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Differential Susceptibility to DL-α-Difluoromethylornithine in Clinical Isolates of *Trypanosoma brucei rhodesiense*

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DL-α-Difluoromethylornithine is an enzyme-activated inhibitor of ornithine decarboxylase and an antagonist of polyamine metabolism that has been successful in clinical trials against West African sleeping sickness caused by *Trypanosoma brucei gambiense*. Its potential for use against the more virulent East African form of the disease, caused by *T. brucei rhodesiense*, is not certain. We examined 14 East African clinical isolates from the Kenya Trypanosomiasis Research Institute strain bank plus 2 established isolates for susceptibility to DL-α-difluoromethylornithine and to standard trypanocides. Seven of 16 strains were partially or totally refractory to DL-α-difluoromethylornithine in our test system. Four strains were also refractory to arsenical drugs, and five were refractory to diamidines. The results indicate that other novel agents or combinations of established agents may be needed for chemotherapy of East African disease.

Protozoa of the genus *Trypanosoma* cause debilitating disease in humans and animals in Africa. *T. brucei rhodesiense*, endemic throughout Central and East Africa, is one of the most virulent. It causes n'gana, a fatal wasting disease in domestic animals, and sleeping sickness in humans. The closely related *T. brucei gambiense* causes West African sleeping sickness, a more slowly progressing disease although also invariably fatal if not treated.

Drugs available for treatment of both of the human African trypanosomiases did not change for over 40 years (27). Suramin and, more rarely, pentamidine are used during the early nonencephalitic stages of the disease. The arsenical drug melarsoprol (Arsobal) has been the only agent available for late-stage, central nervous system involvement. Chemotherapy is complicated by the need for intravenous administration of suramin and melarsoprol, by a reactive encephalopathy in 1 to 5% of melarsoprol-treated cases, and by the inability of suramin or pentamidine to cure late-stage disease (24, 27).

Recently an inhibitor of ornithine decarboxylase, DL-α-difluoromethylornithine (DFMO; eflornithine; Ornidyl) (2) has proven highly effective in clinical trials against late-stage *T. b. gambiense* infections in the Ivory Coast, Sudan, and Zaire (for a review, see reference 22). In many instances, patients had failed one or more courses of melarsoprol before DFMO treatment. Side effects due to DFMO therapy were mild and transient. DFMO has recently been approved in the United States for treatment of *T. b. gambiense* human trypanosomiasis (P. P. McCann, personal communication).

This success gave hope for similar efficacy with *T. b. rhodesiense*, but the utility of DFMO therapy for East African sleeping sickness remains questionable. In limited clinical trials in Kenya, Tanzania, and Mozambique, results with DFMO have been inconsistent (27; P. de Raadt and S. von Nieuwenhove, personal communication).

Most of the preclinical work with DFMO and African trypanosomiasis utilized laboratory model infections of *T. brucei brucei*, a closely related subspecies only infective to animals. Aside from the few clinical cases noted above, studies of the susceptibility of *T. b. rhodesiense* to DFMO are limited to three studies with established laboratory strains that indicated that *T. b. rhodesiense* was less susceptible than *T. b. brucei* (10, 11, 20). We therefore examined the susceptibility of a group of *T. b. rhodesiense* clinical isolates to DFMO and to standard trypanocides. Primary isolates with their inherent mixed populations of parasites were used rather than established and characterized stocks or clones. Despite the variability in results caused by mixed populations, this approach was chosen because the intention was to provide an estimate of the range of drug susceptibilities that will be found in clinical trials and to provide a panel of isolates for evaluation of new therapies before clinical trials. In particular, such results could guide clinical trials planned for DFMO in the near future (19a). All but two of the *T. b. rhodesiense* isolates were from the Kenya Trypanosomiasis Research Institute (KETRI) strain bank. They were chosen to obtain a representative geographic distribution in East Africa with emphasis on major endemic areas in Uganda (16).

