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Naphthylisoquinoline Alkaloids against Malaria: Evaluation of the Curative Potentials of Dioncophylline C and Dioncopeltine A against *Plasmodium berghei* In Vivo†

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Naphthylisoquinoline alkaloid-containing extracts from species of the families *Dioncophyllaceae* and *Ancistrocladaceae* and purified alkaloids derived therefrom were shown to exhibit antiparasitic activity in *Plasmodium berghei*-infected mice. Several extracts and alkaloids, especially dioncophylline C and dioncopeltine A, isolated from *Triphyophyllum peltatum* (*Dioncophyllaceae*), displayed high levels of activity. Dioncopeltine A was able to suppress parasitemia almost totally, while dioncophylline C cured infected mice completely after oral treatment with 50 mg kg of body weight^{-1} day^{-1} for 4 days without noticeable toxic effects. Analysis of the dose-response relationship of dioncophylline C revealed a 50% effective dosage (ED_{50}) of 10.71 mg kg^{-1} day^{-1} under these conditions. Although four daily treatments with 50 mg kg^{-1} day^{-1} are needed to achieve radical cure, one oral dose is sufficient to kill 99.6% of the parasites. Intravenous application of dioncophylline C is even more effective, with an ED_{50} of 1.90 mg kg^{-1} day^{-1} and no noticeable toxic effects. The compound also suppressed more established *P. berghei* infections when orally applied at day 3 after infection. Both dioncopeltine A and dioncophylline C are active against the chloroquine-resistant *P. berghei* Anka CRS parasites. Sustained release of these compounds at 20 mg kg^{-1} day^{-1} by implanted miniosmotic pumps exhibited curative effects. The naphthylisoquinoline alkaloids are therefore promising new antimalarial agents.

More than ever, malaria is one of the most threatening tropical diseases and causes inexpressible suffering in more than 90 countries. Official estimates range between 1.5 million and 2.7 million fatal cases and between 300 million and 500 million clinical cases of malaria per year, and these are accompanied by considerable socioeconomic damage (18, 49). Control of the disease is, among other factors, hampered by resistance of the vector (*Anopheles* spp.) to insecticides and an ongoing spread of drug-resistant strains of *Plasmodium falciparum*, the malaria parasite most dangerous to humans. Resistance to chloroquine has become very common not only in Southeast Asia but also in considerable parts of Africa, South America, and Oceania. At present, there is no country in sub-Saharan Africa from which chloroquine resistance has not been reported (1, 38). Multidrug resistance increases in intensity and spreads geographically. In Southeast Asia and, more specifically, in the border regions between Thailand and both Myanmar (Burma) and Cambodia, *P. falciparum* has developed resistance to all commonly available antimalarial drugs (16, 28, 32, 39, 47, 48). In addition, the emerging resistance of *Plasmodium vivax* to a variety of drugs has been reported (35, 36, 41). On the basis of these facts, it is obvious that new antimalarial agents are urgently needed (37).

Extracts from species of the families *Dioncophyllaceae* and *Ancistrocladaceae* (6), tropical lianas containing naphthylisoquinoline alkaloids, a new class of natural products with special structural and biosynthetic characteristics (3, 5, 10), have been widely applied in traditional medicine of several African and Asian countries. *Dioncophyllum thollonii* (*Dioncophyllaceae*) is known for its activity against leprous skin disease (31), *Ancistrocladus abbreviatus* (*Ancistrocladaceae*) is known for its activity against measles and fevers (27), and *Ancistrocladus tectorius* is known for its activity against dysentery and malaria (43). Several extracts from such species and naphthylisoquinoline alkaloids derived therefrom, as well as synthetic mono- and dimeric alkaloids, exhibit a variety of biological activities (10). They are especially active against asexual erythrocytic stages of *P. falciparum* NF 54 and K1 and *Plasmodium berghei* Anka in vitro (2, 11–13, 20–23, 25, 26). These intracellular stages are of particular interest, being responsible for all clinical malaria symptoms (46). Besides, the potential of naphthylisoquinoline alkaloids against exoerythrocytic stages of *P. berghei* in human hepatoma cells has recently been demonstrated (24, 26). These results, combined with the low cytotoxicity of the compounds (23), make them candidates for in vivo testing. The availability of a selection of alkaloids permitted experiments on their antimalarial activities in *P. berghei*-infected rodents (30, 34) as part of the preclinical phase of their development.

