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ADVERSE EFFECTS IN PATIENTS WITH ACUTE FALCIPARUM MALARIA TREATED WITH ARTEMISININ DERIVATIVES

RIC PRICE, MICHELE VAN VUGT, LUCY PHAIPUN, CHRISTINE LUXEMBURGER, JULIE SIMPSON, ROSE McGREADY, FEIKO TER KUILE, AM KHAM, TAN CHONGSUPHAJAISIDDHI, NICHOLAS J. WHITE, AND FRANÇOIS NOSTEN

Shoklo Malaria Research Unit, Mae Sod, Tak Province, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford, United Kingdom

Abstract. In prospective studies of acute uncomplicated, multidrug-resistant falciparum malaria on the western border of Thailand, the oral artemisinin derivatives were used alone in the treatment of 836 patients (artesunate 630, artemether 206), were combined with mefloquine (15–25 mg base/kg) in 2,826 patients, and mefloquine alone was used in 1,303 patients. The combined regimens of mefloquine plus an artemisinin derivative were associated with more side effects than those with an artemisinin derivative alone; acute nausea (31% versus 16%), vomiting (24% versus 11%), anorexia (51% versus 34%), and dizziness (47% versus 15%) ($P < 0.001$). Oral artesunate and artemether alone were very well tolerated. There was no difference in the incidence of possible adverse effects between the two drugs, and no evidence that either derivative caused allergic reactions, neurologic or psychiatric reactions, or cardiovascular or dermatologic toxicity. Blackwater fever occurred in three patients treated with mefloquine plus artesunate regimens. Oral artesunate and artemether are safe and well tolerated antimalarial drugs.

The antimalarial artemisinin (qinghaosu) is the active principle extract of the plant qinghao (*Artemisia annua L*). This plant has been used for centuries in traditional Chinese medicine although its specific antimalarial properties were only discovered in China in 1972. In the last two decades, more than two million patients have been treated with artemisinin or one of its derivatives (artesunate or artemether), predominantly in China and southeast Asia. Although these antimalarials have proved highly efficacious in clinical trials with very few reported adverse effects,[1–3] there are few systematic large detailed clinical studies of the toxicity of artemisinin derivatives in clinical practice. The general toxicity profile in experimental animals has been good, but in all mammal species tested to date, these compounds have produced an unusual selective pattern of damage to certain brain stem nuclei, particularly those involved in auditory processing. Although there have been no reported neurotoxic reactions to artemisinin or its derivatives in humans, the relevance of these observations in animals to toxicity in humans is unresolved.

Plasmodium falciparum has developed resistance to chloroquine, sulfadoxine-pyrimethamine, and mefloquine on the borders of Thailand.[4] Sensitivity to quinine has also decreased. The rapid decrease in mefloquine efficacy in this region, and the lack of effective alternatives, has necessitated the introduction of treatment regimens containing artemisinin derivatives. A series of comparative drug studies have been conducted on the western border of Thailand with artesunate and artemether that have established their safety and efficacy.[5] Since 1994, these drugs have become an integral part of the treatment of uncomplicated falciparum malaria in this area.[6] The combination of mefloquine plus three days of artesunate is currently the standard first-line treatment for uncomplicated falciparum malaria. The extensive use of the artemisinin derivatives in this area provided us with the opportunity to review the human toxicity of these compounds in a cumulative experience of more than 3,500 prospectively studied treatment courses.

METHODS

Study site. This review is based on a series of large prospective chemotherapeutic trials aimed at optimizing antimalarial treatment regimens in an area of multidrug-resistant falciparum malaria. The studies took place between 1991 and 1995 in a Karen community living in an area of malarious hill forest along the Thai-Burmese border. Studies involving 2,593 of the patients described here have been reported previously.[6–13] The epidemiology of malaria at this site has also been described in detail recently.[14] Transmission of malaria is low (approximately one vivax, and one falciparum malaria infection per person every two years) and seasonal. Nearly all falciparum malaria infections are symptomatic.