TABLE 1. Origin of KETRI *T. b. rhodesiense* stabilates

KETRI strain	Passage	Location	Yr of isolation
243	1	Busoga, Uganda	1961
265	7	Numbuzi, Tanzania	1959
269	2	Kitanga, Tanzania	1960
1765	2	Ngamiland, Botswana	1960
1992	1	Buyinja, Uganda	1972
2002	1	Tororo, Uganda	1972
2285	1	Busoga, Uganda	1976
2482	7	Lumino, Uganda	1969
2538	21	Tete Province, Mozambique	1980
2545	4	Lambwe Valley, Kenya	1981
2562	14	Lumino, Uganda	1959
2636	1	Tete Province, Mozambique	1983
2708	8	Busoga, Uganda	1964
2772	1	Alupe, Kenya	1985

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TABLE 2. Susceptibilities of *T. b. rhodesiense* isolates to DFMO

Dose[a] (%)	Days	Mean survival in days[b] (no. cured/total)						
		KETRI strains						
		243	265	269	1765	1992	2002	2285
1.0	3	47.7 (1/5)	14.7 (0/10)	15 (0/5)	ND	19.4 (0/5)	24.8 (0/5)	ND
2.0	3	35.0 (0/10)	17.0 (0/5)	13.8 (0/5)	52.6 (2/5)	17.4 (0/5)	28.0 (1/5)	17.2 (1/15)
2.0	6	41.0 (4/10)	22.2 (0/5)	17.0 (0/5)	80.3 (2/5)	15.6 (0/5)	29.2 (0/5)	26.0 (2/15)
2.0	9	56.0 (1/10)	26.6 (0/5)	23.2 (0/5)	68.0 (3/5)	22.7 (2/10)	33.4 (0/5)	35.0 (1/5)
4.0	3	35.6 (0/10)	17.8 (0/5)	13.4 (0/5)	ND	17.0 (0/5)	28.0 (0/5)	26.5 (0/5)
4.0	6	37.0 (2/10)	23.8 (0/5)	12.0 (0/5)	ND	18.8 (0/5)	35.0 (0/5)	34.0 (4/5)
4.0	9	(5/5)	31.5 (2/5)	21.6 (0/5)	ND	(5/5)	(5/5)	(5/5)
Control		21.1 (11–55)[c] (40)[d]	13.4 (10–19) (10)	12.8 (10–37) (5)	51.8 (17–70) (5)	11.6 (6–25) (10)	19.2 (17–52) (5)	14.0 (7–20) (15)

[a] Animals were given DFMO in drinking water ad libitum at the dose indicated for the time in days indicated. Control animals received no DFMO.
[b] Mean survival time of animals dying of trypanosomiasis; this does not include cured animals. ND, Not determined.
[c] Range of survival time of controls.
[d] Total number of control animals.

MATERIALS AND METHODS

Animals. Female 20- to 25-g Swiss-Webster mice were obtained from Royal Hart Animal Laboratories, New Hampton, N.Y., and Ace Animals, Inc., Boyertown, Pa.

Trypanosome strains. *T. b. brucei* Lab 110/EATRO was originally obtained from W. Trager of the Rockefeller University and has been maintained at Pace University and New York University for many years. This strain has mistakenly been labeled EATRO 110 in previous publications by us and others. *T. b. rhodesiense* EATRO 105 (ATCC 30119) and *T. b. rhodesiense* Wellcome CT (ATCC 30027) strains were obtained from the American Type Culture Collection, Rockville, Md. The remaining *T. b. rhodesiense* isolates had been stored as frozen stabilates in the KETRI strain bank at Muguga, Kenya, which had evolved from the East African Trypanosomiasis Research Organization (EATRO) strain bank. All stabilates were isolated from human blood or cerebrospinal fluid and had at least one passage in mice. Upon arrival in New York, the stabilates from KETRI were passaged once in rats, and additional stabilates were prepared (Table 1) (7).

Drugs. DFMO (Merrell Dow Research Institute); Arsobal and melarsen oxide (Rhone Poulenc, Paris); and Berenil (diminazene aceturate) and pentamidine (May & Baker, Ltd., Dagenham) were gifts from the manufacturers. Suramin was purchased from Mobay Chemical Co., New York, N.Y.

Experimental design. Groups of 50 mice were infected with 2.5×10^5 trypanosomes taken from the first parasitemia peak of rats inoculated with frozen stabilate. The infection was allowed to develop for 24 h before treatment was begun. Mice were separated into groups of five including a group of five inoculated but untreated control animals for each experiment. DFMO was administered ad libitum in the drinking

TABLE 3. Susceptibilities of *T. b. rhodesiense* isolates to standard trypanocides

