ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1996, p. 2447–2451 Vol. 40, No. 11
0066-4804/96/$04.00+0

In Vivo Efficacy of Oral and Intralesional Administration of 2-Substituted Quinolines in Experimental Treatment of New World Cutaneous Leishmaniasis Caused by *Leishmania amazonensis*

ALAIN FOURNET,[1,2]* MARIA ELENA FERREIRA,[2] ANTONIETA ROJAS DE ARIAS,[2] SUSANA TORRES DE ORTIZ,[2] SANDRA FUENTES,[2] HECTOR NAKAYAMA,[2] ALICIA SCHININI,[2] AND REYNALD HOCQUEMILLER[3]

ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération,[1] *and Department of Tropical Medicine, Instituto de Investigaciones en Ciencias de la Salud,*[2] *Asunción, Paraguay, Laboratoire de Pharmacognosie, Laboratoire Associé au Centre National de la Recherche Scientifique, Faculté de Pharmacie, 92296 Châtenay-Malabry Cédex, France*[3]

Received 27 November 1995/Returned for modification 22 May 1996/Accepted 13 August 1996

The antileishmanial efficacies of 2-*n*-propylquinoline, chimanines B and D, 2-*n*-pentylquinoline, 2-phenylquinoline, 2-(3,4-methylenedioxyphenylethyl)quinoline, and two total alkaloidal extracts of *Galipea longiflora* were evaluated in BALB/c mice infected with *Leishmania amazonensis* or *Leishmania venezuelensis*. Animals were treated for 4 to 6 weeks postinfection with a quinoline by the oral route at 50 mg/kg of body weight twice daily for 15 days or by five intralesional injections at intervals of 4 days with a quinoline at 50 mg/kg of body weight. The reference drug, *N*-methylglucamine antimonate (Glucantime), was administered by subcutaneous or intralesional injection (regimens of 14, 28, or 56 mg of pentavalent antimony [Sb^v] per kg of body weight daily). Twice-daily oral treatment with chimanine B at 50 mg/kg resulted in a decrease in lesion weight by 70% ($P < 0.001$) and a decrease in the parasite loads by 95% ($P < 0.001$). Five injections of chimanine B at intervals of 4 days reduced the lesion weight by 74% and the parasite loads in the lesion by 90% compared with the values for the group of untreated mice. Subcutaneous administration of *N*-methylglucamine antimonate at 28 mg of Sb^v kg per day for 15 days reduced the parasite burden by 95% ($P < 0.001$), and five intralesional injections at the same concentration reduced the parasite burden by 96% ($P < 0.001$). Other 2-substituted quinolines, 2-*n*-propylquinoline administered by the oral and intralesional routes, 2-phenylquinoline administered by the oral route, 2-*n*-pentylquinoline administered by intralesional injection, and two total alkaloidal extracts of *G. longiflora* administered by the oral route, had intermediate effects. These findings suggest that chimanine B may be chosen as a lead molecule in the development of oral therapy against leishmaniasis.

The increment of in vitro antimony resistance due to intermittent drug exposure (11, 12, 20), the isolation of antimony-resistant leishmania strains from patients with unresponsive cutaneous leishmaniasis (13, 15), and recently, the numerous cases of visceral leishmaniasis among patients infected with the human immunodeficiency virus (2, 3, 5, 19) indicate the necessity of finding new therapeutic agents for the treatment of the leishmaniasis. The great variability observed in the parasite strains has been conducive to the assessment of different drugs against different parasite strains. For example, in 1993 drugs trials in Guatemala indicated that oral azoles proved to be more effective than antimonial treatments against cutaneous leishmaniasis caused by *Leishmania mexicana* (20), but they offered little advantage against cases of disease caused by *Leishmania braziliensis*. Currently, specific drugs (pentavalent antimony [Sb^v], pentamidine, or amphotericin B) used for the treatment of leishmaniasis are administered by the parenteral route, and the main disadvantages of these treatments are the need for an adequate health care infrastructure and the long period of patient hospitalization. Oral administration has the advantage of reducing treatment-related socioeconomic difficulties that are present in areas where the disease is endemic and health facilities are lacking. On the basis of the results obtained in studies with 2-substituted quinolines (7), the Integrated Chemotherapy Committee of the Tropical Disease Research Program of the World Health Organization has established as a priority the ability to provide oral treatment for cutaneous leishmaniasis. This treatment would provide a clear advantage and cost savings over present drugs for treating patients from areas where leishmaniasis is endemic.

