ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1991, p. 1403–1407
0066-4804/91/071403-05$02.00/0

Vol. 35, No. 7

Suppression of *Leishmania donovani* by Oral Administration of a Bis(benzyl)polyamine Analog

R. JEFFREY BAUMANN,* PETER P. McCANN, AND ALAN J. BITONTI

Marion Merrell Dow Research Institute, 2110 East Galbraith Road, Cincinnati, Ohio 45215

Received 28 January 1991/Accepted 26 April 1991

We reported previously that intraperitoneal administration of a bis(benzyl)polyamine analog, MDL 27,695, suppressed both pentavalent antimony (Sb^v)-susceptible and -resistant *Leishmania donovani* in vivo. The present studies were performed to optimize parasite suppression by parenteral administration and to evaluate the efficacy of oral treatment with MDL 27,695. *L. donovani* infections in BALB/c mice were suppressed >99% after intraperitoneal dosing for 20 days with a total dose of 150 mg of MDL 27,695 per kg of body weight or 560 mg of Sb^v per kg. Suppression was not increased by a total dose of 400 mg of MDL 27,695 per kg given for 20 days. In mice treated for 2, 4, or 7 days with either MDL 27,695 or Sb^v (total doses of 60, 120, and 210 mg/kg, respectively), more liver parasites were killed with MDL 27,695 than with Sb^v. Assessment of livers posttreatment showed that parasite killing continued for at least 3 days in MDL 27,695-treated mice but not for longer than 1 day in Sb^v-treated mice. Intramuscular administration of drugs resulted in 92% parasite suppression by MDL 27,695 (15 mg/kg three times per day for 5 days) and 64% suppression by Sb^v (60 mg/kg once per day for 5 days). Dosing of mice by oral gavage with 100 mg of MDL 27,695 per kg twice per day for 14 days resulted in 99.7% parasite suppression, and the 50% effective dose was approximately 11 mg of MDL 27,695 per kg. MDL 27,695 represents an effective new drug potentially useful for oral or parenteral treatment of visceral leishmaniasis.

Leishmaniasis causes considerable morbidity and mortality worldwide (3). Three types of the disease are recognized: cutaneous, mucocutaneous, and visceral leishmaniases, all of which are transmitted to humans from sand flies during blood meals taken by these insects. *Leishmania donovani*, the etiologic agent of visceral leishmaniasis, is an obligate intramacrophage trypanosomatid infecting the reticuloendothelial systems of humans and other mammals (21). The first-line drugs for treating leishmanial infections are pentavalent antimonial agents (Sb^v) such as sodium stibogluconate (Pentostam) and *N*-methylglucamine antimonate (Glucantime). Although these drugs remain generally useful, there are several drawbacks to their use. They must be administered parenterally for relatively long periods, and they sometimes produce toxic reactions (3). A more rapidly acting, orally effective drug seems desirable. Allopurinol has some activity against visceral leishmaniasis when administered orally but is much more effective when used in combination with Sb^v (8). The availability of a drug that is effective alone would be preferable for helping to control this disease. Also, leishmaniasis clinically refractory to Sb^v has been reported (3, 7), and resistance is frequently considered to be due to Sb^v-resistant parasites (15) by a mechanism(s) not clearly defined. Therefore, a continued search for new chemotherapeutic strategies is warranted.

Interferences with polyamine biosynthesis and function are two approaches that have led to discovery of antiprotozoal agents. Eflornithine, an irreversible inhibitor of ornithine decarboxylase, the first enzyme in polyamine biosynthesis, has been used successfully in hundreds of cases of human trypanosomiasis, even those infections refractory to standard trypanocidal drugs (22). More recently, we showed that administration of an irreversible inhibitor of another key enzyme in polyamine biosynthesis, *S*-adenosyl-L-methionine decarboxylase, cured mice infected with a multidrug-resistant strain of *Trypanosoma brucei rhodesiense* and was approximately 100 times as potent as eflornithine against murine *T. brucei brucei* (4). Bis(benzyl)polyamine analogs, which may interfere with the intracellular function of the natural polyamines, were found to inhibit proliferation of the malarial parasite *Plasmodium falciparum* in vitro and, in combination with eflornithine, cured *P. berghei* infections in mice (5). *L. donovani* has also been shown to be susceptible to eflornithine (12, 18), as well as to the bis(benzyl)polyamine analog MDL 27,695 (1).