Drug[a]	Dose (mg/kg)	Mean survival in days[b] (no. cured/total)						
		KETRI strains						
		243	265	269	1765	1992	2002	2285
Diminazene aceturate	10	28.0 (0/5)	(5/5)	(5/5)	ND	29.5 (0/10)	(5/5)	(5/5)
Melarsen oxide	1	16.3 (0/5)	(5/5)	33.2 (0/5)	(5/5)	22.8 (0/5)	40.7 (1/5)	(5/5)
	5	20.5 (6/20)	(5/5)	47.6 (2/5)	(5/5)	22.0 (4/5)	(5/5)	(5/5)
	10	18.0 (5/15)	14.0 (9/10)	(5/5)	(5/5)	21.0 (4/5)	70.0 (4/5)	(5/5)
Melarsoprol	1	25.8 (0/20)	11.0 (4/5)	14.6 (2/5)	ND	13.4 (0/5)	8.6 (0/5)	4.0 (1/5)
	5	26.7 (0/20)	14.0 (3/5)	5.0 (0/5)	8.0 (3/5)	13.0 (2/5)	7.5 (3/5)	6.5 (2/5)
	10	17.1 (0/10)	13.5 (6/10)	ND	7.0 (2/5)	10.0 (2/5)	(5/5)	(5/5)
Suramin	5	16.0 (2/5)	(5/5)	(5/5)	ND	(5/5)	9.0 (4/5)	(5/5)
Pentamidine	2	26.4 (0/5)	26.0 (2/5)	27.0 (0/5)	ND	17.2 (0/10)	(5/5)	(5/5)
Control		19.1 (11–30)[c] (40)[d]	13.4 (10–19) (10)	23.1 (14–37) (10)	51.8 (17–70) (5)	15.2 (7–25) (10)	21.7 (8–52) (10)	13.1 (7–20) (10)

[a] Animals were treated once daily (intraperitoneal injection for 3 days beginning 24 h after inoculation. Control animals were untreated.
[b] Mean survival time of animals dying of trypanosomiasis; this does not include cured animals. ND, Not determined.
[c] Range of survival time of controls.
[d] Total number of control animals.

TABLE 2—*Continued*

Mean survival in days[b] (no. cured/total)									
KETRI strains							Wellcome CT	EATRO 105	*T. b. brucei* Lab/110
2482	2538	2545	2562	2636	2708	2772			
10.8 (0/5)	13.6 (0/5)	ND	10.2 (0/15)	26.6 (0/5)	ND	11.6 (2/5)	ND	ND	18.5 (8/15)
18.6 (0/5)	17.8 (0/5)	ND	16.5 (10/15)	26.0 (0/5)	27.6 (1/10)	13.6 (2/5)	9.3 (0/10)	16.0 (2/10)	33.0 (14/15)
22.0 (9/10)	25.0 (0/5)	29.8 (1/10)	18.0 (14/15)	25.6 (1/10)	(10/10)	(5/5)	19.0 (3/10)	17.5 (2/10)	(15/15)
(5/5)	23.6 (0/5)	29.0 (4/5)	17.0 (14/15)	33.5 (3/10)	(5/5)	(5/5)	15.5 (6/10)	17.5 (2/10)	(15/15)
ND	20.5 (0/5)	25.4 (0/5)	21.5 (7/10)	28.0 (0/5)	ND	(5/5)	11.5 (1/15)	25.5 (10/15)	(15/15)
(5/5)	29.0 (0/5)	27.6 (7/10)	16.0 (18/20)	33.0 (4/5)	ND	(5/5)	16.5 (8/10)	32.0 (9/10)	(15/15)
(5/5)	27.6 (0/5)	(5/5)	ND	(5/5)	ND	ND	(5/5)	(5/5)	(15/15)
7.0 (3–10)	14.1 (8–28)	12.5 (7–19)	4.2 (4–7)	19.6 (14–21)	18.9 (17–21)	4.2 (4–5)	4.1 (4–5)	5.2 (4–7)	4.0 (4)
(20)	(5)	(15)	(30)	(5)	(10)	(15)	(15)	(15)	(15)

water over the time indicated. All other drugs were given once daily. Melarsoprol as the commercially available propylene glycol preparation Arsobal, was injected intravenously into tail veins. All other drugs were given by intraperitoneal injection.

Mice were monitored daily for deaths and weekly for parasites by examination of tail vein blood films. An animal was considered cured if it survived more than 30 days beyond the death of the last untreated control with no evidence of parasites in the blood. Since the control animals never became aparasitemic and most treated animals were observed for 100 days after the death of the last control with no deaths or parasitemia relapses occurring beyond the minimum 30 day observation period, this 30-day aparasitemic period is a valid criterion of cure. For animals that were treated but not cured, the mean time to death is reported for comparison with that of the controls.