The efficacies of 2-substituted quinolines (10), isolated from a Bolivian medicinal plant, *Galipea longiflora* Kr of the family *Rutaceae*, in the experimental treatment of cutaneous leishmaniasis of the New World (7) have already been reported. We have also described their activities in the treatment of experimental visceral leishmaniasis by the oral and parenteral routes (10). Several 2-substituted quinolines, especially 2-substituted three-carbon-chain or phenylethyl-chain quinoline alkaloids, have been synthesized in our laboratory (17). Oral administration of two of these compounds, namely, 2-*n*-propylquinoline and chimanine D, had the same efficacy as the reference antimonial drug. The aims of the present study were to evaluate the antileishmanial activities of these 2-substituted quinolines against *Leishmania amazonensis* and *Leishmania venezuelensis* when the drugs were administered by the oral and intralesional routes and to select a leading drug for use in clinical trials in

* Corresponding author. Mailing address: ORSTOM, Casilla de Correo 97, Asuncion, Paraguay. Phone: 595 21 609 181. Fax: 595 21 609 181. Electronic mail address: fournet@ui131.una.py.

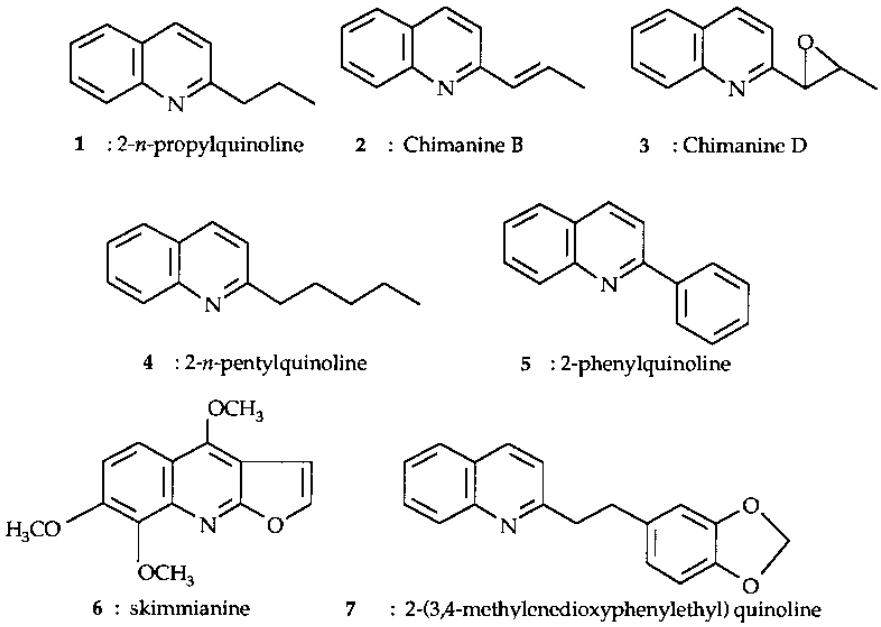

FIG. 1. Chemical structures of 2-substituted quinolines.

veterinary and human medicine. Alkaloidal extracts of stem bark and root bark of *G. longiflora*, an original Bolivian plant used topically and orally to treat cutaneous and mucocutaneous leishmaniasis caused by *L. braziliensis*, were evaluated as well.

MATERIALS AND METHODS

Chemicals. The 2-substituted quinolines were synthesized as described previously (16) or were isolated from the stem bark and leaves of *G. longiflora* by fractionation and purification, monitored by bioassay, as described previously (8, 11). Physical and spectral data (proton and ^{13}carbon nuclear magnetic resonance and mass spectrometry) were used to control the purities of the 2-substituted quinolines. The structures of these compounds are shown in Fig. 1. *N*-Methylglucamine antimonate (Glucantime), equivalent to 0.28 mg of Sb^v per ml, was purchased from Rhône-Poulenc, Paris, France.

Experimental animals. Female and male BALB/c mice were supplied by the IFFA-CREDO, Lyon, France, and were bred at the Instituto de Investigaciones en Ciencias de la Salud, Asuncion, Paraguay. Golden hamsters (*Mesocritus auratus*) were used to maintain the parasites.