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† This is part 9 in the series Antiprotozoal Activity of Naphthylisoquinoline Alkaloids (for Part 8, see reference 26). This is part 97 in the series Acetogenic Isoquinoline Alkaloids (for part 96, see reference 14).

TABLE 1. Treatment of *P. berghei* Anka-infected OF1 mice with extracts from *T. peltatum* (*Dioncophyllaceae*) and *A. abbreviatus* (*Ancistrocladaceae*)[a]

Treatment	Day 4			Day 7		
	% Parasitemia[b]	*P*[c]	No. of dead mice/ total no. of mice	% Parasitemia	*P*	No. of dead mice/ total no. of mice
Control	3.18 (2.82–3.54)		0/6	4.30 (0.00–8.62)		4/6
T. peltatum						
Root (CH_2Cl_2)	3.78 (3.01–4.54)	0.099[d]	0/6	6.99		5/6
Stem bark (CH_2Cl_2)	3.05 (2.14–3.97)	0.723[d]	0/6	6.52 (0.00–29.32)	0.349[d]	4/6
Stem bark (CH_2Cl_2-NH_3)	0.80 (0.40–1.19)	<0.0001[e]	0/6	3.85 (1.47–6.22)	0.714[d]	2/6
A. abbreviatus						
Root (CH_2Cl_2-NH_3)	3.50 (2.35–4.64)	0.520[d]	0/6	5.35 (0.00–14.43)	0.318[d]	4/6
Stem bark (CH_2Cl_2-NH_3)	3.57 (2.81–4.33)	0.260[d]	0/6	7.72 (0.00–17.38)	0.055[d]	4/6

[a] Mice were orally treated with 50 mg of extract kg^{-1} day^{-1} in a 4-day test.
[b] Values are mean levels of parasitemia (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Not significantly different from controls in one-way ANOVA.
[e] Significantly different from controls in one-way ANOVA.

MATERIALS AND METHODS

Extracts. Specimens of *Triphyophyllum peltatum* and *A. abbreviatus* were collected by L. Aké Assi in western Ivory Coast in January 1988 and July 1990, respectively. Voucher specimens were deposited at the Conservatoire et Jardin Botaniques de l'Université d'Abidjan, Abidjan, Ivory Coast. The air-dried, ground root and stem bark material was first lyophilized and was subsequently extracted in a Soxhlet apparatus by consecutively using CH_2Cl_2 and CH_2Cl_2-NH_3 (98:2) as eluents. The solvents were removed under reduced pressure.

Naphthylisoquinoline alkaloids. The following naturally occurring alkaloids (see Fig. 1) were isolated from *T. peltatum* and were characterized as described before: dioncophyllines A (compound 1) (4), B (compound 2) (7), and C (compound 3) (9) and dioncopeltine A (compound 4) (8).

***P. berghei* strains.** *P. berghei* Anka and K173 (15, 29, 33) erythrocytic stages were obtained from cryopreserved samples kept at the Prins Leopold Instituut voor Tropische Geneeskunde, Antwerp, Belgium, and the Sint Radboud Ziekenhuis, Nijmegen, The Netherlands, respectively. The chronic chloroquine-resistant CRS line was derived from the Anka strain by serial passages in Wistar rats and OF1 mice (44, 45).