RESULTS

Growth of strains. All strains produced infections that were invariably fatal in mice. Mean survival times of untreated mice ranged from 4.2 days (KETRI 2772) to 51.8 days (KETRI 1765). Most strains produced several peaks of parasitemia before killing the host, but some, KETRI 2285, 2482, 2562, and 2772, produced a fulminant infection that resulted in a single parasitemia peak and rapid death of the host.

DFMO resistance. Since none of the 16 isolates tested had previously been exposed to DFMO, the refractoriness observed is constitutive. The susceptibility of the isolates to DFMO was judged by comparison to the reference susceptible *T. b. brucei* Lab 110/EATRO isolate (Table 2) in which DFMO produced a greater than 80% cure rate when administered as a 2% solution in drinking water for 3 days (drug dose, 5.3 g/kg per day) (3). At the same dose of 2% for 6 or 9 days, DFMO invariably cured this reference strain.

Five *T. b. rhodesiense* strains, KETRI 2482, 2545, 2562, 2708, and 2772, were similar in susceptibility to the reference susceptible strain; for these, 2% DFMO for 6 or 9 days produced a high cure rate. Eight strains, KETRI 243, 1765, 1992, 2002, 2285, and 2636, EATRO 105, and Wellcome CT, were more resistant than the *T. b. brucei* Lab 110/EATRO

TABLE 3—*Continued*

Mean survival in days[b] (no. cured/total)									
KETRI strains							Wellcome CT	EATRO 105	*T. b. brucei* Lab/110
2482	2538	2545	2562	2636	2708	2772			
ND	18.3 (0/10)	ND	(5/5)	20.0 (3/5)	ND	18.0 (9/10)	(5/5)	(5/5)	(5/5)
ND	15.6 (0/5)	(5/5)	ND	(5/5)	24.4 (0/5)	ND	(5/5)	29.0 (4/5)	(5/5)
ND	16.5 (6/15)	(5/5)	ND	(5/5)	21.0 (0/5)	ND	(5/5)	(5/5)	(10/10)
ND	21.0 (7/10)	(5/5)	ND	(5/5)	25.0 (0/5)	ND	(5/5)	(5/5)	(5/5)
ND	12.0 (0/10)	31.0 (4/5)	12.0 (4/5)	9.4 (0/5)	16.8 (0/5)	16.0 (4/5)	(5/5)	(5/5)	16.5 (7/10)
ND	13.5 (0/15)	7.0 (4/5)	ND	7.6 (2/5)	16.8 (0/5)	ND	19.5 (2/5)	10.5 (1/5)	(10/10)
ND	17.7 (0/10)	(5/5)	ND	6.0 (4/5)	17.2 (0/5)	ND	ND	(5/5)	23.0 (2/5)
(5/5)	(10/10)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(10/10)	(10/10)
(5/5)	14.6 (0/5)	27.0 (4/5)	(5/5)	33.0 (2/5)	ND	ND	(5/5)	(5/5)	(5/5)
7.0 (7)	12.3 (8–22)	12.5 (7–19)	5.0 (5)	17.7 (11–22)	18.9 (17–21)	4.0 (4)	4.1 (3–7)	5.2 (4–7)	4.0 (3–6)
(5)	(15)	(15)	(15)	(10)	(10)	(10)	(20)	(15)	(10)

TABLE 4. Drug susceptibility patterns in *T. b. rhodesiense* strains

Strain	Susceptibility[a] to:		
	Arsenical drugs	Diamidine	Suramin
DFMO-susceptible strains			
Lab 110/EATRO	S	S	S
KETRI 2482		S[b]	S
KETRI 2545	S	S[b]	S
KETRI 2562	S	S	S
KETRI 2708	R		S
KETRI 2772	S	S[c]	S
Strains partially refractory to DFMO			
KETRI 243	R	R	R
KETRI 1765	S		
KETRI 1992	R	S/R	S
KETRI 2002	S	S	S
KETRI 2285	S	S	S
KETRI 2636	S	R	S
Wellcome CT	S	S	S
EATRO 105	S	S	S
DFMO-refractory strains			
KETRI 265	S	S	S
KETRI 269	S	R	S
KETRI 2538	R	R	S

[a] S, Susceptible; S/R, partially refractory; R, refractory.
[b] Data for pentamidine only.
[c] Data for diminazene only.

and were characterized as moderately refractory. For these, regimens employing 2% DFMO for 3, 6, or 9 days provided either no cures or a lower cure rate than for the susceptible strains. However, dose regimens using 4% DFMO for 9 and sometimes 6 days were curative. Three strains, KETRI 265, 269, and 2538, were refractory to DFMO at all doses tested. However, even for the most resistant strains in which there were no cures, the animals treated with higher doses of DFMO survived longer than the untreated controls.

