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Effect of some bisbenzylisoquinoline alkaloids on American *Leishmania* sp. in BALB/c mice

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Four bisbenzylisoquinoline alkaloids, antioquine, berbamine, gyrocarpine and isotetrandrine were tested in BALB/c mice infected with *Leishmania amazonensis* (IFLA/BR/67/PH8 or MHOM/GF/84/CAY-H-142) or *L. venezuelensis* (VE/74/PM-H3). The treatments were initiated 1 day after the parasitic infection, with alkaloid at 100 mg/kg/day for 14 days and the reference compound, meglumine antimonate (Glucantime^R) at 200 mg/kg/day. Antioquine, berbamine and gyrocarpine were less potent than Glucantime against *L. amazonensis* (PH8). Only isotetrandrine exhibited activity approximately equal to or greater than Glucantime in BALB/c mice infected with *L. amazonensis* (PH8 or H-142) and showed significant activity against *L. venezuelensis*. Experiments with a single local treatment on the footpad, 2 weeks after parasitic infection with *L. amazonensis* (PH8), showed that isotetrandrine at 200 mg/kg was less active than Glucantime at 400 mg/kg.

Keywords: bisbenzylisoquinoline alkaloids; antioquine; berbamine; gyrocarpine; isotetrandrine; BALB/c mice; *Leishmania amazonensis*; *L. venezuelensis*.

INTRODUCTION

The cutaneous and mucosal forms of leishmaniasis due to *Leishmania braziliensis* species constitute an important public health problem in South America, particularly in the areas of colonization of the humid lowlands in Bolivia. Although significant advances in the chemotherapy of leishmaniasis have been identified with new potential drugs, liposomal amphotericin B (Croft *et al.*, 1991) and interferon-γ in liposomes (Hockertz *et al.*, 1991), the classic treatments used to cure leishmaniasis remain the pentavalent antimonials, Pentostam^R and Glucantime^R. Generally, antimony treatment failures are cured with further courses of antimony, with amphotericin B or with pentamidine (Saenz and Paz, 1990).

In Bolivia, we established a programme to identify new effective drugs, principally natural compounds, for the treatment of cutaneous leishmaniasis. In previous studies, we reported the *in vitro* antileishmanial and trypanocidal activities of bisbenzylisoquinoline alkaloids (Fournet *et al.*, 1988a, 1988b). Bisbenzylisoquinoline alkaloids constitute a series of almost 400 phenylalanine-derived metabolites with a rich and varied chemistry and pharmacology (Schift, 1991). For this study, we chose four bisbenzylisoquinolines which displayed *in vitro* activity at 10 µg/mL against promastigote forms of three strains of *Leishmania* ssp.: *L. braziliensis*, *L. amazonensis* and *L. donovani*. The aims of this study were to evaluate the activity of these bisbenzylisoquinoline alkaloids in BALB/c mice infected with parasites of the *Leishmania mexicana* complex; *Leishmania amazonensis* and *L. venezuelensis*.

MATERIALS AND METHODS

Animals. Female or male BALB/c mice were supplied by Charles River Breeding Laboratory and then were bred in IBBA (Bolivia). Mice weighed 18–20 g and were 8 weeks old when bioassays were initiated.

***Leishmania* strains.** *Leishmania amazonensis* IFLA/BR/67/PH8 and MHOM/GF/84/CAY-H-142 and *L. venezuelensis* VE/74/PM-H3 were used. The source and history of this isolate have been described by Bonfante-Garrido (1983). It is an effective parasite strain producing severe single and multiple cutaneous lesions in human infection (Hanham *et al.*, 1990), and in BALB/c mice and hamsters a rapid growth of lesions in the footpad. The mouse footpad infection has been used as model for these experiments (Avila *et al.*, 1990; Coleman *et al.*, 1989). BALB/c mice ($n=10$, $n=8$ or $n=6$) were infected subcutaneously in the right rear footpad with 1×10^6 amastigotes obtained from donor hamsters. The parasites were inoculated in 200 µL phosphate buffered saline (PBS).