Laboratory animals and drug administration. Six-week-old, outbred female OF1 mice were purchased from Iffa Credo, Brussels, Belgium. Drugs were orally administered with the aid of a stainless steel probe (0.8 by 3.2 cm) directly introduced into the esophagus. In part of the experiments, drugs were injected subcutaneously in the neck region or intravenously in one of the lateral tail veins. Six-week-old, inbred female C57Black/6J and outbred male and female Swiss mice were obtained from local colonies of the Central Animal Facility of the Katholieke Universiteit Nijmegen and were raised and kept under specific-pathogen-free conditions. The C57Black/6J mice were used in the experiments with implanted osmotic pumps. All mice were housed and handled in conformity with national and international legislation and guidelines. Drinking water and food were available ad libitum.

Antimalarial activity in vivo. The antimalarial activities of extracts and alkaloids were determined by a standard 4-day test (40, 42) applied to *P. berghei*-infected mice.

The OF1 mice were divided into groups of six mice each. At day 0, they were infected intraperitoneally with 10^6 erythrocytic forms of *P. berghei* Anka obtained from a heavily infected donor mouse. Two hours later, all but the control mice received an oral dose varying from 3.125 to 100 mg of extract kg of body $weight^{-1}$ or pure naphthylisoquinoline alkaloid dissolved in a minimal volume of dimethyl sulfoxide (DMSO) adjusted to 0.2 ml with saline. The controls received 0.2 ml of the same concentration of DMSO in saline. The treatment was given daily for 4 consecutive days (days 0 to 3). At days 4 and 7, thin smears of tail blood were made, fixed with methanol, and stained with Giemsa. The level of parasitemia (percentage of infected erythrocytes) was determined microscopically (magnification, ×1,000; Leitz) by examination of 10,000 cells. In modified regimens, the drugs were administered starting at different days after the infection for various periods of time and by different routes.

Sustained release by mini-osmotic pumps. Mini-osmotic pumps with a flow rate of 1 μl/h (Alzet, type 2001; Charles River Deutschland GmbH, Kislegg, Germany) were filled with dioncophylline C and dioncopeltine A, dissolved in a minimal amount of DMSO, and diluted with physiological saline to obtain a concentration of 16.6 mg/ml, providing a sustained release of 20 mg kg^{-1} day^{-1}. Those prepared for the control group contained the same concentration of DMSO in saline.

The pumps were implanted subcutaneously in the backs of C57Black/6J mice at day −1, and all mice were inoculated intraperitoneally with 10^6 *P. berghei* K173 parasites at day 0. The level of parasitemia was determined as described above. The surviving mice were sacrificed at day 28 after infection.

The absence of circulating parasites in treated, smear-negative C57Black/6J mice was verified at day 14 after infection by the transfer of 0.1 ml of blood into naive Swiss mice that were examined for the development of parasitemia for a 4-week period.

TABLE 2. Treatment of *P. berghei* Anka-infected OF1 mice with naphthylisoquinoline alkaloids from *T. peltatum* (*Dioncophyllaceae*)[a]

Treatment	Day 4			Day 7		
	% Parasitemia[b]	*P*[c]	No. of dead mice/ total no. of mice	% Parasitemia	*P*	No. of dead mice/ total no. of mice
Control	3.50 (3.02–3.97)		0/6	8.70 (0.00–45.80)		4/6
Dioncophylline A	2.96 (2.61–3.30)	0.127[d]	0/6	8.97 (8.09–9.85)	0.957[d]	3/6
Dioncophylline B	1.86 (0.66–3.07)	0.009[e]	0/6	8.49 (2.70–14.29)	0.959[d]	1/6
Dioncophylline C	0.00 (0.00–0.00)	<0.0001[e]	0/6	0.00	0.001[e]	0/6
Dioncopeltine A	0.03 (0.01–0.06)	<0.0001[e]	0/6	2.21 (1.35–3.06)	0.005[e]	0/6

[a] Mice were orally treated with 50 mg of alkaloid kg^{-1} day^{-1} in a 4-day test.
[b] Values are mean levels of parasitemia (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Not significantly different from controls in one-way ANOVA.
[e] Significantly different from controls in one-way ANOVA.

