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Cure of Murine *Trypanosoma brucei rhodesiense* Infections with an *S*-Adenosylmethionine Decarboxylase Inhibitor

CYRUS J. BACCHI,[1]* HENRY C. NATHAN,[1]† NIGEL YARLETT,[1] BURT GOLDBERG,[1] PETER P. McCANN,[2] ALAN J. BITONTI,[2] AND ALBERT SJOERDSMA[2]

Haskins Laboratories and Department of Biology, Pace University, New York, New York 10038,[1] and Marion Merrell Dow Research Institute, Cincinnati, Ohio 45215[2]

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The compound 5′-{[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (MDL73811), a potent inhibitor of *S*-adenosylmethionine decarboxylase, was effective in mice against six of eight clinical isolates of *Trypanosoma brucei rhodesiense*, the causative agent of East African sleeping sickness. In combination with the ornithine decarboxylase inhibitor DL-α-difluoromethylornithine (DFMO; Ornidyl), MDL73811 acted synergistically to cure seven of eight infections. MDL73811 was effective when given singly at 50 to 100 mg/kg of body weight per day for 7 days (osmotic pumps). In combination with subcurative DFMO levels (0.25 to 1.0% in drinking water for 7 days), the curative MDL73811 dose could be lowered to 25 or 50 mg/kg, depending on the isolate. Oral administration of the MDL73811-DFMO combination was also effective in an acute infection and in a long-term central nervous system model of *Trypansoma brucei brucei* infection. These data indicate that MDL73811 may be effective therapeutically in drug-refractory and late-stage East African trypanosomiasis.

Polyamine biosynthesis and related pathways in African trypanosomes have been the target of recently developed antitrypanosomal agents (1, 4, 6). Such agents include DL-α-difluoromethylornithine (DFMO; Ornidyl), an irreversible inhibitor of ornithine decarboxylase, and 5′-{[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (MDL73811), an irreversible inhibitor of *S*-adenosylmethionine (AdoMet) decarboxylase (8). AdoMet is a branch point in metabolism; it is the methyl group donor for most cellular methylation reactions, and if decarboxylated, it is committed irreversibly to serve as an aminopropyl group donor in polyamine biosynthesis (16). Recent studies concerning the effects of these novel trypanocidal agents in African trypanosomes have demonstrated that significant elevation of intracellular AdoMet is a common element in the growth inhibition obtained with MDL73811 as well as with DFMO (6, 7, 21, 23). In one case, this is due to inhibition of ornithine decarboxylase by DFMO, with the resulting depletion of putrescine, the acceptor for decarboxylated AdoMet; for MDL73811, this is due to direct inhibition of AdoMet decarboxylase (7, 21).

DFMO is a clinically effective agent for the treatment of early- and late-stage *Trypanosoma brucei gambiense* infections in West Africa (18, 20); however, it has not been as effective against East African disease caused by *Trypanosoma brucei rhodesiense* (5, 20). In light of the demonstrated resistance of *T. brucei rhodesiense* to DFMO and standard trypanocidal agents (3, 20), there is a need for a novel agent which is effective against this form of the disease.

MDL73811 was previously found (6) to be active against isolates of *T. brucei rhodesiense* and *Trypanosoma brucei brucei*. We demonstrate here that this agent alone and in combination with DFMO is active against seven clinical isolates of *T. brucei rhodesiense* and a long-term central nervous system (CNS) model of *T. brucei brucei* infection.

MATERIALS AND METHODS

Chemicals. MDL73811 and DFMO were synthesized at the Marion Merrell Dow Research Institute (8).

Trypanosomes. The strains used in the study were the LAB 110 EATRO isolate of *T. brucei brucei* and clinical isolates of *T. brucei rhodesiense* acquired from the Kenya Trypanosomiasis Research Institute (KETRI), located at Muguga, Kenya. The maintenance of these strains has been described previously (3). One aspect of the study was also done with the TREU 667 strain of *T. brucei brucei*. This strain is a CNS model infection and was obtained from F. Jennings of the University of Glasgow (11, 12).

