Effects of difluoromethylornithine after intravenous administration and its combination with diminazene aceturate against *Trypanosoma brucei* in experimentally infected dogs in Nigeria

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Key words

Dog - *Trypanosoma brucei* - Anti-protozoal agent - Experimental infection - Nigeria.

Summary

Difluoromethylornithine (DFMO, eflornithine) was administered intravenously (IV) to *Trypanosoma brucei* experimentally infected dogs. A dosage of 400 mg/kg/day in three divided doses daily for either 7 days for the primary infection or 21 days in cases of relapse was not curative. Treatment of both primary and relapse infections was characterized by slow induction of action (parasite clearance achieved in 4 to 5 days of treatment) and short aparasitemic periods (6 days). Simultaneous administration of DFMO and a single dose of Berenil (7 mg/kg) in primary infections achieved a higher chemotherapeutic level compared to the monotherapy, as no relapses followed. Oral dosing of DFMO to the dogs caused within four days of administration anorexia, vomiting, profuse diarrhea and severe dehydration. These serious disadvantages make the IV route a plausible option to oral therapy.

■ INTRODUCTION

Difluoromethylornithine (DFMO), which might be available at a high cost on the market, has been clinically tested for some years with promising results in the treatment of human trypanosomosis (8, 9, 23, 27). It is effective against the Gambian sleeping sickness, but has only a variable activity against *Trypanosoma brucei rhodesiense* (5, 28). Also, mouse model *T. brucei* infections were susceptible to the drug as well as pathogens of veterinary importance (*T. congolense* and *T. evansi)* (4, 20).

Trypanosoma brucei infection in dogs is common in the Southeastern part of Nigeria (18). Treatment of the disease with available trypanocides including diminazene aceturate is believed to have variable success (2, 6, 13, 21). In *T. b. brucei* infected dogs DFMO was not curative at an oral dosage of 300 mg/kg/day (divided into three doses) for six days (19). The therapy resulted in a slight improvement in red cell values and remission of symptoms but the infection relapsed ten days after treatment. There are reports of synergism between DFMO and several standard trypanocides (suramin, pentamidine, diminazene aceturate) in acute *T. b. brucei* infections (4). Also, combinations of suramin and DFMO have been shown to be curative in the late-stage African trypanosomosis (7).

The aim of this investigation was to improve DFMO concentrations in the blood, and thus the trypanocidal effect by intravenous application of the drug, and to avoid gastrointestinal adverse effects occurring after using the oral route (19). Additionally, the efficacy of DFMO in combination with diminazene aceturate was assessed.

■ MATERIALS AND METHODS

Experimental animals

A total of 14 young local dogs weighing between 3.5 and 6 kg were used. They were kept in fly-proof housing, fed twice daily and watered *ad libitum*. All dogs were parasitologically negative for trypanosomes by wet blood films, Giemsa-stained thin blood

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smears and hematocrit buffy coat method (17). The dogs were dewormed with pyrantel pamoate at a dosage of 10 mg/kg orally. They were allowed a conditioning period of two weeks before the study started.

Trypanosome infections

Two local isolates of *T. brucei* (isolates I and II) from clinically infected dogs were used in two different studies, respectively. Each dog was inoculated intraperitoneally (IP) with 2.5 x 10^5 or 10^5 trypanosomes per ml of saline diluted infected blood in the first and second experiments, respectively. The trypanosomes were identified morphologically on Giemsa-stained thin film preparations (25), and the number of parasites assessed by the method of Herbert and Lumsden (10). Onset of parasitemia was determined by a daily examination of wet blood films, Giemsa-stained thin blood smears and the hematocrit buffy coat method (17).

Administration of drugs

DFMO (Merrel Dow Research Institute, Cincinnati, OH, USA) was administered intravenously (IV) *via* cephalic or saphenous veins or orally at the dosage of 400 mg/kg in three divided doses per day. The same dosage was applied with respect to combination treatments. For the IV DFMO treatment, dry powder of the drug was dissolved in sterile water and administered as a 4% solution.

Diminazene aceturate (Berenil, Hoechst AG, Frankfurt, Germany) was given as a single intramuscular (IM) injection at the dosage of 7 mg/kg. For combined treatments, diminazene aceturate was administered immediately after the initial DFMO treatment.

Hematological and clinical parameters

The hemoglobin (Hb) level and packed cell volume (PCV) were estimated by cyanmethemoglobin and microhematocrit methods, respectively. Red blood cell (RBC) and white blood cell (WBC) counts were performed using the improved Neubauer hemocytometer. Rectal temperature (using a clinical thermometer) and clinical observations were also recorded.