Patients. All patients treated at the Shoklo Malaria Research Unit (SMRU) with an artemisinin derivative during the study period were included in the analysis. To determine whether the coadministration of an artemisinin derivative altered the toxicity profile of mefloquine therapy, adverse effects in all patients receiving mefloquine monotherapy were also studied for comparison. Patients of all ages were eligible to enroll in these prospective chemotherapeutic studies if they presented with slide-confirmed falciparum malaria to the SMRU or the outpatient clinics of Médecins Sans Frontières (MSF). They were recruited into these prospective studies provided that they or their accompanying relatives gave fully informed consent. Pregnant women, children weighing < 5 kg, and patients with signs of severity[15] or concomitant disease requiring hospital admission were all excluded. Therapeutic studies in Shoklo were carried out in three prospectively defined patient groups: 1) primary episodes of falciparum malaria, 2) recrudescent cases of malaria (recurrence of malaria within 63 days of a previous episode), and 3) hyperparasitemic falciparum malaria ($> 4\%$ parasitized red blood cells) as each of these required a different treatment approach. Apart from this categorization, the inclusion and exclusion criteria were identical between studies. All studies were approved by the Ethics Committee of the

TABLE 1
Antimalarial treatments (completed courses)*

Drug regimen	Number of patients (studies)	Dose
Mefloquine		
M25	n = 1,303 (316,[7] 278,[8] 315,[6] 182,[10] 212†)	Mefloquine, 25 mg/kg
Artesunate plus mefloquine		
MA	n = 323 (274,[8] 49‡)	As 10 mg/kg (3 doses in 24 hr) + M15
MAS1	n = 152 (151,[6] 1‡)	As 4 mg/kg (1 dose) + M25
MAS3	n = 1,972 (179,[6] 180,[10] 30,[13] 1,583†)	As 4 mg/kg daily for 3 days + M25
MAS5	n = 60 (49,[11] 11‡)	As 12 mg/kg over 5 days + M25
MAS7	n = 139 (3,‡ 136§)	As 12 mg/kg over 7 days + M25
Artemether plus mefloquine		
MAM3	n = 180 (178,[10] 1,‡ 1§)	Am 4 mg/kg daily for 3 days + M25
Artesuante		
AS5	n = 153 (50,[11] 105‡)	12 mg/kg over 5 days
AS7	n = 454 (207, 207,‡ 40§)	12 mg/kg over 7 days
AS7T7	n = 23 (23‡)	As 12 mg/kg over 7 days + 7 days tetracycline
Artemether		
AM7	n = 206 (204,[12] 2‡)	12 mg/kg over 7 days

* M15 = mefloquine, 15 mg/kg; M25 = mefloquine, 25 mg/kg; As = artesunate; Am = artemether. Superscript numbers give study reference.
† Routine monitoring of patients with primary falciparum malaria infections.
‡ Routine monitoring of patients with recrudescent falciparum malaria infections.
§ Routine monitoring of patients with hyperparasitemic falciparum malaria infections.

Faculty of Tropical Medicine, Mahidol University and the Karen Refugee Committee.

Study procedures. Clinical and laboratory procedures and patient follow-up was similar irrespective of whether they were enrolled into a comparative drug study or monitored routinely. On admission a questionnaire was completed recording details of symptoms and their duration and the history of previous antimalarial medication (since SMRU and MSF are the only sources of antimalarial drugs in this area, the history is generally a reliable guide to pretreatment). A clinical examination was completed and blood was taken for routine hematology and quantification of peripheral parasitemia. Treatment with one of the drug regimens (Table 1) was assigned to patients in comparative randomized studies as described previously.[6–8,10–13]

Drug administration was observed in all cases. If vomiting occurred < 30 min after drug administration, the full dose was repeated, and if vomiting occurred between 30 and 60 min, half the dose was repeated. No re-treatment was given for vomiting after 60 min.