We reported recently that *L. donovani* was suppressed 99.9% by a total dose of 300 mg of MDL 27,695 per kg of body weight given for 10 days (1). In the present studies, some dosing regimens were extended to 20 days, and even though parasite suppression did not reach 100%, we did achieve 99.8% suppression with a total dose of 150 mg of MDL 27,695 per kg, one-half of the dose needed previously for comparable suppression. We further demonstrated that MDL 27,695, when given alone by oral gavage, is effective against visceral leishmaniasis in mice, with suppression as high as 99.7%.

MATERIALS AND METHODS

Drugs. MDL 27,695, a bis(benzyl)polyamine analog, is the tetrahydrochloride salt of *N*,*N*′-bis{3-[(phenylmethyl)amino]propyl}-1,7-diaminoheptane and was synthesized in our laboratories (11). The molecular weight of MDL 27,695 is 571; see reference 1 for its chemical structure.

Parasites. *L. donovani* (Ethiopian strain) was obtained from Peter F. Bonventre, University of Cincinnati Medical Center, and maintained by passage every 6 to 8 weeks in Syrian golden hamsters.

Leishmaniasis and assessment of drug efficacy. Methods for inducing visceral leishmaniasis in mice are described elsewhere (1, 6, 19). Male BALB/c mice were infected on day 0

* Corresponding author.

TABLE 1. Suppression of visceral leishmaniasis in mice treated orally with MDL 27,695

Single dose (mg/kg)[a]	Total dose (mg/kg)	No. of liver amastigotes (mean ± SE, 10^7)[b]	Parasite suppression (%)
Vehicle		63.4 ± 0.8	
10	280	32.6 ± 3.6	48.6
25	700	6.4 ± 1.2	89.9
50	1,400	1.4 ± 0.45	97.8
100	2,400	0.18 ± 0.04	99.7

[a] Mice (n = 8) were given 0.2 ml of an aqueous solution by drug of gastric gavage twice per day for 14 days, beginning on day 10. The 100-mg/kg dose was not administered on day 15 or 16.

[b] Liver impression smears were made 3 days after the end of treatment. The number of amastigotes per 500 liver nuclei was counted under oil immersion and the approximate amastigote count per liver = number of amastigotes per nucleus × liver mass in mg × (2×10^5).

TABLE 2. Suppression of visceral leishmaniasis in mice treated for 20 days with MDL 27,695

Treatment[a]	Single dose (mg/kg)	Total dose (mg/kg)	No. of liver amastigotes (mean ± SE, 10^7)[b]	Parasite suppression (%)
Vehicle			56.3 ± 4.3	
MDL 27,695	0.63	25	14.7 ± 2.8	74
	3.75	150	0.13 ± 0.03	99.8
	10	400	0.07 ± 0.03	99.9
Sodium stibogluconate	28	560	0.19 ± 0.07	99.7

[a] Mice (n = 6) were treated intraperitoneally with either MDL 27,695 twice per day or sodium stibogluconate (expressed as milligrams of Sb^V per kilogram) once per day for 20 days, beginning on day 13.

[b] Liver impression smears were made 2 days after the end of treatment, and amastigote counts per liver were determined as described in Table 1, footnote b.

by intravenous injection of 5×10^6 to 8×10^6 amastigotes which had been isolated from the spleens of hamsters (13) and then suspended in Hanks balanced salt solution. Treatments were begun on day 10 or 13, at which time the mean parasite burden in the mice was approximately 1.5×10^8 to 2.5×10^8 amastigotes per liver (see Fig. 1). MDL 27,695 or Pentostam was dissolved in 0.85% saline and administered intraperitoneally; MDL 27,695 was also administered in water by gastric gavage. To assess parasite burdens in treated mice, livers were removed and weighed and impression smears from cut sections were prepared and stained with Diff-Quik reagents. The number of amastigotes per 500 liver nuclei was counted under oil immersion and the number of amastigotes per nucleus × liver mass in mg × (2×10^5) is equal to approximate total number of amastigotes per liver (23). Parasite suppression was calculated from the ratio of the mean liver amastigote counts of drug-treated groups to the mean liver amastigote counts of untreated groups. Parasite killing was calculated from the ratio of the mean liver amastigote counts of drug-treated groups after the end of treatment to the mean liver amastigote counts on the day when treatment was begun.