Infection. *L. amazonensis* MHOM/IFLA/BR/67/PH8 and *L. venezuelensis* MHOM/VE/74/PM-H3 were used. These strains were obtained from the Instituto Boliviano de Biologia de Altura (La Paz, Bolivia) and were identified by isoenzyme analysis. All strains were maintained by passage every 6 to 8 weeks in hamsters. BALB/c mice ($n = 8$) were inoculated in the right hind footpad with 2×10^6 amastigotes obtained from donor hamsters. The parasites were delivered in 100 μl of phosphate-buffered saline (PBS). Disease progression was monitored by the measurement of lesion diameters weekly for up to 7 to 12 weeks.

Drug treatment. In all experiments, treatment was initiated 4 or 5 weeks after inoculation, when the infection was well established and lesions were obvious. Two days before administration of drug, the mice were randomly divided into groups of eight. *N*-Methylglucamine antimonate was dissolved in 50 μl of PBS and was administered to the BALB/c mice in regimens of 56, 28, or 14 mg of Sb^v per kg of body weight daily for 15 days by the subcutaneous route or by five intralesional injections of 28 mg of Sb^v per kg of body weight in the infected footpad at intervals of 5 days. The 2-substituted quinolines or total alkaloidal extracts of *G. longiflora* root bark or stem bark were tested at a dose of 50 mg/kg of body weight and were made up in 50 μl of PBS–5 μl of polysorbate (Tween 80; OSI, Elancourt, France). The drugs were administered twice daily by the oral route for 15 days or five times by intralesional injection into the infected footpad at intervals of 5 days. The untreated group received 50 μl of PBS and 5 μl of Tween 80 daily.

Effect of treatment. The animals were sacrificed 1 week after the cessation of treatments to assess parasitological loads in the infected footpad. Briefly, the mice were killed, and the lesions of the infected footpad were excised, weighed, and homogenized in a tissue glass grinder and then homogenized in 5 ml of RPMI 1640 (GIBCO, Paris, France) tissue culture medium supplemented with 10% fetal calf serum, 1 ml of glutamine (29.4 μg/ml; GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/ml). After 7 days of incubation at 27°C, the plates (25 cm^2; Falcon T) were examined with an inverted microscope (Olympus) at a magnification of ×400. The number of parasites per gram in the lesion was calculated by the following equation: parasite burden = geometric mean of the number of parasites in each duplicate/(number of microscope field counted × weight of lesion × hemocytometer correction factor [25,000]). Also, smears were prepared from the infected lesions, fixed with absolute methanol, and stained with Giemsa (OSI), and amastigotes were counted with an inverted microscope at a magnification of ×400 (6, 14). Five hundred cell nuclei from each animal

TABLE 1. Inhibitory effects of treatments with *N*-methylglucamine antimonate by the subcutaneous and intralesional routes and 2-*n*-propylquinoline on *L. venezuelensis*-infected BALB/c mice

Drug (dosage)	Route of administration	Lesion wt (g) (mean ± SD)[a]	% Suppression of lesion wt	% Suppression of parasite burden in lesion	Mean no. of parasites in lesion/g
None (control)		0.094 ± 0.020			1.6×10^7
N-Methylglucamine antimonate (56 mg of Sb^v for 15 days)	Subcutaneous	0.020 ± 0.013	−79[b]	−88[b]	1.9×10^6
N-Methylglucamine antimonate (56 mg of Sb^v for 15 days)	Intralesional	0.008 ± 0.013	−91[b]	−96[b]	5.6×10^5
2-*n*-Propylquinoline (50 mg twice daily for 15 days)	Oral	0.053 ± 0.022	−44[c]	−77[c]	3.6×10^6
2-*n*-Propylquinoline (50 mg for 5 days)	Intralesional	0.096 ± 0.052	+2	−81[c]	3.0×10^6

[a] Values represent the means ± standard deviations for eight mice per group.
[b] $P < 0.001$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.
[c] $P < 0.01$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.

