



Full Length Research Paper

Trypadim[®], Trypamidium[®] and Novidium[®] can eliminate the negative effects on the body temperature and serum chemistry in Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain)

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ABSTRACT

Effect of intramuscular administration of Trypanocides on body temperature and serum chemistry in Wistar rats infected with *Trypanosoma brucei brucei* was investigated. 25 Wistar rats with average body weights of 200-240±20 g were randomly divided into five groups. Group A was administered 0.5 mL of normal saline only, while group B was given 0.1 × 10⁶ of *T. brucei brucei* only. Group C, D and E were inoculated the same dose of the parasite as in group B and they were intramuscularly administered with 3.5 mg/kg b.w. of Trypadim, 1 mg/kg b.w. of Trypamidium and 1 mg/kg b.w. of Novidium respectively. Body temperature increased consistently in all groups except group A. However, there was a significant (p < 0.05) difference in all treated groups compared to group B on 28 day post-infection (DPI). All serum indicators increased significantly (p < 0.05) in group B when compared with all other groups. Values observed for creatinine in all treated groups was only significantly (p < 0.05) higher in group E when compared to group A. In conclusion, administered trypanocidal drugs possess anti-pyrexia activity and could off-set the negative effect of *T.brucei.brucei* on the measured serum chemistry in Wistar rats.

Keywords: Body temperature; Serum chemistry; Trypanocides; *Trypanosoma brucei brucei*; Wistar rats.

INTRODUCTION

Trypanosomiasis has been reported to affect humans and domestic animals. It is described as a complex debilitating and often-fatal condition caused by infection with one or more of the pathogenic tsetse-transmitted protozoan parasites of the genus *Trypanosoma spp.* (Anene, 2001; Kneeland et al., 2012; WHO, 2013). The causative agents of this disease include *Trypanosoma congolense* (*T.congolense*), *T. vivax* and *T. brucei brucei* in cattle, sheep and goats; *T. evansi* in horses and camels, and *T. simiae* in pigs (Finelle, 2002; Grebaut et al., 2009). Amongst these parasites, the sub-species *T. b. brucei* is acclaimed to be the most virulent in attacking

domestic animals because it is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of specific antibodies, thus enabling the parasites to evade the consequences of the host immune reactions by switching the VSG, a phenomenon known as antigenic variation causing severe, often fatal disease in domestic animals (Deitsch et al., 2009). Antigenic variation appears to be the primary mechanism for parasite survival in an immunocompetent host. All domestic animals are susceptible to *T brucei brucei* infection causing clinical

signs such as undulating fever, listlessness, emaciation, hair loss, discharge from the eyes, edema, anemia, paralysis, lacrimation, jaundice, wasting of muscles, infertility and low milk production in cattle (Ekanem and Yusuf, 2008; Akanji et al., 2009). Nowadays, there exist several ways employed in combating this menace which are basically through a) Vector control (the use of insecticides, tsetse traps, aerial wide spraying and sterile insect technology) b) Parasite control (chemotherapy and chemoprophylaxis, alternative medicine or ethno-medicine) and c) Biological control (vaccination, production of apathogenic entomophagal bacteria). The best option remains elimination of the vectors, but its attainability remains elusive as there are multifaceted factors hindering the overall success of this alternative, topmost of which is the non-availability of huge capital outlay needed to execute this program in the existing sub-Saharan African region. The biological control is actively in progress, particularly the production of an effective vaccine, and breakthrough is not given to the nearest future because of the antigenic variation complex of the parasite.

Therefore, the only available alternative is host-drug application. It has been documented that trypanosomiasis continues to be controlled primarily by trypanocides (Holmes et al., 2004; Olurode et al., 2009; Eghianruwa and Anika, 2012), though treatment is not without serious drawbacks because most farmers do not have adequate knowledge on diagnosis and the appropriate drug to use even in areas of high prevalence of trypanosomiasis. In addition, trypanocides are frequently used in the absence of diagnosis or used to treat conditions for which they are not effective leading to waste scarce resources, on some occasion avoidable sickness and death, mask poor production and promote drug resistance leading to exacerbated disease. However, when properly administered, it permits higher level of production, and improves animal welfare (Holmes et al., 2004).

