

STUDIES ON THE CURATIVE AND PROPHYLACTIC CONCENTRATIONS OF ISOMETAMIDIUM CHLORIDE IN THE TREATMENT OF EXPERIMENTAL *Trypanosoma congolense* INFECTION IN RED SOKOTO BUCKS

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ABSTRACT

The effectiveness of therapeutic and prophylactic drug concentrations of isometamidium chloride (ISMTX) was studied in *Trypanosoma congolense* infected Red Sokoto bucks (RSB). Sixteen R.S.B were divided into four groups of four animals each: Group I (1 % ISMTX), Group II (2 % ISMTX), Group III (3% ISMTX) and Group IV (uninfected and untreated-Control). Groups I, II and III bucks were inoculated with approximately 1×10^6 trypanosomes intravenously each. Parasitemia was monitored on wet-mount and mHCT throughout the study. Clinical signs, rectal temperature, PCV, Total white blood cell count (TWBCC), differential leucocyte count were monitored. Group I, II and III were treated with 1 %, 2 %, and 3 % isometamidium chloride at the dose rate of 0.5 mg/kg body weight intramuscularly. 2 % and 3 % ISMTX groups were re-infected post-treatment to test their chemoprophylaxis. Statistical analysis using One way -ANOVA was employed $P < 0.05$ considered significant. The 1 % ISMTX had relapse post-treatment while the parasite in 2 % and 3 % ISMTX groups appeared following re-infection. Pale mucous membranes, pyrexia, inappetance, depression, epiphora and nasal discharges were among the clinical signs observed. There was a significant difference ($P < 0.001$) in the daily mean rectal temperature post-infection and post-treatment between the test groups and the control group. A significant difference ($P < 0.001$) in the PCV was also noted in all the three groups post-infection and post-treatment. The TWBCC in all the three test groups differed significantly ($P < 0.001$) post-infection and post-treatment compared with the control group. A significant ($P < 0.01$) lymphopenia was noted post-infection and post-treatment respectively. The 1 % isometamidium was effective in the treatment of trypanosomiasis according to this study but not curative while the 2 % and 3 % isometamidium chloride were both effective and curative for the period of observation. The chemoprophylaxis of the 2 % and 3 % isometamidium chloride based on this study was five weeks and four weeks respectively.

INTRODUCTION

Trypanosomiasis is a haemo-parasitic disease of both animals and humans, caused by protozoan parasites of the genus *Trypanosoma*. They are mainly cyclically transmitted by *Glossina* spp (Kuzoe and Schofield, 2004) which are found worldwide. In Nigeria, animal trypanosomiasis still constitutes a major obstacle against food security in spite of previous attempts towards chemotherapeutic and tsetse control (Abenga *et al.*, 2004). Isometamidium chloride is the main drug that is used for curative and prophylaxis against trypanosome infections in livestock in Africa. A number of studies have been carried out to investigate the use of isometamidium chloride in sheep and goats under natural tsetse challenge (Masiga, *et al.*, 2001). The studies showed that sheep and goats could be protected against trypanosomiasis by isometamidium chloride for periods ranging from 6 to 16 weeks. Isometamidium chloride is usually administered to trypanosome infected animals by deep intramuscular (IM) injection of 1 % or 2 % solution of the drug at the dose rate of 0.25-0.5 mg/kg for curative and prophylactic rates, respectively (Mamman, 1993). Recently, treatment

of both *T. brucei* and *T. congolense* single and mixed experimental infections in Red Sokoto bucks using the recommended dosage of 0.5 mg/kg (however, with an unspecified concentration) resulted in reappearance of the parasites two weeks after treatment (Karaye, 2012). This study is aimed at determining the efficacy of therapeutic and prophylactic drug concentrations of isometamidium chloride using 1 %, 2 % and 3 % in *T. congolense* infected Red Sokoto bucks (RSB).

