ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1996, p. 1448–1453 Vol. 40, No. 6
0066-4804/96/$04.00+0
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In Vivo Trypanocidal Activities of New *S*-Adenosylmethionine Decarboxylase Inhibitors

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Received 18 December 1995/Returned for modification 20 February 1996/Accepted 4 April 1996

A series of novel aromatic derivatives based on the structure of methylglyoxal bis(guanylhydrazone) (MGBG) was examined for trypanocidal activities in human and veterinary trypanosomes of African origin. One agent, CGP 40215A, a bicyclic analog of MGBG which also resembles the diamidines diminazene (Berenil) and pentamidine, was curative of infections by 19 isolates of *Trypanosoma brucei* subspecies as well as a *Trypanosoma congolense* isolate. Several of these isolates were resistant to standard trypanocides. Curative doses were ≤25 mg/kg of body weight/day for 3 days in these acute laboratory model infections. In addition, CGP 40215A also cured a model central nervous system infection in combination with the ornithine decarboxylase inhibitor DL-α-difluoromethylornithine (DFMO; Ornidyl, eflornithine). Curative combinations were 14 days of oral 2% DFMO (~5 g/kg/day) plus 5, 10, or 25 mg/kg/day for 3 or 7 days given by intraperitoneal injection or with a miniosmotic pump. Combinations were most effective if CGP 40215A was given in the second half or at the end of the DFMO regimen. MGBG has modest activity as an inhibitor of trypanosome *S*-adenosylmethionine decarboxylase (50% inhibitory concentration [IC_{50}], 130 μM), while CGP 40215A was a more active inhibitor (IC_{50}, 20 μM). Preincubation of trypanosomes with CGP 40215A for 1 h caused a reduction in spermidine content (36%) and an increase in putrescine content (20%), indicating that one possible mechanism of its action may be inhibition of polyamine biosynthesis.

Chemotherapy of African trypanosomiasis remains a significant problem, since existing agents have been in continuous use for over 40 years and the incidence of clinical resistance is increasing (28, 31). The only recently developed novel agent to be tested clinically for African trypanosomiasis has been DL-α-difluoromethylornithine (DFMO; Ornidyl, eflornithine [26]), an enzyme-activated inhibitor of ornithine decarboxylase, the initial enzyme of polyamine biosynthesis (22). This agent, although developed as an antitumor agent, has been used successfully against *Trypanosoma brucei gambiense*, the agent of west African trypanosomiasis, but was not curative of *Trypanosoma brucei rhodesiense* infection, the agent of the more acute east African disease (26, 28) and an organism which has exhibited clinical resistance to DFMO as well as standard trypanocides (5, 7, 28, 31).

Recently, Ciba-Geigy Ltd. (Basel, Switzerland) has undertaken synthesis and development of a series of aryl and heteroaryl bis(guanylhydrazones) (24, 25, 27), some of which have shown potential as antitumor agents (23). These agents are inhibitors of *S*-adenosylmethionine (AdoMet) decarboxylase, a key enzyme in polyamine synthesis, and are analogs of methylglyoxal bis(guanylhydrazone) (MGBG), used previously in cancer chemotherapy but now in limited use because of its toxicity (21, 29). The new agents closely resemble trypanocidal diamidines (Fig. 1), and a series of them have been tested against trypanosome isolates in vitro (10). Several of these agents have shown significant levels of activity (50% inhibitory concentrations [IC_{50}s], <1 μM) as potent inhibitors of trypanosome growth.

In this report we describe the activities of the most active bis(guanylhydrazones) against trypanosome isolates in model laboratory infections with clinical isolates of *T. brucei rhodesiense* and *T. brucei gambiense* and with veterinary isolates of *Trypanosoma congolense* and *Trypanosoma brucei brucei.*

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MATERIALS AND METHODS

Animals. Female Swiss Webster mice (20 to 25 g) were purchased from Taconic Farms, Germantown, New York, and Swiss ICR mice (25 to 30 g females) were purchased from the Institut für Zuchthygiene, Zurich, Switzerland.