Experimental design

In the first experiment, 11 dogs after infection with isolate I were randomly assigned to three treatment groups. Group A (3 dogs) was treated with diminazene aceturate alone. Groups B and C (4 dogs each) received DFMO alone and a combination of diminazene aceturate plus DFMO, respectively. The dogs were infected on day 0 and treatment started on day 8 post-infection (PI). The DFMO was administered IV for seven consecutive days. The PCV, rectal temperature and parasitemia were monitored every fourth day for 32 days PI and subsequently every seventh day. Relapses of infection and mortality were recorded. Relapses were retreated as indicated in table I.

In the second experiment, 3 dogs (Nos. 1, 2 and 3) were infected with isolate II. Inoculum per dog was 10^5 trypanosomes per ml and after the onset of parasitemia each dog was bled every fourth day for WBC, RBC, PCV and Hb determinations. Treatment started five days PI with DFMO daily for 12 days (5 days IV/7 days orally). The dogs were monitored for parasitemia for 123 days PI. Relapses of infection were retreated as indicated in table II.

■ RESULTS

Clinical signs of T. brucei *infection*

The prepatent period of infection was 4 to 5 days and 3 days in the first and second experiments, respectively. Infections were characterized by anorexia, pyrexia, weakness, loss of body condition, anemia and, in some cases of relapse, by symptoms involving the central nervous system (CNS), i.e. opisthotonos and loss of balance.

Response to treatment

Trypanosomes disappeared from the dogs' blood within 24h of diminazene aceturate administration and 4 to 5 days after DFMO treatment. This was followed by remission of clinical signs of infection.

Table I

Comparative trypanocidal efficacy of DFMO, diminazene aceturate and a combination of DFMO and diminazene on *T. brucei* infected dogs

		Parasitemia Number of animals positive/number infected															
Group	**Treatment**	**1***	**4***	**8+**	**12**	**16**	**20**	**24**	**28**	**32**	**39**	**46**	**53**	**60**	**67**	**74--**	**102**
A	Diminazene aceturate	0/3	0/3	3/3	0/3	0/3	0/3	0/3	1/3[a]	0/3	0/2	0/2	0/2	0/2	0/2	0/2--	0/2
B	DFMO	0/4	1/4	4/4	0/4	0/4	4/4[b]	0/4	0/4	0/4	0/4	0/3	0/3	0/3	1/3	0/2--	0/2
C	DFMO plus diminazene aceturate	0/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4--	0/4

+ Immediately before drug treatment (0 day after treatment)

* Day 1 post-infection, day 4 post-infection, etc.

a The relapse was retreated with diminazene aceturate

b Two of the relapses (the most critical cases) were retreated with a combination of diminazene aceturate and DFMO IV for seven days, while the other two dogs received a single dose of diminazene aceturate alone

Table II

Trypanocidal efficacy of DFMO alone and in combination with diminazene aceturate in *Trypanosoma brucei* infected dogs

Dog number	Prepatent period (days)	Post-treatment* relapses (days PI)		
		Initial**	Second	Third
1	3	19	N	N
2	3	19	51+	
3	3	19	35	91

* All dogs were treated with DFMO for 9 days (5days IV/4 days oral) starting from day 5 PI

** All dogs were retreated with DFMO IV daily for 3 weeks (omitting days 10 and 11) starting from day 23 PI (i.e. 4th day of relapse). Only dog No. 1 received additionally diminazene aceturate on the first day of retreatment

\+ Died 65 days PI (2 weeks after retreatment with diminazene aceturate)

N = Nil

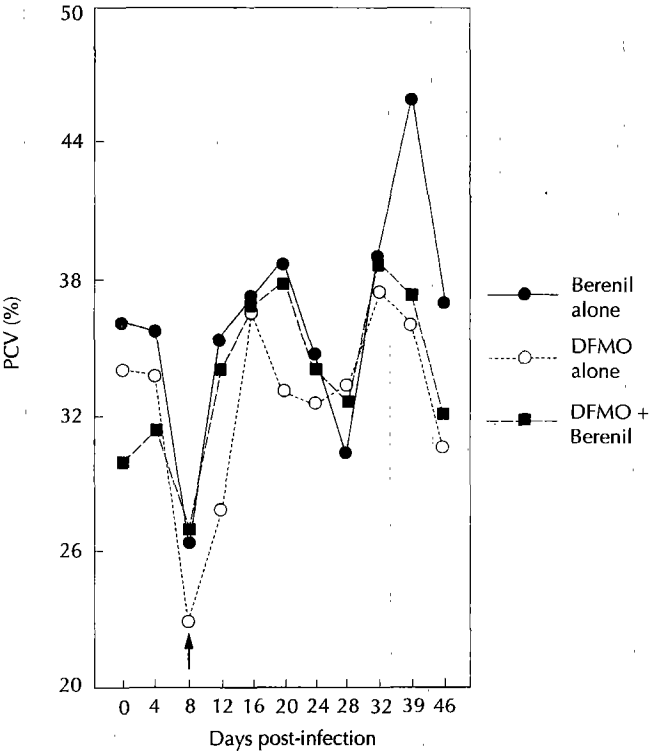

***Figure 1**: PCV of dogs infected with* Trypanosoma brucei *and treated with Berenil, DFMO or DFMO + Berenil (arrow indicates start of initial treatment).*

