in a similar study reported shorter survival times for diabetic *T. brucei* infected rat. Possible factors that may have confounded the results of this study and thus the ability to demonstrate significant differences may include the multiple sub groupings and the few animals in each experimental group.

Conclusion

Diabetes mellitus did not appear to have caused any significant alterations in the course of trypanosome infection in rats, and may thus not aggravate trypanosomosis in animals nor confound the results of blood glucose screening exercise.

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