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The activity of 2-substituted quinoline alkaloids in BALB/c mice infected with *Leishmania donovani*

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Potent antileishmanial activity has recently been described *in vivo* when certain 2-substituted quinoline alkaloids are administered to mice with cutaneous leishmaniasis. We now report the antileishmanial activity of four 2-substituted quinoline alkaloids, namely chimanine D or 2-(1′,2′-*trans*-epoxypropyl) quinoline (I), 2-*n*-propylquinoline (II), 2-styrylquinoline (III) and 2-(2′-hydroxypropyl) quinoline (IV), for experimental treatment of visceral leishmaniasis in infected BALB/c mice. Subcutaneous treatment with chimanine D for 10 days at 0·54 mmol/kg per day resulted in 86·6% parasite suppression in the liver. Oral administration of 0·54 mmol/kg of 2-*n*-propylquinoline once daily for 5 or 10 days to *L. donovani*-infected mice suppressed parasite burdens in liver by 87·8 and 99·9%, respectively. Cutaneous administration of meglumine antimonate for 10 days resulted in 97·4% parasite suppression in the liver. This study is, to our knowledge, the first to demonstrate the activity of 2-substituted quinoline alkaloids in experimental treatment of visceral leishmaniasis. Further biological and chemical studies of these products might yet prove helpful for the development of new antileishmanial drugs.

Introduction

Visceral leishmaniasis or kala-azar is a fatal human disease caused by *Leishmania donovani*. This disease is endemic in many parts of the tropical and subtropical regions of the world. The estimated global prevalence is 12 million, with 400,000 to 2,000,000 new cases reported each year. The World Health Organization estimates that approximately 350 million people live in endemic areas (WHO, 1990). Drugs currently available for treatment of kala-azar are potentially toxic, are inconvenient to administer and frequently (10–15%) give rise to clinical resistance (Berman, 1988; Croft, 1988; Kuhlencord *et al.*, 1992). Drugs of first choice are pentavalent antimonials such as sodium stibogluconate and meglumine antimonate. Second-line drugs are diamidines (pentamidine) and amphotericin B, which although effective in kala-azar, are of limited use because of their toxicity.

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Other treatments for kala-azar are now under investigation. These include liposomal amphothericin B (Croft, Davidson & Thornton, 1991), allopurinol and derivatives (Kager *et al.*, 1981; Chung *et al.*, 1985) bis(benzyl) polyamine analogues (Baumann, McCann & Bitonti, 1991), formycin B (Berman *et al.*, 1983), 8-aminoquinoline derivatives (Kinnamon *et al.*, 1978; Neal, 1987), an alkylphosphocholine compound, hexadecylphosphocholine (Croft *et al.*, 1987; Kuhlencord *et al.*, 1992), acivicin (Mukherjee, Roy & Bhaduri, 1990) and recently hydroxynaphthoquinones, whose activity *in vivo* is limited (Croft *et al.*, 1992).

Recent reports of resistance to antimonial drugs in India and in Sudan (WHO, 1991), and numerous cases of visceral leishmaniasis in patients with AIDS have been described (Peters *et al.*, 1990). For these reasons, new chemotherapeutic agents active against visceral leishmaniasis are urgently required, and such new compounds should be administered by the oral route in order to facilitate their use.

We have already described the efficacy of 2-substituted quinolines for treatment of cutaneous New World leishmaniasis, *Leishmania amazonensis* and *Leishmania venezuelensis* and the synthesis of several 2–substituted quinolines (Fournet *et al.*, 1992), including a 2-substituted three-carbon chain quinoline and a 2-substituted phenylethyl chain. The aim of this study was to evaluate the activity of 2-substituted quinolines in BALB/c mice intravenously infected with *L. donovani*. Different routes of administration, namely intraperitoneal, subcutaneous and oral were investigated.

