Antiprotozoal Activity of Quinoline Alkaloids Isolated from Galipea longiflora, a Bolivian Plant Used as a Treatment for Cutaneous Leishmaniasis

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The stem bark of Galipea longiflora is used by the Chimane Indians in Bolivia for the treatment of cutaneous leishmaniasis produced by Leishmania braziliensis. Petroleum ether and chloroform extracts of stem, root bark and leaves were found active in vitro against Leishmania ssp and Trypanosoma cruzi at 100 µg/mL. The activity guided fractionation of the extracts by chromatography afforded 12 active compounds identified as 2-substituted quinoline alkaloids. BALB/c mice were infected with Leishmania amazonensis (strain PH8 or H-142) and treated 24 h after infection with the major alkaloids from the crude alkaloidal extract; 2-phenylquinoline and 2-n-pentylquinoline. 2-phenylquinoline was as potent as Glucantime (Rhône-Poulenc) against the strain H-142, but less active than the reference drug against the virulent strain PH8 of L. amazonensis. 2-n-pentylquinoline did not exhibit any activity. Assays of single local treatments on the rear footpad infection, 2 weeks after the parasitic inoculation, indicated an effect for 2-phenylquinoline by reducing the severity of lesion. However, this activity was found to be slightly lower than that obtained using Glucantime.

Keywords: alkaloids; quinoline; Galipea longiflora; Rutaceae; Leishmania ssp.; Trypanosoma cruzi.

INTRODUCTION

Cutaneous and mucosal leishmaniasis, caused by the protozoan Leishmania braziliensis, are common infections in the subtropical areas of the Department of La Paz (Yungas and Alto-Beni) and the foothills of the Andes (Department of Beni) where the Chimane Indians live. Cutaneous leishmaniasis is known as espundia by the natives of this region of Bolivia called Oriente. The infection is classically treated with pentavalent antimony (Glucantime, Rhône-Poulenc) for cutaneous leishmaniasis or with amphotericin B for mucosal leishmaniasis. These drugs are administered parenterally to patients in hospitals which are often far from their colonies. These treatments are also too expensive or unavailable for the population suffering from espundia. The dispersion of cutaneous leishmaniasis is accentuated in subandean tropical regions by the influx of people descending from higher areas. The use of medicinal plants is commonplace, especially among the Chimane Indians, a group that inhabits the gallery forest and sandbars of the Rio Maniqui and its tributary streams. We have collected and studied many medicinal plants (Fournet, 1991) and most notably a tree called evanta, used by the Chimane Indians for the treatment of cutaneous leishmaniasis and botanically identified as Galipea longiflora Krause (Rutaceae). The stem bark, in the form of a poultice, is applied on the cutaneous leishmaniasis until complete cicatrization of the wound. In a preliminary screening, the crude alkaloidal extracts of the stem bark, root bark and leaves of Galipea longiflora displayed activity in vitro at a concentration of 100 µg/mL against three strains of promastigote forms of Leishmania species, L. braziliensis, L. amazonensis and L. donovani, and three strains of epimastigote forms of Trypanosoma cruzi (Tulahuen, CSCL1 and Tehuentepec), another trypanosomatid responsible for Chagas’ disease. Activity-directed fractionation and purification of crude alkaloidal extracts in petroleum ether and in chloroform of root bark, stem bark and leaves gave 12 active compounds, identified by their physical data and spectral data as 2-aryl and 2-alkyl quinolines alkaloids (Fournet et al., 1989, 1991).

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Received 19 November 1993
Accepted 19 March 1993

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pentyquinoline in BALB/c mice infected with *Leishmania amazonensis* (IFLA/BR/67/PH8) or MHOM/GF/84/CAY-H-142. We sought to verify the effect of the most abundant alkaloid of *Galipea longiflora*, 2-phenylquinoline, when administered directly in the rear footpad of infected BALB/c mice by *Leishmania amazonensis*.

**MATERIALS AND METHODS**

**Isolation and chemistry**

General experimental procedures. UV spectra were recorded on a Unicam SP 1800 spectrophotometer. IR spectra were measured in KBr with a Perkin Elmer 257 spectrometer. All 1H and 13C-NMR spectra were recorded in CDCl3 (δ ppm) on a Bruker AC200 P spectrometer operating at 200 and 50 MHz respectively. Eims spectra were obtained at 70 eV on a Nermag R 1010 C mass spectrometer.

**Plant material.** Stem bark (3.5 kg), root bark (350 g) and leaves (3.5 kg) of *Galipea longiflora* Krause were collected by A. Fournet (A.F. 850) in August 1989, at Fatima de Chimane, in the Department of Beni, altitude 400 m, Bolivia. The plant material was identified by Dr J. A. Kallunki of the New York Botanical Garden. A reference specimen is deposited in the National Herbarium of Bolivia (La Paz).