In the first experiment (table I) relapses occurred in groups A and B by days 13 (day 28 PI) and 5 (day 20 PI), respectively, after completion of treatment. Group C dogs had not relapsed by the end of the experiment. The relapse case in group A died by day 36 PI (i.e. day 8 after retreatment). The two deaths recorded in group B were the most critical cases of relapse retreated with DFMO plus diminazene aceturate (table I).

In the second experiment the 7 day oral DFMO therapy was discontinued after day 4 of dosing because of severe gastrointestinal effects: anorexia (day 2 of treatment), loose stool (day 3) and profuse diarrhea with dehydration (day 4). Vomiting was variable. Relapse parasitemia occurred in all three dogs by day 6 after treatment (day 19 PI) and all were retreated (table II). After retreatment dog No. 1 did not relapse by the end of the experiment. Dog No. 2 relapsed once again 6 days after retreatment (day 51 PI). The associated symptoms were anemia, ascites, emaciation, hematuria and uncoordinated movements. Dog No. 3 relapsed by day 35 PI following the two day break in DFMO retreatment. Diminazene aceturate was administered in addition to the ongoing DFMO therapy. However, it relapsed again fifty-six days later (day 91 PI).

Hematological parameters and body temperature

The PCV values of dogs in the first experiment are shown in figure 1. Both the initial infection and post-treatment relapses caused a fall in the PCV which rose following trypanocidal administration. Similar results were obtained in the second experiment (figure 2) and the PCV results corresponded with those of Hb and RBC (not shown). Results further indicated that patent parasitemia was associated with transient leucocytosis and relapses with leukopenia, all reversed by trypanocidal therapy.

The initial infection caused elevation of the body temperature. Subsequently, temperatures fluctuated with peaks corresponding to periods of parasitemia and decreases of red cell values.

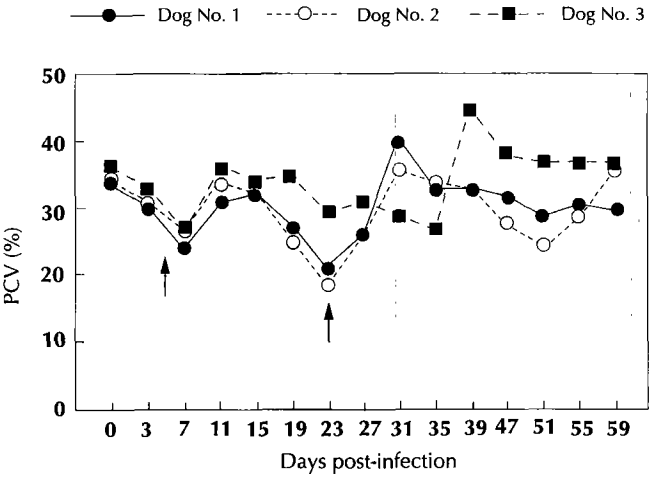

***Figure 2**: PCV of dogs infected with* Trypanosoma brucei *and treated with DFMO singly or in combination with Berenil (arrows indicate start of DFMO treatments).*

■ DISCUSSION

The results of this study are similar to those of Onyeyili and Anika (19) who attributed the ineffectiveness of DFMO in their study to gastroenteropathy associated with oral administration which may have disturbed drug absorption and treatment duration, and was considered insufficient for the elimination of parasites. Attempts in the present study to enhance the trypanocidal effect of DFMO through the use of the IV route to avoid gastrointestinal effects and

extended duration of DFMO treatment gave unsatisfactory results. The DFMO therapy did not cure but merely suppressed the infection as parasitemia recrudesced soon after treatment. The comparatively shorter duration of aparasitemia in this study, despite the higher dosage of DFMO and prolonged therapy, may be associated with differences in the strains of trypanosomes used. Trypanosome isolates are known to differ in their sensitivity to trypanocides (29).

