COMPARATIVE PROPHYLACTIC EFFICACY OF PENTAMIDINE AND ISOMETAMIDIUM IN MICE EXPERIMENTALLY-INFECTED WITH *Trypanosoma brucei brucei*

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ABSTRACT

The development of resistance in trypanosomes to commonly-used trypanocides has an important implication in the chemotherapy and prophylaxis of both human and animal trypanosomosis. This study compared the prophylactic activity of pentamidine isethionate and isometamidium chloride in mice experimentally-infected with *Trypanosoma brucei brucei.* The 96 mice were divided into four groups with 24 animals each: a) Group I - untreated control group; b) Group II - treated with pentamidine isethionate at 4 mg/kg body weight for 3 alternate days; c) Group III - treated with pentamidine isethionate at 8 mg/kg body weight for 3 alternate days; and d) Group IV - treated once with isometamidium chloride at 1 mg/kg body weight. Thereafter, three mice from each group were infected intraperitoneally with *T. brucei brucei* at weekly intervals and monitored for parasitaemia. The entire control group was positive for *T. brucei brucei* by day 6 post infection. Parasitaemia was recorded in Group II at 3 weeks post infection, at 4 weeks in Group III and at 8 weeks in Group IV. Group IV had the highest survival rate followed by Group III. The results showed that isometamidium chloride provides better prophylaxis than pentamidine isethionate.

Keywords: isometamidium chloride, mice, pentamidine isethionate, trypanosome

INTRODUCTION

Trypanosomosis in both humans and animals is a major health and animal production problem in Sub-saharan Africa (Losos and Ikede, 1972; Ohaeri and Eluwa, 2011). New epidemics of human and animal trypanosomosis are reported annually from various parts of Africa, like, Nigeria. Diminazene aceturate and isometamidium chloride have remained the commonly used trypanocides in domestic and pet animals in Africa (Leach and Roberts, 1981).

Isometamidium chloride is the drug of choice for chemoprophylaxis of animal trypanosomosis even in areas of constant heavy challenges. When used for treatment, it may be administered every 6-8 days until recovery (Morrison *et al*.,

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1981; Sones *et al*., 1988). However, its continual use and abuse by Fulani herdsmen and local farmers have led to the development of strains of trypanosomes which are strongly resistant to the drug, resulting in frequent relapses of animals treated against trypanosomosis in the field (Marasco *et al.,* 2002; Anene, 2006).

Pentamidine isethionate is an anti-microbial agent used primarily in the treatment of pneumocystic pneumonia (PCP), leishmaniasis and *Candida albicans* infections in humans (VA Classification, 2011; Wikipedia, 2011). Pentamindine can also be used as an antibiotic in children receiving leukaemia therapy (VA Classification, 2011; Wikipedia, 2011). It has a good activity against the early stage of human *Trypanosoma gambiense* infection (VA Classification, 2011; Wikipedia, 2011). Pentamidine is also used prophylactically in cases of human trypanosomosis and PCP, especially in patients with drug-induced immunosuppression. It has a long lasting prophylactic effect against Gambian sleeping sickness and had been used successfully as a mass prophylactic agent against the disease and so far had shown no resistant trypanosomes (Harding and Hutchinson, 1950; Williamson, 1970). Its anti-protozoal activity is not well-known but is thought to be associated with the action of ubiquitin (Nguewa *et al*., 2005). Trypanosomes take up pentamidine isethionate by its purine receptors in the process of its uptake of adenine from the host. It accumulates to a high level within the parasite and this inhibits certain trypanosomes enzymes and the synthesis of its DNA (WHO, 2011). However, there are some reports of cases of resistant strains of *T. rhodesiense* against pentamidine (Benhard *et al*., 2007). Currently, pentamidine is seldom used as a prophylactic trypanocide in animals. Therefore, the occurrence of strains of animal trypanosomes that are resistant to pentamidine is limited or low when compared with the incidence of resistance to the more commonly used drugs such as diminazene aceturate and isometamidium chloride.

The present emergence of drug resistant trypanosomes makes research into alternative medication and prophylaxis against animal trypanosomosis imperative (Nok, 2003). Hence, the relevance of this study to compare the prophylactic efficacy of pentamidine isethionate and isometamidium chloride in mice experimentally-infected with T*. brucei brucei*.

METHODOLOGY

Ninety-six (96) 8-week old inbred albino mice of both sexes weighing between 30-35 g were used in this study. The mice were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka and kept in clean cages housed in the laboratory animal house. The mice were fed and watered *ad libitum* during one week of acclimatization prior to the commencement of the study. Only mice found to be free from pathogens were included in the study. Each mouse was identified with picric acid stain.