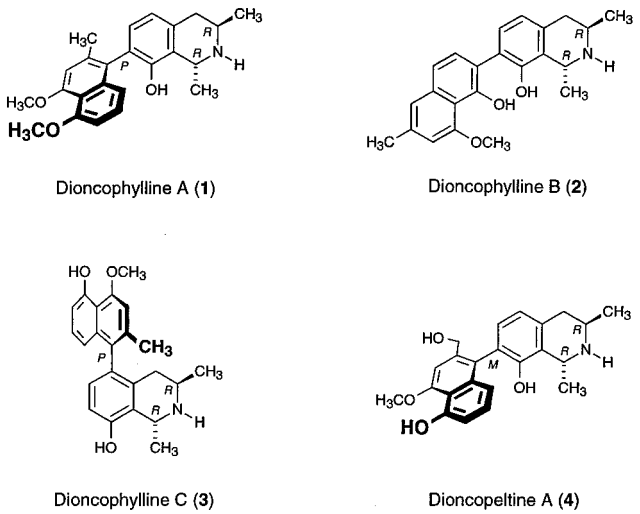

FIG. 1. Structures of the naphthylisoquinoline alkaloids (compounds 1 to 4).

Analysis of the results. Statgraphics (Graphic Software Systems) was used for statistical analysis of treatment versus control data (one-way analysis of variance [ANOVA]; threshold, $P = 0.050$). The sigmoid dose-response curves were linearized by probit transformation (17, 19) for the determination of effective dosages (EDs).

RESULTS

P. berghei-infected OF1 mice were orally treated with three extracts from *T. peltatum* and two extracts from *A. abbreviatus* in the 4-day test (Table 1). The CH_2Cl_2-NH_3 stem bark extract from *T. peltatum* suppressed the parasitemia significantly (day 4), while the other four extracts were inactive. Several pure naphthylisoquinoline alkaloids from *T. peltatum* were highly active (Table 2). Dioncophylline B and dioncopeltine A suppressed the parasitemia significantly (47 and 99% at day 4, respectively), while dioncophylline C was the most active compound tested and completely cleared the parasites from the peripheral blood. Dioncophylline A had no effect (Fig. 1).

Administration of dioncophylline C at dosages ranging from 3.125 to 100 mg kg^{-1} day^{-1} to *P. berghei*-infected mice by the 4-day test revealed significant inhibitory activities at 12.5 mg kg^{-1} day^{-1} and higher dosages (Table 3). The calculated EDs (with their corresponding 95% confidence intervals given in parentheses) at day 4 are as follows: 25% ED (ED_{25}), 6.81 mg kg^{-1} day^{-1} (5.48 to 8.46 mg kg^{-1} day^{-1}); ED_{50}, 10.71 mg kg^{-1} day^{-1}, (8.94 to 12.83 mg kg^{-1} day^{-1}); and ED_{95}, 32.44 mg kg^{-1} day^{-1} (23.40 to 44.98 mg kg^{-1} day^{-1}). The effective range for oral application of dioncophylline C is 12.5 to 50 mg kg^{-1} day^{-1}, since dosages below 12.5 mg kg^{-1} day^{-1} did not suppress proliferation of the parasite and 100 mg kg^{-1} day^{-1} caused a mild but transient neurotoxic effect in one of six treated mice. The initial reduction of the parasitemia by dosages of 12.5 and 25 mg kg^{-1} day^{-1} up to day 4 was not sufficient to prolong the mean survival times of the animals, in contrast to the complete cure caused by 50 and 100 mg kg^{-1} day^{-1}. No obvious acute or chronic side effects could be noted in OF1 mice treated with four dosages of 50 mg kg^{-1} day^{-1}. They looked normal and behaved absolutely normally during several months of observation after the treatment.