Drug testing. Female Swiss-Webster mice (weight, 20 g) were infected (intraperitoneally) with 2.5×10^5 trypanosomes from rat blood, and the infection was allowed to develop for 24 h before drug treatment was begun. Groups of five mice each were tested with each drug concentration, and all experiments included a group of untreated controls. Untreated mice died 5 to 13 days after infection, depending on the isolate. Animals were monitored weekly for parasites in tail vein blood smears. Mice were considered cured if they survived >30 days after the death of the last control with no parasites in tail vein blood smears.

A study was also undertaken to determine the activity of MDL73811 on a CNS model infection. The procedures used have been outlined by Jennings and associates (11, 12), and we have previously used the procedures (2, 9). Briefly, mice were infected with 10^4 trypanosomes, and the infection was allowed to develop for 21 days before beginning therapy. Animals were checked weekly for parasites, and those animals that relapsed were removed from their cages. Animals that survived >180 days beyond the last day of treatment with no evidence of parasites in their blood were considered cured.

MDL73811 was administered in most experiments by using 7- or 14-day Alzet osmotic minipumps (Alza Corp.). These were implanted under pentobarbital anesthesia following the manufacturer's instructions (4). In one experiment, MDL73811 was given per os by using gavage needles. DFMO was given orally in the drinking water at 0.5, 1, or

* Corresponding author.
† Deceased.

TABLE 1. Susceptibilities of *T. brucei rhodesiense* isolates to MDL73811 and DFMO

Drug (dose[a])	No. of mice cured/total no. tested (mean survival time [days]) for mice infected with the following KETRI strains[b]:							
	243	269[c]	1992	2002	2285	2537	2538	2636
Control	0/15 (10.0)	0/15 (9.0)	0/5 (13.2)	0/5 (9.8)	0/5 (8.2)	0/5 (9.0)	0/5 (5.0)	0/5 (10.2)
DFMO								
0.5%	ND	ND	ND	ND	ND	ND	0/5 (8.8)	ND
1%	0/5 (18.4)	0/10 (17.0)	0/5 (21.0)	0/5 (14.2)	0/5 (27.0)	0/5 (18.2)	5/5 (NA)	0/5 (13.6)
2%	2/10 (19.9)	5/10 (27.4)	0/5 (21.8)	0/5 (16.6)	0/5 (23.7)	ND	5/5 (NA)	0/5 (14.0)
MDL73811								
10 mg/kg	ND	0/10 (14.0)	0/5 (22.0)	0/5 (21.0)	2/5 (29.0)	0/10 (23.1)	4/9[d] (22.0)	0/5 (17.8)
25 mg/kg	2/15 (21.2)	0/15 (18.4)	1/5 (31.0)	1/5 (24.7)	3/4[d] (26.0)	2/10 (22.9)	5/10 (22.0)	0/5 (20.4)
50 mg/kg	2/5 (31.3)	0/15 (20.1)	1/5 (30.2)	4/5 (25.0)	5/5 (NA)	6/10 (21.5)	3/5 (24.0)	0/5 (21.8)
100 mg/kg	4/5 (32.0)	1/10 (20.8)	4/5 (38.0)	5/5 (NA)	5/5 (NA)	7/10 (24.6)	4/5 (23.0)	0/5 (26.4)
DFMO (0.5%) + MDL73811 (10 mg/kg)	ND	ND	ND	ND	ND	ND	5/5 (NA)	ND
DFMO (1%) + MDL73811 (10 mg/kg)	ND	0/5 (17.8)	ND	ND	ND	3/5 (28.0)	ND	ND
DFMO (1%) + MDL73811 (25 mg/kg)	4/5 (29.0)	5/10 (20.0)	4/5 (33.0)	5/5 (NA)	5/5 (NA)	5/5 (NA)	2/5 (20.0)	2/5 (20.0)
DFMO (1%) + MDL73811 (50 mg/kg)	5/5 (NA)	9/10 (22.0)	5/5 (NA)	5/5 (NA)	5/5 (NA)	5/5 (NA)	ND	1/5 (26.2)
DFMO (1%) + MDL73811 (100 mg/kg)	5/5 (NA)	ND	ND	ND	ND	ND	ND	ND
DFMO (2%) + MDL73811 (25 mg/kg)	ND	5/5 (NA)	ND	ND	ND	ND	ND	ND
DFMO (2%) + MDL73811 (50 mg/kg)	5/5 (NA)	5/5 (NA)	ND	ND	ND	ND	ND	ND