MATERIALS AND METHODS

The parasite

T. congolense was used in this study and it was obtained from the Centre of Biotechnology Research and Training (CBRT) Ahmadu Bello University, Zaria, Kaduna state, Nigeria. Four donor albino rats were inoculated with the *T. congolense* (CBRT) stabilates intraperitoneally in order to multiply and harvest the parasites in sufficient numbers. The donor rats were sacrificed at a reasonable parasitemia and their bloods collected into clean ethylene diamine tetra-acetate (EDTA) containing sample bottles.

Experimental animals

Sixteen RSB of between the ages of 10-12 months old were purchased from the open market in Maiadua, Katsina State Nigeria. The animals were confined in a fly-proofed pen and fed on maize and guinea corn bran, cowpea haulms and guinea corn haulms, salt lick, and hay. Water was provided *ad libitum*. Acclimatization was done for three weeks during which base line data were collected. The animals were dewormed with Albendazole (WormKiller®-essential animal care, China) at the dose rate of 7.5 mg/kg PO and cypermethrin pour-on (Inothrine® 5 % pour on-Laprovvet, France) were used to treat for ectoparasites.

Experimental design

The animals were divided into four groups (I, II, III and IV) after proper identification. Groups I, II and III served as the experimental (test) Groups with four animals each while, Group IV had four animals also and it served as the control group. 1×10^6 parasites/ml of blood harvested from the donor rats were inoculated into each of the experimental animals intravenously after which parasitemia was monitored and estimated using wet mount and or mHCT of the jugular blood (reported as described in Herbert and Lumsden (1976) parasitemia score chart) two days post-inoculation then once every day until disease was established, while parasite clearance following treatment was determined using the same technique. Daily morning and evening rectal temperatures were taken by the use of a digital thermometer. Following the establishment of infection and development of clinical disease in all the infected goats in Groups I, II and III, chemotherapy was instituted using isometamidium chloride (Trypamidium-Samorin®-merial) at three different concentrations; 1 %, 2 % and 3 % respectively but, using the same dosage of 0.5 mg/kg intramuscular while Group IV was not given anything. Following an aphasitemic phase, blood collected from each animal, seven days post treatment was sub-inoculated into three mice. 2 % and 3 % ISMTX groups were re infected with 1×10^6 parasites I.V post treatment of the primary infection.

Sample collection

Blood (2 ml) was collected via jugular venepuncture into EDTA tubes daily for parasitemia post infection and post treatment while PCV, TWBCC and absolute differential leucocyte count were monitored on weekly basis.

Statistical analysis

One way-Analysis of variance (ANOVA) with Turkey's multiple comparison post-hoc test using Graph Pad Prism® version 5.0 for Windows® was

used to compare the level of significance between the test groups. Values of $P < 0.05$ were considered significant at 95 % confidence interval.

RESULTS AND DISCUSSIONS

Clinical observation: Pale mucous membranes, depression, purulent nasal discharges and epiphora, rough hair coats, pyrexia and prescapular lymph node enlargement were noticed in all the infected experimental animals and they all survived the experimental period. This is in agreement with the findings of Karaye (2012) and Bissalla *et al.* (2009) who found similar clinical signs in Bucks infected with *T. congolense* and sheep infected with *T. congolense* respectively.

Parasitemia, Rectal Temperature, PCV, TWBCC and Differential leucocyte counts

The pre patent period in all the animals in the test groups was four days but very few of them did not show parasitemia until the fifth day. The mean parasitemia score on wet-mount post infection and post treatment of Groups I, II and III are 5.96 ± 1.14 , 5.96 ± 1.14 , 4.97 ± 0.99 and 3.11 ± 1.62 , 0.13 ± 0.07 0.13 ± 0.07 respectively. A significant difference ($P < 0.05$) in the mean parasitemia score was noted in 2 % ISMTX group a day post treatment while in 1 % and 3 % ISMTX groups a decrease was observed two days post treatment with a further decrease in parasitemia in 2 % ISMTX group. This is due to the fact that the 2 % isometamidium chloride achieved a higher plasma concentration faster than the 1 % and 3 %. This is in agreement with the work of Braide and Eghianruwa (1980) who reported a reasonable amount of serum isometamidium concentration (2 % solution) at 24 hours after intramuscular administration of the drug in goats at a dosage rate of 0.5 mg/kg body mass. A further significant decrease ($P < 0.001$) in parasitemia in all the three groups was noted three days post treatment on HCT with the values dropping to 5.96 ± 1.98 , 0.99 ± 0.99 , 7.94 ± 2.80 from the previous values of 11.90 ± 1.61 , 8.93 ± 0.99 and 10.91 ± 1.89 respectively. 2 % ISMTX group had an aphasitemic phase on both wet-mount and HCT earlier than the 1 % and 3 % ISMTX groups. 1 % ISMTX group had a relapse of the infection two weeks post treatment. The 2 % and 3 % ISMTX groups had patency following re infection five weeks and four weeks respectively post treatment. Therefore the durations of their prophylaxis are five weeks for 2 % and four weeks for 3 % isometamidium chloride respectively. Schad *et al.* (2008) reported a similar observation where it was pointed out that isometamidium had two isomers; the red and blue isomers, the most abundant by-

