A Controlled Trial to Assess the Effect of Quinine, Chloroquine, Amodiaquine, and Artesunate on *Loa loa* Microfilaremia

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Abstract. Onchocerciasis control is currently based on mass ivermectin treatment. Unfortunately, this drug can induce serious adverse events (SAEs) in persons with high levels of *Loa loa* microfilaremia (> 30,000 microfilaria/mL). A means of preventing SAEs would be to treat at risk populations with a drug that would progressively reduce the microfilarial loads before administering ivermectin. Antimalarial drugs are a potential solution because they have shown some activity against various filarial species. A controlled trial was conducted to assess the effect of standard doses of quinine, chloroquine, amodiaquine, and artesunate on *L. loa* microfilaremia. Ninety-eight patients were randomly allocated into five groups (one for each drug and a control group) after stratification on microfilarial load. *Loa loa* microfilaremia was monitored on days 0, 3, 7, 15, 30, 60, and 90. No significant change in the loads was recorded in any of the treatment groups. A comprehensive review of the effects of antimalarial drugs against filariae is also provided.

INTRODUCTION

Community-directed treatment with ivermectin is the most cost-effective and sustainable strategy to control onchocerciasis.[1] However, the participation of persons in distribution campaigns is limited in some areas because of the occurrence of rare but sometimes fatal serious adverse events (SAEs). The worst presentation of these events involves encephalopathy with neurologic disorders and coma.[2] The main risk factor for these SAEs is the presence of a high *Loa loa* microfilaremia.[3] Loiasis is highly endemic in rainforest areas of central Africa and its main clinical manifestations include migrating angioedema known as Calabar swelling, pruritus, and passage of the adult worm under the conjunctiva. Although the pathophysiology of the SAEs is not fully understood, it is known that ivermectin has a steady effect on *L. loa* microfilaremia, and clinical observations suggest that the neurologic disorders may be caused by obstruction of brain capillaries by paralyzed *L. loa* microfilaria (mf), or penetration into the central nervous system of mf trying to escape the effects of ivermectin.[4,5] The risk of post-ivermectin encephalopathy is significantly increased when the *L. loa* count exceeds 30,000 mf/mL, and then increases exponentially with microfilaremia.[3]

One way of preventing SAEs would be to progressively reduce the *L. loa* microfilaremia below the risk level before treating with ivermectin. To achieve this reduction, a treatment that eliminates *L. loa* mf and that can easily be distributed on a large scale needs to be identified. Albendazole given at a dose of 200 mg twice a day for 21 days may reduce *L. loa* microfilaremia by 80% after 6 months,[6] but this regimen is logistically challenging for mass treatment programs. Unfortunately, when this drug is given in a single dose of 600 mg, 400 mg once a day for 3 days, or 400 mg twice a day for 3 days, it has little or no effect on *L. loa* microfilaremia.[7–9] Use of ivermectin at low single doses (25–50 μg/kg) do not give significantly different results from that observed with a standard dose (150 μg/kg).[10,11]

A number of studies have been conducted to evaluate the effects of antimalarial drugs on filariae. We have undertaken an exhaustive literature review on the topic, the results for which are presented in the form of detailed supplementary material (Supplementary Tables 1–6, available at www.ajtmh.org). The drugs tested included aryl-amino-alcohols (quinine, mefloquine), 4-aminoquinolines (chloroquine, amodiaquine, quinacrine), and primaquine. Different drugs showed variable efficacies against different filarial species (see Supplementary Tables 1–6 and Discussion for details). Results of *in vivo* trials of chloroquine and amodiaquine are of particular interest. Chloroquine resulted in a marked decrease in *Onchocerca volvulus* microfilarial loads, and had a macrofilaricidal effect when injected into the onchocercal nodules, but not when administered orally.[12–14] Amodiaquine showed a macrofilaricidal effect on *Wuchereria bancrofti* in one patient treated with a high dose (2,400 mg over 4 days).[15]

Artemisinins are effective against *Plasmodium* spp. and various trematodes such as *Schistosoma mansoni* and *Fasciola hepatica*.[16] To the best of our knowledge, these drugs have been tested only once against filariae. Artemisinin, when given at a dose of 100 mg/kg/day for 5 days in the rodent *Meriones unguiculatus* transplanted with adult *Acanthocheilonema vitae* and *Brugia malayi*, appeared to have little effect on the parasites.[17] However, it is worth further exploring antifilarial activity of artemisinins because their mode of action against sensitive helminths is still not well known.[18,19]

Because of results of trials of chloroquine[12–14] and amodiaquine[15] on filariae, we selected these two compounds as good candidates for the present trial. Quinine has never been tested *in vivo* against filariae, but was selected because of its moderate effect *in vitro* on *B. pahangi*.[20] Artesunate was chosen for the reason given in the preceding paragraph.