The growth of the lesion was determined weekly by measuring the diameter of both rear feet with a direct reading vernier caliper (Kroelin 1ODI 00T6). The size of the lesion in millimetres (index of leishmaniasis) was

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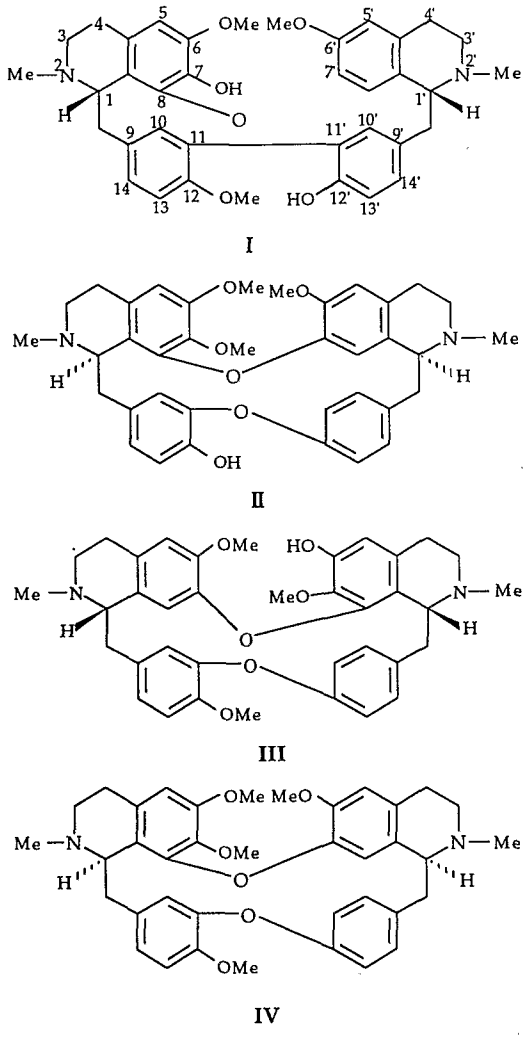

Figure 1. Structures of antioquine (I), berbamine (II), gyrocarpine (III) and isotetradrine (IV).

calculated by subtracting the measurements obtained for the uninfected foot from that of the infected foot. Measurements were commenced 1 day before the inoculation of amastigotes and continued for 8 or 9 weeks. For each experiment, the mean and standard error of the mean (SEM) were calculated.

Drug treatment. Glucantime (meglumine antimonate) from Rhône-Poulenc, France was used as the reference drug. The structures of bisbenzylquinoline alkaloids used in this study are shown in Fig. 1. Antioquine was isolated from *Pseudoxandra sclerocarpa* (Annonaceae) (Cortes *et al.*, 1985), berbamine from *Berberis boliviana* (Berberidaceae) (Weber *et al.*, 1989), gyrocarpine from *Gyrocarpus americanus* (Hernandiaceae) (Chalandre *et al.*, 1986) and isotetrandrine from *Limaciopsis loangensis* (Menispermaceae) (Fournet, 1979).

Two experiments were conducted. Mice in the first experiment were treated by the subcutaneous route. Glucantime was given at a dose of 200 mg/kg/day and bisbenzylisoquinoline alkaloids (antioquine, berbamine, gyrocarpine and isotetrandrine) at 100 mg/kg/day. The bisbenzylisoquinoline alkaloids were dissolved in 40 µL of polysorbate (Tween 80, Prolabo), Glucantime in PBS. The untreated mice received PBS and Tween 80. Drug treatment commenced 1 day after the inoculation of amastigotes and was continued once daily for 14 days.

In the second experiment, mice were treated directly on the infected rear footpad with a single treatment 14 days after the inoculation of parasites. For this experiment, the mice were treated with Glucantime at 400 mg/kg/day and with isotetrandrine at 200 mg/kg/day.

RESULTS

Effects of bisbenzylisoquinoline alkaloids on *Leishmania amazonensis* PH8

The activities of antioquine, berbamine and gyrocarpine against cutaneous leishmaniasis caused by *Leishmania amazonensis* (PH8) in BALB/c mice are presented in Table 1. Six weeks after infection, mice treated with antioquine or with berbamine had an average lesion size of 2.25 mm and 2.52 mm respectively, compared with 1.69 mm in the mice treated with Glucantime and 3.83 mm in the untreated mice. During the last 3 weeks of the experiment, the lesion size increased twice as rapidly in mice treated with antioquine (4.05 mm) or with berbamine (2.74 mm) than in mice treated with Glucantime (1.97 mm). Gyrocarpine was found to be less effective than the standard pentavalent antimonial drug and the other bisbenzylisoquinoline alkaloids. The lesion sizes measured in mice BALB/c treated with gyrocarpine and untreated mice did not differ significantly after 9 weeks (6.38 mm and 6.91 mm, respectively).