Administration of a single dosage of 50 mg of dioncophylline C kg^{-1} day^{-1} at day 0 suppressed the parasitemia by 99.6% until day 4 postinfection, but four daily treatments were required for complete cure (Table 4). The proportion of mice that cleared the parasitemia increased with the number of daily applications: none of six, two of six, and five of six mice after one, two, and three applications, respectively, indicating a strict linear dose-response relationship ($r = 0.984$) and resulting in 2.38 daily treatments to cure 50% of the mice.

Dioncophylline C was also administered via other routes by using the 4-day schedule. Both the subcutaneous and intravenous routes were proven to be effective, although the effects were not strong enough to persist until day 7. Subcutaneous application of 20 mg kg^{-1} day^{-1} reduced the level of parasitemia by ca. 84% ($P < 0.001$) at day 4, while dosages of 5 and 1.25 mg kg^{-1} day^{-1} had no effect. These results are comparable to those obtained after oral administration of dioncophylline C (Table 3). Intravenous injection of the compound was more effective, since dosages of 20, 5, and 1.25 mg kg^{-1} day^{-1} suppressed the parasitemia at day 4 by 89% ($P < 0.0001$), 38% ($P = 0.007$), and 43% ($P = 0.006$), respectively. The corresponding calculated EDs (with their 95% confidence intervals given in parentheses) at day 4 are as follows: ED_{25}, 0.56 mg kg^{-1} day^{-1} (0.11 to 2.95 mg kg^{-1} day^{-1}); ED_{50}, 1.90 mg kg^{-1} day^{-1} (0.72 to 5.00 mg kg^{-1} day^{-1}); and ED_{95}, >20 mg kg^{-1} day^{-1}. It should be noted that even in the case of intravenous injection of dioncophylline C, no signs of acute or subchronic toxicity were observed.

Administration of dioncophylline C to OF1 mice with estab-

TABLE 3. Correlation between suppression of parasitemia of *P. berghei* Anka-infected OF1 mice[a] and applied doses of dioncophylline C

Applied dose of dioncophylline C (mg kg^{-1} day^{-1})	Day 4			Day 7			Survival time (days)	
	% Parasitemia[b]	*P*[c]	No. of dead mice/ total no. of mice	% Parasitemia	*P*	No. of dead mice/ total no. of mice	Mean[d]	*P*[c]
Control	5.66 (3.81–7.51)		0/6	7.72 (0.30–15.13)		3/6	10.00 (3.99–16.01)	
3.125	5.36 (3.96–6.75)	0.748[e]	0/6	8.69 (7.18–10.19)	0.616[e]	3/6	6.17 (5.38–6.96)	0.135[e]
6.25	4.42 (2.77–6.06)	0.226[e]	0/6	9.10 (7.44–10.75)	0.261[e]	2/6	5.83 (5.04–6.62)	0.108[e]
12.5	3.36 (2.35–4.37)	0.019[f]	0/6	7.49 (5.67–9.32)	0.891[e]	1/6	13.33 (5.54–21.12)	0.413[e]
25	0.62 (0.00–1.34)	<0.001[f]	0/6	5.24 (2.66–7.83)	0.224[e]	0/6	13.33 (9.90–16.76)	0.244[e]
50	0.000 (0.000–0.000)	<0.0001[f]	0/6	0.000 (0.000–0.000)	<0.001[f]	0/6	>85	<0.0001[f]
100	0.000 (0.000–0.000)	<0.0001[f]	0/6	0.000 (0.000–0.000)	<0.0001[f]	0/6	>85	<0.0001[f]

[a] Mice were orally treated with dioncophylline C in a 4-day test.
[b] Values are mean levels of parasitemia (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Values are mean survival times (95% confidence intervals).
[e] Not significantly different from controls in one-way ANOVA.
[f] Significantly different from controls in one-way ANOVA.