products (40 %) of the commercial products, have lower chemoprophylactic activities providing only partial protection at one month following re challenge of previously treated mice (post-primary infection). The mean rectal temperatures post infection in 1 % ISMTX, 2 % ISMTX and 3 % ISMTX were $39.23 \pm 0.14^{\circ}\text{C}$, $39.24 \pm 0.05^{\circ}\text{C}$ and $39.17 \pm 0.12^{\circ}\text{C}$ which denote pyrexia compared with that of control group $37.54 \pm 0.07^{\circ}\text{C}$. The difference was significant ($P < 0.001$). A decrease in the rectal temperature was observed following treatment with a significant difference ($P < 0.001$). Two weeks post treatment the rectal temperature in 1 % ISMTX group increased as a result of re appearance of the parasites. A similar observation was made by Karaye (2012) who reported an increase in temperature due to relapse in Red Sokoto Bucks following treatment with therapeutic dose of isometamidium chloride. 2 % and 3 % ISMTX groups experienced an increase in the rectal temperature five weeks and four weeks post treatment respectively. This was attributable to the appearance of the parasites following re infection. There was a significant difference ($P < 0.001$) in the mean PCV in all the three test groups when compared to the control group both at post infection and post treatment. This was due to anemia following hemolysis of red blood cells through diverse mechanisms. There was a significant difference ($P < 0.001$) in the PCV of the 2 % and 3 % ISMTX groups following appearance of the parasite post re infection which was due to marked hemolysis of the red blood cells. The work of Abenga *et al.* (2005) with *T. congolense* and *T. brucei* suggested that trypanosomes were responsible for the progressive development of anaemia. A significant leucopenia ($P < 0.001$) post infection and post treatment was noted in all the test groups compared with the control group. The leucopenia was due to lymphopenia. The decrease in the TWBCC may be due to immunosuppression as a result of the overwhelming nature of the disease. This is in agreement with the work of Adah *et al.* (1993) who reported a contrasting finding in the same study in *T. congolense* infected Red Sokoto goats with leucopenia characterized by neutrophilia and lymphopenia. The absolute neutrophil count, eosinophil, monocyte and basophil count were not significantly different ($P > 0.05$) with that of the control group.

The mice sub-inoculated with the blood from the treated groups were negative of the parasite up to five weeks post sub inoculation. This was evident that the treatment instituted was effective in clearing the parasite from blood.

Conclusion

The 1 % isometamidium chloride that is supposedly curative was only able to clear the parasites temporarily from circulation but two weeks post treatment a relapse was found despite the institution of treatment early enough. This implied that the 1 % isometamidium chloride at 0.5 mg/kg body weight was only effective but not curative. The 2 % and 3 % isometamidium chloride at 0.5 mg/ body weight were both more effective and curative vis-à-vis the 1 % isometamidium chloride solution. The duration of chemoprophylaxis for 2 % isometamidium chloride based on our findings when used at the dose rate of 0.5 mg/kg body weight was five weeks while, that of 3 % isometamidium chloride, was four weeks. This is evident from the re infection of the bucks treated with 2 % and 3 % isometamidium chloride where an aparasitemic phase was observed for five weeks and four weeks respectively.

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