Mouse spleen amastigotes. To determine whether viable amastigotes were present in the spleens of mice after treatment, infected mice were treated with 15 mg of MDL 27,695 per kg twice per day on days 10 to 14, once per day on days 15 and 16, and again twice per day on days 17 to 21 (22 doses, totaling 330 mg of MDL 27,695 per kg). On days 24, 31, and 38, spleens from small groups of mice (n = 3 to 5) were homogenized and amastigotes were isolated by methods used for hamster spleens (13). Spleens, rather than livers, were homogenized for amastigote isolation, since we were concerned that liver enzymes would be detrimental to amastigotes during the isolation procedure. Moreover, we have shown that MDL 27,695-mediated parasite suppression in the liver was paralleled by equal suppression in the spleen (1). The pellets were suspended in 1.5 ml of Hanks balanced salt solution, and 0.5 ml was combined with 4.5 ml of Eagle minimal essential medium supplemented as reported elsewhere (2) and incubated at 26°C for 72 h to induce amastigote-to-promastigote transformation. Infectivity was determined by injecting groups of three naive hamsters per infected mouse via the intracardial route with 0.2 ml of homogenate, and 5 to 6 weeks later, liver impression smears were prepared and examined for amastigotes.

RESULTS

Oral treatment. Preliminary experiments indicated that MDL 27,695 was orally active against visceral leishmaniasis in mice. These data were confirmed in a dose-response study in which 48.6 to 99.7% parasite suppression was achieved following a 14-day dosing regimen, with a 50% effective oral dose of approximately 11 mg of MDL 27,695 per kg per single dose (Table 1). The drug had been administered twice per day by gavage to maximize parasite suppression.

Twenty-day dosing. We recently showed that treatment with MDL 27,695 for 5 days suppressed parasites 96% and treatment for 10 days suppressed parasites 99.9% (1). Those data suggested that if animals were dosed for 20 days, parasite suppression might reach 100%, and if not, the 50% effective dose of MDL 27,695 might be lowered by the longer dosing regimen. After 20 days of dosing with a total of 400 mg of MDL 27,695 per kg, parasite suppression remained

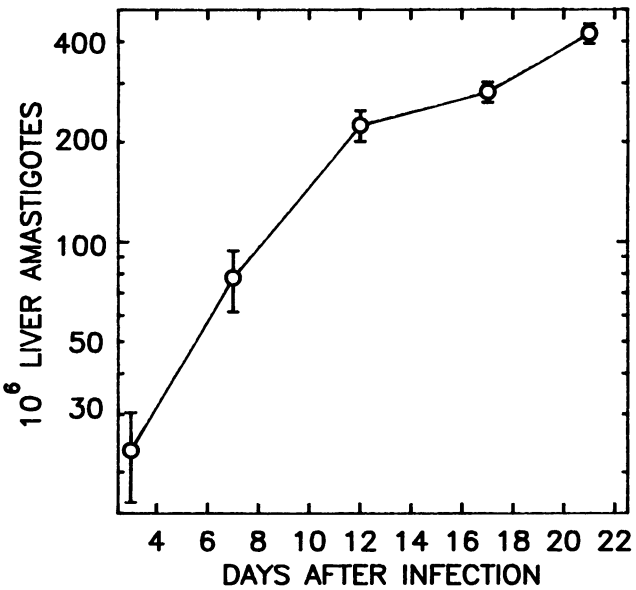

FIG. 1. Male BALB/c mice (n = 6) were infected intravenously with 7×10^6 *L. donovani* amastigotes (day 0). Liver impression stains (Diff-Quik) were prepared on the indicated days postinfection. Amastigote counts per liver were determined as described in Table 1, footnote b. Bars represent standard errors of the means.

