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

Alain Fournet*
Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Département Santé, 213, rue La Fayette, 75480 Paris, Cedex 10, France

Alcira Angelo Barrios and Victoria Muñoz
Instituto Boliviano de Biologia de Altura (IBBA), CP 717, La Paz, Bolivia

Reynald Hocquemiller and André Cavé
Laboratoire de Pharmacognosie, associé au CNRS, Faculté de Pharmacie, Université Paris XI, 92296 Châtenay-Malabry, Cedex, France

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®) 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.

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 × 10^5 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 IODI 00T6). The size of the lesion in millimetres (index of leishmaniasis) was calculated by

\[ \text{Index} = \frac{\text{width} + \text{height}}{2} \times \text{length} \]

the activity of these bisbenzylisoquinoline alkaloids in BALB/c mice infected with parasites of the Leishmania mexicana complex; Leishmania amazonensis and L. venezuelensis.
Figure 1. Structures of antioquine (I), berbamine (II), gyrocarpine (III) and isotetrandrine (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 5 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 boli-viana (Berberidaceae) (Weber et al., 1989), gyrocarpine from Gyrocarpus americanus (Hernandiaceae) (Chalandre et al., 1986) and isotetrandrine from Limacopsis 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.

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

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>Antioquine (I)</th>
<th>Berbamine (II)</th>
<th>Gyrocarpine (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.31 (0.15)</td>
<td>0.34 (0.08)</td>
<td>0.20 (0.14)</td>
</tr>
<tr>
<td>4</td>
<td>1.64 (0.27)</td>
<td>0.86 (0.23)</td>
<td>0.73 (0.24)</td>
</tr>
<tr>
<td>6</td>
<td>3.83 (0.60)</td>
<td>1.69 (0.29)</td>
<td>2.25 (0.66)</td>
</tr>
<tr>
<td>8</td>
<td>6.01 (0.88)</td>
<td>3.23 (0.87)</td>
<td>4.82 (0.92)</td>
</tr>
<tr>
<td>9</td>
<td>6.91 (0.70)</td>
<td>3.66 (1.03)</td>
<td>6.30 (0.49)</td>
</tr>
</tbody>
</table>

* Average measurement (in mm) for 8 mice and ±SEM.
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.

**DISCUSSION**

This study on the evaluation of the *in vivo* antileishmanial activity of some bisbenzyisoquinoline 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/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 3. Effect of Glucantime (200 mg/kg/day), and isotetrandrine (1) (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***

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>Control</th>
<th>Glucantime</th>
<th>Isotetrandrine (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.53 (0.12)</td>
<td>0.42 (0.10)</td>
<td>0.18 (0.07)</td>
</tr>
<tr>
<td>4</td>
<td>0.88 (0.12)</td>
<td>0.68 (0.18)</td>
<td>0.34 (0.11)</td>
</tr>
<tr>
<td>6</td>
<td>1.43 (0.44)</td>
<td>0.66 (0.24)</td>
<td>0.68 (0.21)</td>
</tr>
<tr>
<td>8</td>
<td>2.02 (0.45)</td>
<td>1.30 (0.30)</td>
<td>1.41 (0.67)</td>
</tr>
<tr>
<td>10</td>
<td>2.96 (0.48)</td>
<td>1.98 (0.34)</td>
<td>1.73 (0.62)</td>
</tr>
</tbody>
</table>

* Average measurement (in mm) for 6 mice and ±SEM.

**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***

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>Control</th>
<th>Glucantime</th>
<th>Isotetrandrine (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.62 (0.30)</td>
<td>0.45 (0.19)</td>
<td>0.28 (0.08)</td>
</tr>
<tr>
<td>4</td>
<td>2.07 (0.19)</td>
<td>0.95 (0.21)</td>
<td>1.27 (0.12)</td>
</tr>
<tr>
<td>6</td>
<td>4.12 (0.22)</td>
<td>2.48 (0.34)</td>
<td>2.80 (0.52)</td>
</tr>
<tr>
<td>8</td>
<td>6.60 (0.51)</td>
<td>4.25 (0.44)</td>
<td>5.05 (0.66)</td>
</tr>
</tbody>
</table>

* 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 leishmaniasis, 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 bisbenzylisoquinoline 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.

**Acknowledgements**

We thank Professor J. Bruneton of the Laboratory of Pharmacognosy of the University of Angers, France who provided the gyrocarpine and Professor D. Cortes of the laboratory of Pharmacognosy of University of Rouen, France for supplying the antioquine.

**REFERENCES**