All patients were seen daily until they became asymptomatic and aparasitemic, and were then seen weekly during the follow-up period; for the randomized comparative studies this was for nine weeks, but for routine monitoring of treatment response follow-up was for six weeks. At each visit the following symptoms were specifically asked about illness, headache, a history of fever, weakness, muscle/joint pain, abdominal pain, anorexia, dizziness, nausea, vomiting, stool consistency and frequency, rash/itching, or strange behavior. Any other complaints were also recorded. A physical examination was completed and the patient's temperature was recorded. A blood smear was also taken at each weekly visit and parasite counts were determined on Giemsa-stained thick and thin blood films and recorded per 500 white cells or 1,000 red blood cells, respectively. A blood smear was taken from any patient complaining of fever or illness during the follow-up. Blood samples were taken for the measurement of hematocrit on admission and then weekly throughout the follow-up period.

A brief neurologic examination was also performed at baseline, on day 2 and then on days 7 and 28. This included tests for coordination (heel-toe ataxia), fine finger dexterity (ability to pick up a 500 mg paracetamol tablet), hearing (using a 256 Hz tuning fork), as well as an assessment for nystagmus and balance (assessment of sway when standing with feet together and eyes shut).

Electrocardiographic studies. Two studies were undertaken to look for potential cardiotoxic manifestations following therapy with an artemisinin derivative. In the first study (conducted between June 1993 and July 1994) electrocardiographs (ECGs) were recorded on admission and on days 3, 7, 14, 28 and 63.[10] A second study (Price R, Nosten F, unpublished data) was conducted specifically to look for ECG changes immediately after administration of oral artesunate or artemether. In the latter study, ECGs were recorded on each day of treatment before the dose of artesunate or artemether was taken and again after dosing 1 hr later. Follow-up records were also taken on days 7, 28, and 63. A single strip ECG was recorded on a Fukada Denshi (Tokyo, Japan) Auto-cardiner™ FCP 2201 ECG machine, sampling 12 leads at a paper speed of 50 mm/sec and a sensitivity of 20 mm/mv.

The QT, PR, QRS, and RR intervals were calculated and reported by the machine, but to validate these readings ECGs were also read manually in a random selection of 40 patients by a physician blind to the treatment and to the values recorded by the machine. U waves were not included as part of the QT interval, which was calculated from, when possible, 3–5 consecutive cycles in leads I, V2, and V5. There was no significant difference in the manual or machine measures of the RR, QRS, PR, or QT intervals ($P > 0.1$ for each). The machine recordings were therefore used for the purposes of analysis. The QT interval was then corrected for heart rate by Bazets formula ($QT/\sqrt{RR}$).

Patients were classified into five treatment groups for the purposes of this analysis: mefloquine monotherapy, mefloquine with artesunate, mefloquine with artemether, artesunate monotherapy, and artemether monotherapy (Table 1).

Statistical analysis. Data were analyzed using SPSS for Windows (SPSS Software, Gorinchem, The Netherlands). Categorical data were compared by calculating the chi-square value with Yates' correction or by Fisher's exact test. Normally distributed continuous data were compared by Student's *t*-test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test, or the Kruskal-Wallis one-way analysis of variance on ranks.

Logistic regression. Symptoms and side effects elicited by follow-up questionnaire were assessed on admission and thereafter by their presence on days 1 to 2 and again by their presence on day 7. After starting treatment, independent risk factors were assessed after stratifying by the presence of the symptom on admission. Young children (< 5 years old), who accounted for 16% (813 of 4,965) of all cases, were unable to answer questions about dizziness and nausea, and were therefore excluded from analysis of these side effects.

Forward stepwise logistic regression was used to assess the relationship between drug therapy and possible side effects while controlling for confounding factors. A post-treatment model was constructed for each symptom in which the presence or absence of the symptom between days 1 and 2 or day 7 was entered as the dependent variable and the effect of a different derivative or dosage was evaluated while controlling for the presence or absence of the symptom on admission. Some of the symptoms were significantly associated with age, sex, administration of mefloquine; these factors were therefore entered into the model as potential confounders. Age stratification was done using the following strata: young children < 5 years of age, older children 5–14 years of age, and adults ≥ 14 years of age. The type of infection (primary or recrudescent), body temperature (fever defined as an oral temperature >38°C or an axillary temperature >37.5°C) and a parasitemia > 10,000/μl were significantly associated with symptomatology independent of age and were included in the model as markers of disease severity. The effect of the dose and artemisinin derivative used on symptoms/side effects was assessed by multivariate analysis after correcting for the significant confounding factors. In view of the multiple comparisons, a significance level of $P < 0.01$ was chosen.

