MAJOR ARTICLE

Randomized Comparison of Chloroquine plus Sulfadoxine-Pyrimethamine versus Artesunate plus Mefloquine versus Artemether-Lumefantrine in the Treatment of Uncomplicated Falciparum Malaria in the Lao People's Democratic Republic

Mayfong Mayxay,[1,2] Maniphone Khanthavong,[3] Niklas Lindegårdh,[6,7] Siamphay Keola,[4] Marion Barends,[7,8] Tiengkham Pongvongsa,[5] Ratsuda Yapom,[7,8] Anna Annerberg,[7] Samlane Phompida,[3] Rattanaxay Phetsouvanh,[3] Nicholas J. White,[1,6,7,8] and Paul N. Newton[1,6]

[1]Wellcome Trust–Mahosot Hospital, Oxford Tropical Medicine Research Collaboration, [2]Department of Medicine, Faculty of Medical Science, National University of Laos, and [3]Centre of Malariology, Parasitology and Entomology, Vientiane, and [4]Phalanxay District Clinic and [5]Savannakhet Provincial Malaria Station, Savannakhet Province, Lao People's Democratic Republic; [6]Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, United Kingdom; [7]Faculty of Tropical Medicine, Mahidol University, Bangkok, and [8]Shoklo Malaria Research Unit, Mae Sot, Tak Province, Thailand

Background. Recent clinical trials in the Lao People's Democratic Republic have demonstrated that chloroquine and sulfadoxine-pyrimethamine, which are national malaria treatment policy, are no longer effective in the treatment of uncomplicated *Plasmodium falciparum* malaria.

Methods. A randomized comparison of 3 oral antimalarial combinations—chloroquine plus sulfadoxine-pyrimethamine versus artesunate plus mefloquine versus artemether-lumefantrine—with 42-day follow-up period, was conducted among 330 patients with acute uncomplicated falciparum malaria in southern Laos.

Results. The 42-day cure rates, as determined by intention-to-treat analysis and adjusted for reinfection, were 100%, 97%, and 93% for the groups receiving artesunate plus mefloquine, artemether-lumefantrine, and chloroquine plus sulfadoxine-pyrimethamine, respectively. Of 8 patients receiving chloroquine plus sulfadoxine-pyrimethamine who experienced treatment failure, 6 had early treatment failure. The mean parasite clearance time was significantly longer in patients treated with chloroquine plus sulfadoxine-pyrimethamine (2.9 days; 95% confidence interval [CI], 2.8–3.0 days) than in those treated with artesunate plus mefloquine (2.07 days; 95% CI, 2.0–2.1 days; $P < .001$) and artemether-lumefantrine (2.08 days; 95% CI, 2.0–2.1 days; $P < .001$). Cure rates with artemether-lumefantrine were high despite low mean daily dietary fat intake (13.8 g; 95% CI, 12.5–15.1 g) and day 7 plasma lumefantrine concentrations (0.47 μg/mL; 95% CI, 0.38–0.56 μg/mL).

Conclusion. Oral artesunate plus mefloquine and artemether-lumefantrine are highly effective for the treatment of uncomplicated falciparum malaria in Laos.

Malaria due to *Plasmodium falciparum* remains an important public health problem in the Lao People's Democratic Republic (Laos). Although chloroquine and sulfadoxine-pyrimethamine have been considered ineffective in all adjoining countries for decades [1], they have remained the first- and second-line nationally recommended treatments for uncomplicated falciparum malaria in Laos. During 2000–2002, clinical trials of oral chloroquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria in 5 different areas of Laos recorded unacceptably high treatment failure rates (40%–80% for chloroquine and 18%–35% for sulfadoxine-pyrimethamine) [2–6]. These results suggested that the Lao national strategy for treatment of uncomplicated falciparum malaria required urgent review.

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Reprints or correspondence: Dr. Paul N. Newton, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR (paul@tropmedres.ac).

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There is increasing evidence that antimalarial drugs should be given in combinations, which should include an artemisinin derivative [7]. Artesunate-mefloquine has good efficacy in adjoining countries [8–13]. Artemether-lumefantrine is a better-tolerated and less-expensive combination (~US$2.4 vs. ~$3.5 per adult treatment course), but some studies have demonstrated lower efficacy, probably as a consequence of poor bioavailability of lumefantrine when taken without fatty food [8–10, 12, 14, 15]. The inexpensive combination of oral chloroquine plus sulfadoxine-pyrimethamine is a potential alternative [16–20]. We therefore conducted a randomized, open-label clinical trial of chloroquine plus sulfadoxine-pyrimethamine versus artesunate-mefloquine versus artemether-lumefantrine to assist in determining the optimum combination treatment regimen in Laos.

PATIENTS AND METHODS

Study site, patients, and clinical procedures. The study was conducted from June to October in 2002 and 2003 at Phalanxay District Clinic, Savannakhet Province, Laos [3]. Patients presenting with acute uncomplicated malaria and a blood smear positive for *P. falciparum* were enrolled in the study provided that they, or their attending relatives, gave fully informed written consent, had a density of asexual *P. falciparum* of 5000–200,000 per microliter of blood, were not pregnant or lactating, were aged ⩾1 year, had an axillary temperature of ⩾37.5°C or history of fever in the previous 3 days, were likely to stay in the hospital until parasite clearance and complete the 42-day follow-up period, had not taken a full course of any antimalarials in the previous 3 days, did not have signs of severe malaria [21], and did not have a history of allergy or contraindication to the study drugs. Venous blood samples were obtained for parasite count and determination of hematocrit, and 3 blood spots were collected on filter paper [3]. A urine sample was collected at the time of admission and stored at −30°C. If the study inclusion criteria were met, patients were randomized (in blocks of 15; the treatment choice was kept in a sealed opaque envelope that was opened only after the decision to recruit had been made) to receive 1 of the following regimens: chloroquine sulfate (Government Pharmaceutical Organization; Bangkok, Thailand), 10 mg base/kg of body weight, followed by 10 mg base/kg 24 h later, followed by 5 mg base/kg at 48 h, plus sulfadoxine-pyrimethamine (Fansidar; Roche), 25 mg/1.25 mg/kg on day 1; artesunate (Guilin Pharmaceutical Co.; People's Republic of China), 4 mg/kg/day for 3 days (days 0–2), plus mefloquine, 15 mg/kg on day 1 and 10 mg/kg on day 2 (Lariam; Roche); or artemether-lumefantrine (Coartem; Novartis), 20 mg of artemether and 120 mg of lumefantrine per tablet, 1 dose q12h for 3 days (doses were 1 tablet for patients weighing <15 kg, 2 tablets for patients weighing 15–24 kg, 3 tablets for patients weighing 25–34 kg, and 4 tablets for patients weighing ⩾35 kg; patients were advised to take the drug with fatty food).