Safety considerations were also taken into account to select the drugs to be tested. This criterion was most important because should a compound show a significant effect on *L. loa*, then mass treatment using this drug may be used to reduce the *L. loa* microfilaremias in the population before distributing ivermectin. The main adverse reactions after quinine treatment is a complex of symptoms, known as cinchonism, which resolve with discontinuation of treatment.[21] Quinine may induce serious adverse events such as blackwater fever or hypoglycemia. However, blackwater fever occurs mainly in persons with no or low immunity against malaria, which is exceptional in persons from malaria-endemic areas,[22] and hypoglycemia occurs

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in patients with severe malaria[23] or after high doses of quinine.[24] Standard doses of chloroquine are generally well tolerated.[21] Extrapyramidal symptoms and psychiatric reactions have been reported during chloroquine treatment but are exceptional.[25] A study conducted in the United Kingdom indicated that amodiaquine may induce agranulocytosis and hepatitis with fatality rates of 1/31,300 and 1/15,650, respectively.[26,27] However, because the combination amodiaquine plus artesunate is a first-line therapy for treatment of patients with uncomplicated malaria in Cameroon, we decided to keep amodiaquine in our trial. Lastly, artesunate, as are other derivatives of artemisinin, is remarkably safe and well tolerated.[21] Following the above considerations, we decided to assess the effect of quinine, chloroquine, amodiaquine, and artesunate on *L. loa* microfilaremia.

PATIENTS AND METHODS

Study area and patient selection. The trial was conducted in six villages 40–80 km east of Yaoundé, in the Mefou and Afamba Division, Center Region, Cameroon. Because loiasis is highly endemic and onchocerciasis is hypoendemic in the area,[11] no mass ivermectin distribution has ever been implemented in this region.

The first phase of the trial consisted of identification and recruitment of microfilaremic persons. Information on the objectives and procedures of the survey was given to the population of each selected village. Persons ≥ 15 years of age who volunteered to participate in the study underwent a parasitologic examination. Blood was collected between 11:00 AM and 3:00 PM by fingerprick and a calibrated thick blood film (CTBF) of 50 μL was prepared for each volunteer. Slides were stained using Giemsa and *L. loa* mf were counted under a microscope. Subjects harboring ≥ 200 *L. loa* mf/mL and who signed an informed consent form underwent a clinical examination and were interviewed regarding their medical history. Those persons who had received antifilarial treatment during the previous year, persons with epilepsy, and pregnant or breastfeeding women were excluded from the trial. All remaining persons, when in good health, were enrolled in the trial.

Study design, randomization, treatment, and follow-up. This was an open trial. All patients enrolled were stratified according to their *L. loa* microfilaremia by using the following classes: 200–7,999, 8,000–30,000, and > 30,000 mf/mL). Persons were then randomly allocated, for each stratum, into five treatment groups: one for each of the four antimalarial drugs tested and a control group.

On the first day (D0), a CTBF was prepared for every patient by using the same procedure as in the preliminary survey to re-assess their baseline *L. loa* microfilaremia immediately before treatment. Patients treated with quinine received 600 mg twice a day for 5 days, those treated with chloroquine received 600 mg once a day for 3 days, those treated with amodiaquine received 800 mg once a day for 3 days, and those treated with artesunate received 200 mg once a day for 3 days. We used generic quinine and chloroquine tablets and Falcimon kits® (Cipla, Mumbai, India) containing separate amodiaquine and artesunate tablets. Patients in the control group received a single dose of iron-folic acid (four tablets each containing 65 mg of iron and 0.25 mg of folic acid). Treatments were administered under the direct observation of investigators. Surveillance of potential adverse events was conducted on a daily basis during the treatment period and then at each subsequent visit.