The lack of DFMO curative effect in this study may be due to its inability at the dosage used to diffuse in high enough concentrations into the cryptic sites from where the parasites relapsed (12). On the contrary, IV DFMO has been successfully used even in comatose human patients (24). The drug is reputed to have the ability to enter the CNS (brain and cerebrospinal fluid) after infusion (15) and cure late-stage infections (9, 24, 27). The cure in human patients was achieved at a higher IV oral dosage daily for at least six weeks (22, 23, 24, 27). This dose, route and duration of treatment proved to be inconvenient in dogs. The severe gastrointestinal disorder recorded in this study precludes prolonged oral dosing. Furthermore, perivenous leakage of drug in intractable dogs caused extreme discomfort and phlebitis which impeded venipuncture in protracted treatment.

No relapses followed the combination of DFMO and diminazene aceturate in the primary infections. Similar combinations of DFMO chemotherapeutic regimen with diminazene aceturate (12), or with other trypanocides, Suramin (7), melarsoprol (11) and melarsen oxide (13) in mice, have been shown to be preferable to monotherapy. Results of the combination of DFMO and diminazene aceturate in this study were variable in relapse infections. Higher blood concentrations of the drug were probably needed to completely eliminate the parasites sequestered in some tissues (12).

Trypanosomosis produced typical symptoms (2, 16, 19). The marked decrease in red cell values following infection of the dogs correlates with anemia, which is a characteristic feature of African trypanosomosis (3, 16). The cause of hemoconcentration present on day 39 PI in dogs treated with Berenil (figure 1) could not be ascertained. Hematuria which appears to be a rare side effect of DFMO treatment (9) was observed in one dog. Other side effects associated with oral dosing, vomiting, anorexia, diarrhea and dehydration concurred with other reports (1, 19, 23).

■ CONCLUSION

DFMO monotherapy as applied in this study was not curative for *T. brucei* infection in dogs. However, in combination with diminazene aceturate it was effective in the treatment of the primary infection but variable in cases of relapse. Severe gastrointestinal effects associated with oral administration were a serious drawback making the IV route more desirable. However, its high cost and administration constraints might not favor many veterinary prospects.

Acknowledgements

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Résumé

Anene B.M., Anika S.M., Chukwu C.C. Effets de la difluorométhylornithine après administration intraveineuse et son association avec de l'acéturate de diminazène contre *Trypanosoma brucei* chez des chiens infectés expérimentalement au Nigeria

De la difluorométhylornithine (DFMO, eflornithine) a été donnée par voie intraveineuse (IV) à des chiens infectés expérimentalement par *Trypanosoma brucei*. Une posologie de 400 g/kg/jour répartie en trois doses quotidiennes pendant 7 jours pour les primo-infections ou 21 jours lors de rechutes ne s'est pas révélée curative. Le traitement des primo-infections aussi bien que de leurs récidives s'est caractérisé par un déclenchement lent de l'action (le coefficient d'épuration parasitaire a été atteint après 4 à 5 jours de traitement) et de courtes périodes sans parasitémie (6 jours). L'administration simultanée de DFMO et d'une seule dose de Berenil (7 mg/kg) pour les primo-infections a permis d'obtenir des résultats chimiothérapeutiques plus importants que ceux de la monothérapie car elle n'a pas été suivie de rechute. L'administration orale de DFMO a provoqué, dans les quatre jours qui ont suivi, une perte d'appétit, des vomissements, une diarrhée abondante et une déshydratation avancée. Ces inconvénients importants font de la voie IV une véritable alternative à celle de la thérapeutique orale.

Mots-clés : Chien - *Trypanosoma brucei* - Antiprotozoaire - Infection expérimentale - Nigeria.

Resumen

Anene B.M., Anika S.M., Chukwu C.C. Efecto de la difluorometilornitina post administración intravenosa y su combinación con aceturato de diminazeno contra *Trypanosoma brucei* en perros infectados experimentalmente en Nigeria

Difluorometilornitina (DFMO, eflornitina) se administró por vía intravenosa (IV) en perros infectados experimentalmente con *Trypanosoma brucei*. Una dosis de 400 mg/kg/día, dividida en tres dosis diarias, durante siete días para la infección primaria o 21 días en casos de recurrencia, no representaron cura. Tanto en caso infección primaria como recurrente, el tratamiento se caracterizó por una inducción lenta de la acción (la desaparición del parásito se alcanzó en 4 a 5 días de tratamiento) y cortos períodos aparasitémicos (6 días). La administración simultánea de DFMO y de una dosis única de Berenil (7 mg/kg) en infecciones primarias, logró un nivel quimioterapéutico superior, en comparación con la monoterapia, sin recurrencias. La dosificación oral de DFMO en perros provocó, cuatro dias post administración, anorexia, vómitos, diarrea profusa y deshidratación severa. Estas desventajas hacen la ruta IV una opción posible con respecto a la terapia oral.

Palabras clave: Perro - *Trypanosoma brucei* - Medicamento contra protozoarios - Infección experimental - Nigeria.