Axillary temperature was measured every 6 h. Drug administration was observed directly by study physicians. For children who could not swallow tablets, the appropriate drug dose in milligrams per kilogram of body weight was crushed and mixed with water and given in a syringe. If vomiting occurred within 1 h of dosing, the medication was readministered. Patients were reviewed daily until parasites cleared and then weekly for 42 days from the start of treatment or at other times if they felt unwell. At each visit, blood was obtained by fingerprick for a malaria smear and hematocrit determination. Three blood spots were collected onto filter paper from those with reappearance of asexual parasitemia [3, 22]. A heparinized venous blood sample was requested from patients treated with artemether-lumefantrine on day 7 for determination of plasma concentrations of lumefantrine. Patients who received chloroquine plus sulfadoxine-pyrimethamine or artemether-lumefantrine and had recurrent parasitemia were retreated with artesunate plus mefloquine. Those who had a further falciparum malaria infection following artesunate plus mefloquine or had a second reappearance of falciparum malaria or those who developed severe disease were retreated with artesunate at 2 mg/kg/day for 7 days. Those who received additional courses of therapy were also observed for 42 days. Potential drug side effects were recorded. Ethics clearance for the study was granted by the Ethical Committee of the Faculty of Medical Sciences, National University of Laos, and Oxford Tropical Research Ethics Committee UK.

Laboratory investigations and outcome measures. Parasite counts were done daily until parasite clearance was detected for 2 consecutive days and then weekly from day 7 [3]. Urinalysis for the presence of chloroquine, quinine, and pyrimethamine was done by dipstick test [3, 23]. PCR amplification was performed on paired samples for genotyping of parasites [22], to distinguish between reinfection and recrudescence [3].

Primary end points of the study were the parasitological and clinical responses to treatment [3, 24, 25]. Analysis was done for each treatment until either day 42 or the reappearance of falciparum malaria. Secondary end points were parasite clearance time (time in days from first treatment dose to the first negative result of thick film testing for falciparum parasites after checking ⩾200 oil fields), fever clearance time (time in hours from the start of treatment at which the axillary temperature first decreased to <37.5°C and remained at <37.5°C for 48 h), gametocytemia, and changes in hematocrit following antimalarial treatment.

Dietary assessment. All food items consumed by patients during the 3 days of artemether-lumefantrine therapy were recorded. Approximate weights per serving were estimated and