The effects of isotetrandrine are shown in Fig. 2. The

Table 1. Effect of Glucantime (200 mg/kg/day), antioquine (I) (100 mg/kg/day), berbamine (II) (100 mg/kg/day) and gyrocarpine (III) (100 mg/kg/day) on the development of *L. amazonensis* (PH8) in BALB/c mice (±SEM). Drug were given for 14 day period commencing 1 day after inoculation of *L. amazonensis*

	Diameter of lesion[a]				
Weeks post-infection	Control	Glucantime	Antioquine (I)	Berbamine (II)	Gyrocarpine (III)
2	0.31 (0.15)	0.34 (0.08)	0.20 (0.14)	0.30 (0.12)	0.14 (0.04)
4	1.64 (0.27)	0.86 (0.23)	0.73 (0.24)	0.77 (0.19)	1.39 (0.32)
6	3.83 (0.60)	1.69 (0.29)	2.25 (0.66)	2.52 (0.73)	3.28 (0.62)
8	6.01 (0.88)	3.23 (0.87)	4.82 (0.92)	4.57 (0.74)	5.48 (0.65)
9	6.91 (0.70)	3.66 (1.03)	6.30 (0.49)	5.26 (1.23)	6.38 (0.67)

[a] Average measurement (in mm) for 8 mice and ±SEM.

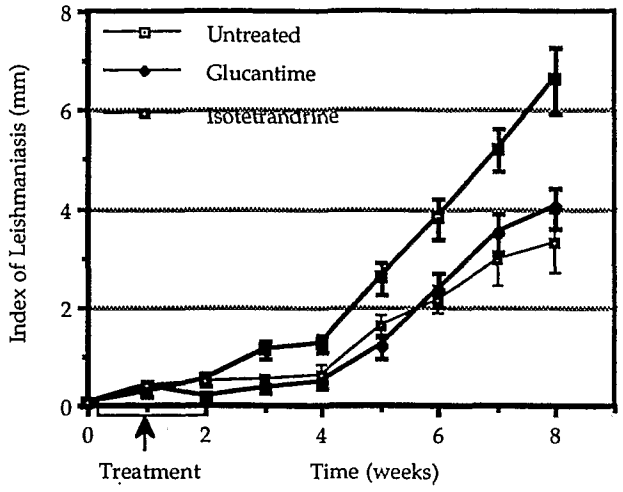

Figure 2. Effects of isotetrandrine (IV) (100 mg/kg/day) and Glucantime (200 mg/kg/day) on the development of *L. amazonensis* (PH8) in BALB/c mice ($n=10$, ±SEM)/Treatments were given for a 14 day period commencing 1 day after inoculation of *L. amazonensis*.

mice treated with isotetrandrine and with Glucantime developed similar lesion sizes for 6 weeks, 2.14 mm and 2.35 mm. In the last 2 weeks of the experiment, an inhibition of the increase in lesion size in mice treated with isotetrandrine (1.15 mm) in comparison with mice treated with Glucantime (1.67 mm) was observed. Table 2 presents the results obtained with a local treatment near the site of the lesion on the infected footpad, 14 days after the parasitic infection with amastigotes of *Leishmania amazonensis* (PH8). Eight weeks after infection, the lesion sizes were 2.83 mm and 3.95 mm, respectively, for isotetrandrine and Glucantime and 4.90 mm in the untreated mice.

Effects of isotetrandrine on *Leishmania amazonensis* H-142

Only the bisbenzylisoquinoline isotetrandrine was tested in BALB/c mice infected with *Leishmania amazonensis* H-142. The results of this experiment are shown in Table 3. The growth of experimental cutaneous leishmaniasis with strain H-142 was slower than with strain PH8. After 9 weeks, mice treated with isotetrandrine had an average lesion size of 1.73 mm, 1.98 mm in mice treated with Glucantime and 2.96 mm in the untreated mice. With this strain of cutaneous leishmaniasis, after 10 weeks there was a beginning of ulceration or healing of the lesions.

Table 2. Effect of Glucantime (400 mg/kg/day), and isotetrandrine (IV) (200 mg/kg/day), on the development of *L. amazonensis* (PH8) in BALB/c mice (±SEM). Drugs were given on the infected rear footpad with a single treatment 14 days after the inoculation of *L. amazonensis*

	Diameter of lesion[a]		
Weeks post-infection	Control	Glucantime	Isotetrandrine (IV)
2	0.35 (0.08)	0.45 (0.05)	0.55 (0.15)
4	1.02 (0.20)	0.53 (0.23)	1.05 (0.33)
6	2.87 (0.29)	1.52 (0.38)	2.45 (0.75)
8	4.90 (0.42)	2.83 (0.58)	3.95 (1.12)

[a] Average measurement (in mm) for 6 mice and ±SEM.

Table 3. Effect of Glucantime (200 mg/kg/day), and isotetrandrine (I) (100 mg/kg/day), on the development of *L. amazonensis* (H-142) in BALB/c mice (±SEM). Drugs were given for a 14 day period commencing 1 day after inoculation of *L. amazonensis*

	Diameter of lesion[a]		
Weeks post-infection	Control	Glucantime	Isotetrandrine (IV)
2	0.53 (0.12)	0.42 (0.10)	0.18 (0.07)
4	0.88 (0.12)	0.67 (0.18)	0.34 (0.11)
6	1.43 (0.44)	0.66 (0.24)	0.68 (0.21)
8	2.02 (0.45)	1.30 (0.30)	1.41 (0.67)
9	2.96 (0.46)	1.98 (0.34)	1.73 (0.62)

[a] Average measurement (in mm) for 10 mice and ±SEM.