Trypanosome strains. The following clinical isolates of *T. brucei rhodesiense* were obtained from A. R. Njogu of the Kenya Trypanosomiasis Research Institute (KETRI; Muguga, Kenya): KETRI 243, 269, 1992, 2002, 2285, 2537, 2538, 2636, 2708, and 2772. Strains refractory to DFMO are KETRI 243 and 269; arsenical-drug-refractory strains are 243, 1992, and 2708; strains 243, 269, 1992, and 2636 are resistant to diamidines (5).

KETRI 243 As3 is a cloned subpopulation of KETRI 243, which is refractory to arsenical drugs as well as the diamidines diminazene (Berenil) and pentamidine (6). Two *T. brucei rhodesiense* isolates were obtained from the American Type Culture Collection: ATCC 30119, the EATRO 105 strain isolated from a patient in Uganda in 1959, and ATCC 30027, the Wellcome CT strain isolated from a patient in 1934. *T. brucei rhodesiense* STIB 900 is a cloned population isolated in 1982 from a patient in Tanzania. It is susceptible to standard trypanocides.

T. brucei brucei isolates used were the Lab 110 EATRO strain (4) and STIB 950, which is a cloned population showing multidrug resistance (to diminazene, isometamidiam, and quinapyramine). It is a derivative of CP 2469 isolated in 1985 from a bovine in Somalia. S427 is a clone derived from an isolate from a Ugandan sheep (13). Infection of mice with *T. brucei brucei* TREU 667 provides a central nervous system (CNS) model infection (18, 19), which we have used previously (4, 6), and is the only CNS model used in this study. *T. brucei gambiense* (STIB 930) is a derivative of TH-1/78E(031), which was isolated in 1978 from a patient in Ivory Coast (20). One other veterinary parasite was also tested: *T. congolense* (STIB 910), which is a cloned derivative of STIB 249 originally isolated in 1971 from a lion in Tanzania.

Drug studies. For studies of acute infections, groups of five mice were usually used. Animals were infected intraperitoneally (i.p.) with 2.5×10^5 trypanosomes from an infected rat or from culture, and the infection was allowed to progress for 24 h before treatment was begun. Unless otherwise stated, i.p. dosing was used. Animals were monitored daily for deaths and weekly for trypanosomes in blood smears (6). Those surviving >30 days beyond the death of untreated controls with no parasites in blood samples were considered cured (5).