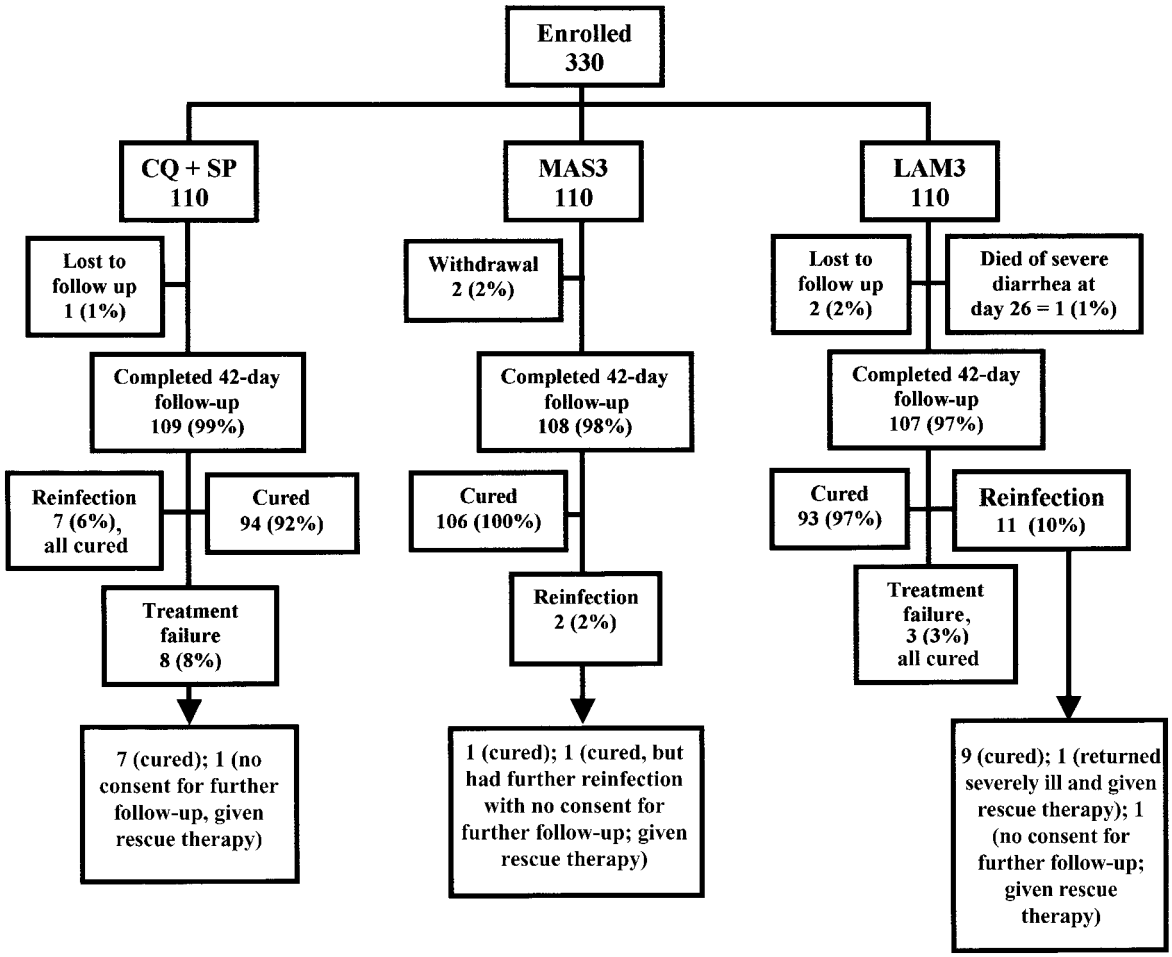

Figure 1. Patient enrollment and outcome in a study comparing chloroquine plus sulfadoxine-pyrimethamine (CQ + SP), artesunate plus mefloquine (MAS3), and artemether-lumefantrine (LAM3) for the treatment of falciparum malaria in Laos.

corrected by the approximate amount of fat by use of data contained in the Food and Agriculture Organization Food Composition Table used in East Asia [26]. Meals were classified as either drinks only (no fat), light meal (10 g of fat), or normal meal (20 g of fat) [27].

Plasma lumefantrine assays. Lumefantrine concentrations were determined by solid-phase extraction and liquid chromatography [28]. Plasma (0.25 mL) was precipitated with acidic acetonitrile (containing, as the internal standard, a hexyl analogue of desbutyllumefantrine; catalog no. TA 213/435/16; Novartis) before being loaded onto a C8 solid-phase extraction column (3M Empore). The eluates were evaporated, reconstituted, and injected into a liquid chromatography system (SB-CN column; Zorbax) and a mobile phase containing 0.05 M sodium perchlorate and acetonitrile-phosphate buffer (pH, 2; 0.1 M) (55:45, v/v). Within-day coefficients of variation were 6.6% and 2.1% at 0.042 μg/mL and 8.02 μg/mL, respectively. Between-day coefficients of variation were 12.0% and 2.9% at 0.042 μg/mL and 8.02 μg/mL, respectively.

Statistical analysis. Data were analyzed with SPSS, version 8.0 (SPSS). Comparisons between 2 groups were made by the Mann-Whitney *U,* Student's *t,* χ^2, and Fisher's exact tests, as appropriate.

RESULTS

Figure 1 provides a flow chart of enrollment and outcomes. Blood smears from 3767 febrile patients were examined, of which 758 (20%) were positive for malaria (686 [91%] were positive for *P. falciparum,* 55 [7%] were positive for *Plasmodium vivax,* 1 [0.1%] was positive for *Plasmodium malariae,* and 16 [2%] indicated mixed infections [15 involving *P. falciparum* and *P. vivax* and 1 involving *P. falciparum* and *P. malariae*]). Of the 686 patients with falciparum malaria, 330 were eligible for and willing to participate in the study. The reasons for ineligibility were parasite load of <5000 parasites/μL (52%), low probability of completing follow-up (14%), severe malaria (13%), inability to take oral drugs (6%), parasite load of

>200,000 parasites/μL (4%), pregnancy (4%), age <1 year (4%), and unwillingness to consent to the study (3%).

The demographic and clinical features at admission between the 3 study groups were similar, except for the proportion of the patients with previous malaria attacks, which was lower in the group receiving artemether-lumefantrine than in the group receiving artesunate plus mefloquine (table 1). Of all study patients, 207 (63%) of 330 were aged ≤15 years old (73 receiving chloroquine plus sulfadoxine-pyrimethamine, 71 receiving artesunate plus mefloquine, and 63 receiving artemether-lumefantrine). Only 1 person (1%) receiving chloroquine plus sulfadoxine-pyrimethamine, 3 (3%) receiving artesunate plus mefloquine, and 6 (5%) receiving artemether-lumefantrine vomited in the first hour after drug administration; they were readministered medication successfully. The mean duration of hospital stay was 3.3 days (95% CI, 3.2–3.3 days). One patient in the group receiving chloroquine plus sulfadoxine-pyrimethamine was lost to follow-up after day 35. Two children (from the same parents) who received artesunate plus mefloquine withdrew from the study on day 21 because their parents did not consent to further blood sampling. In the group receiving artemether-lumefantrine, 2 patients were lost to follow-up after day 28 and 35, and 1 child died of severe diarrhea at home on day 26. Antimalarial drugs were detected in the urine of 89 patients (81%). The proportion of patients with antimalarial drug detected in their urine was higher in those receiving chloroquine plus sulfadoxine-pyrimethamine than in those receiving artesunate plus mefloquine or artemether-lumefantrine (table 1). Quality-control slide microscopy by an independent microscopist gave a κ statistic of 0.96 (95% CI, 0.93–0.99; $n = 509$).

Table 1. Demographic, clinical, and laboratory details at admission for patients enrolled in a study comparing chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine for the treatment of falciparum malaria in Laos.