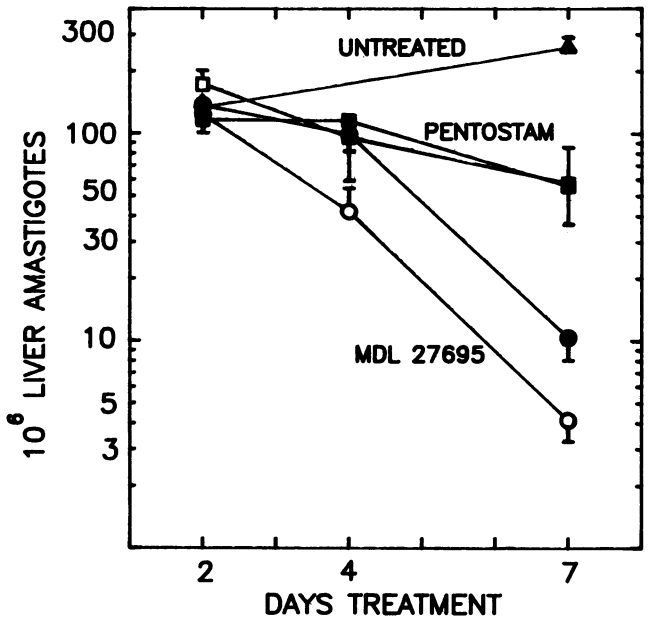

FIG. 2. Male BALB/c mice (n = 5) were infected intravenously with 7×10^6 *L. donovani* amastigotes (day 0). On day 10, treatment was begun intraperitoneally with 15 mg of MDL 27,695 per kg twice per day or 105 mg of sodium stibogluconate (Pentostam) (30 mg of Sb^v) per kg once per day. Liver impression stains for parasite counts were prepared 1 (● and ■) or 3 (○ and □) days after the end of treatment. Parasites in untreated mice (▲) were counted on days 10 and 19. Amastigote counts per liver were determined as described in Table 1, footnote *b*. Bars represent standard errors of the means.

99.9% (Table 2). However, a total dose of 150 mg/kg given over the 20-day period suppressed parasites 99.8%. The 50% effective dose for this regimen was estimated to be 0.4 mg/kg per single dose, considerably lower than the previous value of 2.5 mg/kg per single dose in mice dosed three times per day for 5 days (1).

Parasite suppression and killing. The increase in liver amastigotes in untreated mice for the 3 weeks following infection is shown in Fig. 1. This experiment shows that on days 10 and 13, which correspond to the times when treatments were begun in all experiments, liver amastigote numbers continued to increase and that the level of infection was at least 10^8 amastigotes per liver. To ascertain the suppressive activity of MDL 27,695 in comparison with Sb^v over a time course, mice were treated with equal total doses of MDL 27,695 and Sb^v for 2, 4, or 7 days. As shown in Fig. 2, the mean liver parasite counts declined more rapidly in mice treated with MDL 27,695 than in mice treated with Sb^v when parasite counts were made either 1 or 3 days after the end of treatment. After the 2-day treatment period, parasite counts were about the same for each drug at either 1 or 3 days posttreatment. However, after the 4- and 7-day treatment periods, parasite counts continued to decline between 1 and 3 days posttreatment in mice treated with MDL 27,695 but not in mice treated with Sb^v. The percentage of parasites killed was calculated by using the amastigote count on day 10 (1.47×10^8 per liver; Fig. 1) and counts obtained 3 days posttreatment (Fig. 2). The data in Table 3 show that parasites were killed more rapidly in mice treated with MDL 27,695 than in mice treated with Sb^v.

Intramuscular administration. MDL 27,695 and Sb^v were administered to mice infected by the intramuscular route in the hind leg. MDL 27,695 (15 mg/kg three times per day for 5 days) suppressed parasites 92%, while Pentostam (60 mg of Sb^v/kg once per day for 5 days) suppressed parasites only 64%. Parasite suppression of 92% by MDL 27,695 compares favorably with the suppression we reported previously (83 to 96%) after intraperitoneal administration of the same total dose of MDL 27,695 (225 mg/kg). However, to suppress parasites within that range after oral administration required a total dose of 700 mg/kg (Table 1).