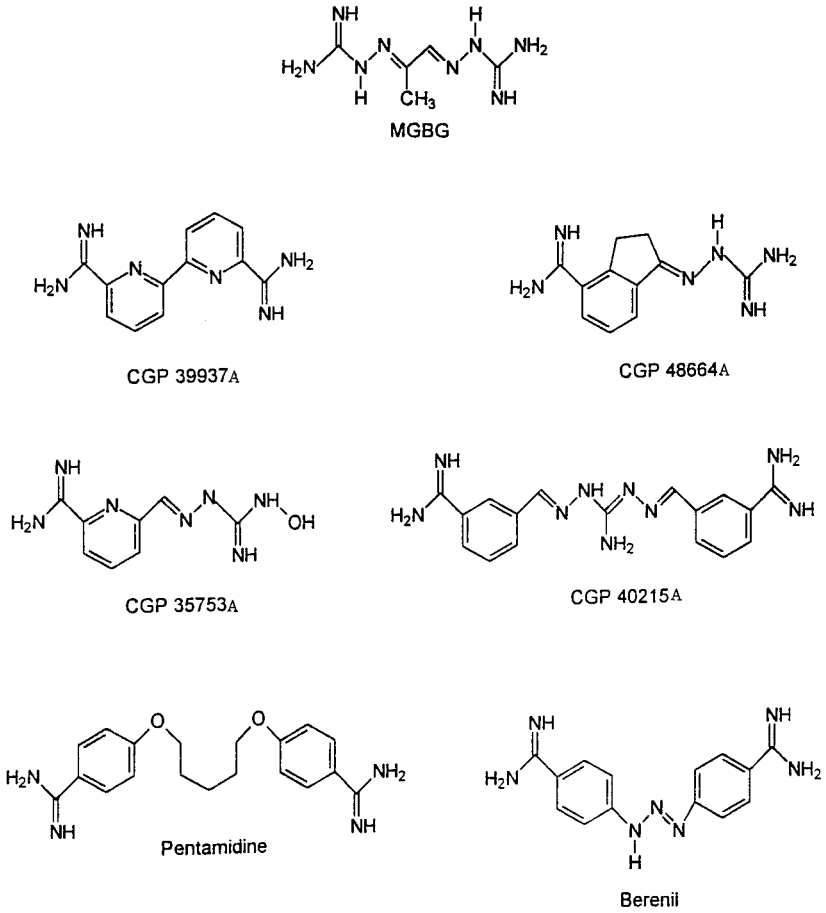

FIG. 1. Structural formulas of Ciba-Geigy analog MGBG and diamidine trypanocides diminazene (Berenil) and pentamidine.

The TREU 667 model was used to gauge the potential efficacy of agents against late-stage CNS infections (18, 19). In these studies, mice were infected i.p. (10^4 trypanosomes per mouse) and the infection was allowed to develop for 21 days before treatment was initiated. After checking each animal for parasitemia, mice were randomly placed in groups of five for treatment. Each experiment included a control group treated with diminazene, which initially clears parasites from peripheral blood but is noncurative for late-stage infection (18). As the blood parasitemia reoccurred during treatment, animals were removed from cages and sacrificed. Animals were considered cured if they survived and were aparasitemic for at least 180 days after the end of treatment. Most cured animals were observed for more than 210 days posttreatment. In these studies, DFMO was administered in the drinking water for 14 days. Animals administered a dose of 2% DFMO consume an average of 5 ml/day for a dose rate of 5 g/kg of body weight/day (6). CGP 40215 in these experiments was administered once daily i.p. or in subcutaneously implanted miniosmotic pumps (Alza Corp., Palo Alto, Calif.), which dispense a measured volume of drug continuously for 7 or 14 days. These pumps contain 200 μl of concentrated drug solution and dispense 1 μl/h for 7 days or 0.5 μl/h for 14 days. The solubility of CGP 40215A in distilled water was sufficient to allow a dosage of 50 mg/kg/day for 7 days to be prepared.

Chemicals. CGP compounds were synthesized and made available by the Pharmaceuticals Division, Ciba-Geigy Ltd. DFMO was synthesized by and a gift of the Marion Merrell Dow Research Institute (Cincinnati, Ohio). Diminazene aceturate (the active agent of Berenil) was purchased (Calbiochem-Novabiochem Corp., San Diego, Calif.). Pentamidine isethionate was a gift of May & Baker Ltd. (Essex, United Kingdom).

RESULTS

Survey of CGP analogs for in vivo activity. Initial in vivo studies examined those AdoMet decarboxylase inhibitors which were found to be active in vitro (10). These included CGP 35753A, 39937A, 40215A, and 48664A (Fig. 1), which were examined in *T. brucei brucei* model infections (Table 1). The *T. brucei brucei* strains used, Lab 110 EATRO, STIB 950, and S427, are stock laboratory strains used for initial screening and biochemical studies.

Screening of the Ciba-Geigy analogs indicated that CGP 35753A, 40215A, and 48664A were curative of the Lab EATRO 110 strain. CGP 40215A had substantial activity (a 50% cure rate) at 5 mg/kg/day for 3 days and a 100% cure rate at 10 mg/kg/day for 3 days, while CGP 35753A was 80 and 100% curative at 25 and 50 mg/kg/day for 3 days, respectively. CGP 48664A was 60% curative if given for 5 days at a single daily dose of 25 mg/kg or as a daily dose of 25, 50, or 100 mg/kg for 3 days with the dose split into three injections. CGP 40215A was also active at 5 to 20 mg/kg/day against the S427 and STIB 950 isolates. A single dose of 20 mg/kg/day was sufficient for a 75% cure rate (STIB 950), while 10 mg/kg/day for 2 (STIB 950) or 4 (S427) days yielded a 100% cure rate. CGP 39937A was marginally active (with a 20% cure rate) against the Lab 110 EATRO isolate at 25 and 50 mg/kg/day for 3 days (Table 1).