[a] Animals were treated daily beginning 24 h after inoculation. Control animals were untreated. Treated animals were given DFMO in drinking water for 3 days, and MDL73811 was given in surgically implanted osmotic minipumps that released 1 μl of drug per h for 7 days.
[b] Mean survival time for animals dying of trypanosomiasis. ND, not done; NA, not applicable.
[c] DFMO was given to KETRI 269-infected mice for 7 days in all cases.
[d] One animal from the group died from the implanted pump.

2%. Animals consumed an average of 5 ml/day, a dose rate of 5,000 mg/kg of body weight per day for a 2% solution. Short-term *T. brucei rhodesiense* infections were treated for 3 days, while treatment for the CNS model infection was for 14 days.

RESULTS

Susceptibility to MDL73811. Eight strains of *T. brucei rhodesiense* were tested for their susceptibilities to MDL73811 alone and in combination with DFMO (Table 1). The data obtained for one strain, KETRI 2538, have been reported previously (6), but susceptibility studies were expanded, and the results are included here for comparison with the results for the other isolates tested. For mice infected with six of the eight strains tested, cure rates were 70% or better with treatment with 100 mg of MDL73811 per kg for 7 days, and for mice infected with four of these strains, cure rates were >60% when the dose was decreased to 50 mg/kg. At 25 mg/kg, both KETRI 2285 and KETRI 2538 remained susceptible, with cure rates for mice infected with these strains of 75 and 50%, respectively. Only two strains, KETRI 269 and KETRI 2636, were not eradicated by MDL73811.

Drug combinations. Six of the strains used in the present study were previously found to be refractory to DFMO; these were KETRI strains 243, 269, 1992, 2002, 2285, and 2636. These strains retained their resistance in the present study, as indicated by 50% or lower cure rates for mice infected with these strains and treated with 2% DFMO for 3 days (Table 1). Mice infected with KETRI 269 were dosed for 7 days with DFMO in all cases. KETRI 2538 appeared to lose its resistance, as seen by complete cures in KETRI 2538-infected mice treated with 1 and 2% DFMO for 3 days. Previously, this strain had been refractory to 2% DFMO for 6 days (3). Mice infected with seven of eight strains studied were cured by combinations of DFMO and MDL73811 (Table 1). For many successful combinations, doses of each agent that were not curative when used singly were used. For example, MDL73811 at 25 mg/kg and 1% DFMO, used singly, were not curative for infections caused by KETRI strains 243, 1992, 2002, and 2537, yet they were 100% curative when used as a combination. Experiments with KETRI 269 involved 1 or 2% DFMO given for 7 days and yielded 0 and 50% cure rates, respectively, yet high cure rates were obtained with 1% DFMO plus 25 or 50 mg of MDL73811 per kg. Because these cures were obtained with generally ineffective single-agent levels, these agents appeared to act synergistically.

TABLE 2. Activity of oral administration of MDL73811 and DFMO against *T. brucei brucei* LAB 110 EATRO[a]

Drug (dose)	Survival time (days)		No. of mice cured/total no. tested
	Mean	Range	
Control	3.2	3–4	0/10
MDL73811			
10 mg/kg	7.8	7–9	0/5
25 mg/kg	10.6	9–13	0/5
50 mg/kg	13.6	11–17	5/10
100 mg/kg	12.3	9–15	7/10
DFMO			
0.25%	6.6	6–8	0/5
0.5%	10.3	9–11	2/5
DFMO (0.25%) + MDL73811 (10 mg/kg)	11.6	11–13	2/5
DFMO (0.25%) + MDL73811 (25 mg/kg)	18.0	18	4/5
DFMO (0.5%) + MDL73811 (10 mg/kg)	NA[b]	NA	5/5
DFMO (0.5%) + MDL73811 (25 mg/kg)	NA	NA	5/5

[a] Mice were infected as described in the text, and treatment was begun 24 h postinfection. MDL73811 was given by gavage twice daily (9 a.m. and 4:30 p.m.) for 3 days at the total daily dose listed. DFMO was given ad libitum in the drinking water for the same period.
[b] NA, not applicable.

