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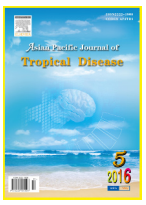

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A comparative study of the chemotherapeutic effects of diminazene aceturate and Ivermectin on *Trypanosoma brucei brucei* infected rats

Fredrick Osondu[1], Chukwuebuka Iniobong Ikenna Ugochukwu[2*], Emmanuel Ikenna Ugochukwu[1]

[1]*Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria*

[2]*Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria*

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ABSTRACT

Objective: To investigate the comparative effect of diminazene aceturate (DA) or ivermectin in albino rats experimentally infected with *Trypanosoma brucei brucei*.
Methods: A total of 21 adult male albino rats were divided into three groups consisting 7 albino rats each and all the members of the groups were infected intraperitoneally with 6.3 × 10^6 trypanosomes in infected mouse blood diluted with normal saline. By 7 days, post-infection when parasitaemia was fully established and Groups A and C were treated with DA and ivermectin respectively, while Group B served as the control (untreated). Parameters assessed included rectal temperature, body weight changes, packed cell volume, total leucocyte counts, differential leucocyte counts and parasitaemia.
Results: The results showed that following the treatment with DA and ivermectin at the peak of parasitaemia, the ivermectin in treated group remained parasitaemic till the end of the experiment. The survivability of ivermectin treated group was longer than those of the control group. DA on the other hand was able to effect a complete plasma clearance of the parasites within 48 h post-treatment at a dose of 3.5 mg/kg body weight. In the untreated control group, parasitaemia peaked on Day 7 post-infection, dropped transiently on Day 28 post-infection and peaked again with the second wave of parasitaemia showing no remission until the end of the experiment.
Conclusions: It was concluded from the results of this present study that DA has a better efficacy than ivermectin which has no chemotherapeutic effect against *Trypanosoma brucei brucei* infection. The efficacy of DA is on the decline because of drug resistance and incidence of relapse, therefore a search for effective alternative chemotherapy or drug combinations should be encouraged.

1. Introduction

High incidence of infectious diseases constitutes a major constraint to livestock production in most developing countries[1]. Parasitic infections are of great worldwide significance[2]. African trypanosomiasis which is also called nagana disease is an infectious disease of humans and animals of similar aetiology and epidemiology[3].

The etiologic agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies of the *Glossina* species[3]. It produces the following clinical signs of pyrexia, apathy, anaemia and corneal opacity[4].

African trypanosomes are protozoan parasites responsible for both animal and human trypanosomosis. The disease is fatal if left untreated and chemotherapy which is the major means of control in Africa is faced with problems of toxicity and the ever increasing incidence of resistance[5-7]. Animal trypanosomosis continues to constitute a major threat to food security in several parts of sub-Saharan Africa including Nigeria where it is a menace in the livestock industry despite the length of years puts in an attempt to control the disease[8-11]. Diminazene aceturate (DA) commonly known as berenil, isometamidium chloride commonly known as trypamidium, homidium salt (ethidium), cymelarsan and suramin are the drugs commonly used for the treatment of African animal trypanosomiasis. Of these drugs, DA is the most commonly used therapeutic agent[12,13]. While ivermectin, a macrolide antibiotic produced from a fungus *Streptomyces avermitilis* and as a broad spectrum antiparasitic agent has also been previously reported, and its potent effects

*Corresponding author: Chukwuebuka Iniobong Ikenna Ugochukwu, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.
Tel: +2348037322220
E-mail: iniobongugochukwu@gmail.com
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has been clearly demonstrated using different hosts, doses and methods of administration[14,15] .

Despite increases in the incidence of many parasitic infections in recent years, the number of studies designed to improve the treatment of these infections have not been able to address the situation[2]. The burgeoning problem of resistance to effective antiparasitic agents which are in use in the last decade has added urgency to the need to discover new antiparasitic agents and to make better use of existing ones[2]. In the light of the above problem of undue resistance by trypanosomes to trypanocides coupled with the mechanism of relapse, this study was undertaken to compare the chemotherapeutic effect of DA and ivermectin on *Trypanosoma brucei brucei* (*T. brucei brucei*) infection.

