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CHEMOTHERAPY OF CNS — TRYPANOSOMIASIS: THE COMBINED USE OF ISOMETAMIDIUM AND DIFLUOROMETHYLORNITHINE (DFMO) IN IMMUNOSUPPRESSED RABBITS

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The therapeutic efficacy of difluoromethylornithine (DFMO) and isometamidium chloride combinations were investigated in immunosuppressed rabbits infected with Trypanosoma brucei brucei. Treatments with DFMO in drinking water plus isometamidium intramuscularly were observed to be ineffective, even in combinations which were 100 percent effective in immunocompetent animals. It is therefore suggessted that a competent immune system is important in the chemotherapy of T. b. brucei using DFMO/ isometamidium combinations.

Key words: chemotherapy, trypanosomiasis, immunosuppression, rabbits.

INTRODUCTION

Of all the livestock diseases in Africa trypanosomiasis is regarded as one of the main factors which limits the numbers and productivity of cattle, sheep and goats. Isometamidium, one of the veterinary trypanocides, is used in therapeutic and prophylactic management of trypanosome infections (Gray & Stephen, 1962; Braide and Eghianruwa, 1980) despite its toxicity. Moreover, the drug is not curative in the late-stage trypanosomiasis since cases of relapses have been observed following therapy (Toupe, 1970; Anika et al; 1987; Kagwa et al; 1988).

The non-effectiveness and toxicity of isometamidium necessitated the utilization of isometamidium - dextran complex (Aliu and Sannusi, 1979) to reduce the incidence of adverse drug reactions and to increase the efficacy of the agent. Earlier synergism was achieved with the combination of difluoromethylornithine (FMO) and isometamidium in the treatment of CNS trypanosomiasis (Egwu Et al.1993). DFMO, an irreversible inhibitor of ornithine decarboxylase (Metcalf, et al. 1978) has been used effectively in the early stage trypanosomme infections (Bacchi et al; 1980; Schillinger and Gorton

1984). However, it is not curative in CNS infections (Clarkson et al; 1983; Onyeyili and Anika, 1990). A competent immune system seems to be important in the chemotherapy of African trypanosomiasis, since there are suggestions that most routinely used trypanocides do not directly kill the trypanosomes, but instead, make them accessible to the defence mechanisms (Maxie and Losos, 1977; deGee et al., 1983). The response of hosts to trypanosome parasites can be modified by use of immunosuppressive agents like cyclophosphamide and the corticosteroids (Karlu and Esuruoso, 1985). It is important to note that trypanosomiasis is endemic in areas where prevailing socioeconomic conditions result in a depressed immune status in both man and animals resulting from malnutrition and infection with other parasites. The objective of this study was to determine the effectiveness of isometamidium - DFMO combinations in the treatment of *Trypanosoma brucei brucei* in immunosuppressed rabbits in view of the strong possibility of the important association between an immune response to trypanosomes and parasite elimination following treatment.

MATERIALS AND METHODS

Experimental Animals:

Twenty young New Zealand rabbits of both sexes, 3 to 4 months old and weighing 1.0 to 1.8 kg were obtained from the University of Maiduguri. All animals were housed in appropriate cages, fed fresh vegetables, and dry legumes and given water *ad libitum.*

Trypanosoma brucei brucei (Gboko strain) obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, was used for the study. The organisms were maintained by serial passages in rats. The rabbits were inoculated through the intraperitoneal (I.P.) route. After trypanosome counts using the haemocytometer technique (Sannusi, 1977), serial dilutions of infected rat blood were made using phosphate glucose buffered saline solution, in such a way that the required number of trypanosomes (1×10^6) was present in 0.4 ml of inoculum. The development of parasitaemia in experimental rabbits was checked in two days intervals by wet blood film prepared from the ear vein at x 400 magnification.

Drug Treatments:

The DFMO (Merrel Dow Research Centre, Cincinnati, Ohio) was given as a 2 and 4 percent solution in drinking water while isometamidium chloride (Samerin (R), May and Baker, Dagenham, Essex, England) was administered at the rate of 0.125, 0.25 and 0.5 mg/kg intramuscularly.

Treatments were initiated 12 days post infection. To induce immunosuppression, dexamethasone (Dexadresson, Intervet International, Boxmer, Holland) was given intramuscularly (I.M) at the dose rate of 0.5 mg/kg for 4 days before infection. Thereafter, 0.3 mg/kg was given at 7 day intervals to maintain immunosuppression.