Effects of isotetrandrine on *Leishmania venezuelensis* (H-3)

The BALB/c mice infected with the effective strain *L. venezuelensis* and treated with 100 mg/kg/day of isotetrandrine for 14 days or with pentavalent antimonial developed identical lesion sizes (Table 4). An increase in lesion size was observed in all mice between week 4 and the last week of the experiment, 4.53 mm in untreated mice, and in mice treated with Glucantime and isotetrandrine 3.30 mm and 3.78 mm.

DISCUSSION

This study on the evaluation of the *in vivo* antileishmanial activity of some bisbenzylisoquinoline alkaloids, on BALB/c mice infected with New World strains of cutaneous leishmaniasis, shows the efficacy of one compound, isotetrandrine. With the data obtained in our models, we observed that isotetrandrine administered at 100 mg/kg in BALB/c mice, is approximately as effective as pentavalent antimonials at 56 mg/Sb^V/kg against two strains of *Leishmania amazonensis* (PH8 and H-142) and slightly less active against *L. venezuelensis*.

The *in vitro* activity of isotetrandrine against *Leishmania* ssp. was not described in our previous study (Fournet *et al.*, 1988b). Antioquine, berbamine and gyrocarpine were not active against *L. amazonensis* (PH8) infected BALB/c mice. They had good *in vitro* activity against the promastigote forms of *Leishmania* ssp. We have observed variations in the infectivity of parasite strains of *Leishmania amazonensis*. With the same inoculum in the footpad, the strain

Table 4. Effect of Glucantime (200 mg/kg/day) and isotetrandrine (100 mg/kg/day) on the development of *L. venezuelensis* (H-3) in BALB/c mice (±SEM). Drugs were given for 14 day period commencing 1 day after inoculation of *L. venezuelensis*

	Diameter of lesion[a]		
Weeks post-infection	Control	Glucantime	Isotetrandrine (IV)
2	0.62 (0.30)	0.45 (0.19)	0.28 (0.08)
4	2.07 (0.19)	0.95 (0.21)	1.27 (0.12)
6	4.12 (0.22)	2.48 (0.34)	2.80 (0.52)
8	6.60 (0.51)	4.25 (0.44)	5.05 (0.66)

[a] Average measurement (in mm) for 6 mice and ±SEM.

PH8 of *L. amazonensis* and *L. venezuelensis* produced the fastest growing lesion. For the evaluation of new potential drugs active against the New World cutaneous leishmaniases, the choice of fast growing strains of *Leishmania* ssp. (PH8) are more sensitive to drugs and therefore better indicators of activity compared with the slow growing strain (H-142).

The two bisbenzylisoquinoline alkaloids, berbamine (**II**) and isotetrandrine (**IV**) have two diphenyl ether linkages in positions 8–7′ and 11–12′ (Fig. 1) with the configuration R,S. In position 12, isotetrandrine is substituted by a methoxyl-group and berbamine by a hydroxyl group. Only isotetrandrine is as effective as meglumine antimonate against *Leishmania amazonensis* and *L. venezuelensis*. The growth of experimental leishmaniasis is slower in mice treated with berbamine than in untreated mice. This small difference in the chemical structure of these two alkaloids shows that it will not be easy to find a structure–activity relationship with bisbenzylisoquinoline alkaloids. The other compounds tested present a different chemical structure. Gyrocarpine (**III**) has two diphenyl ether linkages in positions 7–8′ and 11–12′ and antioquine has a diphenyl ether linkage in position 8–7′ and one diphenyl linkage in position 11–11′. The antiprotozoal activities of bisbenzylisoquinolines have been described by different authors against *Plasmodium falciparum* (Partridge *et al.*, 1988; Ye and VanDyke, 1989), *Plasmodium berghei berghei* (Dreyfuss *et al.*, 1987b) and *Trypanosoma brucei brucei* (Dreyfuss *et al.*, 1987a).

This is the first reported *in vivo* study of the antileishmanial activity of bisbenzyloquinoline alkaloids against New World cutaneous leishmaniasis, *Leishmania amazonensis* and *L. venezuelensis*. The study also identified a compound, isotetrandrine, with promising antileishmanial activity. Further studies will examine activity against infection with visceral leishmaniasis (*Leishmania donovani*) and continue to investigate the *in vivo* activity against *Leishmania* ssp. of new bisbenzylisoquinoline alkaloids.

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