Oral dosing. Table 2 lists the data obtained by using twice-daily oral administration of MDL73811 to animals infected with the standard laboratory isolate *T. brucei brucei* LAB 110 EATRO. These studies were done to determine whether oral administration of MDL73811 to mice infected with LAB 110 EATRO could be used in place of the 7-day pump regimen and whether oral dosage regimens of MDL73811 and DFMO were synergistic. At 50 and 100 mg/kg for 3 days, MDL73811 was 50 to 70% curative for this acute infection, and at 10 and 25 mg/kg, the agent was synergistic with 0.25 and 0.5% DFMO. Results of this study indicate the potential utility of short-term oral therapy with these agents used in combination. The currently approved regimen for DFMO requires intravenous dosing for 2 weeks, which is not an optimal route of administration in most clinical settings in less-developed countries (20).

CNS model of infection. An important criterion of the clinical utilities of potential trypanocides is the ability to cure CNS infections. Therefore, an experiment was initiated by using the CNS model of *T. brucei brucei* TREU 667 infection (Table 3). This model has been used to demonstrate the potential efficacy of DFMO alone or in combination with suramin against CNS infections (2, 9). MDL73811 was not highly effective when used at 50 to 150 mg/kg for 7 days as a single agent against this infection, and no significant improvement was observed when the dose period was doubled to 14 days (Table 3). However, if MDL73811 was administered concurrently with 1 or 2% DFMO, the combinations were curative in all cases except the dosage regimens with 50 mg of MDL73811 per kg plus 1% DFMO.

TABLE 3. Activities of MDL73811 and DFMO singly and in combination against a CNS model of *T. brucei brucei* TREU 667 infection[a]

Treatment (dose)	Duration of treatment (days)	Avg day of relapse (range)	No. of mice cured/total no. tested
DFMO			
1%	14	65	0/5
2%	14	64.8 (40–94)	0/9[b]
MDL73811			
25 mg/kg	14	40	0/5
50 mg/kg	7	65	1/5
50 mg/kg	14	65	0/5
100 mg/kg	7	65	2/5
100 mg/kg	14	65	2/5
150 mg/kg	14	65	1/5
DFMO (1%) + MDL73811 (50 mg/kg)	14	65	0/5
	7		
DFMO (1%) + MDL73811 (50 mg/kg)	14	74 (65–93)	1/5
	14		
DFMO (1%) + MDL73811 100 mg/kg	14	NA	5/5
	7		
DFMO (1%) + MDL73811 (100 mg/kg)	14	72	4/5
	14		
DFMO (2%) + MDL73811 (25 mg/kg)	14	40	3/4[b]
	14		
DFMO (2%) + MDL73811 (50 mg/kg)	14	NA	10/10
	14		
DFMO (2%) + MDL73811 (100 mg/kg)	14	NA	9/9[b]
	14		
DFMO (2%) + MDL73811 (150 mg/kg)	14	NA	5/5
	14		

[a] Mice were infected (intraperitoneally) on day 0 with 10^4 trypanosomes, and the infection was allowed to develop for 21 days, when treatment was started. Animals were examined weekly for parasites in tail vein blood smears, and animals that relapsed were removed from the cages. Untreated control animals survived for 45 days. Animals that survived >180 days posttreatment were considered cured. MDL73811 was given in 7- or 14-day osmotic minipumps (Alzet), while DFMO was given in drinking water. NA, not applicable.
[b] Indicates that one animal died at the beginning of treatment.