TABLE 3. Estimates of parasite killing during treatment[a]

Days of treatment	No. of parasites per liver (10^8)		No. of parasites killed (%)[b]		Parasite suppression (%)	
	MDL 27,695	Sb^v	MDL 27,695	Sb^v	MDL 27,695	Sb^v
2	1.23	1.72	16	0	37	12
4	0.42	0.95	71	35	81	58
7	0.04	0.57	97	61	99	79

[a] Details were described in the legend to Fig. 2, except that all values are for 3 days after the end of treatment.
[b] Based on a mean of 1.47×10^8 parasites per liver on the day when treatment was started (day 10 [Fig. 1]).

Viability and infectivity of amastigotes. Few, if any, amastigotes were seen in liver impression smears from mice treated with sufficient MDL 27,695 to suppress parasites >99%, and most of those amastigotes were smaller or morphologically atypical in comparison with amastigotes in untreated mice. This curious observation raised some concern regarding amastigote viability. We therefore examined spleen homogenates to determine whether viable amastigotes could be recovered qualitatively from at least one component of the reticuloendothelial system following drug treatment (Table 4). In liver smears from 11 treated mice, only two amastigotes were seen, i.e., one at 10 days and one at 17 days posttreatment. These data were presumptive evidence that nine mice were cured by the drug, because no amastigotes were seen per 500 liver nuclei. However, the absence of viable amastigotes in these nine mice was only apparent, since amastigote-to-promastigote transformation occurred in all of the culture broths inoculated with spleen homogenates. Furthermore, all naive hamsters injected with spleen homogenates became infected, as shown by the

TABLE 4. Viability of parasites in mouse spleen homogenates

Day posttreatment[a]	Drug treatment (no. of mice)[b]	Mean no. of liver amastigotes[c] (10^7)
3	+ (3)	0
	− (1)	4.7
10	+ (3)	0.02
	− (1)	1
17	+ (5)	0.01
	− (1)	0.7

[a] Mice were dosed intraperitoneally with 15 mg of MDL 27,695 per kg twice per day for 12 days (days 10 through 21), except on days 15 and 16, when the dose was given once per day.
[b] +, treated; −, not treated.
[c] Liver impression smears were made at the indicated days posttreatment; numbers of amastigotes were calculated as indicated in Table 1, footnote *b*. Amastigote-to-promastigote transformation occurred in culture media inoculated with spleen homogenates, and spleen homogenates induced infections in naive hamsters, under all of the conditions tested.

presence of amastigotes in impression smears prepared from the livers of these animals.

DISCUSSION

In a previous report (1), we showed that parenteral administration of MDL 27,695 significantly suppressed both Sb^v-susceptible *L. donovani* in mice and hamsters and Sb^v-resistant *L. donovani* in hamsters. We now show that oral administration of this polyamine analog to mice suppressed parasites by as much as 99.7%. Since the acute oral 50% lethal dose is >500 mg/kg (unpublished data) and the 50% effective oral dose is 11 mg/kg (a therapeutic index of approximately 50), MDL 27,695 seems to have potential as an orally effective antileishmanial drug. Significant oral activity is an unexpected property of the polyamine analog, since few other orally effective antileishmanial drugs are known. The purine analog allopurinol and allopurinol ribonucleoside are weakly orally active antileishmanial agents (9, 20). In *L. donovani*-infected mice, total estimated doses of 3 to 13 g of allopurinol per kg (given for 14 days in drinking water) resulted in 47% parasite suppression (19). In contrast, the lowest total dose of MDL 27,695 administered by oral gavage (280 mg/kg) for 14 days suppressed *L. donovani* 48%. Administration of allopurinol as a primary agent for treating human visceral leishmaniasis in clinical trials has been correlated with 80% relapses (16). Nevertheless, allopurinol in combination with Sb^v was shown to be useful in curing human visceral leishmaniasis (8, 17). A primaquine analog, WR6026 (19), also has oral activity in mice but has not been tested in humans.