2. Materials and methods

2.1. Experimental animals

A total of 21 male albino rats of uncharacterized sexes were used in this study. The rats which weighed from 45 g to 105 g, were purchased from the breeding stock of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed with commercially prepared, standard rat feed (Vital feeds®) and given water *ad-libitum* throughout the duration of the experiment. The animals were allowed to acclimatize for 7 days pre-infection.

2.2. T. brucei brucei

The strain of trypanosome used for this study was initially isolated from a naturally infected dog presented at the Veterinary Teaching Hospital, University of Nigeria, Nsukka. It was identified as *T. brucei brucei* based on morphological characteristics as described by Soulsby[16].

2.3. Research methods

A drop of blood was aseptically collected from the tail vein of the albino rats and used for the preparation of a wet mount using methods described by Herbert and Lumsden[17].

2.4. Experimental design

2.4.1. Infection of experimental animals

The male albino rats were divided randomly into 3 groups of 7 each. They were then infected intraperitoneally with 6.3×10^6 trypanosomes in normal saline diluted infected blood of mice. By 7 days, post-infection (PI) when parasitaemia was fully established and they were divided into three groups (A, B and C) consisting of 7 albino rats each. Groups A and C were treated with DA and ivermectin respectively while Group B served as the control (untreated). The blood collected from these albino rats was examined daily PI and post-treatment to establish the onset of parasitaemia and parasite clearance time.

2.4.2. Estimation and monitoring of parasitaemia

The degree of parasitaemia in the infected blood of mice was estimated using standard procedures used in performing wet mount technique as described by Herbert and Lumsden[17] and microhematocrit buffy coat microscopy as described by Murray *et al*.[18].

2.4.3. Administration of DA and ivermectin

All animals in Group A were treated with DA at a dose of 3.5 mg/kg body weight as a single intraperitoneal injection. Also, all animals in Group C were also treated with ivermectin at the approved dose rate of 0.2 mg/kg body weight intraperitoneally as a single injection.

2.4.4. Haematological studies

Blood samples were collected from each albino rat through the tail vein. This was done by gradually massaging the snipped tails of the rats into 21 different sterile vials containing anticoagulant, ethylene diamine tetraacetic acid for haematological analysis. The following indices were determined using routine laboratory methods. Packed cell volume (PCV) was used as described by Schalm *et al*.[19-21]. Leucocytes counts were determined by the method described by Jain[22], while differential count was determined as described by Schalm *et al*.[19]. The determination of haematological parameters was done pre-infection, at the peak of parasitaemia and post-treatment.

2.5. Statistical analysis

The collected data were subjected to descriptive statistical analysis to obtain mean and mean ± SD. Differences between treatment group's changes overtime were determined using the Student's *t*-test. The *P* values less than 0.05 and 0.01 were considered statistically significant.

3. Results

3.1. Parasitaemia

Results of daily estimation of parasitaemia were presented in Figure 1. All infected animals showed detectable parasitaemia within 5 days PI. Parasitaemia increased rapidly in all groups and the groups attained the first peak of parasitaemia on 7 days post-infection. Barring drug effects, parasitaemia was sustained in Groups B and C till the death of all the animals in these two groups.

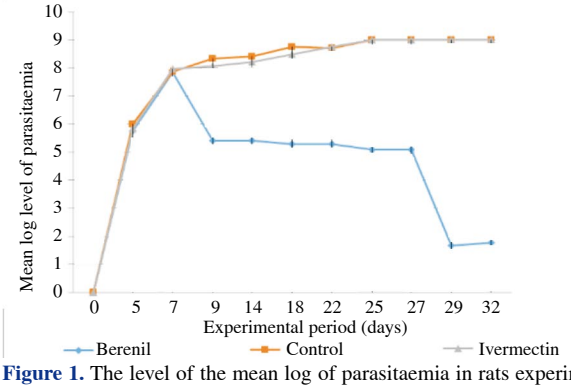

Figure 1. The level of the mean log of parasitaemia in rats experimentally infected with *T. brucei brucei* and treated with berenil or ivermectin.

3.2. Effects of drug treatments

Following the treatment of DA and ivermectin at the peak of parasitaemia as shown in Figure 1, the ivermectin in treated group remained parasitaemic till the end of the experiment. The ivermectin was able to depress the level of parasitaemia though it was an insignificant extent ($P > 0.05$). DA on the other hand was able to clear the infection within 48 h post-treatment at a dose of 3.5 mg/kg body weight. However, relapse was observed in two of the