DISCUSSION

The development of DFMO for treatment of West African trypanosomiasis marked the first breakthrough in the chemotherapy of sleeping sickness in over 40 years (18, 20). This agent is effective against late-stage arsenical-refractory disease with minor reversible side effects, but it requires a relatively high dose and a long duration of treatment and lacks activity against East African disease (5, 20). The observations reported here highlight MDL73811 as another significant lead in trypanosome chemotherapy. MDL73811 is

effective at about 1/50 of the normal DFMO dose (5 ml of a 2% DFMO solution for 3 days = 300 mg per mouse, whereas 100 mg of MDL73811 per kg/day = 6 mg per mouse) and has not shown any signs of toxicity; most cured animals were held and observed for 45 to 60 days beyond the 30 days necessary to demonstrate cures. Moreover, TREU 667-infected animals were held for >6 months after therapy ended. All mice had normal weight gains and no apparent ill effects.

DFMO and MDL73811 appeared to be synergistic against seven of eight strains. Only KETRI 2636 was not affected by a DFMO-MDL73811 combination. The reason for this lack of susceptibility to both agents is not apparent, but it is under investigation. The synergism between DFMO and MDL73811 observed with the other isolates is significant since many of the *T. brucei rhodesiense* strains used were refractory to standard trypanocides, including arsenical drugs, diamidines, and, in one instance (KETRI 243), suramin (3). In this type of study, we considered it important to use clinical isolates rather than extensively passaged laboratory strains or clones in screening systems to allow for testing of the genetically divergent populations expected to be encountered in clinical treatment settings. For example, KETRI 243 is composed of at least two subpopulations that have moderate and complete resistance to lysis by arsenical drugs (22).

In terms of the relationship of the present findings to clinical applications, use of an agent such as MDL73811 in conjunction with DFMO should reduce by at least 50% the amount of DFMO necessary for successful treatment in most cases. Since the routine DFMO dosage regimen used in West Africa is 400 mg/kg/day (18), a reduction to 200 mg/kg/day or less is likely.

The biochemical actions of DFMO and MDL73811 have generally been attributed to depletion of polyamines caused by blockade of ornithine decarboxylase (1) and AdoMet decarboxylase (6) by DFMO and MDL73811, respectively. However, biochemical studies have revealed that both agents cause a significant (>40-fold) increase in AdoMet in trypanosomes. In vivo, this increase in DFMO-treated trypanosomes takes 12 to 36 h and is probably delayed because of a need for putrescine depletion (21), while a 20-fold increase occurs after 1 h of treatment with MDL73811 (7). The specificity of this response in trypanosomes appears to be a critical factor in the activity of these agents, since mammalian cells treated with these agents do not exhibit significant increases in AdoMet (7, 13, 14). Additionally, α-monofluoromethyl-3,4-dehydroornithine ethyl ester, an ornithine decarboxylase inhibitor which does not cure trypanosome infections, depletes polyamines but does not significantly increase AdoMet levels (7).

An advantage of MDL73811 over DFMO appears to be its relatively rapid action. Within 1 h of a single dose in vivo, trypanosome AdoMet decarboxylase activity was completely inhibited, while putrescine and spermidine levels remained unchanged (7). One difference in the action of DFMO and MDL73811 in vivo is that while polyamines coadministered with treatment could reverse the action of DFMO (15), they had no effect on the curative doses of MDL73811 (6). The precise mechanism for the activity of MDL73811 is not known; however, the significant increase in intracellular AdoMet alters the methylation index (ratio of AdoMet to its transmethylation product *S*-adenosylhomocysteine) of DFMO-treated trypanosomes (21) and may result in hypermethylation of nucleic acids, proteins, or other cell components, as is the case in mammalian cells (10, 19). Recently, agents such as methylhydrazines were found to be therapeutically effective in model trypanosome infections and were shown to be active on the basis of their ability to generate reactive methyl groups (17).

In summary, this report described the activity of MDL73811 alone and in combination with DFMO against drug-refractory clinical isolates of *T. brucei rhodesiense*. Administered orally alone or in combination with MDL73811, DFMO was curative for a *T. brucei brucei* infection and also acted synergistically in combination with DFMO in curing a CNS model of infection. The low doses of MDL73811 and apparent lack of toxicity (the 50% lethal dose in mice was 300 to 400 mg/kg) indicate that this agent has significant potential as a trypanocide.

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