The ability of CGP 40215A to clear potentially overwhelming infections with *T. brucei brucei* Lab 110 EATRO was examined by delaying the start of therapy until 48 or 72 h postinfection, when blood parasitemias averaged 3.9×10^7/ml and 8.1×10^8/ml, respectively (Table 2). When treatment was begun at 48 h, cures were obtained at 10 (a 60% cure rate) and 25 mg/kg/day (a 100% cure rate) for 3 days while treatment begun at 72 h yielded a 40% cure rate with a dosage of 25 mg/kg/day for 3 days. It is important that in this model, death

TABLE 1. Activities of Ciba-Geigy compounds against *T. brucei brucei* isolates[a]

Isolate	Treatment	Dosage (mg/kg/day)	Day(s) of treatment	Mean survival (days)	Survival range (days)	No. of mice cured/total
T. brucei brucei Lab 110 EATRO	None			3.9	3–4	0/10
	CGP 35753A	10	3	9	7–11	0/5
		25	3	11	11	4/5
		50	3			5/5
	CGP 39937A	10	3	6.6	4–10	0/5
		25	3	10.3	10–12	1/5
		50	3	7	7	1/5
	CGP 40215A[b]	1	3	6.5	3–14	0/15
		2.5	3	12.2	8–19	5/10
		5	3	19.5	17–22	13/15
		10	3			10/10
		1	5	10.1	4–21	0/10
		2.5	5	32	32	4/5
		5	5	32	32	9/10
		10	5			5/5
	CGP 48664A	25 (i.p.)	3	8	7–11	1/5
		50 (i.p.)	3	10.5	5–14	1/5
		100 (i.p.)	3	7	3–11	2/5
	CGP 48664A	5 (i.p. BID)	3	4	4	0/5
		10 (i.p. BID)	3	4.2	4–5	0/5
		25 (i.p. BID)	3	10	8–12	1/5
		10 (i.p.)	5	9.2	4–17	0/5
		25 (i.p.)	5	14	4–24	3/5
		25 (i.p. TID)	3	12	12	4/5
		50 (i.p. TID)	3			5/5
		100 (i.p. TID)	3			5/5
T. brucei brucei S427[c]	None			4	4	0/5
	CGP 40215A	10 (s.c.)	4			5/5
T. brucei brucei STIB 950	None			11.3	6–17	0/4
	CGP 40215A	5	4			4/4
		10	2			4/4
		20	1	21	21	3/4

[a] Mice (2.5×10^5 parasites) were infected, and the infections were allowed to progress for 24 h before treatment began. CGP compounds were dissolved in distilled water and injected i.p. or subcutaneously (s.c.) once, twice (BID), or three times (TID) daily for 3, 4, or 5 days as indicated. Animals surviving >30 days beyond the death of the last control with no parasites in tail blood smears were considered cured. Mean survival (days) is exclusive of uncured animals.

[b] The Wilcoxon rank test was applied to data for CGP 40215A and indicated that at 2.5 mg/kg, this agent is statistically significantly active ($P = 0.02$, $n = 10$) and that at the curative dose of 5 mg/kg, P improves to 0.01 ($n - 10$).

[c] Inoculum was 5×10^4 parasites.

TABLE 2. Effects of delayed dosing on ability of CGP 40215A to cure *T. brucei brucei* Lab 110 EATRO[a]

Treatment (mg/kg/day)	Time started (h postinfection)	Mean survival (days)	Survival range	No. of mice cured/total
None		3.6	3–6	0/5
5	24			5/5
10	24			5/5
25	24			5/5
5	48	6.1	4–24	7/15
10	48	4	4	8/15
25	48			15/15
5	72	3.4[b]	3–4	0/5
10	72	4[c]	4	1/5
25	72	4[d]	4	2/5

[a] Mice, in groups of five, were infected with 2.5×10^5 trypanosomes. Treatment was begun 24, 48, or 72 h after infection and consisted of three single daily, i.p. injections of drug. Hemocytometer counts of parasites in tail vein blood were done daily. Mean survival (in days) is exclusive of uncured animals.

[b] Two animals received one dose; none lived to receive three doses.

[c] Four animals received one dose, one lived to receive three doses.