	Treatment group			
	All ($n = 330$)	Chloroquine plus sulfadoxine-pyrimethamine ($n = 110$)	Artesunate plus mefloquine ($n = 110$)	Artemether-lumefantrine ($n = 110$)
Sex, no. of patients				
Male	202	70	70	62
Female	128	40	40	48
Age, years	15.4 (13.9–16.9)	15.0 (12.3–17.7)	15.4 (12.8–17.9)	15.9 (13.2–18.7)
Body weight, kg	29.8 (27.9–31.7)	28.2 (24.8–31.5)	30.9 (27.5–34.3)	30.2 (27.0–33.4)
No. of days ill	3.2 (3.0–3.4)	3.2 (2.8–3.6)	3.2 (2.9–3.5)	3.1 (2.8–3.4)
Previous malaria attack[a]	97 (30)	33 (31)	39 (37)	25 (23)
Axillary temperature, °C	38.2 (38.0–38.3)	38.2 (37.9–38.5)	38.2 (37.9–38.5)	38.1 (37.8–38.3)
Systolic blood pressure, mm Hg[b]	102 (100–104)	103 (99–106)	102 (98–105)	101 (98–104)
Diastolic blood pressure, mm Hg[b]	67 (65–68)	68 (65–71)	65 (63–68)	67 (64–70)
Pulse, beats/min	98 (96–99)	99 (96–102)	97 (94–100)	98 (95–101)
Respiratory rate, breaths/min	28 (27–28)	28 (27–29)	28 (26–29)	28 (26–29)
Splenomegaly	70 (21)	24 (22)	19 (17)	27 (25)
Hepatomegaly	28 (8)	9 (8)	6 (5)	13 (12)
Parasitemia, parasites/μL	26,779 (24,138–29,703)	26,122 (21,994–31,031)	25,026 (20,811–30,095)	29,363 (24,361–35,392)
Hematocrit, %	35.2 (34.6–35.9)	35.4 (34.2–36.5)	35.3 (34.2–36.4)	35.1 (34.0–36.1)
Presence of drug in urine				
Any antimalarial	234 (71)	89 (81)	72 (65)	73 (66)
Chloroquine	222 (67)	84 (76)	70 (64)	68 (62)
Quinine	3 (1)	2 (2)	1 (1)	0
Pyrimethamine	2 (0.6)	1 (1)	0	1 (1)
Chloroquine and quinine	4 (1)	1 (1)	1 (1)	2 (2)
Quinine and pyrimethamine	2 (0.6)	1 (1)	0	1 (1)
Chloroquine, quinine, and pyrimethamine	1 (0.3)	0	0	1 (1)

NOTE. Data are mean (95% CI) or no. (%) of patients, unless otherwise indicated. Parasitemia is expressed as geometric mean.

[a] Defined as patient or patient's guardian reporting that the patient had had a febrile illness with a blood smear positive for malaria. Data were available from only 106, 106, and 109 patients in chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine groups, respectively

[b] Data were available from only 56, 66, and 70 patients in chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine groups, respectively

For patients who received artemether-lumefantrine, the mean estimated fat intake per meal was 4.8 g (95% CI, 4.4–5.2 g); per day, it was 13.8 g (95% CI, 12.5–15.1 g); and per 3 days of therapy, it was 41.5 g (95% CI, 37.7–45.3 g). Of all meals taken during the 3 days of therapy, 13% contained no fat and 84%, 3%, and 0% contained an estimated 0.1–10 g, 10–20 g, and >20 g of fat, respectively. The mean fat content in each meal was significantly lower for children than for adults (3.6 g [95% CI, 3.2–3.9 g] vs. 6.6 g [95% CI, 6.0–7.1 g]; $P < .001$).

Cure rates, fever and parasite clearance, and changes in hematocrit. Of 26 paired samples from 25 patients (1 patient had 2 episodes of posttreatment parasitemia) who had recurrent parasitemia after day 7, PCR results showed matching genotypes consistent with recrudescent infections in 5 paired samples. In the other 20 patients, the recurrent parasitemia was of a genotype different than the primary infection, suggesting a new infection. Cure rates, excluding patients who were lost to follow-up or experienced reinfections, were 100% (106 of 106 patients), 97% (93 of 96), and 92% (94 of 102) for subjects receiving artesunate plus mefloquine, artemether-lumefantrine, and chloroquine plus sulfadoxine-pyrimethamine, respectively. By use of intention-to-treat analysis, the 42-day cure rates, adjusted for reinfections, were 100% (110 of 110 patients), 97% (107 of 110), and 93% (102 of 110) for the groups receiving artesunate plus mefloquine, artemether-lumefantrine, and chloroquine plus sulfadoxine-pyrimethamine, respectively (table 2). The cure rate was significantly higher for those receiving artesunate plus mefloquine than for those receiving chloroquine plus sulfadoxine-pyrimethamine ($P = .007$). The cure rates for the artemether-lumefantrine group and the chloroquine plus sulfadoxine-pyrimethamine group were not statistically significantly different ($P = .12$). The frequency of early treatment failures in the group receiving chloroquine plus sulfadoxine-pyrimethamine was 6% (6 of 102 patients), compared with nil for those receiving artesunate plus mefloquine and artemether-lumefantrine ($P = .029$ for both). All patients with late treatment failure (2 receiving chloroquine plus sulfadoxine-pyrimethamine and 3 receiving artemether-lumefantrine) had recurrent parasitemia on or after day 21, with a mean interval to recrudescence of 28.0 days (95% CI, 27.1–29.1 days). Only 1 patient, in the group receiving chloroquine plus sulfadoxine-pyrimethamine, had recrudescence after 28 days (on day 30). There were no differences between parasitological and clinical assessments (6 patients with early treatment failure were classified as R-III and 5 patients with late treatment failure were classified as RI [3, 25]).

Of 11 patients with treatment failure (8 receiving chloroquine plus sulfadoxine-pyrimethamine and 3 receiving artemether-lumefantrine), 10 were successfully retreated with artesunate plus mefloquine; the remaining patient was re-treated with artesunate alone for 7 days but declined further follow-up. Of the 20 patients with reinfections (7 receiving chloroquine plus sulfadoxine-pyrimethamine, 2 receiving artesunate plus mefloquine, and 11 receiving artemether-lumefantrine), 16 were treated successfully with artesunate plus mefloquine (15 patients) or artesunate alone for 7 days (1 patient) Four patients were treated with either artesunate plus mefloquine or arte-

Table 2. Outcome measures for the treatment of patients enrolled in a study comparing chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine for the treatment of falciparum malaria in Laos.