TABLE 2. Effects of treatments with *N*-methylglucamine antimonate by the subcutaneous route and with 2-*n*-propylquinoline, chimanine B, 2-phenylquinoline, chimanine D, and 2-*n*-pentylquinoline by the oral or intralesional route on *L. amazonensis*-infected BALB/c mice

Drug (dosage)	Route of administration	Lesion wt (g) (mean ± SD)	% Suppression of lesion wt	% Suppression of parasite burden in lesion	Mean no. of parasites in lesion/g
None (control)[a]		0.096 ± 0.042			8.3×10^6
N-Methylglucamine antimonate (28 mg of Sb^v for 15 days)[b]	Subcutaneous	0.010 ± 0.013	−90[c]	−95[c]	4.3×10^{5c}
N-Methylglucamine antimonate (14 mg of Sb^v for 15 days)	Subcutaneous	0.085 ± 0.031	−11	−17	6.9×10^6
2-*n*-Propylquinoline (50 mg twice daily for 15 days)	Oral	0.055 ± 0.018	−43[d]	−77[d]	1.9×10^6
2-*n*-Propylquinoline (50 mg for 5 days	Intralesional	0.013 ± 0.013	−86[c]	−96[c]	3.0×10^5
Chimanine B (50 mg twice daily for 15 days)[a]	Oral	0.029 ± 0.016	−70[c]	−95[c]	4.3×10^5
Chimanine B (50 mg for 5 days)[b]	Intralesional	0.025 ± 0.009	−74[e]	−90[e]	8.3×10^5
2-Phenylquinoline (50 mg twice daily for 15 days)[a]	Oral	0.031 ± 0.016	−68[c]	−94[c]	4.7×10^5
2-Phenylquinoline (50 mg for 5 days)[b]	Intralesional	0.086 ± 0.036	−10	−10	7.9×10^6
Chimanine D (50 mg twice daily for 7 days)[b]	Oral	0.061 ± 0.040	−36	−52	4.0×10^6
Chimanine D (50 mg for 2 days)[b]	Intralesional	0.082 ± 0.056	−15	+13	9.4×10^6
2-*n*-Pentylquinoline (50 mg twice daily for 15 days)[b]	Oral	0.077 ± 0.018	−20	−45	4.6×10^6
2-*n*-Pentylquinoline (50 mg for 5 days)[b]	Intralesional	0.033 ± 0.036	−66[f]	−84[e]	1.3×10^6

[a] Values represent the means ± standard deviations for two pooled experiments or for 16 mice per group.
[b] Values represent the means ± standard deviations for one experiment with eight mice per group.
[c] $P < 0.001$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.
[d] $P < 0.002$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.
[e] $P < 0.01$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.
[f] $P < 0.05$ for treated versus control mice.

were examined under oil immersion. A good correlation ($R = 0.835$) was found between the two methods of determining the parasite burden, and data from first method are presented in Tables 1 to 4.

A corrected parasite suppression index was calculated by the following formula:

$$\frac{\text{Mean number of parasites in (or weight of) treated mice} \times 100}{\text{Mean number parasites in (or weight of) untreated mice}} - 100$$

Statistical analysis. The mean and standard deviation were calculated by using Microsoft EXCEL software. Comparisons of parasite suppression in the infected footpads of the untreated and drug-treated groups were done by analysis of variance (ANOVA) and Student's *t* test. Data were considered statistically significant at $P < 0.05$.

RESULTS

Treatment with 2-*n*-propylquinoline. Table 1 presents the results of the effect of 2-*n*-propylquinoline in vivo on lesion weight and parasite numbers in *L. venezuelensis*-infected BALB/c mice. In this experiment 2-*n*-propylquinoline was administered by the oral or intralesional route and *N*-methylglucamine was administered by the subcutaneous or intralesional route. *N*-Methylglucamine produced a significant reduction in the number of parasites in the lesion: by 88% ($P < 0.001$) and 96% ($P < 0.001$) by the subcutaneous and intralesional routes, respectively. Treatment with 2-*n*-propylquinoline suppressed lesional parasites by 77% ($P < 0.05$) and 81% ($P < 0.05$) the by the oral and intralesional routes, respectively.

Two independent experiments were performed in order to determine the effects of twice-daily oral treatment with 2-*n*-propylquinoline for 15 days on *L. amazonensis*-infected BALB/C mice. Table 2 presents the results of these experiments and includes the effect of 2-*n*-propylquinoline when it was administered orally once daily or by five intralesional injections of 50 mg/kg at intervals of 4 days. In all the experiments, twice-daily treatment with 2-*n*-propylquinoline produced a significant suppression ($P < 0.002$) of the parasite burden in the lesion. Intralesional treatment with 2-*n*-propylquinoline was efficient as well; in this case, the parasite burden in the infected footpads decreased by 96% ($P < 0.001$) compared with the burden in the footpads of untreated mice. In BALB/c mice treated with 15 subcutaneous injections of *N*-methylglucamine, parasites were suppressed by 95% ($P < 0.001$) compared with the parasite burden in untreated mice.