Experimental Design:

Sixteen rabbits inoculated with *T. b. brucei* (Gboko strain) were randomly separated into 4 groups (A - D) of 4 rabbits each. Four other rabbits, which were neither infected nor treated, were used as the positive control (Group E). The rabbits were treated as follows - Group A: 4% DFMO for 5 days plus 0.5mg/kg isometamidium. Group B: 4% DFMO for 6 days plus 0.25mg/kg isometamidium. Group C: 2% DFMO for 6 days plus 0.125mg/kg isometamidium. Group D:Infected untreated rabbits. Group E: Uninfected control.

Isometamidium was administered 4 days after the initiation of DFMO therapy. Following treatment, examinations for parasites in the blood were performed every 4 days. Parameters used for assessing the effect of immunosuppression on the therapeutic efficacy of the DFMO- isometamidium combination include the presence of parasites, frequency of relapse and death, and erythrocyte (RBC) and total leucocyte (WBC) counts (Schalm et., 1975). Rabbits were scored as "cured" if they survived more than 45 days post treatment, with no parasite in the peripheral blood. Such rabbits were sacrificed and the brain homogenised in a phosphate buffer. It was subinoculated intraperitoneally into uninfected rats. The recipient rats were examined from day 2 to 12 using wet mounts and those without parasites were considered healthy.

RESULTS

The infection of rabbits with 1 x 10^6 *T. b. brucei* organisms resulted in patent parasitaemia within four days in all infected groups. Treatment with the various combinations of DFMO and isometamidium were ineffective in clearing the trypanosomes from the peripheral blood (Table 1).

Table 1. Trypanocidal efficacy of DFMO-Isometamidium combinations *T. b. brucei* infected immunosuppressed rabbits

Group	Parasitaemia*										
	Days Post Infection										
	0	1–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40
A	0	4/4	4/4	4/4	1/4	2/4	3/4	4/4	4/4	4/4	2/2
B	0	4/4	4/4	4/4	3/4	3/4	3/3	3/3	2/2	0/0	0/0
C	0	4/4	4/4	4/4	3/4	4/4	4/4	3/3	0/0	0/0	0/0
D	0	4/4	4/4	4/4	3/3	0/0	0/0	0/0	0/0	0/0	0/0
E	0	0	0	0	0	0	0	0	0	0	0

* No of animals positive/No of survivors.
Day O = Day of infection.
Day 12* Day of treatment commencement.

The mean leucocyte counts of rabbits immunosuppressed with dexamethasone and treated with DFMO - isometamidium combinations after infection with *T. b. brucei* are presented in Table 2. With dexamethasone treatment, circulating leucocyte numbers decreased by 19.1% to 30.5% on the fourth day i. e. on the day of infection (0 day) (Table 2). The leucocyte numbers decreased further with the *T. b. brucei* infections and subsequent dexamethasone treatments.

Table 2. Mean leucocyte counts ($X10^3/mm^3$) of immunosuppressed rabbits infected with *T. b. brucei* and treated with DFMO-isometamidium combinations.

Group	Days Post Infection											
	PDT	0	4	8	12	16	20	24	28	32	36	40
A	6.20 ± 0.3	4.59 ± 0.8	4.62 ± 0.6	4.09 ± 0.4	3.42 ± 0.3	3.32 ± 0.4	3.05 ± 0.3	2.65 ± 0.2	2.42 ± 0.3	2.03 ± 0.2	1.89 ± 0.2	1.81 ± 0.1
B	6.61 ± 0.4	4.68 ± 0.7	4.32 ± 0.7	4.11 ± 0.5	3.86 ± 0.1	3.25 ± 0.5	3.10 ± 0.4	2.76 ± 0.1	2.31 ± 0.2	2.00 ± 0.2	D	D
C	6.42 ± 0.3	4.55 ± 0.5	4.16 ± 0.8	4.45 ± 0.4	4.05 ± 0.2	3.60 ± 0.5	3.10 ± 0.3	2.82 ± 0.4	2.11 ± 0.1	D	D	D
D	6.30 ± 0.2	5.10 ± 0.3	4.44 ± 0.3	4.08 ± 0.7	3.52 ± 0.4	3.20 ± 0.2	D	D	D	D	D	D
E	6.81 ± 0.2	6.92 ± 0.1	6.91 ± 0.3	6.74 ± 0.5	6.77 ± 0.6	6.81 ± 0.2	6.62 ± 0.3	6.67 ± 0.4	6.81 ± 0.4	6.75 ± 0.3	6.63 ± 0.4	6.72 ± 0.5

PDT = Pre-dexamethasone treatment values
O day = Day of infection
Day 12 = Day when treatment was commenced
Day 40 = 28 days post treatment
D = The animals died due to trypanosomiasis.