Materials and methods

Drugs

The quinoline alkaloids, 2-*n*-propylquinoline (I), 2-(1′,2′-*trans*-epoxypropyl) quinoline or chimanine D (II) were isolated from the Bolivian plant, *Galipea longiflora* Krause (Rutaceae) by fractionation and purification monitored by bioassay (Fournet *et al.*, 1991). 2-styrylquinoline (III) and (2-(2′-hydroxypropyl) quinoline (IV) were synthesized as described in PCT Patent (Fournet *et al.*, 1992). The structures of these products are shown in the Figure. *N*-methylglucamine antimonate (meglumine antimonate, Glucantime^{R}) equivalent to 0·28 mg Sb^{v}/mL was obtained from Rhône-Poulenc, France.

Animals

Female BALB/c mice (weight 18–20 g) were supplied by the IFFA-CREDO, France and male hamsters (weight 90–120 g) (*Mesocricetus auratus*) by the Animal Production Centre, France. The latter were used to maintain the parasite.

Parasites

The Ethiopian strain of *L. donovani* (MHOM/ET/67/L82; LV9) was obtained from Dr Simon L. Croft (London School of Hygiene, London, UK) and maintained by serial passage in hamsters at the time of infection. Parasites were obtained by homogenizing the spleen of a freshly killed hamster, infected for approximately 4 weeks in RPMI 1640 medium containing 10% fetal calf serum (Gibco). The hamsters were infected by intracardial injection of 10^{8} amastigotes of *L. donovani* in 100 μL of medium.

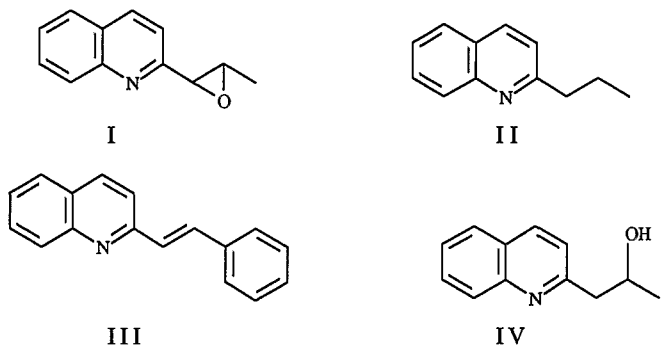

Figure. Structures of chimanine D (I), 2-*n*-propylquinoline (II), 2-styrylquinoline (III) and 2-(2-hydroxypropyl)quinoline (IV).

Parasite suppression and parasite distribution

Ten BALB/c mice were infected via the tail vein (without anaesthetic) by injection of 5×10^6 amastigotes in 100 μL medium derived from homogenates of infected hamster spleens. One day after the last drug administration, the mice were weighed, killed and the livers and spleens removed and weighed. Liver impressions were prepared and stained by Giemsa and the numbers of amastigotes per host liver cell nucleus were counted (500 liver nuclei of each animal were examined under oil immersion). The number of amastigotes per organ per nucleus × liver mass in mg × (2×10^5) is approximately equal to the total number of amastigotes per liver (Stauber, Franchino & Grun, 1958). Parasite suppression was calculated from the ratio of the mean liver amastigote counts of drug-treated mice and the mean liver amastigotes counts of untreated mice multiplied by 100 to obtain the percentage of parasite suppression. Pieces of liver were cultured in 25 cm^2 tissue culture flasks (T25, Falcon) on 90 mL RPMI 1640 medium containing 10% fetal calf serum, 1 mL of solution of meglumine antimonate (29·4 mg/L), 2 mL of Schneider medium and antibiotics (100 UI of penicillin and 100 μg of streptomycin/mL). The cultures were observed daily for 10 days.