RESULTS

Between July 1991 and December 1995, 5,075 patients were recruited into oral drug studies and received either mefloquine monotherapy, an artemisinin derivative, or a combination of the two. Overall, 110 (2%) patients were excluded from the analysis; 71 patients did not complete their course of treatment (51 were allocated a seven-day course of treatment with artesunate or artemether, 19 a short course, and one patient refused to take mefloquine), 26 had concomitant vivax malaria, three had markers of severe malaria and were subsequently treated parenterally, two were found to be pregnant, one was hypertensive, one had a negative blood smear on review, and in six the case records were lost. Of the 4,965 patients who completed a full course of treatment, 2,593 (52%) were recruited into randomized prospective studies (1,041 were treated with artemisinin derivatives plus mefloquine [AM], 461 with artemisinin derivatives alone [A], and 1,091 with mefloquine alone [M]), and an additional 2,372 (48%) patients presented to the clinics. Although the latter group were not part of comparative studies, they were treated in the same way (1,785 AM, 375 A, and 212 M), and therefore included in this analysis. Overall, 3,461 (70%) patients were recruited into studies of primary infections (1,043 M, 2,412 MA, and 6 A), 1,201 (24%) into studies of recrudescent infections (260 M, 197 MA, 744 A), and 303 (6%) into studies of hyperparasitemic infections (217 MA and 86 A). Of the 3,662 patients who received an artemisinin derivative, 3,276 (89%) received a treatment regimen containing artesunate, and 386 (11%) received artemether (Table 1). Mefloquine, when given alone, was given as a split dose (15 mg/kg followed by 10 mg/kg 8–24 hr later) in 353 patients (27%), and as a single dose in the remainder (950; 73%). Of the 2,826 patients of patients who received combination therapy, 4% (102) had mefloquine administered as a split dose. In the remaining patients, mefloquine was given as a single dose either on admission (587, 21%), day 1 (204, 7%), or thereafter (1,933, 68%). Of the 619 patients who received more than one treatment course with an artemisinin derivative, three received a total dosage of artesunate greater than 100 mg/kg (the maximum was 115 mg/kg). Overall, the median cumulative dose of artemisinin derivative that a patient received was 384 mg (range = 30–4,020 mg).

The baseline characteristics are shown in Table 2. There were significant differences between treatment groups. Compliance to follow-up was achieved in 4,696 (95%) by day 7, 4,074 (82%) by day 28, and 2,700 (54%) by day 42.

Early vomiting (within 1 hr). Data on drug vomiting were available for artesunate, artemether, and mefloquine treatments in > 95% of patients on all observed days. Overall, vomiting of artesunate or artemether within 1 hr of administration occurred in 3% (93 of 3,561) of the patients on the day of admission, 1.5% (50 of 3,339) on day 1, and 2% (72 of 3,022) on day 2, and 0.3% (13 of 3,782) of the drug administrations were vomited on subsequent days. After stratifying by day of administration, coadministration of mefloquine increased the risk of vomiting the artemisinin derivative by a factor of 2.8 (95% confidence intervals [CI] = 2.1–3.9, $P < 0.0001$). In those not receiving mefloquine, 2.2% (65 of 2,972) of patients vomited on the day of admission, 1.3% (40 of 3,145) on day 1, and 1.1% (12 of 1,131) on day 2, with 0.2% (9 of 3,674) of the drug administrations vomited on subsequent days. On admission, the following were found to be independent risk factors associated with vomiting artesunate or artemether: age ≤ 14 years (odds ratio [OR] = 3.9, 95% CI = 1.5–9.9, $P = 0.005$), coadministration of mefloquine (OR = 2.8, 95% CI = 1.1–6.9, $P = 0.03$), a history of vomiting (OR = 2.7, 95% CI = 1.4–5.2, $P = 0.005$), or nausea (OR = 2.1, 95% CI = 1.1–4.2, $P = 0.03$). The population attributable risks (PAR) for vomiting an artemisinin derivative (artesunate and artemether pooled) within 1 hr were 65% for an age ≤ 14 years, 69% for coadministration of mefloquine, 29% for a history of vomiting, and 30% for nausea. The overall PAR, calcu-