Treatment with chimanine B. The effect of treatment of BALB/c mice infected with *L. amazonensis* with *N*-methylglucamine or chimanine B by the oral or intralesional route is presented in Fig. 2. The lesions in treated mice did not increase in size during the first 3 weeks. In contrast, untreated mice had rapidly enlarging lesions. Twice-daily oral treatment with chimanine B at 50 mg per kg of body weight resulted in a significant effect (Table 2). A decrease in the lesion weight (70%; $P < 0.001$) and a decrease in the parasite burden of the infected footpads (95%; $P < 0.001$) were observed. The efficacy of this quinoline was similar to that of the regimen of the antimonial agent at 28 mg/kg of body weight per day for 15 days, which reduced the lesion weight by 90% ($P < 0.001$) and the parasite burden by 95% ($P < 0.001$). Nevertheless, once-daily oral treatment with chimanine B at 50 mg/kg was not as

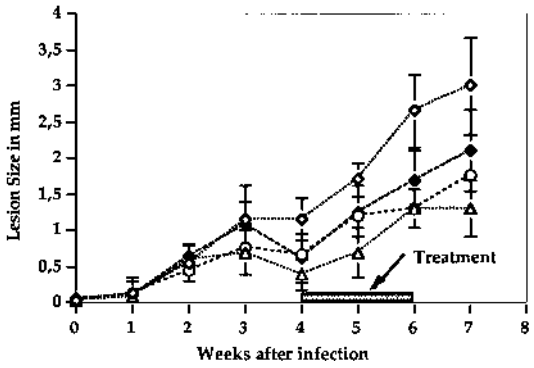

FIG. 2. Effects of treatments with *N*-methylglucamine antimonate (28 mg of Sb^v per kg per day) (△) and chimanine B administered orally at 50 mg/kg twice daily for 15 days (○) and intralesionally for five injections at 50 mg/kg at intervals of 4 days (◆) during the course of infection of BALB/c mice with *L. amazonensis* (◇, untreated mice). Each point represents the mean ± standard deviation of the mean difference in size between infected and uninfected footpads for 16 untreated mice, 16 mice treated orally with chimanine B, and 8 mice treated intralesionally with chimanine B.

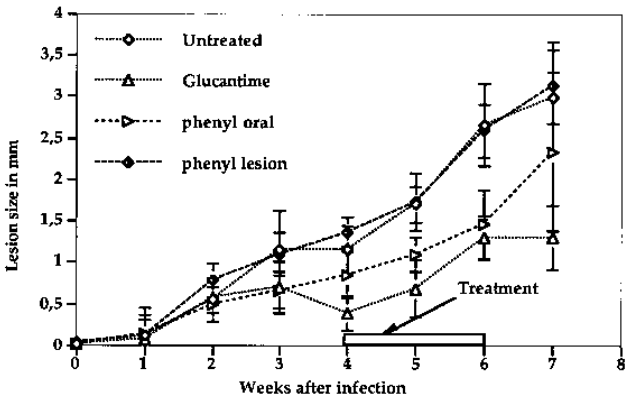

FIG. 3. Effects of treatments with *N*-methylglucamine antimonate (28 mg of Sb^v per kg per day) and 2-phenylquinoline administered orally at 50 mg/kg twice daily for 15 days and intralesionally for 5 injections at 50 mg/kg at intervals of 4 days during the course of infection of BALB/c mice with *L. amazonensis*. Each point represents the mean ± standard deviation of the mean difference in lesion size between infected and uninfected footpads for 16 untreated mice, 16 mice treated orally with 2-phenylquinoline, and 8 mice treated intralesionally with 2-phenylquinoline.

effective as twice-daily oral administration. Subcutaneous treatment with the antimonial agent at 14 mg/kg of body weight per day for 15 days had no effect (reduction of 11% of lesion weight and 17% of parasite burden). When chimanine B was administered by five intralesional injections, it reduced the parasite load by 90% ($P < 0.01$).

Treatment with 2-phenylquinoline. 2-Phenylquinoline is the major quinoline alkaloid of *G. longiflora*, a Bolivian plant used as treatment for cutaneous leishmaniasis. In previous experiments (8), 2-phenylquinoline had no effect when it was administered by subcutaneous injection. However, treatment with 2-phenylquinoline administered orally at 50 mg/kg twice daily for 15 days decreased the lesion weight by 68% ($P < 0.001$) and suppressed the parasite burden by 94% ($P < 0.001$) (Table 2). Five intralesional injections of 2-phenylquinoline at 50 mg/kg at intervals of 4 days did not suppress the parasite burden. The lesions of animals receiving 2-phenylquinoline orally twice daily increased slightly in size (Fig. 3).