TABLE 4. Effect of number of treatments of *P. berghei*-infected OF1 mice with dioncophylline C[a]

No. of treatments with dioncophylline C	Day 4			Day 7		
	% Parasitemia[b]	*P*[c]	No. of dead mice/ total no. of mice	% Parasitemia	*P*	No. of dead mice/ total no. of mice
Control	5.66 (3.81–7.51)		0/6	7.72 (0.30–15.13)		3/6
One[d]	0.02 (0.00–0.05)	<0.0001[e]	0/6	1.23 (0.35–2.10)	0.012[e]	0/6
Two[f]	0.01 (0.00–0.02)	<0.0001[e]	0/6	0.07 (0.00–0.19)	<0.001[e]	0/6
Three[g]	0.000 (0.000–0.000)	<0.0001[e]	0/6	0.001 (0.000–0.002)	<0.001[e]	0/6
Four[h]	0.000 (0.000–0.000)	<0.0001[e]	0/6	0.000 (0.000–0.000)	<0.001[e]	0/6

[a] Mice were orally treated with 50 mg of dioncophylline C kg^{-1} day^{-1}.
[b] Values are mean levels of parasitemia (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Treatment at day 0.
[e] Significantly different from controls in one-way ANOVA.
[f] Treatments at days 0 and 1.
[g] Treatments at days 0, 1, and 2.
[h] Treatments at days 0, 1, 2, and 3.

lished infections (Table 5) revealed that a first oral dosage of 50 mg kg^{-1} day^{-1} at day 3 after infection was sufficient to suppress the parasitemia significantly (45.1% inhibition at day 4). Additional dosages at days 4, 5, and 6 each caused further reductions (91.2, 98.4, and 99.7%, respectively), and the last treatment, at day 7, cured all animals.

Dioncophylline C and dioncopeltine A, the most potent in vitro and in vivo naphthylisoquinoline alkaloids identified until now (20, 22, 23) (Table 2), were administered orally to OF1 mice infected either with chloroquine-sensitive and virulent *P. berghei* Anka parasites or with resistant and chronic *P. berghei* Anka CRS parasites (Table 6). While both compounds suppressed the parasitemia considerably at least until day 7 after infection, in each of the cases, dioncophylline C was more active. The suppressive activity was less pronounced against the resistant line (dioncophylline C, 87% at day 4; dioncopeltine A, 72% at day 4), and complete cure was not obtained in the 4-day regimen.

Sustained administration of both dioncophylline C and dioncopeltine A to C57Black/6J mice was obtained via subcutaneously implanted mini-osmotic pumps (Table 7). A daily dose of 20 mg kg^{-1} resulted in both cases in a rapid and radical cure, confirmed by isodiagnosis with Swiss mice. Very low levels of parasitemia ($\ll$1%) were determined in some of the mice at days 5 and 6 during the dioncopeltine A treatment but only at day 5 during the dioncophylline C treatment.

DISCUSSION

Several extracts derived from *Dioncophyllaceae* and *Ancistrocladaceae* species display strong in vitro activities against *P. falciparum* and *P. berghei* erythrocytic stages (20, 22, 23). To explore their in vivo potentials, a selection was administered to *P. berghei*-infected OF1 mice in a standard 4-day test (40, 42). The highly active *T. peltatum* (*Dioncophyllaceae*) CH_2Cl_2-NH_3 stem bark extract (50% inhibitory concentrations [IC_{50}s], 0.014 and 0.081 μg/ml for *P. falciparum* and *P. berghei* in vitro, respectively) strongly suppressed the development of parasitemia in OF1 mice (Table 1), thus supporting the in vitro observations.