Pentamidine isethionate (Rhone-Poulenirorer, May & Baker, Dagenham, England) was reconstituted with distilled water according to the manufacturer's instructions and administered at a dose of 4 mg/kg body weight or 8 mg/kg body weight intraperitoneally for three alternate days. The isometamidium chloride

(Trypadium Samorin®) used in this study was reconstituted using distilled water and administered once at the dose rate of 1 mg/kg body weight intraperitoneally.

The *T. brucei brucei* parasite used in this study was a "Federe" strain obtained from the National Institute of Trypanosomosis Research (NITR) Vom, Plateaue State, Nigeria. The parasites were cryopreserved in liquid nitrogen from where donor rats were initially infected. The parasites were subsequently maintained by serial passage in mice at the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

An estimated 2.5 x 10^6 trypanosomes suspended in 1 ml of normal saline were used to infect each experimental mice through the intraperitoneal route with the aid of 1 ml tuberculin syringes. The quantity of parasite was estimated using the rapid matching method of Herbert and Lumsden (1976).

The phosphate buffered saline was prepared by mixing 200 ml of a solution containing 8 g of sodium chloride and 0.1 g of hydrated magnesium chloride in 1000 ml of distilled water with 200 ml of a solution containing 0.2 g of potassium chloride and 1.44 g of sodium hydrogen tetraoxosulphate in 1000 ml of distilled water and 800 ml of a third solution containing 0.1 g calcium chloride in 1000 ml of distilled water.

The ninety six mice were divided into 4 groups with 24 mice in each group: 1) Group I, infected control with no treatment; 2) Group II, treated with pentamidine isethionate at a dose of 4 mg/kg body weight for 3 alternate days; 3) Group III, treated with pentamidine isethionate at a dose of 8 mg/kg body weight for 3 alternate days; and 4) Group IV, treated with isometamidium chloride at a dose of 1 mg/kg body weight once. Three mice from each group were infected with 2.5 x 10^6 *T. brucei brucei* parasites suspended in 1 ml of phosphate buffered saline every week. Infected mice were housed in separate cages and screened for parasitaemia using wet mount and buffy coat methods at 7-14 days post infection. Negative samples by 14 days post infection were subjected to the haematocrit buffy coat method (Woo, 1970). The procedure was repeated up to the last three rats of each group in the experiment.

To determine parasitaemia, two methods were used. For wet mount method, blood was collected from the tail vein of the mice after nipping the tail with a sterile razor blade. A wet blood mount was prepared by placing a drop of blood on a clean glass slide and placing a cover slip to allow even spread of the blood. The slide was examined under x 40 objective of the microscope for the presence of trypanosomes (Woo, 1970). For haematocrit buffy coat method, blood from the nipped tail of the mice was allowed to flow into heparinised capillary tube and the other end of the tube was sealed with wax. The filled tubes were balanced in position in the microcentrifuge and centrifuged at 12000 revolutions for 5 min. The centrifuged tubes were cut at the buffy coat level and the buffy coats were expressed into clean glass slides. The expressed buffy coat was covered with cover slips and then viewed under the microscope (Woo, 1970). The end-point of the study was fixed at ten weeks following the experimental infection of the mice with trypanosome parasites.

RESULTS AND DISCUSSION

Table shows the occurrence of parasitemia in treated and non-treated groups. Trypanosomes (*T. brucei brucei*) were readily detected in the blood of all the mice in the control group (Group I) 7 days after infection with the parasite. Trypanosomes were not detected in the blood of mice in Groups II and III by the 14th and 21st days (*i.e* 2nd and 3rd weeks) post infection, respectively. There was no evidence of trypanosome parasites in the blood of mice in Group IV until 7 weeks post infection.

Table. Occurrence of parasitaemia in non-treated mice and mice treated with pentamidine isethionate and isometamidium chloride and infected with *Trypanosoma brucei brucei*.

Experimental period (weeks post treatment)	Number of positive over number of mice			
	Group I (No cover)	Group II	Group III	Group IV
One	3/3	0/3	0/3	0/3
Two	3/3	0/3	0/3	0/3
Three	3/3	1/3	0/3	0/3
Four	3/3	3/3	2/3	0/3
Five	3/3	3/3	3/3	0/3
Six	3/3	3/3	2/3	0/3
Seven	3/3	NA	NA	0/3
Eight	3/3	NA	NA	1/3

Group I: untreated control; Group II: treated with pentamidine isethionate at 4 mg/kg body weight; Group III: treated with pentamidine isethionate at 8 mg/kg body weight; Group IV: treated with isometamidium chloride at 1 mg/kg body weight.
NA: none alive.