TABLE 2
Admission characteristics

	Mefloquine + artesunate	Mefloquine + artemether	Monotherapy			*P**
			Artesunate	Artemether	Mefloquine	
No. of subjects enrolled	2,646	180	630	206	1,303	
Dose of QHS† administered, mg median (range)	276 (30–840)	474 (70–828)	276 (66–816)	396 (60–720)	–‡	<0.001
Males	1,505 (57%)	86 (48%)	344 (55%)	114 (55%)	747 (57%)	0.13
Age (years)						
Median (range) in years	10 (0.2–84)	16 (0.4–60)	10 (0.2–66)	15 (0.6–58)	14 (0.4–88)	<0.001
<5	440 (17%)	15 (8%)	163 (26%)	32 (16%)	163 (13%)	
5–14	1,432 (54%)	62 (34%)	275 (44%)	69 (34%)	522 (40%)	<0.001
>14	774 (29%)	103 (57%)	192 (30%)	105 (51%)	618 (47%)	
Primary infection	2,445 (92%)	179 (99%)	63 (10)	4 (2%)	1,043 (80%)	<0.001
Fever on admission	1,595 (61%)	115 (64%)	327 (52%)	113 (55%)	851 (66%)	<0.001
Hematocrit						
Mean (SD)	35.6 (5.5)	35.9 (5.2)	32.9 (5.9)	33.3 (5.8)	34.6 (5.7)	<0.001
<30%	318 (14%)	18 (11%)	158 (28%)	51 (27%)	162 (20%)	<0.001
Geometric mean (95% confidence intervals) parasite count per μl of blood	7,393 (6,804–8,033)	5,295 (3,936–7,122)	8,953 (7,419–10,805)	4,501 (3,397–5,965)	3,379 (3,038–3,760)	<0.001
Parasitemia >10,000/μl	1,155 (44%)	70 (39%)	292 (46%)	67 (33%)	424 (33%)	<0.001

* Overall difference between treatment groups.
† QHS = artemisin derivative (artesunate and artemether pooled).
‡ – = no QHS administered.

TABLE 3
Development of symptoms/side effects after treatment in those without the symptom on admission

		Mefloquine monotherapy	Mefloquine + QHS*	QHS†	Relative risk [95% CI]‡
Dizziness§	Day 1–2¶	54% (203/376)	47% (123/263)	15% (141/935)	3.4 [2.8–4.1] $P < 0.001$
	Day 7	12% (40/343)	5% (47/872)	3% (6/244)	$P = 0.08$
Nausea§	Day 1–2¶	46% (196/424)	31% (95/305)	16% (175/1,081)	1.9 [1.6–2.4] $P < 0.001$
	Day 7	2% (9/392)	2% (20/990)	2% (7/338)	$P = 0.9$
Vomiting	Day 1–2¶	24% (168/697)	24% (115/485)	11% (166/1,536)	2.3 [1.8–2.7] $P < 0.001$
	Day 7	0.3% (2/671)	0.6% (8/1,440)	0.6% (3/507)	$P = 0.7$
Anorexia	Day 1–2¶	54% (152/284)	51% (102/203)	34% (284/831)	1.5 [1.3–1.7] $P < 0.001$
	Day 7	7% (19/274)	5% (35/736)	5% (13/245)	$P = 0.4$
Diarrhea	Day 1–2¶	3% (28/904)	3% (15/613)	1% (29/2,049)	$P = 0.1$
	Day 7	0.3% (3/988)	0.8% (17/2,111)	1% (8/666)	$P = 0.1$

* QHS = artemisinin derivative (artesunate and artemether pooled). Patients receiving mefloquine on admission or day 1.
† Patients who have only received a qinghaosu derivative, but not mefloquine.
‡ Comparison of groups treated with mefloquine + QHS with those receiving QHS alone.
§ Only in those able to answer questions about dizziness and nausea (>5 years old).
¶ Symptom reported on day 1 or 2.