Loa loa microfilaremia was monitored by examination of CTBF on days 3, 7, 15, 30, 60, and 90. To reduce the effect of daytime variability in microfilaremia,[28] the samples were collected from each patient at approximately the same time as it was collected on D0. The slides were examined independently by two microscopists in a blind manner relative to treatment, and the microfilaremia counts were considered valid when the two results differed by less than 10%. The average value was used for statistical analyses.

Statistical analyses. Microfilaremia was compared between the five groups at each examination round by using the Kruskal-Wallis test. To compare the longitudinal trend in microfilaremia between treatments, we performed a regression analysis using a linear mixed-effects model, which adequately handles cross-sectional measures taken repeatedly on the same persons. The outcome variable was the *L. loa* microfilaremia. The regressors were the treatment group and the day of sampling, which were entered in the model as main factors and as interaction terms. To account for non-normality of microfilarial load distribution, these loads were logarithmically transformed. Intra-individual correlation between consecutive measures and potential inter-individual variation around the average trend were accounted for by a random effect set at the subject level. Analyses were performed using Stata software version 10.0 (Stata Corp., College Station, TX).

Ethical agreement. The trial was reviewed and approved by the National Ethics Committee of Cameroon. Administrative authorization was provided by the Cameroon Ministry of Public Health. All patients benefited from general health checks during the follow-up.

RESULTS

Overall, 901 persons were screened during the preliminary survey. The prevalence of *L. loa* microfilaremia was 27.1%. A total of 142 persons were eligible for the trial (microfilaremia ≥ 200 mf/mL) and allocated into the five groups. Only 98 patients (50 female patients and 48 male patients) were present on D0 and finally enrolled in the trial. The mean age of the study population was 52.6 years (range = 14–80 years).

All patients included in the trial received the full treatment, and no serious adverse events were reported. All events recorded were mild, and related mostly to amodiaquine, chloroquine, and quinine. In the amodiaquine group, six patients had asthenia, one had a headache, one had anorexia and one had ocular itching. Among persons treated with quinine, three reported asthenia, one reported dizziness, and one reported tinnitus. In the chloroquine group, three patients had itching, one had asthenia, one had tinnitus, and one had insomnia. Among the persons treated with artesunate, two reported asthenia and one reported itching. All adverse events spontaneously regressed within 24–48 hours after the last dose of drug.

At D0, microfilaremia levels were similar in the different treatment groups. No statistically significant difference was observed between the groups at any of the subsequent examinations (Table 1). Individual changes with time in each treatment group are shown in Figure 1. No significant decrease in microfilaremia was observed in any of the groups during the 90-day follow-up. This finding was confirmed by results of the mixed-effects linear model (Table 2).

TABLE 1
Loa loa microfilaremia in the five treatment groups, at different times of follow-up*

Treatment group†	Day 0		Day 3		Day 7		Day 15		Day 30		Day 60		Day 90	
	No.	GM (range)	No.	GM (range)	No.	GM (range)	No.	GM (range)	No.	GM (range)	No.	GM (range)	No.	GM (range)
Quinine	17	6,540 (860–140,400)	17	6,104 (500–154,800)	16	6,359 (400–111,760)	15	5,696 (740–100,400)	17	5,010 (480–140,200)	13	4,513 (720–34,540)	11	4,897 (540–147,360)
Chloroquine	25	4,062 (220–87,640)	24	3,933 (100–120,620)	19	4,245 (180–39,280)	23	3,385 (80–93,860)	25	3,379 (100–80,500)	19	1,979 (60–29,980)	18	3,448 (140–53,640)
Artesunate	23	6,266 (600–24,540)	21	5,684 (580–24,180)	18	7,051 (780–31,420)	23	6,659 (620–33,800)	23	6,546 (320–31,000)	15	6,320 (340–31,000)	13	5,359 (500–30,760)
Amodiaquine	18	5,980 (200–30,400)	16	5,731 (1,900–26,320)	16	5,560 (200–41,480)	16	4,776 (180–29,060)	18	5,130 (380–29,700)	11	7,229 (320–28,460)	7	4,641 (520–16,460)
Control	15	3,911 (500–154,800)	14	3,574 (280–21,260)	12	4,235 (340–26,760)	14	3,689 (80–24,800)	15	3,145 (120–15,300)	12	3,659 (260–16,060)	10	4,136 (480–18,820)
P‡	0.68		0.79		0.73		0.72		0.51		0.15		0.95	