The IC_{50}s of naphthylisoquinoline alkaloids derived from *T. peltatum* (dioncophyllines B and C and dioncopeltine A) for *P. falciparum* and *P. berghei* in vitro were far below 1 μg/ml, while the corresponding value for dioncophylline A was about 1 μg/ml in both systems (20, 22, 23). Again, the compounds with the highest in vitro activities were by far the most effective ones in vivo, as shown in the cases of dioncophyllines B and C and dioncopeltine A (Table 2). The best in vivo results were obtained by treatment with dioncophylline C (IC_{50}s, 0.014 and 0.015 μg/ml for blood forms of *P. falciparum* and *P. berghei* in vitro, respectively) of *P. berghei*-infected OF1 mice, causing a complete clearance of parasites after oral administration of 50 mg kg^{-1} day^{-1} (Table 2).

TABLE 5. Effects of treatment with dioncophylline C[a] on established parasitemia in OF1 mice infected with 10^6 *P. berghei* Anka blood forms at day 0

Time after infection (day)	Control		Treatment		
	% Parasitemia[b]	No. of dead mice/ total no. of mice	% Parasitemia	*P*[c]	No. of dead mice/ total no. of mice
3	0.78 (0.59–1.00)	0/6	0.95 (0.72–1.18)	0.197[d]	0/6
4	2.26 (1.79–2.73)	0/6	1.24 (0.80–1.69)	0.002[e]	0/6
5	5.11 (1.51–8.70)	0/6	0.45 (0.24–0.65)	0.008[e]	0/6
6	3.67 (1.17–6.17)	1/6	0.06 (0.02–0.09)	0.002[e]	0/6
7	2.91 (2.15–3.67)	3/6	0.01 (0.00–0.02)	<0.0001[e]	0/6
8	3.30 (0.00–10.40)	3/6	0.00 (0.00–0.00)	0.018[e]	0/6
10	25.61 (5.38–45.83)	3/6	0.00 (0.00–0.00)	<0.001[e]	0/6
13	38.08	5/6	0.00 (0.00–0.00)	<0.0001[e]	0/6

[a] Mice were orally treated with 50 mg of dioncophylline C kg^{-1} day^{-1} at days 3, 4, 5, 6, and 7.
[b] Parasitemia levels were determined prior to the next treatment. Values are means (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Not significantly different from control in one-way ANOVA.
[e] Significantly different from controls in one-way ANOVA.

TABLE 6. Dioncophylline C and dioncopeltine A treatment of OF1 mice[a] infected with chloroquine-sensitive and -resistant strains of *P. berghei*

P. berghei strain and treatment	Day 4			Day 7		
	% Parasitemia[b]	*P*[c]	No. of dead mice/ total no. of mice	% Parasitemia	*P*	No. of dead mice/ total no. of mice
Sensitive (Anka)						
Control	4.17 (2.40–5.93)		0/6	3.18 (0.00–6.82)		3/6
Dioncophylline C	0.000 (0.000–0.000)	<0.001[d]	0/6	0.000 (0.000–0.000)	<0.001[d]	0/6
Dioncopeltine A	0.01 (0.00–0.03)	<0.001[d]	0/6	0.68 (0.00–1.35)	0.008[d]	0/6
Resistant (Anka CRS)						
Control	0.79 (0.68–0.90)		0/6	1.22 (1.02–1.41)		0/6
Dioncophylline C	0.10 (0.05–0.15)	<0.0001[d]	0/6	0.53 (0.20–0.86)	0.001[d]	0/6
Dioncopeltine A	0.22 (0.07–0.36)	<0.0001[d]	0/6	0.50 (0.36–0.64)	<0.0001[d]	0/6

[a] Mice were orally treated with 50 mg of alkaloid kg^{-1} day^{-1} in 4-day test.
[b] Values are mean levels of parasitemia (95% confidence intervals).
[c] Probability level of difference with control values.
[d] Significantly different from controls in one-way ANOVA.