	Treatment group			
	All (n = 330)	Chloroquine plus sulfadoxine-pyrimethamine (n = 110)	Artesunate plus mefloquine (n = 110)	Artemether-lumefantrine (n = 110)
42-Day cure rate[a]	319 (97)	102 (93)	110 (100)[b]	107 (97)
Fever clearance time, h[a,c]	29.5 (27.4–31.5)	40.2 (35.9–44.4)[d]	24.6 (21.8–27.3)	23.1 (20.9–25.3)
Parasite clearance time, days[a,e]	2.3 (2.3–2.4)	2.9 (2.8–3.0)[d]	2.07 (2.0–2.1)	2.08 (2.0–2.1)
Gametocytemia after treatment	37 (11.2)	28 (25.5)[d]	4 (3.6)	5 (4.5)
Gametocytemia, gametocytes/μL	230 (141–375)	312 (174–558)	171 (59–496)	53 (24–114)[d]
Plasmodium vivax appearance after treatment for *Plasmodium falciparum* infection	5 (1.5)	0	0	5 (4.5)

NOTE. Data are no. of patients (%) or mean (95% CI), as appropriate. Gametocytemia is expressed as geometric mean.

[a] Intention-to-treat analysis.

[b] Significant difference from chloroquine plus sulfadoxine-pyrimethamine group ($P = .007$).

[c] Data were available from only 95, 82 and 97 patients in chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine groups, respectively.

[d] Significant difference from the other 2 groups ($P < .05$).

[e] Data were available from only 104 patients in chloroquine plus sulfadoxine-pyrimethamine group.

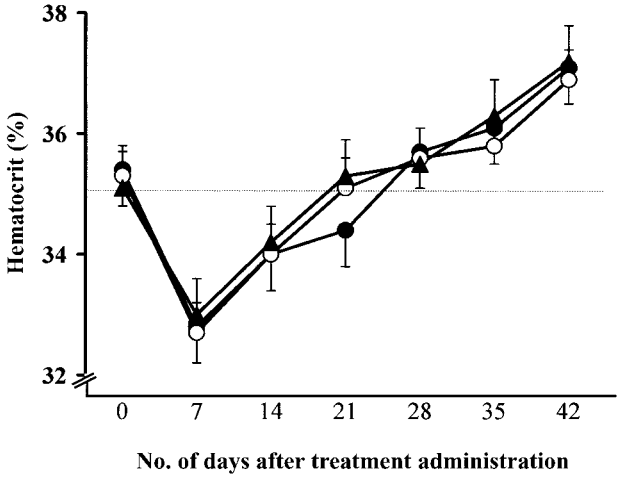

Figure 2. Mean hematocrit (%) before and after administration of study drug in groups receiving chloroquine plus sulfadoxine-pyrimethamine *(solid circles),* artesunate plus mefloquine *(open circles),* and artemether-lumefantrine group *(triangles).* Bars indicate SDs.

sunate for 7 days but declined follow-up. The patient with a second recurrence of parasitemia after 7 days of artesunate treatment was re-treated with artemether-lumefantrine but declined further follow-up. Five patients in the group receiving artemether-lumefantrine had appearance of *P. vivax* during follow-up, at a median of 28 days (range, 27–35 days), compared with none in the other 2 groups ($P = .06$ and .06, respectively). Treatment response did not differ between patients with and those without antimalarial drugs detected in their urine ($P = .43$).

The mean fever clearance time was significantly longer in the group receiving chloroquine plus sulfadoxine-pyrimethamine (40.2 h; 95% CI, 35.9–44.4 h) than in those receiving artesunate plus mefloquine (24.6 h; 95% CI, 21.8–27.3 h; $P < .001$) and artemether-lumefantrine (23.1 h; 95% CI, 20.9–25.3 h; $P < .001$). The mean parasite clearance time was significantly longer in patients treated with chloroquine plus sulfadoxine-pyrimethamine (2.9 days; 95% CI, 2.8–3.0 days) than in those treated with artesunate plus mefloquine (2.07 days; 95% CI, 2.0–2.1 days; $P < .001$) and artemether-lumefantrine (2.08 days; 95% CI, 2.0–2.1 days; $P < .001$). The mean hematocrit after treatment (day 7–42) was not significantly different between the 3 groups ($P > .05$) (figure 2). The proportions of patients given ferrous sulfate and folate were the same in all groups (3%).

Factors associated with treatment response. Patients who experienced treatment failure tended to be younger than those treated successfully (mean age, 10.3 years [95% CI, 3.9–16.5 years] vs. 15.7 years [95% CI, 14.1–17.3 years]; $P = .21$). Those with treatment failure had longer mean clearance times for fever (52.3 h [95% CI, 35.1–69.4 h]) and parasitemia (3.0 days [95% CI, 1.7–4.2 days]) than did those whose infections were cured (29.0 h [95% CI, 26.9–31.1 h]) and 2.3 days [95% CI, 2.2–2.4 days], respectively; $P < .001$ and .012, respectively), and they had significantly lower mean hematocrits on day 28 (33.0% [95% CI, 30.5%–35.5%] vs. 35.7% [95% CI, 35.3%–36.2%]; $P = .03$).

From 110 patients treated with artemether-lumefantrine, 77 plasma samples from day 7 (70%) were available for measurement of lumefantrine concentrations. The mean plasma lumefantrine concentration on day 7 was 0.47 μg/mL (95% CI, 0.38–0.56 μg/mL). Mean day 7 lumefantrine levels were similar for children and adults (0.52 μg/mL [95% CI, 0.39–0.65 μg/mL] vs. 0.41 μg/mL [95% CI, 0.28–0.53 μg/mL]; $P = .24$). Day 7 plasma lumefantrine concentrations were <0.28 μg/mL for 32 patients (42%) and >0.50 μg/mL for 28 patients (36%). For the 3 patients given artemether-lumefantrine who experienced treatment failure, day 7 plasma lumefantrine levels were 0.16, 0.20, and 0.85 μg/mL. There was no correlation between day 7 lumefantrine concentration and the estimated fat intake during the 3 days of therapy ($r = .06$; $P = .58$).