[d] Three animals received one dose, two lived to receive three doses.

occurs when parasitemia reaches $\sim 10^9$/ml and that all animals surviving to receive the full course of three daily doses of 25 mg/kg were cured. In one experiment, trypanosome counts were taken just prior to treatment and 4 h after each dose, using a treatment regimen beginning at 48 h. Parasitemias continued to rise until 4 h after the second dose of 10 or 25 mg/kg and then began to decline. The parasites were cleared 4 h after the third dose of 25 mg/kg and substantially reduced (from 1.2×10^9/ml to $<10^7$/ml) for the 10-mg/kg group. This would indicate that CGP 40215A is toxic to trypanosomes after a 48-h exposure, which is supported by the in vitro finding that a 1- to 2-day exposure to 2 μM is needed to block viability (10).

Activity of CGP 40215A against clinical and veterinary isolates. A group of 15 isolates of *T. brucei rhodesiense*, many resistant to the standard trypanocides pentamidine, melarsoprol, and DFMO (5), were tested for susceptibility to CGP 40215A in acute infection models. Table 3 summarizes the results of these experiments as the lowest doses yielding at least a 75% cure rate. Generally, untreated infected control mice died 3 to 8 days after infection, depending on the isolate. Treatment with CGP 40215A once daily for 3 days with up to 25 mg/kg resulted in cures of all strains. Infections with most strains were cured by three doses of ≤10 mg/kg, although two

TABLE 3. Summary of activities of CGP 40215A against laboratory infections of clinical isolates and a veterinary parasite[a]

Strain	Minimal curative dosage (mg/kg/day)	No. of doses
T. brucei rhodesiense		
KETRI 243	10	3
KETRI 269	10	3
KETRI 1992	25	3
KETRI 2002	5	3
KETRI 2285	5	3
KETRI 2537	25	3
KETRI 2538	5	3
KETRI 2545	5	3
KETRI 2636	25	3
KETRI 2708	25	3
KETRI 2772	5	3
KETRI 243 As3	25	3
EATRO 105	2.5	3
Wellcome CT	2.5	3
STIB 900	10	2
T. brucei gambiense STIB 930	20	1
T. congolense STIB 910	10	2

[a] Mice in groups of four (STIB isolates) or five (all others) were infected (2.5×10^5 trypanosomes per mouse), and treatment was begun 24 h postinfection. Minimal curative doses were those giving ≥75% cure rates. The KETRI, EATRO 105, and Wellcome strains were tested with groups receiving 1, 2.5, 5, 10, and 25 mg/kg i.p. for 3 days. STIB isolates were tested with groups receiving 1×20 mg/kg, 2×10 mg/kg, or 1×5 or 4×5 mg/kg. The data summarize repeated experiments with most isolates. Animals were considered cured after surviving >30 days beyond the death of the last untreated control.

of the arsenical drug refractory strains, KETRI 1992 (arsenical drug and diamidine resistant) and KETRI 243 As3 (an arsenical-drug- and diamidine-resistant clone of KETRI 243), responded to 25 mg/kg/day, the highest dosage used. In model infections, *T. brucei gambiense* and *T. congolense* isolates were also susceptible to CGP 40215A, which was curative at a single daily dose of 20 mg/kg or two daily doses of 10 mg/kg, respectively.

The responses of five of the KETRI isolates to a single 20-mg/kg dose 24 h postinfection were also examined. Infections with KETRI 2537 and 269 (diamidine resistant) were completely cured (five of five animals) by a single 20 mg/kg dose, while KETRI 243 (multidrug resistant)-infected animals had a 60% survival rate with this dose. KETRI isolates 2636 (diamidine resistant) and 1992 (multidrug resistant) were not cured with this dose.

CNS model infection. CGP 40215A was tested for activity against the *T. brucei brucei* TREU 667 CNS model infection. Drug treatment began at 21 days postinfection when a CNS infection was established. Diminazene, which will cure a 1-day infection (one dose of 40 mg/kg), will clear blood parasites if administered on day 21, but the parasites will repopulate the blood from a CNS reservoir of infection. Diminazene is effective against peripheral blood parasitemia but not against parasites in brain tissue (18).