lated as one minus the product of one minus each individual PAR, was 94%. Although fever on admission was a univariate risk factor for vomiting (relative risk [RR] = 2.7, 95% CI = 1.6–4.4, $P < 0.001$), it was not a significant factor in a multivariate model. The presenting parasitemia, type of derivative (artesunate or artemether), or dosage of artemisinin derivative given (2 mg/kg versus 4 mg/kg) did not affect vomiting. Coadministration of an artemisinin derivative did not increase the risk of vomiting mefloquine on admission.

Late side effects (onset > 1 hr). Both the malaria infection itself and mefloquine cause adverse effects on the neurologic and gastrointestinal systems that are difficult to separate and therefore confound assessment of possible artemisinin derivative side-effects.[9]

Confounding factors. On admission, 65% (3,160 of 4,889) of the patients reported anorexia, 46% (1,795 of 3,871) dizziness, 38% (1,525 of 3,979) nausea, 25% (1,250 of 4,943) vomiting, and 2% (110 of 4,949) diarrhea. An admission parasitemia greater than 10,000/μl and fever were taken as markers of disease severity, and were found to be significant independent risk factors for the following signs/symptoms on admission: dizziness, anorexia, nausea, and vomiting (OR = 1.5–2.2, $P < 0.001$). Admission parasitemia and fever were independent risk factors for anorexia on days 1 and 2 (OR = 1.4 and 1.5, respectively, $P < 0.001$), but patients had largely recovered by day 7 and initial disease severity was no longer a confounder at that time. Primary infections were significantly associated with nausea, vomiting, and anorexia on admission (OR = 1.3–1.6, $P < 0.001$) and on days 1 and 2 (OR = 1.4–2.0, $P < 0.001$). They were also associated with dizziness on days 1–2 (OR = 1.4, $P = 0.003$), but with none of the symptoms on day 7.

The administration of mefloquine was a major independent risk factor for the following symptoms/side effects in the first two days of treatment: dizziness (OR = 3.8, 95% CI = 3.1–4.6, $P < 0.001$), vomiting (OR = 1.9, 95% CI = 1.6–2.2, $P < 0.001$), nausea (OR = 2.7, 95% CI = 2.3–3.2, $P < 0.001$), anorexia (OR = 1.7, 95% CI = 1.4–2.0, $P < 0.001$), and diarrhea (OR = 1.8, 95% CI = 1.1–2.8, $P = 0.01$). On day 7, only dizziness was influenced by prior mefloquine administration (OR = 2.0, 95% CI = 1.2–3.2, $P = 0.004$).

There were significant differences in the frequency of reported side effects between adults and children. Vomiting and diarrhea were more common in children than adults on admission (OR = 1.7–4.3, $P < 0.001$). This remained apparent for diarrhea on days 1 and 2 (OR = 2.3, $P = 0.005$) and at day 7 (OR = 11, $P < 0.001$). Conversely, dizziness and nausea (both only assessed reliably in those over 5 years of age) and anorexia were more common in adults from admission until day 7 (OR = 1.4–7, $P < 0.001$). On admission and days 1 and 2, females were significantly more likely than adult males to report dizziness, anorexia, and nausea (OR = 1.2–1.3, $P < 0.01$), but by day 7 this was no longer apparent.

Patients who failed to defervesce within 24 hr were more likely to report the following associated symptoms on days 1 and 2: dizziness (OR = 2.1, 95% CI = 1.6–2.7, $P < 0.001$), vomiting (OR = 2.6, 95% CI = 2.1–3.2, $P < 0.001$), nausea (OR = 2.4, 95% CI = 1.9–3.0, $P < 0.001$), and anorexia (OR = 2.7, 95% CI = 2.1–3.6, $P < 0.001$). By day 7, only anorexia was more common in those still febrile after 24 hr (OR = 1.6, 95% CI = 1.1–2.2, $P = 0.008$). The reporting of symptoms or side effects was not influenced by the parasite clearance time.

