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SHORT COMMUNICATION

Reduced fasting blood glucose levels following relapse in diminazene aceturate (Dinazene®) treated *Trypanosoma brucei* infected albino rats

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Abstract The blood glucose levels of rats were assessed following experimental *Trypanosoma brucei* infection and diminazene aceturate treatment. Ten adult female albino rats were randomly assigned into two groups of five rats each. Group A were infected with 10^6 trypanosomes while group B served as the uninfected control group. Group A rats were treated with 7 mg/kg Dinazene® (diminazene aceturate) at the peak of parasitaemia. Blood glucose level was assayed weekly while parasitaemia level was assessed daily. The blood glucose levels of the infected rats did not vary significantly ($P > 0.05$) from that of control group except following relapse when the values became significantly ($P < 0.05$) low. The implications of blood glucose reduction following relapse infection in rats is therefore highlighted and discussed.

Keywords Fasting blood glucose levels · Relapse infection · Diminazene aceturate · *Trypanosoma brucei* · Rats

Introduction

Trypanosomosis caused by pathogenic tsetse transmitted trypanosomes is recognized as one of the major diseases of animals and man in Africa (Obi et al. 2013). Alterations in the host metabolic system and the release of inflammatory responses and reactive oxygen species (ROS) resulting in degenerative changes have been reported in trypanosomosis (Nwoha and Omamegbe 2016; Mishra et al. 2017). These changes usually correlate with parasitaemia levels and organ/tissue damages. The islet of Langerhans in the pancreas is prone to degenerative changes consequent upon trypanosome infection resulting in alterations in blood glucose level (Corbett et al. 2002). The effect of trypanosomosis on the mammalian host blood glucose levels have been a subject of controversy. This study investigated the effects of experimental *Trypanosoma brucei* infection and treatment on fasting blood glucose levels of albino rats.

Materials and method

Experimental animals

Ten adult female albino rats weighing between 130 and 190 g procured from the Laboratory Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for this study. The rats were kept in rat cages in the Laboratory Animal House of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka and were acclimatized for 2 weeks prior to the commencement of the experiment. Within this period, they were confirmed free of blood parasites and were dewormed with albendazole (Zolat®) for gastrointestinal parasites. They were fed standard rat feed and given water ad libitum.

Parasites/infection

The *T. brucei* used in this experiment was originally obtained from goats during a survey in Gboko Benue State

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and was designated "Gboko strain". They were subsequently maintained in mice in the Laboratory Animal Unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka.

Drug

Diminazene aceturate—Dinazene® (Vetindia Pharmaceuticals Ltd, India) was used at 7 mg/kg body weight.

Experimental design

The rats were randomly assigned into two groups (A and B) of five rats each. Rats in groups A were inoculated intraperitoneally with 1.0×10^6 trypanosomes suspended in 0.4 ml of phosphate buffered saline while rats in group B were neither infected nor treated. Pre-infection fasting blood glucose levels were determined prior to infection. At the peak of parasitaemia i.e. day 11 post inoculation (PI), rats in group A were treated with 7.0 mg/kg body weight Dinazene®.

Parasitaemia was estimated using the rapid matching method (Herbert and Lumsden 1976) daily from 3 days post infection, up to treatment and until total parasite clearance from the blood. Thereafter, it was assessed weekly till the end of the experiment.

Fasting blood glucose levels were assessed weekly using Accu-check® blood glucose kit comprising of a glucometer and test strips. The rats were fasted overnight preceding blood sample collection. The rats were allowed water during fasting. Blood samples for the test were obtained directly from the tail vein of fasted rats on to the strips. The blood glucose levels were then read from the glucometer.

Data obtained were subjected to student's t test. Probability values of < 0.05 were considered significant.

Ethical approval

Valid ethical clearance and approval were obtained from the Ethics Committee for Medical and Scientific Research of the University of Nigeria, Nsukka before the commencement of this study. The University of Nigeria, national and international guidelines for the ethical use of animals were fully observed.

Results

All the infected animals became parasitaemic between days 4 and 7 post infection. The infection was acute in nature reaching peak parasitaemia on day 8 post infection and subsequently increased till the 11th day post infection when the infected rats were treated. Anemia, emaciation or anorexia, pale mucous membrane and dullness were the clinical manifestations observed in the *T. brucei* infected rats. Following treatment, there was a significant decline in parasitaemia 24 h post treatment. However, total parasite clearance from the blood was observed 96 h (4 days) post treatment in all the infected rats (Fig. 1). Relapse infection which led to resurgence of trypanosomes in the blood occurred in the entire infected (group A) rats 24 days post treatment and parasites persisted in blood until the termination of the experiment with the relapsed rats becoming extremely weak.

The blood glucose levels of all the rats did not vary significantly ($P > 0.05$) during the first 6 weeks of the study. However, from the 7th week when relapse infection occurred till the end of the experiment, the blood glucose level of the group A rats was significantly ($P < 0.05$) lower than that of the group B rats (Fig. 2).

Discussion

The short pre-patent period of 7 days observed in this study was comparable to the findings of Egbe-Nwiyi et al. (2003) and Ezeokonkwo et al. (2007) in rats. Relapse of infection observed following treatment with 7.0 mg/kg Dinazene® could be attributed to the invasion of trypanosomes into drug inaccessible tissues/sites, trypanocidal resistance or drug insufficiency (Ezeh et al. 2009).