Our data suggest that caution must be exercised in the evaluation of parasite burdens in drug-treated animals. Examination of spleen homogenates from MDL 27,695-treated mice for viable amastigotes showed that even though no amastigotes were seen in liver impression smears, animals were still infected and that small and/or morphologically atypical amastigotes were probably viable. Nevertheless, most of the parasites in these animals were killed by MDL 27,695. It is uncommon to achieve 100% parasite suppression in rodent models used to evaluate antileishmanial drugs (10, 14, 19). Although Gradoni et al. (12) reported 100% parasite suppression in BALB/c mice after treatment with Sb^v (100 mg of Glucantime per kg per day) for 28 days, they did not report testing organ homogenates for viable amastigotes.

During 7 days of treatment, MDL 27,695 killed parasites more rapidly than did Sb^v, and after 4 or 7 days of treatment, parasite killing continued for at least 3 days in MDL 27,695-treated mice but not in Sb^v-treated mice. This suggested that MDL 27,695 was superior to Sb^v in terms of posttreatment parasite killing, and perhaps a lower total dose of MDL 27,695 than of Sb^v may evoke more rapid total parasite elimination from the host. It is likely that parasite killing after treatment was related to residual MDL 27,695 in tissues (1), as well as to the lysosomotropic property of bis(benzyl) polyamine analogs. We observed this interesting property with a structural analog of MDL 27,695 which suppressed *L. donovani* 93% in mice and was found to be lysosomotropic in rat hepatoma (HTC) cells in vitro (unpublished data). In fact, the latter preliminary observation prompted us to examine polyamine analogs for activity against *Leishmania* parasites, since the lysosome is the site of amastigote proliferation in cells permissive to *Leishmania* infection. This unusual affinity of bis(benzyl)polyamine analogs for the lysosomal compartment may point the way for development of agents with similar properties as antileishmanial drugs. The efficacy of the bis(benzyl)polyamine against *L. donovani* by both parenteral and oral routes indicates that exploration of its potential as a new drug should continue.