CGP 40215A was curative for this strain with treatment for an acute infection (2.5 mg/kg/day for 5 days, beginning 24 h postinfection [data not shown]) but was not curative in the CNS model when used singly at 5 to 25 mg/kg/day i.p. for 7 or 14 days or for 7 days in continuous dosing at 25 or 50 mg/kg/day (Table 4). DFMO, administered at 2% in drinking water for 14 days (5 g/kg/day), was also not curative. However, combinations of DFMO and CGP 40125A were highly synergistic.

TABLE 4. Treatment of *T. brucei brucei* TREU 667 CNS model infections[a]

Drug	Concentration (mg/kg/day)	Day(s) of treatment	No. of mice cured/total	Cure rate (%)
Diminazene	40	1	15/15	100
	40	21	0/15	0
CGP 40215A	5	21–27	0/5	0
	10	21–27	0/10	0
	25	21–27	0/10	0
	25	21–23	0/5	0
	5	21–34	0/5	0
	10	21–34	0/10	0
	25	21–34	0/10	0
	25*	21–27	0/5	0
	50*	21–27	1/5	20
2% DFMO plus CGP 40215A		21–34	0/15	0
	25	21–23	1/5	20
	25	28–30	4/5	80
	25	35–37	4/5	80
	5	21–27	3/5	60
	10	21–27	8/10	80
	25	21–27	7/9[b]	78
	50*	21–27	5/5	100
2% DFMO plus CGP 40215A		21–34		
	5	21–34	3/4[b]	75
	10	21–34	5/5	100
	25	21–34	8/10	80
	5	28–34	1/5	20
	10	28–34	6/10	60
	25	28–34	4/5	80
	5	35–41	4/5	80
	10	35–41	4/5	80
	25	35–41	4/4[b]	100

[a] Mice were inoculated with 10^4 trypanosomes, and the infections were allowed to develop for 21 days before treatment was begun (day 1). Diminazene and CGP 40215A were given once daily i.p. as indicated; doses marked with an * were given through Alza miniosmotic pumps, implanted subcutaneously. DFMO was given in the drinking water at the percentage listed. When two treatment periods are given beside the drug name, the first refers to DFMO and the second refers to CGP 40215A. Animals surviving >180 days beyond the last day of treatment with no parasites in weekly blood examinations were considered cured.

[b] Animals dying within 3 days of the beginning of treatment were removed from the groups and not included in the final results.

Several types of single daily dose schedules were used with CGP 40215A, in combination with a 14-day continuous DFMO dose regimen: 3, 7, or 14 days, beginning concurrently with DFMO (days 21 to 23, 21 to 27, or 21 to 35), and 3 or 7 days, beginning in the middle (days 28 to 30 or 28 to 35) or at the end (days 35 to 37 or 35 to 41) of DFMO administration. Sequential administration of DFMO and CGP 40215A was suggested by the success of recent studies using DFMO in combination with suramin, melarsoprol, or diamidines (6, 16, 17). Dosing with DFMO plus 3-day, 25 mg/kg/day CGP 40215A regimens yielded a 20% cure rate if the agent was given early in the DFMO regimen (day 21 to 23) but an 80% cure rate was obtained when dosing was delayed until day 28 or day 35 (Table 4). Dose regimens of 10 and 25 mg/kg/day at the beginning (days 21 to 27) or middle (days 28 to 34) of DFMO administration for 7 days yielded 60 to 100% cure rates, while a 5 mg/kg, 7-day dose schedule administered at the end of DFMO treatment (days 35 to 41) resulted in an 80% cure rate.

The latter dosage is also consistent with the 100% cure rate obtained with 10 and 25 mg/kg/day at the end of DFMO treatment. Increasing the CGP 40215A administration period to 14 days resulted in a similarly high (80 to 100%) cure rate for dosages of 5, 10, and 25 mg/kg/day. In these studies it is interesting that a 3-day treatment regimen with CGP 40215A in combination with 2 weeks of DFMO produced results equivalent to those obtained with 1- and 2-week CGP 40215A regimens in combination with 2 weeks of DFMO, especially if the CGP 40215A treatment was begun at the middle or end of the DFMO administration period. This suggests that only short regimens of CGP 40215A may be needed if they are delayed at least until the midpoint of DFMO treatment.