Effect of dose and derivative. The administration of mefloquine was associated significantly with all symptoms and side effects reported on days 1 or 2. Thus, all potentially iatrogenic adverse effects in patients given both antimalarial drugs could be attributed to mefloquine (Table 3). After correcting for confounding baseline characteristics, there was no effect of the type of artemisinin derivative used (artesunate or artemether) on dizziness, diarrhea, nausea, vomiting, or anorexia. Of the 2,801 patients treated with two or more days of artesunate therapy, 460 received (16%) a low dose of artesunate in the first 48 hr (2 mg/kg/day) and 1,972 (70%) received 4 mg/kg/day. After correcting for confounding factors, dosage did not independently influence any of the symptoms/side effects. Overall, 2,814 (77%) patients treated with an artemisinin derivative (2,428 with artesunate and 386 with artemether) either received mefloquine on or after day 2 or not at all. In these patients, mefloquine toxicity could not be a confounding factor; 65% (1,528 of 2,363) reported anorexia on days 1–2, 42% (750 of 1,765) dizziness, 29% (493 of 1,710) nausea, 17% (361 of 2,137) vomiting, and 2% (42 of 2,107) diarrhea (after treatment).

TABLE 4
Incidence of recorded neurologic deficits during follow-up in all patients tested*

		Day 2			Day 7		
	Admission	MFQ	MFQ + QHS	QHS	MFQ	MFQ + QHS	QHS
Inability to stand with feet together	0.9% (12/1,319)	0% (0/45)	1.6% (1/62)	1.1% (11/990)	2.5% (2/81)	0.2% (2/1,083)	0% (0/337)
Heel toe ataxia	0.9% (12/1,316)	2.3% (1/44)	4.8% (3/62)	1.5% (15/988)	2.5% (2/81)	0.3% (3/1,084)	0.3% (1/377)
Impaired fine finger dexterity	0.2% (2/1,316)	0% (0/44)	1.6% (1/62)	0.1% (1/989)	0% (0/81)	0.1% (1/1,084)	0% (0/337)
Hearing impairment	0.4% (4/1,046)	NT	0% (0/21)	0.4% (3/798)	NT	0% (0/817)	0% (0/330)
Nystagmus	0.8% (10/1,324)	2.2% (1/45)	1.6% (1/62)	0.7% (7/989)	0% (0/81)	0.4% (4/1,085)	0% (0/337)

* MFQ mefloquine in those patients who have only received mefloquine; MFQ + QHS in patients who have received both mefloquine and a qinghaosu derivative; in most cases mefloquine was taken on or after day 2; QHS in those patients who have only received a qinghaosu derivative without any mefloquine. NT = none tested.

By day 7, 12% (399 of 3,462) of all patients studied complained of anorexia, 3% (80 of 2,834) of nausea, 0.5% (19 of 3,500) of vomiting, and 1% (34 of 3,859) of diarrhea. In those not receiving mefloquine (a significant confounding factor), 10% (45 of 470) reported being dizzy on day 7. There was no difference between short (less than four days) and longer courses of drug treatment or the derivative used in these rates. Furthermore, the incidence of reported diarrhea, vomiting, nausea, and dizziness on day 7 did not differ significantly from the incidence of these complaints on day 14 or day 28. This suggests that these symptoms were not attributable to the artemisinin derivatives. Anorexia was more common on day 7 than on day 14 (RR = 1.4, 95% CI = 1.2–1.7, $P < 0.0001$), but thereafter the incidence did not change significantly.

Overall, 2,151 (43%) patients received mefloquine in the first two days of treatment: 1,303 were treated with mefloquine alone and 848 received mefloquine plus artesunate or artemether. On days 1–2, the incidence of nausea was significantly lower in those receiving combination treatment with artesunate (OR = 0.60, 95% CI = 0.48–0.77, $P < 0.001$). This apparently beneficial effect was no longer significant after controlling for the more rapid fever clearance associated with combination treatment. The addition of an artemisinin derivative did not affect the reported incidence of dizziness, vomiting, anorexia, or diarrhea on days 1–2, or any of the symptoms on day 7.