REFERENCES

1. **Baumann, R. J., W. L. Hanson, P. P. McCann, A. Sjoerdsma, and A. J. Bitonti.** 1990. Suppression of both antimony-susceptible and antimony-resistant *Leishmania donovani* by a bis(benzyl)polyamine analog. Antimicrob. Agents Chemother. **34**:722–727.
2. **Berens, R. L., R. Brun, and S. M. Krassner.** 1976. A simple monophasic medium for axenic culture of hemoflagellates. J. Parasitol. **62**:360–365.
3. **Berman, J. D.** 1988. Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy and future strategies. Rev. Infect. Dis. **10**:560–586.
4. **Bitonti, A. J., T. L. Byers, T. L. Bush, P. J. Casara, C. J. Bacchi, A. B. Clarkson, Jr., P. P. McCann, and A. Sjoerdsma.** 1990. Cure of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* infections in mice with an irreversible inhibitor of *S*-adenosylmethionine decarboxylase. Antimicrob. Agents Chemother. **34**:1485–1490.
5. **Bitonti, A. J., J. A. Dumont, T. L. Bush, M. L. Edwards, D. M. Stemerick, P. P. McCann, and A. Sjoerdsma.** 1989. Bis(benzyl) polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (*Plasmodium falciparum*) in vitro and in combination with α-difluoromethylornithine cure murine malaria. Proc. Natl. Acad. Sci. USA **86**:651–655.
6. **Black, C. D. V., G. J. Watson, and R. J. Ward.** 1977. The use of Pentostam liposomes in the chemotherapy of experimental leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. **71**:350–352.
7. **Bryceson, A. D. M., J. D. Chulay, M. Mugambi, J. B. Were, G. Gachihi, C. N. Chunge, R. Muigai, S. M. Bhatt, M. Ho, H. C. Spencer, J. Meme, and G. Anabwani.** 1985. Visceral leishmaniasis unresponsive to antimonial drugs. II. Response to high-dosage sodium stibogluconate or prolonged treatment with pentamidine. Trans. R. Soc. Trop. Med. Hyg. **79**:705–714.
8. **Chunge, C. N., G. Gachihi, R. Muigai, K. Wasuna, J. R. Rashid, J. D. Chulay, G. Anabwani, C. N. Oster, and A. D. M. Bryceson.** 1985. Visceral leishmaniasis unresponsive to antimonial drugs. III. Successful treatment using a combination of sodium stibogluconate plus allopurinol. Trans. R. Soc. Trop. Med. Hyg. **79**:715–718.
9. **Croft, S. L.** 1988. Recent developments in the chemotherapy of leishmaniasis. Trends Pharmacol. Sci. **9**:376–381.
10. **Croft, S. L., R. A. Neal, W. Pendergast, and J. H. Chan.** 1987. The activity of alkyl phosphorycholines and related derivatives against *Leishmania donovani*. Biochem. Pharmacol. **36**:2633–2636.
11. **Edwards, M. L., D. M. Stemerick, A. J. Bitonti, J. A. Dumont, P. P. McCann, and A. Sjoerdsma.** 1991. Antimalarial polyamine analogs. J. Med. Chem. **34**:569–574.
12. **Gradoni, L., M. A. Iorio, M. Gramiccia, and S. Orsini.** 1989. *In vivo* effect of eflornithine (DFMO) and some related compounds on *Leishmania infantum*. Farmaco **44**:1157–1166.
13. **Haidaris, C. G., and P. F. Bonventre.** 1983. Efficacy of combined immunostimulation and chemotherapy in experimental visceral leishmaniasis. Am. J. Trop. Med. Hyg. **32**:286–295.
14. **Hanson, W. L., W. L. Chapman, Jr., and K. E. Kinnamon.** 1977. Testing of drugs for antileishmanial activity in golden hamsters infected with *Leishmania donovani*. Int. J. Parasitol. **7**:433–447.
15. **Jackson, J. E., J. D. Tally, and D. B. Tang.** 1989. An in vitro micromethod for drug sensitivity testing of *Leishmania*. Am. J. Trop. Med. Hyg. **41**:318–330.
16. **Jha, T. K.** 1983. Evaluation of allopurinol in the treatment of kala-azar occurring in North Bihar, India. Trans. R. Soc. Trop. Med. Hyg. **77**:204–207.
17. **Kager, P. A., P. H. Rees, B. T. Wellde, W. T. Hockmeyer, and W. H. Lyerly.** 1981. Allopurinol in the treatment of visceral leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. **75**:556–559.
18. **Kaur, K., K. Emmett, P. P. McCann, A. Sjoerdsma, and B. Ullman.** 1986. Effects of DL-alpha-difluoromethylornithine on

Leishmania donovani promastigotes. J. Protozool. **33**:518–521.

19. **Neal, R. A., S. L. Croft, and D. J. Nelson.** 1985. Antileishmanial effect of allopurinol ribonucleoside and the related compounds, allopurinol, thiopurinol, thiopurinol ribonucleoside, and of formycin B, sinefungin and the lepidine WR6026. Trans. R. Soc. Trop. Med. Hyg. **79**:122–128.
20. **Nelson, D. J., S. W. LaFon, G. B. Elion, J. J. Marr, and R. L. Berens.** 1980. Comparative metabolism of a new antileishmanial agent allopurinol riboside, in the parasite and the host cell. Adv. Exp. Med. Biol. **122B**:7–12.
21. **Russell, D. G., and P. Talamas-Rohana.** 1989. Leishmania and the macrophage: a marriage of inconvenience. Immunol. Today **10**:328–333.
22. **Schecter, P. J., J. L. R. Barlow, and A. Sjoerdsma.** 1987. Clinical aspects of inhibition of ornithine decarboxylase with emphasis on therapeutic trials on eflornithine (DFMO) in cancer and protozoan diseases, p. 345–364. *In* P. P. McCann, A. E. Pegg, and A. Sjoerdsma (ed.), Inhibition of polyamine metabolism. Biological significance and basis for new therapies. Academic Press, Inc., Orlando, Fla.
23. **Stauber, L. A., E. M. Franchino, and J. Grun.** 1958. An eight-day method for screening compounds against *Leishmania donovani* in the golden hamster. J. Protozool. **5**:269–273.