The first mortality was recorded in Group I by day 14 post infection. In general, in animals pre-treated with pentamidine isethionate, mortality was higher in Group II than in Group III. No mice in Group IV died until the end of the experiment. The Group III mice remained aparasitaemic until the fourth week post-treatment when two of the three pre-treated and infected mice became parasitaemic. By the fifth week post treatment, all the three pre-treated infected mice in Group III were all parasitaemic. The Group IV mice remained aparasitaemic until the eight week post-treatment when only one out of the three infected mice showed parasitaemia.

This study confirms the generally held view that pentamidine isethionate at the dose rate of 4 mg/kg and 8 mg/kg body weight and isometamidium chloride at 1 mg/kg body weight have clinically important prophylactic effects against trypanosome infection in animals and man. The two months prophylaxis conferred on the mice by isometamidium chloride agrees with the observation of McDermott *et al.* (2003) who reported that isometamidium chloride provided prophylaxis in cattle for three

months. Even though isometamidium chloride is the most widely used chemoprophylactic trypanocide in animals (Sones *et al.,* 1988), certain bio-factors such as the species treated with isometamidium chloride *i.e* the tse-tse (glossina), the animal livestock or man, the pre existing resistance status of the trypanosome or perhaps the species of trypanosome involved, seem to affect its prophylactic efficacy (Agu, 1984; Peregrine *et. al*., 1991; Bouyer, 2008).

The high mortality recorded in the control group was attributed to the absence of prophylactic medication, thus enabling the quick proliferation of trypanosomes and their attendant pathology.

This study shows that the duration of the prophylactic cover of pentamidine isethionate against *T. brucei brucei* infection is dose dependent and is inferior to that provided by a single dose of isometamidium chloride in mice and probably other animals. This is in spite of having to administer pentamidine isethionate thrice on alternate days as against once for isometamidium chloride. This factor is of clinical importance in the field where repetition of prophylactic treatment may be impractical, especially in nomadic or pastoral herds and with respect to costs.

The death of all the pentamidine isethionate treated groups (Groups II and III) by 5 weeks post treatment could be attributed to drug toxicity. It has been shown that the use of pentamidine isethionate is contraindicated in patients with renal impairment and those in their third trimester of pregnancy. There have also been unconfirmed reports of spontaneous abortion in humans following inhalation of pentamidine isethionate (Konishi *et al*., 2003).

Pentamidine isethionate has been shown to exhibit good clinical activity against different strains of trypanosomes (Pearson *et al*., 1985). It acts by interfering with the incorporation of nucleotides into RNA and DNA, inhibits oxidative phosphorylation, biosynthesis of DNA, RNA, protein and phospholipids and also interferes with foliate transformation in trypanosomes (Pearson *et al*., 1985). In man, pentamidine isethionate is effective in treating the late stage of the early phase of trypanosomosis and is comparable with melarsoprol or enflornithine, two main drugs used in the treatment of late stages of trypanosomosis in terms of its tolerance and availability (Doua *et al*., 1999). It has a half life of 2-4 weeks (VA Classification, 2011) which is at variance with the 12 months prophylaxis it is reported to confer on humans by a single dose of 4 mg/kg body weight (Harding and Hutchinson, 1950). Nevertheless, it correlates with the findings of this study – conferring prophylactic cover for three to four weeks at 4.0 mg and 8.0 mg/kg body weight, respectively. The reduced strength of the drug left in the system after these periods was unable to effectively inactivate the trypanosomes which then proliferate rapidly, leading to parasitaemia.

Generally, the discrepancies in the duration of prophylaxis conferred by pentamidium isethionate in man and mice and isometamidium chloride in cattle and mice may be attributed to the differences in drug pharmacokinetics in different species (Sones *et al*., 1988). Due to a higher metabolic rate in mice, the drugs are altered and eliminated faster from the system of the mice than in man or cattle, thereby reducing the duration of prophylaxis of the drugs.

In conclusion, isometamidium chloride has a better prophylactic cover in animals than pentamidine isethionate. However, because of the increasing parasite resistance to isometamidium chloride, the result of this work suggests that

pentamidine isethionate should be used strictly by clinicians and should be avoided in animals with renal compromise. However, because of the increasing parasite resistance to isometamidium chloride, pentamidine isethionate at the dose of 8 mg/ kg body weight may be used interchangeably with isometamidium chloride in prophylactic coverage of animals.

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