* No. = number of patients; GM = geometric mean of *Loa* microfilaremia (microfilariae per milliliter).
† Quinine = 1,200 mg/day for 5 days; chloroquine = 600 mg/day for 3 days; artesunate = 200 mg/day for 3 days; amodiaquine = 800 mg/day for 3 days; control = iron-folic acid tablets.
‡ Results of Kruskal-Wallis test for the time point.

DISCUSSION

To the best of our knowledge, the present trial is the first ever conducted to evaluate the effect of antimalarial drugs on *L. loa*. We included patients harboring a wide range of *L. loa* microfilaremias, including some who had less than 8,000 mf/mL, and thus would not be at risk for developing marked or serious adverse events after ivermectin treatment. Because of the initial stratification procedure, the geometric mean *L. loa* microfilaremia did not differ significantly between the groups before treatment.

The drug regimens we used were not the exact recommendations for the treatment of patients with malaria but were adapted so as to be applicable (in terms of number of doses received per day and numbers of days) as part of a mass treatment regimen. The time points of follow-up were chosen so as to be able to detect either a rapid decrease in the microfilarial count (from D1 to D15), as recorded in the trials of chloroquine on *O. volvulus*,[12,13] or a more progressive effect (on D30 and D90), as observed in one patient infected with *W. bancrofti* treated with amodiaquine.[15] The examinations on D90 would have enabled us to detect an effect limited to the adult worms, whether a macrofilaricidal effect or an interruption of the release of new mf. Knowing that the lifespan of *Loa* mf is approximately 6–12 months,[29] the microfilarial load on D90 would be reduced by 25–50%.

In light of our objective (to bring about a slow decrease in the *L. loa* microfilaremia), even a moderate or delayed effect would have been desirable. Unfortunately, our results show that none of the four antimalarial regimens tested has a significant effect on *L. loa* microfilaremia. The fact that no decrease was recorded at any time point suggests that the drugs at the doses administered were inactive on mf and adult worms.

Given the promising results previously obtained with chloroquine, its inefficacy against *L. loa* is particularly disappointing. Chloroquine was first tested *in vitro* against mf and adults of *O. volvulus* and *O. gutturosa*,[20,30–35] and then *in vivo* in onchocerciasis patients.[12–14] This drug, when given *per os*, appeared to bring about a marked decrease in microfilarial loads and had a macrofilaricidal effect when injected into the onchocercal nodules but not when administered orally. In addition, it was shown that after oral treatment, adult *O. volvulus* accumulated the drug to high concentrations.[36] Chloroquine was also tested *in vitro* against *B. pahangi*, *B. patei*, *Dirofilaria immitis* and *A. vitae*,[20,33,34,37] and *in vivo* against *D. immitis*.[38] It was less effective or less active on these species than on *Onchocerca* spp.

