Communication

DL-α-difluoromethylornithine (DFMO^R) - Berenil^R combination : therapeutic and prophylactic activity against *Trypanosoma brucei brucei* infection in mice

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ONYEYILI (P.A.), EGWU (G.O.), ZARIA (L.T.), ORJIUDE (B.A.). Activité thérapeutique et prophylactique de la combinaison Berenil^R et DL-alpha-difluorométhylornithine (DFMO^R) contre l'infection à *Trypanosoma brucei brucei* chez la souris. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (4) : 443-445

Les auteurs ont étudié l'activité thérapeutique et prophylactique de l'association DL-alpha-difluorométhylornithine (à la dose de 2 p. 100 dans l'eau de boisson) et Bérénil^R (à 7 mg par kg de poids vif par voie intrapéritonéale) sur des souris infectées par *Trypanosoma brucei brucei*. Utilisant un modèle sur souris précedemment décrit, de la trypanosomose africaine du système nerveux central, ils ont démontré l'effet curatif de cette association et son action synergétique. Cependant, à titre prophylactique, il n'en résulte aucun avantage par rapport au Bérénil^R employé seul. *Mots clés* : Souris - *Trypanosoma brucei brucei* - Trypanocide - Bérénil^R - DFMO^R - Nigeria.

Introduction

Trypanosomosis is a serious health problem in both man and domestic animals in Africa. The compounds used clinically for the control of trypanosome infections were introduced about 30 years ago, and considerable resistance of trypanosome to these existing drugs has developed (10, 14, 15). The organic arsenical melasoprol (Mel B. Arsobal^R) used in humans and melarsemine (Cymelarsan^R) used in animals are the only effective drugs available for the treatment of late stage trypanosomosis, despite its toxicity (4, 13, 15).

Lack of effective new antitrypanosomal agents (3) forces the exploration of new drug combinations in the chemotherapy of trypanosomosis such as those used in tuberculosis or cancer. Difluoromethylornithine (DFMOR), an irreversible inhibitor of ornithine decarboxylase (ODC) (11) has been effectively combined with bleomycin (an anticancer drug (9, 2) in the treatment of experimental *Trypanosoma b. brucei* infection in mice. Furthermore, DFMO^R was found to be synergistic with some standard trypanocides when examined in acute *T. b. brucei* infection. The agents include suramin, pentamidine and Berenil^R (1). The combination of a DFMO and suramin was also found to be curative in the late state of trypanosomosis (3). DFMO^R and Berenil combination was superior to DFMO^R or Berenil^R alone in the treatment of late stage *T. b. brucei* model in dogs although relapse parasitaemia occurred (12). The purpose of this report is to describe the therapeutic effects of DFMO^R and Berenil^R combination against *T. b. brucei* infection in mice.

Materials and Methods

Male Swiss albino mice (20-28 g) purchased from the National Veterinary Research Institute, Vom, were used for the studies. The animals were fed on mouse cubes (Pfizer) and water was provided *ad libitum*.

The *Trypanosoma brucei brucei* strain 8/18 obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, was used for both the efficacy and prophylactic tests. The trypanosomes were maintained by serial passage in rodents. They produced 100 % mortality and had a prepatent period of 2-4 days.

DFMO^R (Merrel Research Centre, Cincinnati, Ohio) was used as a 2/100 solution in drinking water. Diminazene aceturate (Berenil^R Hoechst AG, Frankfurt am Main, Germany) was administered intraperitoneally at rate of 7 mg/kg body weight.

In the efficiency study twenty mice were inoculated intraperitoneally with 0.5 ml of diluted rat blood containing 5 x 10^5 parasites. The number of parasites was determined using the haemocytometer technique. Wet blood film examinations were carried out daily using blood obtained from the tail. When parasitaemia was established the mice were separated into four groups (A, B, C and D). Mice in group A were treated with DFMO^R, those in group B with Berenil^R, while those in group C were treated with a combination of DFMO^R and Berenil^R. Group C was treated with a combination of DFMO^R and Berenil^R. Mice in group D were left untreated and a group of five non infected mice (E) were used as controls to monitor the course of the disease and the presence of any other infections.

All treatments were initiated 18 days post infection. DFMO^R was administered for a period of 4 days while Berenil^R was given once. The animals were examined daily for the presence of parasites for the first 6 days after treatment, and thereafter every 3 days for 30 days to establish the duration of clearance of parasitaemia. If parasitaemia was not established within the 36 days of observation, the treatment was considered as efficacious.

