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Comparative Therapeutic Efficacy of Imidocarb Di-Propionate and Diminazene Aceturate in Rats Experimentally Infected with *Trypanosoma brucei*

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ABSTRACT

Diminazene aceturate is a yellow to orange aromatic diamidine compound with both antibacterial and protozoal activities. Diminazene aceturate is one of the most important drugs used in the treatment of trypanosomosis in animals. It is often used in treatment of *Babesia* and *Trypanosoma* species in animals. Over the years, consistent use of diminazene aceturate especially at low doses by quacks made provisions for development of resistant strains of trypanosome species and relapses of infection few days post treatment. The present challenge in chemotherapy in trypanosomosis necessitated the search for alternative therapies in both humans and animal patients. This study experimentally demonstrates the efficacy of imidocarb dipropionate in treatment of trypanosomosis. Its administration at the dose of 24 mg kg^{-1} subcutaneously for 2 consecutive days had a curative effect on rats with experimental *Trypanosoma brucei* infection. There was no relapse recorded in the treated group till the last (12th) day of the experiment. The present study threw some light on the potentials of imidazole dipropionate as an alternative therapy in trypanosomosis. It however requires a much detailed research to confirm its potency as a trypanocid.

Key words: *Trypanosoma brucei*, treatment, rats, imidazole dipropionate, diminazene aceturate

INTRODUCTION

African trypanosomosis is an intractable haemoparasitic disease of animals caused by trypanosomes. Over the past 2 decades, trypanosome parasites have developed significant resistance to the existing trypanocides, diminazene aceturate and isomethamidium chloride used in curative and prophylactic regimens, respectively (Silayo, 1990; Mitchell, 1990; Doxsey, 1990; Chigozie *et al*., 2012). The mechanism of drug resistance in trypanosomosis is poorly understood, however several hypotheses were made to explain it (Doxsey, 1990; Mitchell, 1990). The challenges in the existing chemotherapy of trypanosomosis require an urgent alternative in therapy. This prompted the exploration of efficacy of imizol as trypanocide in dogs. Imidazole is an aromatic organic compound classified as an alkaloid (Bogle *et al*., 1994). It consists of a class of heterocycles with similar ring structure but varying substituent (Hochachka and Somero, 2002; Brown, 1998). Imizole® is a brand of imidazole that commonly comes as salt of dipropionate or hydrochloride primarily used in treatment of babesiosis in dogs (Brown, 1998). Other drugs in the class of

imidazole such as nitroimidazole posses both antibacterial and antiprotozoal properties widely used in clinical and veterinary practice (Trunz *et al*., 2011). The anti-babesia efficacy of imizol engineered its study as a possible antitrypanosomal drug in dogs.

MATERIALS AND METHODS

Twenty one, 9 weeks old pathogen-free albino rats of both sexes weighing between 150-300 kg were used in the study. The rats were breed in the laboratory animal house of Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The rats were fed and watered *ad libitum* prior to commencement of the study. Each rat was identified with picric acid stain.

Imidocarb di propionate12% (Imizole®. Intervet/Merck Animal Health NADA 141-071, Approved by FDA Germany) was administered to the group C rats at the dose of 24 mg kg^{-1} subcutaneously for two consecutive days.

Diminazene aceturate (Veribin® CEVA Sante Animale- La Ballasteiére 33501 Libourne Cedex, France) a generic brand of trypanocide was also administered to group B at the dose of 3.5 mg kg^{-1} given intramuscularly stat.

The *T. brucei* parasite used in this study was a "Federe" strain obtained from the National Institute of Trypanosomosis and Onchocerciasis Research (NITOR) Vom, Plateaue State, Nigeria. The parasites were cryopreserved in liquid nitrogen from where donor rats were initially infected. The parasites were maintained by serial passage in rats at the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

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

Care of experiment animals: The care of the laboratory animals was in conformity with the guideline for animals experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The rats were humanely treated throughout the study. They were kept in ventilated cages in good hygienic condition and provided adequate feeding with clean portable drinking water.

Experimental design: Twenty one albino rats were randomly grouped into 3 groups of 7 members each: Group A was un-infected control, group B was infected with *T. brucei* and treated with diminazene aceturate at the dose of 3.5 mg kg^{-1}. Group C was infected with *T. brucei* and treated with Imidazole dipropionate at the dose of 24 mg kg^{-1} for 2 consecutive days.

Parasitaemia was determined using 2 methods. The wet blood mounts and the haematocrit buffy coat methods as described by Woo (1970).

Statistical analysis of data: Data on parasitaemia was presented in logrhythm. The results were analyzed using independent T-test of SPSS version 16.0 software package. The level of significance was accepted at $p<0.05$ (Snedecor and Cochran, 1973) (Table 1).