It is our aim therefore, to evaluate the effectiveness or otherwise of the three most established trypanocides namely: Trypadim[®], Trypamidium[®] and Novidium[®] that have been in existence since the middle of the last century against the backdrop of the current waves of drug resistance saga, on body temperature and some serum chemistry in Wistar rats experimentally infected with *T. b. brucei*. (Federe strain).

MATERIALS AND METHODS

Experimental site

The study was conducted at the Nigerian Institute for Trypanosomiasis Research (NITR) in Kaduna North Local Government Area of Kaduna State which is located between latitude 10° 30' 00" N and longitude 7° 25' 50" in Kaduna, Nigeria.

Experimental animals

The experiment was approved by the Research Ethics Committee of NITR, and the study was conducted under protocols in the guidelines established by the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C. 1996). Twenty five adult Wistar rats of average weight between 200 and 240 g were obtained from the rat colony of NITR. The animals were randomly divided into five groups, of five rats each and kept in standard plastic cages as follows:

Group A: Uninfected, untreated (negative control)

Group B: Infected but not treated (positive control)

Group C: Infected and treated with Trypadim (Diminazene di-aceturate).

Group D: Infected and treated with Trypamidium (Isometamidium chloride).

Group E: Infected and treated with Novidium (Homidium chloride).

The animals were feed with a pelleted basal diet obtained from a commercial feed outlet (Vital Feeds Plc., Kaduna, Nigeria) and water was given *ad libitum*.

Trypanosome infection

Trypanosoma brucei brucei (Federe strain) was obtained from the stabilates kept in the Department of Vector and Parasitology, NITR, Kaduna, Nigeria. The infected blood from donor rat at peak parasitaemia (4 DPI) was collected by means of tail picking and diluted with cold physiological saline. The numbers of parasites in the diluted blood was determined through the method described by Herbert and Lumsden (1976). A volume containing approximately 1×10^6 was injected intraperitoneally into each rats in the infected groups.

Drugs administered

All drugs used were obtained from a commercial outfit in Kaduna, Nigeria. The drugs were products of the same Company (Merial, 29, Avenue Tony Garnier, 69007 Lyon, France) which were all administered as a single dose from the onset of parasitaemia. Briefly, the drugs were dissolved and reconstituted in distilled water according to manufacturer's instruction and given intramuscularly in the following concentrations: Trypadim at 3.5 mg/kg/bodyweight(bw); Trypamidium at 1.0 mg/kg/bw and Novidium at 1.0 mg/kg/bw, respectively.

Measurement of Body temperature

The Body temperature of all animals in the groups were measured once daily between 12:00 and 15:00 h. Briefly,

Table 1. Effect of intramuscular administration of Trypadim®, Trypamidium® and Novidium® on some serum chemistry of Albino Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain) (Means \pm SEM, n = 5).

Group	Uninfected untreated	Infected treated	not treated	Infected treated with Trypadim	and with Trypamidium	Infected treated with Novidium	and with
Initial	37.52 \pm 0.16	37.42 \pm 0.24 ^c		37.49 \pm 0.15 ^c	37.50 \pm 0.19	37.51 \pm 0.14 ^c	
7 DPI	37.56 \pm 0.20	39.86 \pm 0.18 ^a		38.37 \pm 0.14 ^a	38.05 \pm 0.19	38.45 \pm 0.16 ^a	
14 DPI	37.55 \pm 0.16	38.45 \pm 0.12 ^b		37.95 \pm 0.13 ^b	37.80 \pm 0.15	37.96 \pm 0.15 ^b	
21 DPI	37.41 \pm 0.19	38.81 \pm 0.14 ^b		38.13 \pm 0.12 ^{ab}	38.05 \pm 0.16	38.24 \pm 0.19 ^{ab}	
28 DPI	37.53 \pm 0.14	39.94 \pm 0.22 ^a		37.74 \pm 0.13 ^b	37.56 \pm 0.15	38.05 \pm 0.13 ^b	

DPI = Days post-infection; Mean values with different superscripts along the same row are significantly ($p < 0.05$) different.