Treatment with miscellaneous 2-substituted quinolines. No significant difference was observed between untreated BALB/c mice and mice treated orally or intralesionally with various 2-substituted quinolines, including skimmianine and 2-(3,4-methylenedioxyphenylethyl)quinoline (Table 3). However, on both occasions, intralesional treatment with 2-*n*-pentylquinoline or twice-daily oral treatment with chimanine D (Table 2) for 5 days resulted in parasite loads in the infected footpads that were decreased by 84% ($P < 0.002$) and 52% (not significant), respectively. Oral or intralesional combined treatment, including a mixture containing equal parts of 2-phenylquinoline and 2-*n*-pentylquinoline, did not have a leishmanicidal effect on *L. amazonensis.*

Treatment with total alkaloidal extracts of *G. longiflora*. Extracts of stem bark or root bark of *G. longiflora* were administered twice daily by the oral route for 15 days at 50 mg/kg or by five intralesional injections of 50 mg/kg at intervals of 4 days. The results are presented in Table 4. Oral treatment with root bark extract reduced the weights of the lesions by 70% ($P < 0.001$) and the parasite load by 95% ($P < 0.001$). Intralesional treatment reduced the lesion weights and parasites loads by 46% ($P < 0.001$) and 96% ($P < 0.001$), respectively. Intralesional treatment with stem bark extract decreased the lesion weight by 24% and decreased the parasite loads in the infected footpads by 99% ($P < 0.001$). Oral treatment did not show the same efficacy, reducing the parasite load by only 49% ($P < 0.05$).

DISCUSSION

The results indicate that twice-daily oral treatment with chimanine B, 2-*n*-propylquinoline, or 2-phenylquinoline at 50 mg/kg for 15 days produces the same effect as treatment with the classical antimonial drug (*N*-methylglucamine antimonate) in *L. amazonensis*-infected BALB/c mice. The leishmanicidal activities of chimanine B and 2-*n*-propylquinoline administered to experimentally infected mice by the subcutaneous route at 100 mg/kg were described previously (7). While complete cure did not occur, animals treated with these quinolines had small lesions and low *L. amazonensis* loads (3×10^5 to 4×10^5) in the infected footpads. In contrast, we have observed the inexorable development of disease in untreated mice (infected with 10^7 amastigotes). Chimanine B and 2-*n*-propylquinoline are two compounds with a propyl chain for which the synthetic preparation process does not present any inconvenience (17). The reduced toxicities of these products will facilitate treatment for longer durations, perhaps with three daily administrations for 30 or 60 days. We were surprised by the results obtained with oral 2-phenylquinoline. This quinoline is the major alkaloid in the stem bark or root bark of *G. longiflora*. Each kilogram of dried stem bark and root bark contains 15 and 25 g of this quinoline, respectively. The extraction of the compound is very easy, and it could potentially be used to treat those suffering from leishmaniasis in developing countries. The

TABLE 3. Effects of treatments with *N*-methylglucamine antimonate by the subcutaneous route and skimmianine and 2-(3,4-methylenedioxyphenylethyl)quinoline administered by the intralesional or oral route on *L. amazonensis*-infected BALB/c mice

Drug (dosage)	Route of administration	Lesion wt (g) (mean ± SD)[a]	% Suppression of lesion wt[b]	% Suppression of parasite burden in lesion[b]	Mean no. of parasites in lesion/g (10^6)
None (control)		0.046 ± 0.015			5.6
N-Methylglucamine antimonate (14 mg of Sb^v for 15 days)	Subcutaneous	0.040 ± 0.0312	−13	−16	4.7
2-(3,4-Methylenedioxyphenylethyl)quinoline (50 mg twice daily for 15 days)	Oral	0.045 ± 0.018	−2	−29	4.0
2-(3,4-Methylenedioxyphenylethyl)quinoline (50 mg for 5 days)	Intralesional	0.048 ± 0.018	+4	−14	4.8
Skimmianine (50 mg twice daily for 15 days)	Oral	0.039 ± 0.029	−15	−12	4.9
Skimmianine (50 mg for 5 days)	Intralesional	0.049 ± 0.025	+7	−7	5.2

[a] Values represent the means ± standard deviation for eight mice per group.
[b] Treated versus control mice.