Antimicrobial treatment

The animals were treated intraperitoneally, subcutaneously or orally once daily with the experimental drugs for 5 or 10 days during 1 or 2 week periods. The treatments were initiated 1 week after parasite inoculation. The BALB/c mice were weighed before treatment began. One week after infection the mice were randomly divided into groups of ten. Drugs were made up in 100 μL 1% carboxymethylcellulose (CMC) and Tween 80 and administered daily on days 5 and 10 by intraperitoneal, subcutaneous or oral routes. Quinoline alkaloids were tested at dose level of 0·54 mmol/kg body weight/day for 5 or 10 days. Quinoline alkaloids were also administered in CMC-Tween by gavage. In each experiment, mice were treated for 5 or 10 days with the reference drug, *N*-methylglucamine antimonate at a dose of 56 mg of Sb^v/kg/day, which corresponds to 200 mg/kg or 0·54 mmol/kg. The reference drug was dissolved in 100 μL of CMC-Tween and administered by intraperitoneal or subcutaneous routes. In each protocol two groups of ten mice were treated daily for 5 and 10 days and ten infected mice were untreated and served as controls.

Presentation and statistical analysis of data

Parasite suppression was calculated from the ratio of the mean amastigote counts in the drug-treated groups to the mean amastigote counts in the untreated control groups. The Student *t*-test was used for the statistical analysis of all data ($P > 0{\cdot}05$).

Results

Intraperitoneal treatment

Four quinoline alkaloids were used in this study: chimanine D (I), 2-*n*-propylquinoline (II), 2-styrylquinoline (III) and 2-(2′-hydroxypropyl) quinoline (IV). Table I shows that the weights of spleen and liver of all infected mice increased after 2 weeks of experiment and 5 days of treatment with quinoline alkaloids or antimonial compound. The greatest increase in splenic and liver weight was (total weight of 240 mg and 1·96 g, respectively) in mice treated with chimanine D (I). The liver and spleen weights of mice treated with other compounds and meglumine antimonate were identical. Table I also shows that suppression of the parasite burden in livers of BALB/c mice treated with antimony compounds was reduced by 97·2% and 79·6% in mice treated with 2-styrylquinoline (III). With other compounds we obtained suppression of parasites between 62·4% with 2-*n*-propylquinoline (II) and 55·7% with 2-(2-hydroxypropyl)quinoline (IV). We did not observe any side-effects of these quinoline alkaloids during these experiments: necrosis at the site of drug inoculation or loss of weight were not observed. Homogenates of liver of mice treated for 5 days with quinoline alkaloids and meglumine antimonate were cultured and observed for parasite growth. All cultures of homogenates of liver from meglumine antimonate or quinoline alkaloid treated mice were positive after 10 days of incubation.

Table I. Efficacy of four quinoline alkaloids and meglumine antimonate administered intraperitonally to *L. donovani* infected BALB/c mice[a]

Treatment	Dose (mmol/kg)	Liver wt (g) (mean ± S.E.)[b]	Spleen wt (mg) (mean ± S.E.)[b]	% Suppression of parasites load in the liver[c]
Uninfected		1·27 ± 0·07	107 ± 13	
No drug (control)		1·57 ± 0·09	176 ± 14	—
Meglumine antimonate[d]	0·54 × 5	1·58 ± 0·08	168 ± 25	97·2
Chimanine D (I)	0·54 × 5	1·96 ± 0·09	240 ± 30	57
2-*n*-propylquinoline (II)	0·70 × 5	1·53 ± 0·12	190 ± 25	62·4
2-styrylquinoline (III)	0·54 × 5	1·52 ± 0·08	169 ± 30	79·6
2-(2′-hydroxypropyl) quinoline (IV)	0·54 × 5	1·53 ± 0·10	180 ± 30	55·7

[a] Mice ($n = 10$) were treated intraperitoneally with quinoline alkaloids or meglumine antimonate once per day for 5 days, beginning on day 7.

[b] Mean liver or spleen weight ± standard error.

[c] Values including the liver weight (mg) × number of amastigotes/500 liver nuclei (see Stauber *et al.*, 1958), compared with data for mice receiving 100 μL CMC-Tween 80 only. Each treatment and control group included ten mice.

[d] 0·54 mmol = antimony 56 mg/kg.