There is a good correlation between the suppression of parasitemia of *P. berghei* Anka-infected OF1 mice and the dose of dioncophylline C administered in the 4-day test (Table 3). The 4-day schedule used for dioncophylline C is comparable to the chloroquine treatment scheme required to cure *P. berghei* Anka-infected OF1 mice (data not shown). A minimum number of four daily oral treatments with 50 mg of dioncophylline C kg^{-1} day^{-1} was needed to cure all *P. berghei*-infected OF1 mice (Table 4). It is encouraging (Table 4 and data not shown) that one oral treatment reduced the level of parasitemia by 99.6% and produced 50% smear-negative mice at day 4.

The effects of dioncophylline C depend on the pharmacokinetic properties and bioavailability of the compound in the bloodstream. The difference in efficacy between the oral and subcutaneous route on the one hand and the intravenous route on the other hand could relate to chemical instability, limited absorption, a rapid metabolization in the gastrointestinal tract after oral administration, or the appearance of peak levels after intravenous application.

The strong effects of dioncophylline C administered to OF1 mice starting 3 days after the infection (Table 5) suggest that less than five daily treatments could be considered sufficient to cure mice with established malaria infections. The somewhat lower activities of both dioncophylline C and dioncopeltine A against the chloroquine-resistant *P. berghei* Anka CRS strain (Table 6) could possibly be improved in adapted administration schemes as well.

The most effective treatments with dioncophylline C and dioncopeltine A were obtained with subcutaneously implanted osmotic pumps, which allowed for the sustained release of the drugs at a rate of 1 μl/h (Table 7). The high efficacy obtained with osmotic pumps suggests the importance of these and other sustained-release systems for the delivery of naphthylisoquinoline alkaloids in future experiments and applications.

The considerable potential of the naphthylisoquinoline alkaloids as new antimalarial drugs, already strongly suggested by data obtained previously from in vitro experiments with *P. falciparum* and *P. berghei* (20, 22, 23), was confirmed by these results. Dioncophyllines B and C and dioncopeltine A are highly active against the rodent malaria parasite *P. berghei* in vivo, and in particular, dioncophylline C is a promising candidate for further development in the preclinical and clinical phases. On the other hand, many more naphthylisoquinoline alkaloids of natural and synthetic origin are waiting for further evaluation in the in vitro and in vivo malaria models. These new tools for the chemotherapy of malaria are badly needed, considering the deteriorating global malaria situation,

TABLE 7. Inhibitory effects of 20 mg of dioncophylline C and dioncopeltine A kg^{-1} day^{-1} on the course of parasitemia of *P. berghei* K173-infected C57Black/6J mice applied with the aid of mini-osmotic pumps[a]

Time after infection (day)	% Parasitemia[b]								
	Control			Dioncophylline C			Dioncopeltine A		
	Mouse 1	Mouse 2	Mouse 3	Mouse 1	Mouse 2	Mouse 3	Mouse 1	Mouse 2	Mouse 3
4	1	5	5	0	0	0	0	0	0
5	2	10	9	0	≪1	≪1	0	≪1	≪1
6	13	26	14	0	0	0	≪1	0	≪1
7	6	27	33	0	0	0	0	0	0
8	12	18	†	0	0	0	0	0	0
14	25	†	†	0[c]	0[c]	0[c]	0[c]	0[c]	0[c]
18	50	†	†	0	0	0	0	0	0
21	†	†	†	0	0	0	0	0	0
28	†	†	†	0	0	0	0	0	0

[a] Mice were infected intraperitoneally with 10^6 parasites at day 0. Osmotic pumps contained 16.6 mg of alkaloid/ml, released at 1 μl/h, and were implanted subcutaneously in C57Black/6J mice at day −1.
[b] Individual values for each mouse (†, dead).
[c] No parasites present after isodiagnosis with samples from these mice with Swiss mice and observation for 4 weeks.

the lack of commercially available vaccines, and the continuing spread of drug resistance.

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