The discrepancy in the results obtained for *O. volvulus* and *L. loa* may be caused by differences in the localization of parasites, and thus in the level of exposure to the drug. *Onchocerca volvulus* mf live in the dermis, whereas *L. loa* mf circulate in the bloodstream. With regard to the adult stages, *O. volvulus* macrofilaria are located in subcutaneous or deep nodules, whereas those of *L. loa* are found in the connective tissue under the skin and the fascial layers overlying the somatic muscles. Because chloroquine concentrations observed in the skin are much higher than those in plasma,[36,39] one may assume that for a given oral dose of chloroquine mf of *O. volvulus* are more exposed to the drug than those of *L. loa*. With regard to adult worms, it has been shown that after oral treatment, *O. volvulus* accumulates chloroquine to concentrations more than 100 times higher than that measured in the skin or the
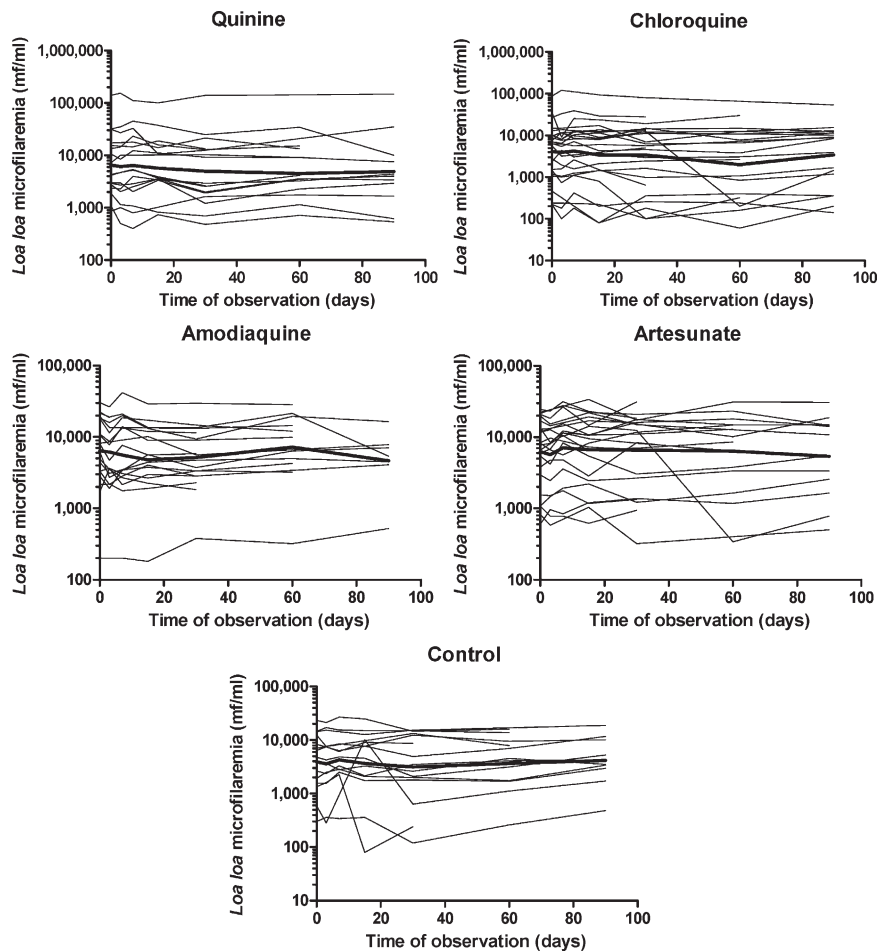
FIGURE 1.   Individual follow-up of *Loa loa* microfilaremia in the five treatment groups (quinine, 1,200 mg/day for 5 days; chloroquine, 600 mg/day for 3 days; amodiaquine, 800 mg/day for 3 days; artesunate, 200 mg/day for 3 days; control group, persons receiving iron-folic acid tablets). Thick lines represent the geometric mean numbers of *Loa loa* microfilaria per milliliter of blood.

nodular tissue around the adult worms.[36] Despite this finding, no macrofilaricidal effect was observed after oral chloroquine treatment,[13] and adult *O. volvulus* were killed only when the drug was injected into the nodule.[14] Thus, high concentrations are required to obtain a macrofilaricidal effect, which may also be true for *L. loa*, whose adults are often located in poorly vascularized tissues.

The lack of effect of amodiaquine on *L. loa* is also disappointing. The possible antifilarial activity of amodiaquine has also been studied *in vitro* against *B. pahangi*[20] and *Breinlia booliati*[40] and *in vivo* against *O. volvulus*,[41] *Wuchereria bancrofti*,[15,42] and *Litomosoides carinii*.[43–45] A macrofilaricidal effect was reported for all species tested, except for *O. volvulus* (see supplementary material for details). With regard to *W. bancrofti*, a progressive decrease in microfilaremia (from 114 mf/mL on D0 to 16 mf/mL on D90) was observed in a patient treated with a dose (2,400 mg over 4 days) similar to that administered as part of the present trial.[15] We have no explanation for the absence of an effect of amodiaquine on *L. loa*.

The lack of an effect obtained with artesunate confirms previous observations in animal models, which showed little antifilarial effect for this drug.[17] This finding may be related to the fact that artesunate has a shorter life-time (less than one hour) than chloroquine or amodiquine.