Table 2. Effect of intramuscular administration of trypadim®, trypamidium® and novidium® on some serum chemistry of Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain) (Means \pm SEM, n-5).

Group	Uninfected untreated	Infected not treated	Infected and treated with Trypadim	Infected and treated with Trypamidium	Infected and treated with Novidium	and with
ALT	22.00 \pm 0.52 ^b	31.50 \pm 0.62 ^a	13.20 \pm 0.47 ^c	10.80 \pm 0.39 ^d	14.00 \pm 0.52 ^c	
AST	33.50 \pm 0.60 ^b	46.90 \pm 0.82 ^a	23.30 \pm 0.73 ^c	18.20 \pm 0.84 ^d	25.10 \pm 0.72 ^c	
ALP	81.70 \pm 1.25 ^c	220.50 \pm 2.37 ^b	233.30 \pm 1.50 ^a	224.80 \pm 1.76 ^b	234.60 \pm 1.51 ^a	
Urea	176.10 \pm 1.78 ^b	332.80 \pm 2.53 ^a	153.80 \pm 1.19 ^{cd}	149.10 \pm 1.68 ^d	156.90 \pm 1.03 ^c	
Creatinine	57.20 \pm 0.73 ^c	111.60 \pm 2.02 ^a	58.20 \pm 0.77 ^{bc}	56.80 \pm 0.53 ^c	61.00 \pm 1.01 ^b	

ALT = Alanine amino-transferase; AST = Aspartate amino-transferase; ALP = Alkaline phosphatase. Mean values with different superscripts along the same row are significantly ($p < 0.05$) different.

each animal was gently caught and a digital thermometer with a maximum gauge of 42 °C (accuracy \pm 0.1°C MODE: ECT-1, MAXICOM), was inserted 3 cm into the wall of the colorectum of each rat and at the sound of a beep, the thermometer was immediately withdrawn and values obtained recorded accordingly.

Sample collection and Serum Analysis

Tail blood was collected daily for monitoring parasitemia as described by Herbert and Lumsden (1976) and PCV by the micro-haematocrit method. On 28 DPI, the rats were sacrificed by humane decapitation prior anesthesia with sterile cotton impregnated chloroform, and blood was collected in plain vacutainers, serum was harvested and used for estimation of alanine amino-transferase (ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) activities using the method described by Bergmeyer et al. (1978) with the aid of commercial reagent kit (Gaselch aft fur Biochemica und Diagnostica, Wiesbgden, Germany). The serum samples were also used for the estimation of Urea and Creatinine by the Diacetylmonoxime and Jaffe's reactions, respectively as described by Kaplan et al. (1988).

Statistical analyses

All data were presented as Means \pm SEM and were analyzed by one way analysis of variance (ANOVA). In

addition, differences between means were compared by Duncan (1955) post-hoc test using the SPSS statistical package version 19. Values of $p < 0.05$ were considered significant.

RESULTS

Table 1 shows the values of body temperature of animals in all groups during the experimental period. Body temperature increased consistently in all groups except group A. Rise in body temperature was observed 7 DPI in all infected groups. But this trend was changed 14 and 21 DPI as body temperature dropped significantly in all infected groups, only to rise again on 28 DPI. However, there was a significant ($p < 0.05$) difference in all treated groups compared to group B on day 28 post-infection, as their values were lower than that obtained in that group.

Table 2 presents mean values of ALT, AST, ALP, Urea and Creatinine measured during the study period. All serum indicators increased significantly ($p < 0.05$) in group B when compared to all other groups. Nevertheless, the values of groups C and E for ALT, AST and Urea significantly ($p < 0.05$) decreased, while decrease in group D was highly significant ($p < 0.01$) when compared to group A. ALP showed similar pattern conversely. Values observed for creatinine in all treated groups was only significantly ($p < 0.05$) higher in group E when compared to group A.