**Extraction and isolation.** The isolation procedure was effected by activity-directed fractionation and purification of the crude alkaloidal extracts of root bark, stem bark and leaves. Extraction, partitioning, chromatographic separation and physicochemical data of the 12 alkaloids identified were performed as described by Fournet et al. (1989) and Fournet (1991).

**Biological assays**

**Parasites.** Cultures of *Leishmania* spp and *Trypanosoma cruzi* were obtained from IBBA (Instituto Boliviano de Biologia de Altura, La Paz) and identified by isoenzyme analysis.

Five strains of *Leishmania* were used during these investigations, *L. braziliensis* (MHOM/BR/75/M 2903), *L. amazonensis* (IFLA/BR/67/PH8), *L. amazonensis* (MHOM/GF/84/CAY H-142), *L. donovani* (MHOM/IN/83/HS-70) and *L. donovani* (MHOM/BR/74/M 2682), and five strains of *Trypanosoma cruzi*: SC 43 CL2 (Bolivian strain), C8 CL1 (Brazilian strain), Tulahuen (isolated from *Triatoma infestans* in Brazil), TeCL2 (Brazilian strain) and 1979 CL1 (Bolivian strain).

**Promastigote forms of *Leishmania* were grown at 28 °C in USMARU medium (Evans, 1987) containing 10% heat inactivated (56 °C for 30 min) fetal bovine serum and epimastigote forms of *T. cruzi* in liver infusion tryptose medium (LIT, Bacto) supplemented with 10% fetal calf serum at 28 °C with an inoculum of 10⁶ cells per mL. Logarithmically growing promastigotes and epimastigotes were maintained by transferring 10⁶ cells per mL. The extracts and the fractions of *Galipea longiflora* were dissolved in a known volume of DMSO (dimethyl sulphoxide) and then in medium, from which aliquots were drawn. Parasites were counted after 48 h of contact with drugs in a haemocytometer and the counts were compared with those of controls grown without drug. The 90% inhibitory concentrations (IC₉₀) were chosen for the comparison of susceptibilities of the strains to drugs tested.

N-methylglucamine antimonate (Glucantime) (Rhône-Poulenc, France) and Pentamidine (May and Baker, England), nifurtimox (Bayer, Germany) and benznidazole (Roche, USA) were used as the baseline drugs against which the efficacy of the quinoline alkaloids of *Galipea longiflora* were compared. Each assay was performed in triplicate.

**In vivo studies**

The mouse footpad infection was used as a model for these experiments (Avila et al., 1990; Coleman et al., 1989).

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

*Leishmania amazonensis* IFLA/BR/67/PH8 and MHOM/GF/84/CAY-H-142 were used. BALB/c mice (n = 10 or n = 8) were infected subcutaneously in the right footpad with 1 × 10⁶ amastigotes obtained from donor hamsters. The parasites were delivered 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 10DI 00T6). The size of lesion in millimetres (Index of Leishmaniasis) was calculated by subtracting the measurements obtained from the uninfected foot from that of the infected foot. Measurements commenced 1 day prior to the inoculation of amastigotes and were continued for 8 weeks. For each experiment, the mean and standard error of the mean (SEM) were calculated.

N-methylglucamine antimonate (Glucantime) with a pentavalent antimony (Sb⁵⁺) content of 28% by weight was obtained from the Rhône-Poulenc, France and was used as a reference drug.

Two experiments were conducted. Mice in the first experiment were treated by the subcutaneous route. Glucantime was given at a dose of 56 mg Sb²⁺ per kg per day and quinoline alkaloids (2-phenylquinoline and 2-n-pentyquinoline) at 100 mg/kg/day. The quinoline alkaloids were dissolved in 40 µL of polysorbate (Tween 80, Prolabo). Glucantime was dissolved in PBS and 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 a rate of 112 mg Sb²⁺ per kg per day and with the major quinoline alkaloid of *Galipea longiflora*, 2-phenylquinoline at 200 mg/kg/day.
technical conditions and effects of altitude (3500 m).

Products are presented in Fig. 1. Chimaniine C was not pounds are shown in Table 1 against five strains of promastigote forms of parasites. All quinoline alkaloids showed activity against all strains noline alkaloids showed activity against all strains.