The blood glucose level of the infected group of rats did not vary statistically with those of the uninfected group prior to relapse. This could be attributed to the acute nature of the infection caused by *T. brucei* in this study.

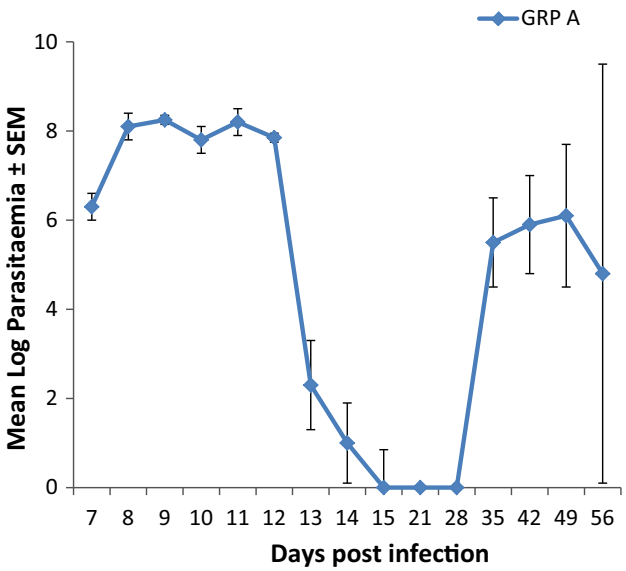

Fig. 1 Mean log parasitaemia of group A rats infected with *T. brucei* and treated with Dinazene®

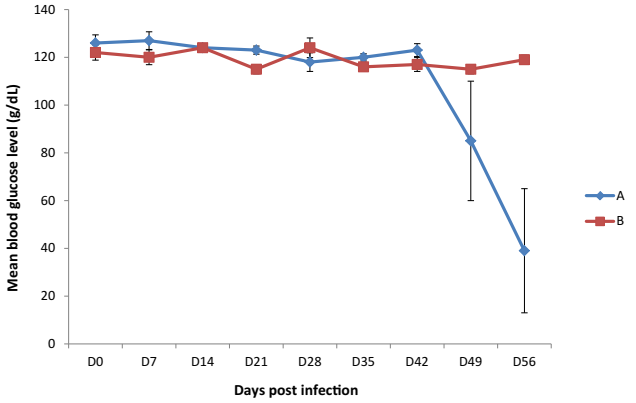

Fig. 2 Mean blood glucose of rats infected with *T. brucei* and treated with Dinazene® (A—infected and treated; B—uninfected and untreated)

Nevertheless, many authors have reported either hyperglycemia (Kadima et al. 2000; Nwoha and Omamegbe 2016) or hypoglycemia (Takeet and Fagbemi 2009; Sivajothi et al. 2015; Mishra et al. 2017) following trypanosomal infection. Iyayi (1996) reported no obvious changes in the plasma glucose concentration of rabbits following *Trypanosoma congolense* infection which Igbokwe (1998) attributed to the fact that the rabbits were unfasted, thus were able to maintain normal blood glucose level through the overriding effect of insulin. However, in the present study, the rats were fasted.

Paucity of information exists pertaining to blood glucose levels following relapse in trypanosome infected and treated animals. Nevertheless, the lowered blood glucose level (hypoglycemia) and extreme weakness observed following relapse could be attributed to fever-induced increased metabolic rate, extensive utilization of host glucose by trypanosomes (Saleh et al. 2009) and/or the increased blood glucose metabolism by the host. Endocrine homeostatic mechanisms involving gluconeogenesis and glycogenolysis are the host's physiological means of maintaining blood glucose levels. However, large decrease in the glycogen content of the liver, suggestive of increased glycogenolysis, decline in the gluconeogenic actions of glucose-6-phosphatase and fructose-1, 6-diphosphatase limiting glucose release into the extracellular fluid, ultimately resulting in hypoglycemia have been reported in trypanosome infected animals (Igbokwe 1995).

Trypanosomes are entirely dependent upon glycolytic phosphorylation for energy generation (Coley et al. 2011) and induce alterations in host's blood glucose levels following infection and/or relapse. This implies that inhibition of glucose metabolism can be a veritable target for trypanosomosis therapeutic intervention. The localization of the first seven enzymes of the glycolytic pathway in a subcellular compartment known as glycosomes and their limited homology with host enzymes (Musanabaganwa and Byiringiro 2014) makes them potential targets. Some of these glycolytic enzymes especially hexokinases (TbHK1); glucose transporters (TbTHT1) and enzymes involved in glycosome replication and development have been explored and validated experimentally (Coley et al. 2011).

In conclusion, *T. brucei* relapse infection in rats is associated with reduced blood glucose levels (hypoglycemia).

Authors' contributions IOE and RE designed the study. NEU, VOE, CFO, MIO and IOE carried out the laboratory experiment. IOE performed the statistical analysis while NEU, CFO and IOE drafted the manuscript. The final version of the manuscript was read, revised critically and approved by all the authors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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