Comparison between adults and children. Of all study patients, 63% were children (age, ≤15 years). The proportion with previous malaria attacks was significantly higher in adults (39%) than in children (24%) ($P = .002$). The mean admission temperature was significantly higher (38.3°C [95% CI, 38.1–38.5°C] vs. 37.9°C [95% CI, 37.7–38.1°C]; $P = .021$) and the frequencies of palpable spleen (30% and 12%; $P < .001$) and liver (6% and 2%; $P < .002$) were significantly higher in children than in adults. Fever clearance times were significantly longer in children (31.5 h; 95% CI, 28.8–34.3 h) than in adults (25.6 h; 95% CI, 22.8–28.5 h; $P = .007$). Parasite clearance times for children and adults were similar (2.4 days [95% CI, 2.3–2.5

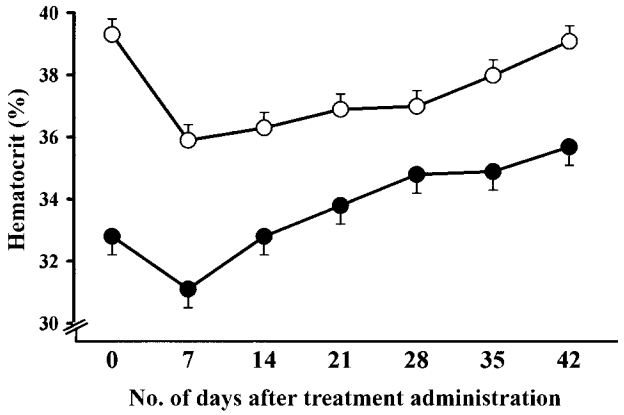

Figure 3. Mean hematocrit (%) before and after administration of study drug in children *(solid circles)* and adults *(open circles).* Bars indicate SDs.

Table 3. Potential adverse effects after treatment in patients enrolled in a study comparing chloroquine plus sulfadoxine-pyrimethamine, artesunate plus mefloquine, and artemether-lumefantrine for the treatment of falciparum malaria in Laos.

		After treatment		
	All (n = 330)	Chloroquine plus sulfadoxine-pyrimethamine (n = 110)	Artesunate plus mefloquine (n = 110)	Artemether-lumefantrine (n = 110)
Weakness	231 (70)	18 (16)	28 (25)[a]	7 (6)[b]
Dizziness	186 (56)	15 (14)	38 (35)[c]	11 (10)
Headache	232 (70)	12 (11)	28 (25)[c]	12 (11)
Nausea/vomiting	123 (37)	8 (7)	19 (17)[c]	1 (1)[b]
Abdominal pain	22 (7)	7 (6)	10 (9)[a]	2 (2)
Diarrhea	15 (4.5)	10 (9)	13 (12)[a]	1 (1)[b]
Urticaria	1 (0.3)	0	0	2 (2)
Labial herpes	0	0	1 (1)	0
Dyspnea	14 (4)	3 (3)	7 (6)	2 (2)
Palpitations	0	0	1 (1)	0
Blurred vision	8 (2)	2 (2)	7 (6)	2 (2)
Tinnitus	4 (1)	4 (4)	2 (2)	2 (2)
Confusion	1 (0.3)	1 (1)	7 (6)[a]	0
Nightmares	0	0	5 (5)	0
Irritable/angry[d]	0	0	7 (6)[c]	0
Neuropsychiatric	0	0	3 (3)	0

NOTE. Data are no. (%) of patients with sign or symptom.

[a] Significant difference from the artemether-lumefantrine group ($P < .05$).

[b] Significant difference from the chloroquine plus sulfadoxine-pyrimethamine group ($P < .05$).

[c] Significant difference from the other groups ($P < .05$).

[d] As reported by the patient's family as uncharacteristic patient behavior.

days] vs. 2.3 days [95% CI, 2.2–2.4 days]; $P = .10$). Mean hematocrit values on admission and between day 7–42 were significantly lower in children than in adults ($P < .001$) (figure 3).

Gametocyte carriage. The proportion of patients with gametocytemia at any time point following treatment was significantly higher in the group treated with chloroquine plus sulfadoxine-pyrimethamine (28 [25.5%] of 110]) than in those treated with artesunate plus mefloquine (4 [3.6%] of 110) and artemether-lumefantrine (5 [4.5%] of 100; $P < .001$ for both). The mean duration was 0.17 person-gametocyte-weeks (95% CI, 0.11–0.23 person-gametocyte-weeks) for all patients and did not differ between groups ($P > .05$). During follow-up, the geometric mean gametocyte counts were significantly lower for patients receiving artemether-lumefantrine (53 gametocytes/μL; 95% CI, 24–114 gametocytes/μL) than for those receiving chloroquine plus sulfadoxine-pyrimethamine (312 gametocytes/μL; 95% CI, 174–558 gametocytes/μL) and artesunate plus mefloquine (171 gametocytes/μL; 95% CI, 59–496 gametocytes/μL; $P = .015$ and .029, respectively). The frequency of patients with gametocytemia at any time point following treatment was significantly higher for children (31 [15%] of 207) than adults (6 [5%] of 123; $P = .005$).

Adverse events. The proportions of patients with symptoms and signs before treatment that may subsequently be confused with drug-related adverse events did not differ significantly between the 3 groups (table 3; $P > .05$). The proportion of patients with $\geqslant 1$ recorded potential side effect was higher in the group receiving artesunate plus mefloquine (57 [52%] of 110) than in those receiving artemether-lumefantrine (30 [27%] of 110) or chloroquine plus sulfadoxine-pyrimethamine (48 [44%] of 110; $P < .001$ and $P = .22$, respectively). The frequency of patients with $\geqslant 1$ recorded potential side effect was significantly lower in the group treated with artemether-lumefantrine than in the group treated with chloroquine plus sulfadoxine-pyrimethamine ($P = .01$).

Fifteen days after the first dose of chloroquine plus sulfadoxine-pyrimethamine, a 17-year-old woman developed fever and a Glasgow Coma Score of 8/15. Blood films demonstrated gametocytes, without asexual forms, throughout this episode. She was treated with parenteral antipyretic and chloramphenicol and recovered completely. Three patients, who were treated with artesunate plus mefloquine, had serious neuropsychiatric effects following treatment. Of these, 2 men had severe nightmares and hallucinations on the third and fourth nights after

treatment, and 1 patient wandered into an area of unexploded ordnance. An 8-year-old girl had hallucinations and uncharacteristic anxiety on day 20 after treatment. However, their symptoms were well controlled with oral diazepam, and they remained well throughout the remaining 42-day follow-up.