The erythrocyte counts following infection was significantly decreased compared to the preinfection values.

Table 3. Mean erythrocyte counts ($X10^6mm^3$) of immunosuppressed rabbits infected with *T. b. brucei* and treathed with DFMO-isometamidium combinations

Group	Days Post Infection										
	0	4	8	12	16	20	24	28	32	36	40
A	5.6 ± 0.3	5.1 ± 0.2	406 ± 0.2	3.8 ± 0.2	4.01 ± 0.3	4.1 ± 0.4	3.9 ± 0.3	3.1 ± 0.2	3.0 ± 0.3	2.4 ± 0.2	2.1 ± 0.4
B	5.9 ± 0.1	5.3 ± 0.2	4.1 ± 0.3	3.6 ± 0.2	3.9 ± 0.4	3.7 ± 0.3	3.2 ± 0.5	3.1 ± 0.1	2.9 ± 0.3	D	D
C	5.0 ± 0.5	5.1 ± 0.2	4.3 ± 0.2	3.6 ± 0.1	3.6 ± 0.2	3.4 ± 0.3	3.0 ± 0.3	2.5 ± 0.2	D	D	D
D	5.7 ± 0.4	5.2 ± 0.1	4.4 ± 0.2	3.7 ± 0.3	3.0 ± 0.3	D	D	D	D	D	D
E	5.6 ± 0.2	6.0 ± 0.3	6.2 ± 0.3	6.1 ± 0.2	6.2 ± 0.4	6.1 ± 0.1	5.9 ± 0.3	6.3 ± 0.5	6.1 ± 0.2	6.0 ± 0.4	6.3 ± 0.3

O day = Day of infection
Day 12 = Day when treatment was commenced
Day 40 = 28 days post treatment
D = Animals died due to trypanosomiasis.

DISCUSSION

The presence of anaemia in trypanosome infection has been well documented (Anosa et al., 1974; Onyeyili and Anika, 1990) and usually is correlated with the degree of parasitaemia (Nguyen et al., 1976). Dexamethasone was not responsible for the anaemia observed in this study. The parasites appeared to be responsible. In an earlier study it was also observed that dexamethasone does not induce anaemia (Kalu and Esuruoso, 1985). It was also noticed that the high degree of anaemia seen in immunosuppressed animals was caused by increased parasitaemia. In the present study, dexamethasone treatments were observed to induce low leucocyte counts. This was similar to the earlier findings (Kalu and Esuruoso, 1995) and may be an indication of a decreased imune system activity.

The present study has shown that the DFMO and isometamidium combinations used were not curative even through these combinations produced 100 percent cures when used previously in immunocompetent animals (Egwu et al., 1993). This may be an indication that antibody responses may be important for the rapid removal of trypanasomes from the blood stream following therapy. A competent immune system therefore appears to be important in

the chemotherapy of trypanosomiasis (Maxie and Losos, 1977), since full doses of the two agents were used as one of the combinations (Group A) without being effective. In conclusion, based on the findings presented, fully competent immune system is very important in the treatment of *T. b. brucei* infection using DFMO/ isometamidium combinations.

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KOMBINOVANA PRIMENA IZOMETADIJUMA I DIFLUOROMETILONITINA (DFMO) U HEMOTERAPIJI TRIPANOZOMIJAZE CNS-a KOD IMUNOSUPRESNIH KUNIĆA

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SADRŽAJ

Terapijska efikasnost difluorometilornitina (DFMO) u kombinaciji sa izometadijum hloridom ispitivana je kod imunosupriniranih kunića inficiranih sa *Trypanosoma brucei brucei.* Pokazalo se da kombinovani tretman DFMO-om u vodi za piće, i intramuskularno izometadijumom nije efikasan čak i u kombinaciji koja je 100% efektivna kod imunokompetentnih životinja. Dobijeni rezultati ukazuju da je kompetentan imunski sistem značajan pri hemoterapiji *T. b. brucei* DFMO/izometadijum kombinacijom.

Ključne reči: hemoterapija, tripanozomijaza, imunosupresija, kunić.