RESULTS

The parasitaemia was first detected in the experimental groups (Group B and C) on the 4th day post-infection. The levels of parasitaemia were estimated at log 4.8 in group B and log 4.9 in

Table 1: Parasitaemia in logarithm (log) and efficacy of diminazene aceturate and imidazole dipropionate on rats with experimental *Trypanosoma brucei* infection

Experimental period (days)	Group A uninfected control (log)	Group B treated with diminazene aceturate/im (log)	Group C treated with imizole subcut (log)
0	ND	ND	ND
1[#]	ND	ND	ND
2	ND	ND	ND
3	ND	ND	ND
4	ND	4.8[a]	4.9[a]
5*+	ND	5.1[a]	5.6[a]
6*	ND	5.1[a]	5.6[a]
7*	ND	ND	ND
8	ND	ND	ND
9	ND	ND	ND
10	ND	ND	ND
11	ND	ND	ND
12	ND	ND	ND

Superscripts (a) in a row indicate significant ($p<0.05$) difference between the groups means, [#]: Day of infection with *Trypanosoma brucei*, ND: Non detected, *Treatment with imidazole dipropionate subcutaneously, 7: No of rats in a group, +: Treatment with Diminazene aceturate intramuscularly

group C. By the 5th day, the levels of parasitaemia increased to log 5.3 in group B and log 5.6 in group C. There was no significant difference in the level of parasitaemia in group B compared to group C and treatment was commenced on the 5th day in both group B and C. Treatment with diminazene aceturate in group B was done only once at the dose of 7 mg kg^{-1}. Group C was treated with imizol® at the dose of 28 mg kg^{-1} once daily for 3 days. By the 7th day, parasitaemia had completely disappeared from the treated groups which remain so till last day 12 of the experiment.

DISCUSSION

The establishment of parasitaemia within 4-5 days post infection in the rats agrees with previous studies done on *T. brucei* infection in animals (Anene *et al*., 2011; Nwoha and Anene, 2011). Imidocarb di propionate given at dose of 24 mg kg^{-1} subcutaneously for 3 consecutive days cleared parasitaemia in the rats without apparent relapse till day 12 of the experiment. The efficacy of imidocarb di propionate was appreciable giving complete clearance of parasitaemia in group C after the 3rd dose of treatment. The repeated doses enhanced the efficacy of Imidocarb on trypanosome parasites. This was somewhat as recorded in the use of nitroimidazole, in the treatment of both acutely and chronically *T. brucei* infection in rats when administered *per os* at the dose of 25-50 mg kg^{-1} for 4 days and at 50-100 mg kg^{-1} for 5 days, respectively (Trunz *et al*., 2011). Similarly, in use of 2-substituted 5-nitroimidazoles such as fexinidazole and 1-[4-(1-methyl-5-nitro-1H-imidazol-2-ylmethoxy)-pyridin-2-yl-piperazine (9e) in treatment of *invitro T. brucei* in Human African Trypanosomosis (Samant and Sukhthankar, 2011). However, doses used in treatment of *Babesia* and *Anaplasma* species maybe tried on trypanosome infections in animals (Hashemi-Fesharki, 1975, 1977; Kuttler, 1980). The mechanism of action is poorly understood but thought to be due to interference with the production and/or utilization of polyamines, or prevention of entry of inositol into *T. brucei* parasitized erythrocytes (EMEA., 2001). Efficacy of imidocarb appears to depend on the route of administration as both salts of imidocarb dipropionate and dihydrochloride are poorly absorbed per os (EMEA., 2001). Both oral and subcutaneous administration of imidazole remains residues in various tissues depending on the animal species (Nimmo-Smith, 1968). The Lethal Dose (LD_{50}) of imidazole in rats was recorded at 450-1200 mg kg^{-1} and at 6.6-9.9 mg kg^{-1} in dogs. Most dogs experience pain at the site of injection and develop acute toxicity when administered in combination with choline esterase inhibitor

(Nolan, 2004). Therefore, the determination of accurate doses is essential in effective use of imidazole in therapy. The use of diminazene aceturate cleared parasitaemia after 48 h of treatment. This was as observed by Sukanto *et al.* (1990) in *T. evansi* infection in mice and in *T. brucei* infection in rats (Egbe-Nwiyi *et al*., 2014). Mortalities recorded in GPB were due to traumatization from inmates. The efficacy of imidazole dipropionate shown in this study when administered subcutaneously compares favorably with that of berenil in the treatment of *T. brucie* infection in rats. The study shows that while imidazole di-propionate did not show any demonstrable superiority to berenil in these trials, the former may be used when trypanosomes develop resistance to the latter in the treatment of *T. brucei* infection in animals. However, similar drug trial studies should be done on other laboratory animals for consistency.

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