Resistance to standard trypanocides. Few studies have compared the drug susceptibilities of *T. b. rhodesiense* clinical isolates. Since resistance to standard trypanocides remains one of the major obstacles to chemotherapy, we felt it was important to determine the susceptibilities of these strains to standard drugs. KETRI 243 and 2538 originated from patients who had failed two to four courses of melarsoprol therapy, and therefore these can be regarded as resistant isolates. The remaining KETRI strains were isolated from patients before chemotherapy. We have no history of the clinical response of the patients from whom EATRO 105 and the Wellcome CT isolates were isolated.

Susceptibilities of the strains to standard trypanocides are shown in Table 3. Resistance to the diamidines diminazene and pentamidine was apparent in three strains: KETRI 243, 1992, and 2538. Only one strain, KETRI 269, was resistant to pentamidine but not diminazene, illustrating a general cross-resistance to diamidines as predicted by the diamidine transport studies of Damper and Patton (9). Some degree of resistance to arsenical drugs was found in four strains, KETRI 243, 1992, 2708, and 2538. Of these, KETRI 243 and 2708 were clearly resistant to both melarsen oxide and melarsoprol, whereas KETRI 2538 was susceptible to 5 and 10 mg of melarsen oxide per kg but refractory to melarsoprol at 5 and 10 mg/kg. KETRI 1992 was susceptible to melarsen oxide but partly refractory to melarsoprol, since only a 40% cure rate was obtained with the higher dose levels of melarsoprol.

All strains were susceptible to suramin at 5 mg/kg with the exception of KETRI 243, for which only 40% of suramin-treated mice were cured (Table 3). Since suramin did not cross the blood-brain barrier, the almost universal efficacy of suramin in these studies implies that the test strains did not establish infections in the brain or other areas inaccessible to the drug within the 24-h incubation period before treatment (18, 19). The drug susceptibility patterns in *T. b. rhodesiense* strains are shown in Table 4.

DISCUSSION

Key general issues that appear to be involved in the response of any trypanosome isolate to any drug are as follows: (i) the number of prior syringe passages, since repeated passage is often associated with increasing virulence; (ii) prior selection of resistant organisms by exposure to drug in vivo before the original samples were collected; (iii) the variable time different isolates require to establish infection in sites not accessible to the test drugs; (iv) the degree to which an isolate is able to evade the host immune response, since this will affect the usual synergism between a chemotherapeutic agent and the immune system (12, 26); and (v) variable innate biochemical resistance to the drug. We attempted to minimize variables i through iv by beginning treatment 24 h postinfection, using outbred Swiss Webster mice throughout, and using inocula no more than two syringe passages removed from the original strain bank isolate. For 9 of 14 KETRI isolates, no more than two passages had occurred since isolation from the patient, and none had undergone more than 21 passages (Table 1). Thus the passage artifact was minimal. Most of the KETRI isolates were made before drug treatment, eliminating a selection for drug resistance. The efficacy of suramin in treating all infections except KETRI 243 indicated that the parasites did not become established in sites inaccessible to drugs and that there was little variation in immune system cooperation. We were therefore primarily examining the interaction of the test drugs and the parasites relatively independently of host-parasite model system peculiarities.

The size of the individual test groups was necessarily restricted to allow a broader range of strains and drugs to be tested. However, an examination of Tables 2 and 3 shows that the differences in drug susceptibility were large enough so that the same group size did not prevent a clear demonstration of the variable susceptibility of different isolates to both DFMO and standard trypanocides.