DISCUSSION

This study demonstrates that oral artesunate plus mefloquine and artemether-lumefantrine are highly effective for the treatment of uncomplicated falciparum malaria in southern Laos. Lower cure rates with artemether-lumefantrine have been reported in some studies, possibly related to low concurrent dietary fat intake [8–10, 12, 14, 15]. However, in southern Laos, despite very low fat intake, the 42-day follow-up cure rate following treatment with 6 doses of artemether-lumefantrine was 97%. Consistent with the low-fat diet, the day 7 plasma lumefantrine concentrations were also low. In a prior analysis of day 7 lumefantrine pharmacokinetic-pharmacodynamic relationships, 49% of patients with lumefantrine concentrations of <0.28 μg/mL had subsequent recrudescence, whereas 94% of those with concentrations of >0.5 μg/mL were cured [29]. If this relationship applied to the situation in Laos, we would have seen considerably more artemether-lumefantrine treatment failures. The differences may be explained by greater parasite susceptibility and perhaps higher levels of background immunity.

In this study, 75% of patients who had failure of treatment with chloroquine plus sulfadoxine-pyrimethamine had early treatment failure or high-grade (R-III) resistance. This result is consistent with recent treatment failure rates of 35% and 18% for chloroquine alone and sulfadoxine-pyrimethamine alone, respectively, in this population [3]. The chloroquine plus sulfadoxine-pyrimethamine combination was significantly inferior to artesunate plus mefloquine or artemether-lumefantrine in terms of initial responses. Patients treated with artesunate plus mefloquine or artemether-lumefantrine recovered from fever and cleared parasitemia more rapidly than did those who received chloroquine plus sulfadoxine-pyrimethamine. In addition, patients treated with chloroquine plus sulfadoxine-pyrimethamine developed gametocytemia more often than did those treated with artesunate plus mefloquine or artemether-lumefantrine, suggesting that these patients may be the source of significantly more further infections in the community. That malaria immunity may be an important contributor to the treatment response [3, 30] is suggested by both the high proportion of early to late treatment failure in the group receiving chloroquine plus sulfadoxine-pyrimethamine and the higher frequency of treatment failure, slower fever clearance, and increase in hematocrit among children.

Most patients tolerated the 3 drug regimens well, but artemether-lumefantrine was associated with significantly fewer adverse effects than were chloroquine plus sulfadoxine-pyrimethamine and artesunate plus mefloquine. More patients who received chloroquine plus sulfadoxine-pyrimethamine or artesunate plus mefloquine developed self-limiting diarrhea, whereas there was only 1 case in artemether-lumefantrine group [9, 11, 31, 32]. In this study, all patients except 1 developed diarrhea within 7 days of drug administration. Although a 1-year-old child who received artemether-lumefantrine died of severe diarrhea on day 26, this was unlikely to be a consequence of the drug therapy.

In this study area, adding chloroquine to sulfadoxine-pyrimethamine significantly improved outcome, compared with sulfadoxine-pyrimethamine alone. The mean times for fever and parasite clearance were shorter in patients treated with chloroquine plus sulfadoxine-pyrimethamine (40.2 h [95% CI, 35.9–44.4 h] and 2.9 days [95% CI, 2.8–3.0 days], respectively) than for those treated in our previous trial in 2001–2002 with sulfadoxine-pyrimethamine alone (61.1 h [95% CI, 50.9–71.3 h] and 3.2 days [95% CI, 2.9–3.5 days], respectively) [3]. The shorter fever clearance time associated with chloroquine plus sulfadoxine-pyrimethamine, compared with sulfadoxine-pyrimethamine treatment alone, may reflect an antipyretic effect of chloroquine [33] or earlier stage specificity of chloroquine's activity.

In conclusion, artesunate plus mefloquine and artemether-lumefantrine are more effective treatments for malaria in southern Laos than is chloroquine plus sulfadoxine-pyrimethamine, but artesunate plus mefloquine is associated with significantly more serious, although transient, adverse effects.

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References

1. White NJ. Drug resistance in malaria. Br Med Bull **1998**; 54:703–15.
2. Mayxay M, Phetsouvanh R, Phompida S, et al. A randomized comparison of oral chloroquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria in the Lao PDR (Laos) Trans R Soc Trop Med Hyg **2003**; 97:343–4.
3. Mayxay M, Newton PN, Khanthavong M, et al. Chloroquine versus sulfadoxine-pyrimethamine for treatment of *Plasmodium falciparum* malaria in Savannakhet province, Lao People's Democratic Republic:

an assessment of national antimalarial drug recommendations. Clin Infect Dis **2003**; 37:1021–8.
4. Pillai DR, Labbe AC, Vanisaveth V, et al. *Plasmodium falciparum* malaria in Laos: chloroquine treatment outcome and predictive value of molecular markers. J Infect Dis **2001**; 183:789–95.
5. Guthmann JP, Kasparian S, Nathan N, et al. The efficacy of chloroquine for the treatment of acute uncomplicated *Plasmodium falciparum* malaria in the Lao People's Democratic Republic. Ann Trop Med Parasitol **2002**; 96:553–7.
6. Schwobel B, Jordan S, Vanisaveth V, et al. Therapeutic efficacy of chloroquine plus sulfadoxine/pyrimethamine compared with monotherapy with either chloroquine or pyrimethamine/sulfadoxine in uncomplicated *Plasmodium falciparum* malaria in Laos. Trop Med Int Health **2003**; 8:19–24.
7. Adjuik M, Babiker A, Garner P, et al. Artesunate combinations for treatment of malaria. Lancet **2004**; 363:9–17
8. van Vugt M, Brockman A, Gemperli B, et al. Randomized comparison of artemether-benflumetol and artesunate-mefloquine in treatment of multidrug-resistant falciparum malaria. Antimicrob Agents Chemother **1998**; 42:135–9.
9. van Vugt M, Looareesuwan S, Wilairatana P, et al. Artemether-lumefantrine for the treatment of multidrug-resistant falciparum malaria. Trans R Soc Trop Med Hyg **2000**; 94:545–8.
10. Lefevre G, Looareesuwan S, Treeprasertsuk S, et al. A clinical and pharmacokinetic trial of six doses of artemether-lumefantrine for multidrug-resistant *Plasmodium falciparum* malaria in Thailand. Am J Trop Med Hyg **2001**; 64:247–56.
11. Krudsood S, Looareesuwan S, Silachamroon U, et al. Artesunate and mefloquine given simultaneously for three days via a prepacked blister is equally effective and tolerated as a standard sequential treatment of uncomplicated acute *Plasmodium falciparum* malaria: randomized, double-blind study in Thailand. Am J Trop Med Hyg **2002**; 67:465–72.
12. Krudsood S, Chalermrut K, Pengruksa C, et al. Comparative clinical trial of two fixed combinations dihydroartemisin-napthoquine-trimethoprim (DNP) and artemether-lumefantrine (Coartem/Riamet) in the treatment of acute uncomplicated falciparum malaria in Thailand. Southeast Asian J Trop Med Public Health **2003**; 34:316–21.
13. Price R, van Vugt M, Phaipun L, et al. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. Am J Trop Med Hyg **1999**; 60:547–55.
14. van Vugt M, Wilairatana P, Gemperli B, et al. Efficacy of six doses of artemether-lumefantrine (benflumetol) in multidrug-resistant *Plasmodium falciparum* malaria. Am J Trop Med Hyg **1999**; 60:936–42.
15. Poravuth Y, Socheat D, Fandeur T, et al. Efficacy and safety of six dose regimen of Coartem in the treatment of uncomplicated falciparum malaria in Cambodia 2002. Mekong Malaria Forum **2002**; 10:54.
16. Bojang KA, Schneider G, Forck S, et al. A trial of Fansidar plus chloroquine or Fansidar alone for the treatment of uncomplicated malaria in Gambian children. Trans R Soc Trop Med Hyg **1998**; 92:73–6.
17. Bustos DG, Canfield CJ, Canete-Miguel, Hutchinson BA. Atovaquone-proguanil compared with chloroquine and chloroquine-sulfadoxine-pyrimethamine for treatment of acute *Plasmodium falciparum* malaria in the Philippines. J Infect Dis **1999**; 179:1587–90.
18. Gogtay NJ, Desai S, Kadam VS, Kamtekar KD, Dalvi SS, Kshirsagar NA. A randomized, parallel-group study in Mumbai (Bombay), comparing chloroquine with chloroquine plus sulfadoxine-pyrimethamine in the treatment of adults with acute, uncomplicated, *Plasmodium falciparum* malaria. Ann Trop Med Parasitol **2000**; 94:309–12.
19. Gasasira AF, Gorsey G, Nzarubara B, et al. Comparative efficacy of aminoquinoline-antifolate combinations for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. Am J Trop Med Hyg **2003**; 68:127–32.
20. McIntosh HM, Greenwood BM. Chloroquine or amodiaquine combined with sulfadoxine-pyrimethamine as a treatment for uncomplicated malaria—a systematic review. Ann Trop Med Parasitol **1998**; 92: 265–70.
21. World Health Organization. Severe falciparum malaria. Trans R Soc Trop Med Hyg **2000**; 94(Suppl 1):1–90.
22. Brockman A, Paul REL, Anderson TJC, et al. Application of genetic markers to the identification of recrudescent *Plasmodium falciparum* infections on the northwestern border of Thailand. Am J Trop Med Hyg **1999**; 60:14–21.
23. Silamut K, Hough R, Eggelte T, Pukrittayakamee S, Angus B, White NJ. A simple method for assessing quinine pretreatment in acute malaria. Trans R Soc Trop Med Hyg **1995**; 89:665–7.
24. World Health Organization. Assessment and monitoring of antimalrial drug efficacy for the treatment of uncomplicated falciparum malaria. Document no. WHO/HTM/RBM2003.50. Geneva: World Health Organization, **2003**.
25. World Health Organization. Chemotherapy of malaria and resistance to antimalarials: report of a WHO Scientific Working Group. World Health Organ Tech Rep Ser **1973**; 529:1–121.
26. Food and Agriculture Organization. Food composition table. Available at: http://www.fao.org/DOCREP/003/X6878E/X6878E00.htm.Accessed 28 January 2004.
27. Ezzet F, van Vugt M, Nosten F, Looareesuwan S, White NJ. Pharmacokinetics and pharmacodynamics of lumefantrine (Benflumetol) in acute falciparum malaria. Antimicrob Agents Chemother **2000**; 44: 697–704.
28. Annerberg A, Lindegårdh N, Blessborn D, Bergqvist Y, White NJ. A bioanalytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of lumefantrine and its desbutyl metabolite in plasma. Journal of Pharmaceutical and Biomedical Analysis (in press).
29. White NJ, van Vugt M, Ezzet F. Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clin Pharmacokinet **1999**; 37:105–25.
30. Mayxay M, Chotivanich K, Pukrittayakamee S, Newton P, Looareesuwan S, White NJ. The contribution of humoral immunity to the therapeutic response in falciparum malaria. Am J Trop Med Hyg **2001**; 65:918–23.
31. Price RN, Nosten F, Luxemburger C, et al. Artesunate versus artemether in combination with mefloquine for the treatment of multidrug-resistant falciparum malaria. Trans R Soc Trop Med Hyg **1995**; 89: 523–7.
32. Bakshi R, Hermeling-Fritz I, Gathmann I, Alteri E. An integrated assessment of the clinical safety of artemether-lumefantrine: a new oral fixed-dose combination antimalarial drug. Trans R Soc Trop Med Hyg **2000**; 94:419–24.
33. Hugosson E, Tarimo D, Troye-Blomberg M, Montgomery SM, Premji Z, Bjorkman A. Antipyretic, parasitologic, and immunologic effects of combining sulfadoxine/pyrimethamine with chloroquine or paracetamol for treating uncomplicated *Plasmodium falciparum* malaria. Am J Trop Med Hyg **2003**; 69:366–71.