Other antimalarial compounds that had been tested against filariae were not used in the present trial because of safety reasons or because they were not available commercially. Quinacrine showed no effect against *O. volvulus* mf *in vivo*,[46] but had a moderate effect against *B. pahangi* adult females *in vitro*.[20] Attempts to increase the antifilarial activity of 4-aminoquinolines were made by synthesizing derivatives,[40,47,48] but the results were disappointing. The activity of mefloquine was evaluated *in vitro* against *B. patei* mf and against adult stages of *O. gutturosa*,[49] *B. malayi*, and *B. patei*.[37] The drug seemed to have a paralyzing effect but for the latter two species this effect disappeared when fetal calf serum and human serum were added to the medium. Lastly, primaquine was shown to have a marked effect against *B. pahangi* adult worms *in vitro*[20] and a delayed effect on *W. bancrofti* microfilaremia in humans.[50]

Despite the disappointing results of the present trial, it would be worth investigating the modes of action of antimalarial drugs against filariae. The detailed modes of action of quinoline-containing drugs on *Plasmodium* spp. seem to differ according to the compounds, but in each case they rely on the mechanisms related to hemoglobin digestion.[51,52] Interference with hemozoin formation also explains at least partly the action of antimalarial drugs on *Schistosoma* spp.[53–57] However, what occurs in blood-feeding helminths such as *Schistosoma*

TABLE 2
Results of the multivariate mixed-effect regression model of microfilaremia by treatment group and time

Variables	Estimates	P	95% confidence interval
Treatment			
Quinine	0.4810	0.321	–0.4684 to 10.4305
Chloroquine	–0.0029	0.995	–0.8784 to 0.8727
Amodiaquine	0.3564	0.456	–0.5811 to 10.2939
Artesunate	0.5466	0.229	–0.3432 to 10.4364
Time, in days	0.0007	0.771	–0.0039 to 0.0052
Interaction terms			
Quinine × time	–0.0013	0.676	–0.0076 to 0.0049
Chloroquine × time	–0.0027	0.357	–0.0085 to 0.0031
Amodiaquine × time	–0.0001	0.981	–0.0066 to 0.0064
Artesunate × time	–0.0017	0.575	–0.0077 to 0.0043
Constant term	50.2484	0.0001	40.5562 to 50.9406
Random effects parameters			
Time	0.0068	–	0.0053 to 0.0088
Intercept	10.3520	–	10.1677 to 10.5654
Residual	0.3753	–	0.3503 to 0.4021

spp. may not be true for filariae. Even if iron was found in the intestinal epithelial cells of adult *Onchocerca* sp.,[58–60] and blood feeding has been observed in young adult *L. sigmodontis*,[61] it is thought that filariae feed mainly through the body wall.[62] Thus, the antifilarial activity of quinoline drugs probably results from mechanisms different from those occurring in other sensitive parasites. To our knowledge, this activity has been investigated only by VandeWaa and others,[63] who evaluated the effects of intermediary metabolites and electron transport inhibitors on the paralyzing effects of chloroquine on *B. pahangi* and *O. volvulus*. Their results suggested that chloroquine may inhibit aerobic energy metabolism in the filariae, possibly at the level of electron transport.[63] Unfortunately, these studies were not continued.

We also think that despite the lack of effect obtained in the present study with artesunate, it would also be worth considering artemisinin-derived compounds for future trials against filariae. This suggestion is justified because artemisinin reacts with translationally controlled tumor protein (TCTP) in *Plasmodium* spp.,[64] and homologs of TCTP are also present in *S. mansoni*,[65] *B. malayi*, and *W. bancrofti*.[66]

As mass distribution of ivermectin extends progressively to cover all areas endemic for onchocerciasis, the probability of observing post-ivermectin SAEs is decreasing because most people living in these foci and at risk would have already taken this drug at least once. Nonetheless, ivermectin is soon to be distributed outside these areas as part of the lymphatic filariasis elimination program. Even if the geographic distribution of lymphatic filariasis in central Africa is not well known, it is likely that many areas are co-endemic with loiasis. For this reason, research should continue to identify a drug able to progressively reduce high *L. loa* microfilarial loads.

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