A range of susceptibilities to standard trypanocides was also observed. Only KETRI 243 showed significant resistance to suramin, which confirms another report showing that suramin resistance is not common in clinical isolates (24). Unfortunately, KETRI 243 was even more resistant to all other drugs tested. For melarsen oxide we have direct evidence that the resistance of KETRI 243 reflects the ability of the organism to survive exposure to the drug and not the avoidance of contact with drug in the experimental host. A previous report indicated that lysis of trypanosomes in the presence of melarsoprol is dose dependent when done in the presence of normal serum or buffer containing physiological Ca^{2+} levels (8). In preliminary studies, purified bloodstream KETRI 243 (5×10^7 cells per ml) suspended in fetal bovine

serum withstood incubation in 100 μM melarsen oxide for 30 min without evidence of lysis; for *T. b. brucei* Lab 110/EATRO, 7.5 μM melarsen oxide caused 80% lysis in only 15 min (N. Yarlett, B. Goldberg, and C. J. Bacchi, unpublished data). Beyond KETRI 243, four other isolates were resistant to the diamidines pentamidine and/or diminazene. Since pentamidine is one of the agents used in early stage East African sleeping sickness, the occurrence of resistance in 25% of the tested isolates is particularly important and agrees with similar findings in the field (15, 24) and the laboratory (28). In addition to KETRI 243, three other isolates were resistant to arsenical compounds.

In spite of the virtual 100% success of DFMO in treating *T. b. gambiense*, the majority of *T. b. rhodesiense* strains were at least moderately resistant when inoculated into mice and three were highly resistant. This variable susceptibility could be due to several causes, since DFMO treatment, with the ensuing polyamine deprivation, has many biochemical effects on cells. Polyamine deprivation rapidly inhibits parasite DNA, RNA, and protein synthesis while decreasing parasite multiplication in blood and tissues (1, 5). Blockage of parasite cell division likely blocks antigenic variation, allowing the parasites to be destroyed by the immune system of the host (6, 11). Trypanosomes have a spermidine-containing redox intermediate, trypanothione, which is unique to kinetoplastids and may cause them to be particularly susceptible to polyamine depletion (13).

Data on the mechanism of DFMO resistance have begun to be collected. Greater than 50-fold resistance was experimentally induced in cultured procyclic trypanosomes (analogous to vector midgut forms) by serial passage with increasing drug concentrations (4, 21). In one strain, the induced resistance was attributed to decreased DFMO uptake (21). In another it was associated with the ability to maintain a normal concentration of trypanothione in the presence of DFMO; wild-type cells exposed to DFMO have a reduced trypanothione concentration, as would be expected if spermidine biosynthesis were blocked by DFMO (4, 13). This strain had an elevated intracellular ornithine concentration, which would allow ornithine to compete with the inhibitor, an analog of ornithine, for the active site of ornithine decarboxylase. Ornithine decarboxylase would be thus protected, and polyamine biosynthesis therefore would be maintained in the presence of the drug (4).

Rather than analyzing laboratory-induced DFMO resistance in the noninfectious vector form of the parasite, this study focuses on clinically relevant infective trypanosomes of wild-type, uncloned isolates that have had no prior exposure to DFMO. The reason(s) for the observed resistance to DFMO is not yet known, although it may in part reflect naturally occurring variations in polyamine metabolism. It is intriguing that several strains that are resistant to DFMO are also resistant to arsenical drugs, since the trypanothione-arsenical drug complex formation is believed to be responsible for the action of melarsoprol because the complex inhibits an enzyme that is important for the oxidant stress protection mechanism of the trypanosome (12a, 14). A naturally occurring excess production of spermidine leading to trypanothione over production might compensate for the arsenical drug-induced loss of trypanothione and confer resistance to both arsenical drugs and DFMO. Further studies with these strains will involve examination of the mechanism(s) for DFMO and arsenical drug resistance and the development of new therapies based on rational combinations of clinically active agents.

In summary, knowledge of the range of susceptibility of wild trypanosome populations to trypanocides is important, since resistance has been a constant problem for the treatment of human and animal trypanosomiases (12, 17, 23–25). This report provides data collected with two established stocks and 14 clinical isolates with limited laboratory passage after isolation from sleeping sickness patients. A range in susceptibility to both standard trypanocides and to the newly approved drug DFMO was observed. Although the number of clinical isolates is not yet large enough to confidently predict the full range of drug responsiveness or frequency of resistance that may be encountered under field conditions, the existence of such a range for DFMO, a drug not yet used in the field against *T. b. rhodesiense*, is clearly demonstrated. For this reason, the choice of isolates used for preclinical drug development must be made carefully to understand the results of clinical trials and to best predict the drug susceptibilities of trypanosomes that will be encountered in ultimate clinical use.

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