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TABLE I Trypanocidal efficacy of DFMO®, Berenil® and a combination of both in **T. b. brucei** *infected mice.*

Group	Drug	Dose and route	Parasitaemia* Days post treatment						
			0-5	6-10	11-15	16-20	21-25	26-30	31-36
A	DFMO®**	2 % orally	5/5	0/5	0/5	2/5	4/5	4/5	2/3***
B	Berenil®	7 mg/kg IP	5/5	0/5	0/5	0/5	0/5	1/5	3/5
C	Berenil® + DFMO®**	7 mg/kg	5/5	0/5	0/5	0/5	0/5	0/5	0/5
D	Infected untreated control	—	5/5	5/5	3/3	2/2	0/0	0/0	0/0***
E	Non infected control	—	0	0	0	0	0	0	0

** : No. of animals positive/No. of survivors.*
*** : DFMO® administered in drinking water for 4 days.*
**** : The animals died due to trypanosomiasis.*
Day 0 : day of commencement of treatment.

Four groups of 18 mice each (3 treated and 1 control) were used for the prophylactic tests. The treatments given included DFMO^R in drinking water for 4 days, Berenil^R on day one or a combination of DFMO^R for 4 days plus Berenil^R on day one.

At the end of DFMO^R treatment (day 4) and therefore on day 11, 18 and 25, five or three mice from each group were I.P. challenged with 0.2 ml of diluted blood containing 0.2 x 10^5 trypanosomes. Tail blood was examined daily for 6 days and thereafter once weekly for a further period of 30 days. The animals were recorded as protected if they remained parasite free for 30 days after challenge.

Results

Table I summarizes the data concerning the therapeutic efficacy of DFMO^R and its Berenil^R combination. Animals receiving the different treatments were compared to the non-treated controls. Prior to the treatment parasitaemia with *T. b. brucei* was detected in all the challenged animals. In mice treated with either Berenil^R, DFMO^R or their combination, the level of parasitaemia was significantly reduced after an initial 24-h period ; all of them became negative by day 5. Those treated with the combination of DFMO^R and Berenil^R remained parasite-free throughout the 36 days of observation. Relapse parasitaemia was detected in mice treated with Berenil^R or DFMO^R alone.

Berenil^R alone and a combination of DFMO^R and Berenil^R conferred a complete protection against *T. b. brucei* infection in mice for 11 days (table II). By day 18 the number of mice protected by Berenil^R and the drug combination declined and by day 25 none of the mice were protected by either Berenil^R or the drug combination. DFMO^R alone conferred no protection against *T. b. brucei* infection in mice.

TABLE II Prophylactic effect of DFMO® and Berenil® alone and in combination in mice infected with **T. b. brucei.**

Day after prophylactic treatment	Drug	Number survived*/ number challenged
4	Nil	5/5
	DFMO®	4/5
	Berenil®	0/5
	DFMO® + Berenil®	0/5
11	Nil	5/5
	DFMO®	5/5
	Berenil®	0/5
	DFMO® + Berenil®	0/5
18	Nil	5/5
	DFMO®	5/5
	Berenil®	3/5
	DFMO® + Berenil®	4/5
25	Nil	3/3
	DFMO®	3/3
	Berenil®	3/3
	DFMO® + Berenil®	3/3

** : Number that survived and remained parasite free 30 days post inoculation/No. inoculated after treatment.*
Nil : not treated.

Discussion

The treatment of *T. b. brucei* with DFMO^R, Berenil^R and the combination of DFMO^R and Berenil^R at the dosage levels employed produced an obvious period of parasitaemia before relapse occurred in DFMO^R and Berenil^R treatment groups. Relapse parasitameia did nor occur in the group treated with the drug combination. This could be taken as evidence for the therapeutic superiority of the drug combination in late-stage of *T. b. brucei* infection in mice, consistent with earlier findings in dogs (12). Similarly, the combination of DFMOR and suramin (a human trypanocide) used in early stage infection was observed to act synergistically in the same mouse model of CNS trypanosomosis as that used in the study (3).

Conclusion

The mechanism of DFMO^R and Berenil^R synergism is unknown. Small amounts of Berenil^R have been observed to cross the blood-brain barrier (12) possibly allowing this drug to act synergistically with DFMO^R which also reaches low but significant concentrations in brain tissue (18).

The protection period offered by Berenil^R against experimental trypanosomosis was not prolonged by DFMO^R. This may be due to the rapid elimination of either drug from the body (5, 12).

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The therapeutic and prophylactic activity of difluoromethylornithine (DFMO^R) (2 % in drinking water for 4 days) and Berenil^R (7 mg/kg live weight intraperitoneally) combination was investigated in mice infected with *Trypanosoma brucei brucei*. Using a previously described mouse model of the African trypanosomosis of the central nervous system, it was demonstrated that the combination was curative and acted synergistically. However, if used prophylactically it had no advantage over Berenil^R alone.

Key words : Mice - *Trypanosoma brucei brucei* - Trypanocide - Berenil^R - DFMO^R - Nigeria.

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