Another dose regimen employed with CGP 40215A was continuous infusion with Alza miniosmotic pumps implanted subcutaneously. The dose regimens used, 25 and 50 mg/kg/day for 7 days, were not curative when used singly. However, in combination with 2% DFMO, CGP 40215A at 50 mg/kg for 7 days was 100% effective.

Polyamine synthesis. The ability of MGBG analogs to inhibit the AdoMet decarboxylase activity of several isolates was examined with crude enzyme preparations of *T. brucei brucei* Lab 110 EATRO by a standard assay (1). CGP 39937A, CGP 40215A, and CGP 48664A are inhibitors of mammalian AdoMet decarboxylase and inhibited the enzyme from trypanosome isolates at 3.4, 20.3, and 6.9 μM, respectively. This was approximately 10- to 100-fold less activity than obtained with the rat liver enzyme (24, 25, 27). CGP 40215A, the most active of the analogs in vivo, was less effective (IC_{50}, 20.3 μM) as an AdoMet decarboxylase inhibitor than the other analogs.

A related study examined the effect of CGP 40215A on polyamine synthesis in intact trypanosomes. Bloodstream trypanosomes were harvested and purified from rat blood (1). Trypanosomes (10^8/ml) were pretreated for 1 h with 10 μM CGP 40215A in PSG-BSA (70 mM phosphate-buffered 43 mM NaCl, 1% glucose, 1% bovine serum albumin plus 50 μl of penicillin per ml plus 50 μl of streptomycin per ml [pH 7.8]) washed and incubated with 3.3 μCi of L[2,3-^{3}H(*N*)ornithine (New England Nuclear) and without drug for 1 h. Acid extracts (10% trichloroacetic acid) were analyzed for polyamines by a standard high-performance liquid chromatography assay with radiodetection (1). Spermidine levels were reduced by an average of 36% in CGP 40215A-treated cells, while putrescine levels increased 20% compared with those of controls (two experiments). AdoMet decarboxylase activity in cell extracts from cells incubated under these conditions was reduced by 68% (137 versus 44 pmol of CO_2/mg of protein/h).

DISCUSSION

Inhibition of polyamine metabolism as a means of antitrypanosomal chemotherapy has been successful with the ornithine decarboxylase inhibitor DFMO and the AdoMet decarboxylase inhibitor MDL 73811 (2, 8, 9). MGBG used alone at up to 25 mg/kg for 3 days, was not curative for the Lab 110 EATRO model of *T. brucei brucei* (3), resulting only in moderate (10- to 15-day) prolongation of life. In combination with DFMO, MGBG was antagonistic (3), presumably by elevating blood putrescine levels through inhibition of diamine oxidase activity (15). In contrast, several of the series of aromatic MGBG analogs used here were curative for laboratory infections, while the most active agent, CGP 40215A, was strongly synergistic with DFMO against a model CNS infection. MGBG was also examined for activity against five KETRI isolates at 25 mg/kg for 3 days. Only marginal prolongation of life was found (~4 days, data not shown).

The strains of *T. brucei rhodesiense* used are noteworthy in that all are clinical isolates and several were refractory to standard agents. KETRI 243 is moderately resistant to arsenical drugs and diamidines, while a clone (KETRI 243 As3) derived from this parent is highly resistant to melarsen oxide, diminazene, and pentamidine (in vivo curative dosage, >25 mg/kg/day for 3 days [data not shown]). KETRI 269 is DFMO and pentamidine refractory, while KETRI 1992 is also arsenical drug resistant (5).

The most active compound in vivo, CGP 40215A, appears to have a selective advantage, since although it was active against rat liver AdoMet decarboxylase (IC_{50}, 0.06 μM [24]), it was far less effective against growth of T24 human bladder carcinoma cancer cells in vitro (IC_{50}, >100 μM) than other derivatives in the same series (27). In data reported elsewhere, CGP 40215A was also a more effective inhibitor of trypanosome metabolism in vitro (IC_{50}, ~0.0045 μM) than were other MGBG analogs in the series (10).