Table II. Efficacy of four quinoline alkaloids and meglumine antimonate administered subcutaneously to *Leishmania donovani* infected BALB/c mice[a]

Treatment	Dose (mmol/kg)	Liver wt (g) (mean ± S.E.)[b]	Spleen wt (mg) (mean ± S.E.)[b]	% Suppression of parasites load in the liver[c]
Untreated		1·61 ± 0·20	270 ± 95	—
Meglumine antimonate[d]	0·54 × 5	1·69 ± 0·18	210 ± 35	89·9
	0·54 × 10	1·37 ± 0·20	200 ± 120	97·4
Chimanine D (I)	0·54 × 5	1·52 ± 0·13	160 ± 30	69·5
	0·54 × 10	1·46 ± 0·20	200 ± 60	86·6
2-*n*-propylquinoline (II)	0·54 × 5	1·57 ± 0·13	190 ± 20	76·3
	0·54 × 10	1·69 ± 0·11	250 ± 40	67·8
2-styrylquinoline (III)	0·20 × 5	1·85 ± 0·27	240 ± 80	37·1
	0·20 × 10	1·83 ± 0·29	350 ± 130	26·3

[a]Mice ($n = 10$) were treated subcutaneously with quinoline alkaloids or meglumine antimonate once per day for 5 or 10 days, beginning on day 7.
[b]Mean liver or spleen weight ± standard error.
[c]Values including the liver weight (mg) × number of amastigotes/500 liver nuclei (see Stauber *et al.*, 1958), compared with data for mice receiving 100 μL CMC-Tween 80 only. Each treatment and control group included ten mice.
[d]0·54 mmol = antimony 56 mg/kg.

Subcutaneous treatment

The liver and spleen weights of mice treated with the antimonial compound and with chimanine D (I) and 2-*n*-propylquinoline (II) were equivalent. Subcutaneous treatment with chimanine D (I) at 0·54 mmol/kg once per day for 5 days caused 69·5% suppression in the spleen parasite count compared with untreated mice (Table II). Prolonging the treatment by 5 days produced a better effect with 86·6% suppression of liver parasites. In this study, subcutaneous meglumine antimonate treatment for 5 and 10 days suppressed spleen parasites respectively by 89·9% and 97·4% compared with infected untreated mice. Treatment with 2-*n*-propylquinoline at 0·54 mmol/kg for 5 or 10 days resulted in suppression of the parasites by 76·3% and 67·8%. Preliminary toxicological evaluation of quinoline alkaloids I and II in mice indicated that the acute intraperitoneal 50% lethal dose was greater than 400 mg/kg. The third quinoline alkaloid 2-styrylquinoline (III) tested did not show efficacy against *L. donovani* when it was administered at 46 mg/kg once a day for 5 or 10 days. We decreased the dose of this compound because of fatal toxicity when it was administered at the same molecular concentration (0·54 mmol/kg) as the other quinoline alkaloids and reference drug (meglumine antimonate).

Oral treatment

Two quinoline alkaloids were administered by the oral route, chimanine D (I) and 2-*n*-propylquinoline (II) at 0·54 mmol/kg for 5 and 10 days, 1 week after parasitic infection. The other compound, 2-styrylquinoline (III) was given at 0·2 mmol/kg under the same conditions. As shown in Table III, both chimanine D (I), 2-*n*-propylquinoline (II) and meglumine antimonate were effective in reducing liver and spleen weight when

Table III. Parasite suppression in leishmania-infected mice treated with quinoline alkaloids orally for 5 or 10 days.

Treatment[a]	Dose (mmol/kg)	Liver wt (g) (mean ± S.E.)[b]	Spleen wt (mg) (mean ± S.E.)[b]	% Suppression of parasites load in the liver[c]
Untreated		1·61 ± 0·20	270 ± 95	—
Meglumine antimonate[d]	0·54 × 5	1·69 ± 0·18	210 ± 35	89·8
	0·54 × 10	1·37 ± 0·20	200 ± 120	97·4
Chimanine D (I)	0·54 × 5	1·40 ± 0·14	140 ± 20	72·9
	0·54 × 10	1·45 ± 0·14	200 ± 50	62·0
2-*n*-propylquinoline (II)	0·54 × 5	1·51 ± 0·06	150 ± 20	87·8
	0·54 × 10	1·37 ± 0·13	200 ± 40	99·9
2-styrylquinoline (III)	0·20 × 5	1·46 ± 0·13	200 ± 90	42·6
	0·20 × 10	1·62 ± 0·28	330 ± 110	6·1

[a]Mice ($n = 10$) were treated orally with quinoline alkaloids and subcutaneously with meglumine antimonate once per day for 5 or 10 days, beginning on day 7.