TABLE 4. Effect of treatments with alkaloidal extracts of root bark or stem bark of *G. longiflora* administered by the intralesional or oral route on *L. amazonensis*-infected BALB/c mice

Drug (dosage)	Route of administration	Lesion wt (g) (mean ± SD)	% Suppression of lesion wt[a]	% Suppression of parasite burden in lesion[a]	Mean no. of parasites in lesion/g
None (control)		0.127 ± 0.064			1.0×10^7
Alkaloid extract of root bark of *G. longiflora* (50 mg twice daily for 15 days)	Oral	0.038 ± 0.022	−70[b]	−95[b]	5.3×10^5
Alkaloid extract of root bark of *G. longiflora* (50 mg for 5 days)	Intralesional	0.068 ± 0.019	−46[b]	−96[b]	3.7×10^5
Alkaloid extract of stem bark of *G. longiflora* (50 mg twice daily for 15 days)	Oral	0.064 ± 0.026	−50[b]	−49[c]	5.1×10^6
Alkaloid extract of stem bark of *G. longiflora* (50 mg for 5 days)	Intralesional	0.097 ± 0.036	−24	−99[b]	1.1×10^5

[a] Values represent the means ± standard deviations for one experiment with eight mice per group.
[b] $P < 0.001$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.
[c] $P < 0.05$ for treated versus control mice; values were confirmed to be <0.05 by ANOVA and Student's *t* test.

activities of extracts of *G. longiflora* stem bark and root bark demonstrate that this folk remedy for cutaneous leishmaniasis is effective. The other major 2-substituted quinoline, 2-*n*-pentylquinoline, did not show any activity when it was administered by the oral route, but it had significant activity when it was administered by intralesional injection. We cannot explain these variable leishmanicidal activities because the modes of action of these compounds are unknown. However, it seems that the active 2-substituted quinolines, chimanine B or 2-*n*-propylquinoline, have good absorption (they are oily compounds) and are metabolized at low levels. *L. amazonensis*-infected BALB/c mice are not the perfect model for testing the effectiveness of compounds against leishmaniasis, but the production of high numbers of parasites at the sight of the lesion allows for the evaluation of new drugs (1, 4, 18).

We conclude that of the 2-substituted quinolines tested, chimanine B is the more effective compound and is the lead molecule in this group of natural chemical compounds. The results obtained in the present study are sufficiently encouraging to continue the development of 2-substituted quinolines as antileishmanial drugs, especially chimanine B, 2-*n*-propylquinoline, and perhaps 2-phenylquinoline, and pharmacokinetic and toxicological studies with these compounds are warranted. In the coming months, clinical trials of oral treatments with chimanine B and 2-*n*-propylquinoline will be in progress in Bolivia with the support of the Bolivian Ministery of Health and the World Health Organization.

The present study is an example of the development of antileishmanial products based on ethnomedical information obtained from populations which use traditional remedies with a long history of efficacy and tolerance in humans (16).

ACKNOWLEDGMENTS

This investigation was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (grant WHO 940572) Steering Committee on Drugs for African Trypanosomiasis, Chagas' Disease and Leishmaniasis (I-CHEM).