Used alone, CGP 40215A was curative for 15 *T. brucei rhodesiense* strains as laboratory infections, as well as for one *T. brucei gambiense* and one *T. congolense* isolate. The 3-day dose regimens used for most experiments proved adequate for cures; however, single doses of 20 mg/kg were also curative and are of prime interest since they indicate that shorter treatment regimens are possible. In these studies, we observed no overt toxicity (e.g., ruffling of hair or lethargy) at the highest dosages utilized, 50 or 100 mg/kg for 3 days for acute infections, and 25 mg/kg for 7 or 14 days i.p. or 50 mg/kg for 7 days administered with Alza pumps (CNS infection). At i.p. dosages of 50 or 100 mg/kg/day for 14 days, deaths were observed toward the end of the dosage period (data not shown).

Although CGP 40215A was inactive when used singly against a model CNS infection, it was highly synergistic when used in combination with noncurative doses of DFMO. Three-day dosing with CGP 40215A in combination with 2 weeks of DFMO appeared to be as active as 7- or 14-day dosing if CGP 40215A was given in the middle or at the end of the DFMO regimen. The synergism appears to be unique to CNS infections, since subcurative doses of DFMO (0.25 or 0.5% for 3 days in drinking water) and CGP 40215A (0.5, 1.0, or 2.5 mg/kg daily for 3 days [data not shown]) were not synergistic against the *T. brucei brucei* Lab 110 EATRO model or against four of the KETRI isolates. Strong synergism between DFMO and other trypanocides in model CNS infections has been noted in studies utilizing DFMO plus suramin (4, 6, 12), DFMO plus melarsen oxide (6, 16), and DFMO plus diminazene or pentamidine (17). Interestingly, suramin alone or the diamidines are also inactive against laboratory CNS infections in clinical studies (4, 30).

CGP 40215A is a bicyclic analog of MGBG, which resembles the diamidine trypanocides diminazene and pentamidine (Fig. 1). The Ciba-Geigy agents were developed to take advantage of MGBG's activity as a potent AdoMet decarboxylase inhibitor while minimizing toxicity due to nonspecific antimitochondrial effects and inhibition of diamine oxidase (24, 25, 27). Since the nonspecific and cytotoxic effects have been reduced, these MGBG analogs tend to have low IC_{50}s for AdoMet decarboxylase (<1.0 μM) and more specific growth inhibitory properties for tumor cells (23). The strong resemblance between CGP 40215A and the diamidines led us to include the multidrug-resistant strain KETRI 243 As3 in the study. This arsenical-drug-resistant strain is also refractory to pentamidine at >50 mg/kg/day for 3 days, yet infections were cured by CGP 40215A dosages of 25 mg/kg/day for 3 days. At 10 mg of CGP 40215A per kg per day, 30% cures were also obtained with this strain (data not shown). Since diamidines and melarsen-based

arsenical drugs appear to enter trypanosomes via a common purine transporter (11, 14), CGP 40215A may enter trypanosomes through a different site. This is an important consideration in developing new agents for human and veterinary parasites which have been treated for over 40 years with existing agents.

In summary, members of a series of aromatic MGBG analogs were active trypanocides in model infections. CGP 40215A, a bicyclic MGBG analog and inhibitor of AdoMet decarboxylase, was effective in curing laboratory infections of *T. brucei brucei*, *T. brucei rhodesiense*, *T. brucei gambiense*, and *T. congolense* when used singly at 5 to 25 mg/kg/day. In combination with the ornithine decarboxylase inhibitor DFMO, CGP 40215A was strongly synergistic in curing a model CNS infection at dosages which alone were not effective against this model. Since several isolates used in the study were resistant to standard trypanocides, CGP 40215A appears to be an excellent candidate for continued studies with human and veterinary trypanosomiases.

ACKNOWLEDGMENTS

This work was supported in part by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (920204, to C.J.B.; 930707, to R.B.; and 920117, to S.L.C.) and by National Institutes of Health grant AI 17340 (to C.J.B.).

We thank Michael Huening, Louis Jimenez, Peter Lyte, Edward Milman, Donna Rattendi, Diane Snowdon, Louis Vargas, and Vanessa Yardley for technical assistance.

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