DISCUSSION

The body temperature observed in all infected groups was very high and the value of $39.85 \pm 0.17^\circ\text{C}$ recorded 28 DPI in group B was outside the established physiological value of $37.00 \pm 1.5^\circ\text{C}$ for this specie (Stammers, 1926). The undulating waves of increase and decrease observed in body temperature in all infected groups agrees with the report of Karle (1974) who observed that the undulating fever associated with trypanosomiasis may contribute in causing erythrocyte destruction. This view is supported by the observation that exposure of erythrocytes to temperatures above the normal body temperature increased their osmotic fragility, membrane permeability and decreased their plasticity such that their life span was decreased *in vivo* (Karle, 1974). Apart from increasing the metabolic rate to hasten the exhaustion of the metabolic resources of the cell, temperature elevation may increase the rate of immunochemical reactions and may affect membrane lipids by thermal initiation of lipid peroxidation. Furthermore, Igbokwe (1994) reported that trypanosomiasis infection elevates body temperature and therefore increased the rate of immunochemical reactions thereby initiating lipid peroxidation of erythrocytes. Therefore, the undulating thermal value observed in all infected groups is a direct reflection of the host response to successive waves of parasitaemia (Stephen, 1986).

Group B showed a higher increase between the mean values with a significant difference at $p < 0.05$. The thermal increment observed in the infected and untreated group particularly at 7 DPI and 28 DPI may be due to the body temperature setting point in the hypothalamus which changes under the influence of pyrogenic stimuli released during infection, it has been reported that increase in body temperature can lead to pyrexia which correlates with the presence of high levels of trypanosomes in blood (Taylor and Authie, 2004). In contrast, pyrexia were reduced significantly in the infected animals treated with trypadim[®], trypanidium[®] and novidium[®], with the greatest reduction observed for trypanidium treated group. Results suggest that administered drugs suppressed the pyrexia triggering mechanism of the proliferating parasites.

In this study, serum ALT, AST and Urea levels increased in infected rats when compared to normal rats. This agrees with studies where ALT was elevated in *Trypanosoma evansi*-infected camels (Sazmand et al., 2011) and AST, Urea and Creatinine in *T. brucei brucei*-infected animals (Yusuf et al., 2012). Several other studies have reported elevated serum enzymes (Umar et al., 2007; Allam et al., 2011; Abd El-Baky and Salem, 2011). The elevation of these enzymes is usually indicative of liver damage, being the major liver marker enzymes; or partly due to cellular damage caused by lysis or destruction of the trypanosomes (Yusuf et al., 2012). ALP showed a significant increase in groups B, C,

D and E compared to group A. This characteristic increase may be attributed to disseminated anomalies occasioned by the parasitic infection other than in the liver, since ALP is also found in many other tissues (intestinal mucosa, kidneys, and blood vessels), increased activities sometimes can also be found under other pathological conditions such as peritonitis (Andersson et al., 1991). Furthermore, the significant difference ($P < 0.05$) of ALP activities of the infected untreated and prophylactic treated compared to normal group, may be attributed to interaction between toxin released by the parasite and the constituents of the drugs administered. The elevated creatinine level in the *T. b. brucei*-infected rats agrees with previous findings (Allam et al., 2011; Ezeokunkwo et al., 2012) and could be due to destruction of kidney cells resulting in the inability of the kidneys to excrete creatinine (Ezeokunkwo et al., 2012). In addition, the elevated serum creatinine level in infected but not treated group may be associated with impaired kidney function or as a result of sequestration of the trypanosomes in the muscle tissues of the heart leading to damage to the cardiac muscles and release of creatine phosphokinase with attendant increase in the circulating creatinine (David and Michael, 2003). However, all serum chemistry parameters observed in the infected and treated groups were significantly ($P < 0.05$) reduced compared to the infected group only.

In conclusion, our observation has clearly demonstrated the modulating effect of the trypanocides used on body temperature and serum chemistry in trypanosome infected rats, and evidence have shown the order of their effectiveness with trypanidium[®] leading the pack and closely followed by trypadim[®] and novidium[®].

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