REFERENCES

1. **Afonso, L. C. C., and P. Scott.** 1993. Immune responses associated with susceptibility of C57BL/10 mice to *Leishmania amazonensis*. Infect. Immun. **61:**2952–2959.
2. **Albrecht, H., H. J. Stellbrink, G. Gross, B. Berg, U. Helmchen, and H. Mensing.** 1994. Treatment of atypical leishmaniasis with interferon γ resulting in progression of Kaposi's sarcoma in an AIDS patient. Clin. Invest. **72:**1041–1047.
3. **Altes, J., A. Salas, M. Riera, M. Udina, A. Galmes, J. Balanzat, A. Ballesteros, J. Buades, F. Salva, and C. Villalonga.** 1991. Visceral leishmaniasis: another HIV-associated opportunistic infections? Report of eight cases of the litterature. AIDS **5:**201–207.
4. **Barral-Netto, M., J. Santana da Silva, A. Barral, and S. Reed.** 1995. Up-regulation of T-helper 2 and down-regulation of T helper 1 cytokines during murine retrovirus-induced immunodeficiency syndrome enhances susceptibility of a resistant mouse strain to *Leishmania amazonensis*. Am. J. Pathol. **146:**635–642.
5. **Berenguer, J., S. Moreno, E. Cercenado, J. C. L. Bernaldo de Quiros, A. Garcia de la Fuente, and E. Bouza.** 1989. Visceral leishmaniasis in patients infected with human immunodeficiency virus (HIV). Ann. Intern. Med. **111:**129–132.
6. **Buffet, P. A., A. Sulahan, Y. J. F. Garin, N. Nassar, and F. Derouin.** 1995. Culture microtitration: a sensitive method for quantifying *Leishmania infantum* in tissue of infected mice. Antimicrob. Agents Chemother. **39:**2167–2168.
7. **Fournet, A., A. Angelo Barrios, V. Muñoz, R. Hocquemiller, A. Cavé, and J. Bruneton.** 1993. 2-Substituted quinoline alkaloids as potential antileishmanial drugs. Antimicrob. Agents Chemother. **37:**859–863.
8. **Fournet, A., A. Angelo Barrios, V. Muñoz, R. Hocquemiller, R., F. Roblot, A. Cavé, P. Richomme, and J. Bruneton.** 1994. Antiprotozoal activity of quinoline alkaloids isolated from *Galipea longiflora*, a Bolivian plant used as a treatment for cutaneous leishmaniasis. Phytother. Res. **8:**174–178.
9. **Fournet, A., J. C. Gantier, A. Gautheret, L. Leysalles, M. H. Munos, J. Mayrargues, H. Moskowitz, R. Hocquemiller, and A. Cavé.** 1994. The activity of 2-substituted quinoline alkaloids in BALB/c mice infected with *Leishmania donovani*. J. Antimicrob. Chemother. **33:**537–544.
10. **Fournet, A., R. Hocquemiller, and J. C. Gantier.** 1995. Combattre la leishmaniose. Recherche **26:**424–429.
11. **Fournet, A., R. Hocquemiller, F. Roblot, A. Cavé, P. Richomme, and J. Bruneton.** 1993. Les chimanines, nouvelles quinoléines substituées en 2, isolées d'une plante bolivienne antiparasitaire: *Galipea longiflora*. J. Nat. Prod. **56:**1547–1552.
12. **Grogl, M., A. M. J. Odula, L. D. C. Cordero, and D. E. Kyle.** 1989. *Leishmania* spp: development of pentostam-resistant clones in vitro by discontinuous drug exposure. Exp. Parasitol. **69:**78–90.
13. **Grogl, M., T. N. Thomason, and E. D. Franke.** 1992. Drug resistance in leishmaniasis: its implication in systemic chemotherapy of cutaneous and mucocutaneous leishmaniasis. Am. J. Trop. Med. Hyg. **47:**117–126.
14. **Hill, J. O., R. J. North, and F. M. Collins.** 1983. Advantages of measuring changes in the number of viable parasites in murine models of experimental cutaneous leishmaniasis. Infect. Immun. **39:**1087–1094.
15. **Ho, J. L., R. Badaro, D. Hatzigeorgiou, S. G. Reed, and W. D. Johnson, Jr.** 1994. Cytokines in the treatment of leishmaniasis: from studies of immunopathology to patient therapy. Biotherapy **7:**223–235.
16. **Iwu, M. M., J. E. Jackson, and B. G. Schuster.** 1994. Medicinal plants in the fight against leishmaniasis. Parasitol. Today **10:**65–68.
17. **Munos, M. H., J. Mayrargue, A. Fournet, J. C. Gantier, R. Hocquemiller, and H. Moskowitz.** 1994. Synthesis of an antileishmanial alkaloid isolated from *Galipea longiflora* and of related compounds. Chem. Pharm. Bull. **42:**1914–1916.
18. **Silva, E. M., A. L. Betho, and C. F. Mendoça.** 1993. Effect of *in vivo* depletion of $CD4^+$ T cells on experimental infection of susceptible BALB/c mice with *Leishmania amazonensis*. Acta Trop. **56:**111–120.
19. **Torre-Cisneros, J., and J. L. Villanueva.** 1995. Efficacy of liposomal amphotericin B in the treatment of visceral leishmaniasis in patients coinfected with the human immunodeficiency virus. Clin. Infect. Dis. **20:**191 (Letter.)
20. **United Nations Development Program/World Bank/World Health Organization.** 1995. Tropical disease research. Progress 1975–94. Highlights 1993–94, p. 137. United Nations Development Program/World Bank/World Health Organization, Geneva.