[b]Mean liver or spleen weight ± standard error.

[c]Values including the liver weight (mg) × number of amastigotes/500 liver nuclei (see Stauber *et al.*, 1958), compared with data for mice receiving 100 μL CMC-Tween 80 only. Each treatment and control group included ten mice.

[d]0·54 mmol = antimony 56 mg/kg.

given for 5 or 10 days compared with untreated groups. Treatment with 2-styrylquinoline (III) alone for 10 days resulted in a great increase of spleen weight. Oral treatment with 2-*n*-propylquinoline (0·54 mmol/kg) (II) and meglumine antimonate (56 mg of Sb^v/kg) for a 5-day period produced an equivalent suppression of parasite load in the liver by 87·8% and 89·8%, respectively. Extending oral treatment to 10 days with 2-*n*-propylquinoline (II) increased the effect of parasite suppression in the liver to 99·9% 10 days' subcutaneous treatment of infected mice with meglumine antimonate did not produce greater parasite suppression. In these experiments we did not observe any side-effects when mice were orally treated with chimane D (I) or 2-*n*-propylquinoline (II). Examination of the liver and spleen did not show any apparent toxicity.

Discussion

In a previous study (Fournet *et al.*, 1991) we reported that subcutaneous treatment with quinoline alkaloids was effective against New World cutaneous leishmaniasis, *L. amazonensis* and *L. venezuelensis* in BALB/c mice. We now show that oral administration of one of these compounds, 2-*n*-propylquinoline (II), suppressed 99·9% of liver parasites and that another quinoline alkaloid, chimanine D (I) resulted 86·6% parasite suppression when it was given for 10 days at 0·54 mmol/kg by the subcutaneous route. In contrast, we found suppression of *L. donovani* by 97·4% in the liver when mice were treated with meglumine antimonate by the subcutaneous route for 10 days at 56 mg/Sb^v/kg/day. Oral administration of chimanine D (I) for 5 days resulted in lower parasite suppression (72·9%). Attempts to administer quinoline alkaloids by the parenteral route did not produce a similar effect on mice infected with *L. donovani*. Treatment of infected mice by the peritoneal route with quinoline alkaloids was not as effective as the antimony compound. 2-styrylquinoline (III) alone suppressed 79·6% of parasites in the liver.

The interesting oral activity of 2-*n*-propylquinoline (II) in the liver of mice may probably be explained by its excellent distribution within the reticuloendothelial system. In this study we have observed better antileishmanial activity of quinoline alkaloids which include a propyl chain, such as compounds I and II. Longer therapy or administration twice a day might enhance the efficacy of these compounds because of parasite suppression in the spleen or the liver. The reduced toxicity of oily compounds (such as quinoline alkaloids with 2-substituted three-carbon chain) will facilitate treatments of longer duration. The *in vivo* efficacy of oral 2-*n*-propylquinoline (II) and chimanine D (I) against *L. donovani* suggests that these agents should be evaluated for their therapeutic effects in dogs infected with visceral leishmaniasis.

The efficacy of the 2-substituted quinoline alkaloids against cutaneous leishmaniasis of the New World and visceral leishmaniasis by parenteral and oral routes suggests that exploration of their potential should continue. This study is the first to our knowledge, to show the activity of 2-substituted quinoline alkaloids for treating experimental visceral leishmaniasis. We continue to explore the activity of these oral compounds and new analogues against *L. donovani* even though commercial motivation to develop drugs for most tropical diseases may be limited (Del Mar Sanz *et al.*, 1991).

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