The crude alkaloidal extracts of stem bark, root bark and leaves of Galipea longiflora showed an activity on promastigote forms of Trypanosoma cruzi. The fractionation and purification, monitored by bioassays, led to isolation of 12 active quinoline alkaloids. The new products, the four chimanines (A, B, C and D) were described by Fournet in 1991. The chemical structures of these products are presented in Fig. 1. Chimanie C was not tested against parasites since it was not isolated following the bioassay purification procedure.

The results of the in vitro activity of these compounds are shown in Table 1 against five strains of promastigote forms of Leishmania species. Three quinoline alkaloids showed activity against all strains of both parasites at 50 µg/mL; 2-n-propylnquinoline (III), chimanie B (XI) and chimanie D (XII). All these compounds are substituted in position 2 with a propylic chain. We did not notice any different activity against Leishmania ssp. and T. cruzi or the same type of parasites. All quinoline alkaloids are more potent than Glucantime, but 25-100 times less active than pentamidine against the promastigote forms of Leishmania species. It was impossible to confirm in vitro activity against the intracellular forms of Leishmania either with mouse peritoneal macrophages (Neal and Croft, 1984) or with cell lines U937 (Martinez et al., 1988). This failure seemed to be due to a lack of good technical conditions and effects of altitude (3500 m).

The in vitro effects of the quinoline alkaloids, nifurtimox and benznidazole against five strains of epimastigote of Trypanosoma cruzi are presented in Table 2. Nine quinoline alkaloids were less active than nifurtimox and benznidazole, and three (2-n-propylquinoline (III), chimanie B (XI) and chimanie D (XII) showed a similar activity to the reference drugs.

We chose to determine the in vivo activity of two major quinoline alkaloids of the crude alkaloidal extracts of Galipea longiflora, in BALB/c mice infected with the strain PH8 or H-142 of Leishmania amazonensis but not with the endemic cutaneous strain, L. braziliensis. The in vivo model of infection with this strain was obtained by coinfection with saliva and parasites isolated from infected sand fly (Samuelson et al., 1991). The major quinoline, 2-phenylquinoline,
Table 4. Effect of N-methylglucamine antimonate (56 mg of Sb per kg per day), 2-phenylquinoline (I) (100 mg/kg/day) on the development of Leishmania amazonensis H-142 in BALB/c mice (±SEM). Treatments were given for 14 days period commencing 1 day after inoculation of L. amazonensis

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>Diameter of lesion</th>
<th>Control</th>
<th>Glucantime</th>
<th>2-Phenylquinoline (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.07 (0.19)</td>
<td>0.91 (0.17)</td>
<td>0.76 (0.14)</td>
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</tr>
<tr>
<td>2</td>
<td>1.37 (0.14)</td>
<td>1.32 (0.10)</td>
<td>0.92 (0.15)</td>
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</tr>
<tr>
<td>4</td>
<td>2.02 (0.22)</td>
<td>1.74 (0.15)</td>
<td>1.54 (0.16)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.85 (0.21)</td>
<td>2.17 (0.24)</td>
<td>2.08 (0.36)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.49 (0.28)</td>
<td>2.51 (0.26)</td>
<td>2.54 (0.21)</td>
<td></td>
</tr>
</tbody>
</table>

* Average measurement (in mm) for 10 mice and ±SEM.

Table 5. Effect of N-methylglucamine antimonate (112 mg of Sb per kg per day), 2-phenylquinoline (I) (200 mg/kg/day) on the development of Leishmania amazonensis H-142 in BALB/c mice (±SEM). Treatments were given on the infected rear footpad with a single treatment 14 days after the inoculation of L. amazonensis

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>Diameter of lesion</th>
<th>Control</th>
<th>Glucantime</th>
<th>2-Phenylquinoline (I)</th>
</tr>
</thead>
<tbody>
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<td>0.45 (0.05)</td>
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<td>1.52 (0.38)</td>
<td>2.62 (0.22)</td>
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<tr>
<td>8</td>
<td>4.90 (0.42)</td>
<td>2.83 (0.58)</td>
<td>3.62 (0.52)</td>
<td></td>
</tr>
</tbody>
</table>

* Average measurement (in mm) for 8 mice and ±SEM.
The investigations on the antileishmanial activity of *Galipea longiflora* continue in our laboratory to determine *in vivo* activities of the other quinoline alkaloids isolated towards cutaneous leishmaniasis of the New World (*Leishmania amazonensis* and *L. venezuelensis*) and the parasite of visceral leishmaniasis, *L. donovani*.

**Acknowledgements**

We express our sincere thanks to Padre Martin Bauer of the Mission Redentorista of Fatima (Department of Beni, Bolivia), to the Chimane Indians who gave essential logistic support and Dr J. A. Kallunki (New York Botanical Garden, NY, USA) for the identification of plant material.

**REFERENCES**