Neurologic side effects. Neurologic examinations could be performed reliably only in patients > 5 years old. Examinations were carried out in 66% (1,971 of 3,003) of the patients receiving an artemisinin derivative (307 following treatment with artemether and 1,664 with artesunate). Of these, 1,512 (77%) also received mefloquine, and in 181 (9%) this was on admission or day 1. An additional 134 (12%) patients treated with mefloquine alone were also tested using similar procedures.

Data were recorded in 1,327 (63%) patients on admission, 1099 (52%) on day 2, 1,503 (71%) on day 7, and 1,438 (68%) on or after day 28. Of the patients in whom neurologic examinations were not possible on admission, 45% (353 of 778) were tested instead on day 2 (Table 4). On day 2, reporting of any neurologic disturbance was associated with dizziness (RR = 6.2, 95% CI = 3.1–12, $P < 0.001$). Of the 746 (35%) patients tested on both admission and day 2, six of the 733 patients (0.8%) without neurologic deficit on admission developed neurologic signs on day 2 (0 of 23 who had received mefloquine alone, 1 of 20 who had received mefloquine plus an artemisinin derivative, and 5 of 690 who had received an artemisinin derivative alone [$P = 0.1$]). All six patients had disturbed balance (one had heel toe ataxia, three were unable to stand with their feet together, and two had both signs). One patient also developed nystagmus and another had impaired finger dexterity. Although all patients were aparasitemic at the time of testing, three reported headache and fever before testing and the other three reported feeling dizzy. The signs had resolved in all cases by day 7. A total of 955 (45%) patients were tested on both admission and on day 7. Of the 938 patients without neurologic deficit on admission, four (0.4%) had developed neurologic signs on day 7 (two had heel toe ataxia, one was unable to stand with his feet together and one had both signs). All had received prior mefloquine as part of their treatment regimen. One of these patients had also developed nystagmus, and another reported dizziness, headache, and weakness. In all but one case the neurologic disturbance had resolved by day 14: in the remaining patient (treated with three days of artesunate plus mefloquine [MAS3]) the disturbance persisted until day 42. There was no apparent association between neurologic side effects and type or dose of derivative (either on the episode in question or total cumulative dose). No patient developed deafness or permanent neurologic abnormalities.

Rare adverse effects. Rare adverse effects that could have been related to antimalarial medication were reported in 17 patients: 15 following treatment with mefloquine plus artesunate/artemether, none following artesunate monotherapy, and two following mefloquine monotherapy (Table 5). Five patients had generalized tonic clonic seizures. In two of these cases the seizure occurred in children < 6 years old on the day of admission 6 and 10 hr after starting antimalarial treatment with artesunate and prior to receiving mefloquine. At the time of the seizures, both were febrile (one had a temperature of 39.8°C and the other a temperature of 38°C). One patient (a 57-year-old man) had an episode of hypertonia, generalized shaking, and brief unresponsiveness associated with a high fever on day 1, 24 hr after receiving mefloquine plus artesunate (MAS1). This could have been a rigor. A six-year-old boy with a history of epilepsy was treated with artemether plus mefloquine (MAM3) for three days and had a seizure two weeks later. A 31-year-old woman

TABLE 5
Incidence of rare adverse following treatment of uncomplicated falciparum malaria with an artemisinin derivative (QHS) +/− mefloquine

	Mefloquine monotherapy incidence per 100,000	Mefloquine + QHS incidence per 100,000	QHS monotherapy incidence per 100,000	*P**
Urticaria	0 (0/1,303)	71 (2/2,826)	0 (0/836)	0.5
Hemoglobinuria	0 (0/1,303)	106 (3/2,826)	0 (0/836)	0.3
Seizures	0 (0/1,303)	177 (5/2,826)	0 (0/836)	0.6
Neuropsychiatric reactions	154 (2/1,303)	177 (5/2,826)	0 (0/836)	0.5
Development of reversible neurology on day 2	0 (0/23)	5,000 (1/20)	725 (5/690)	0.2

* Overall difference between treatment groups.

with a history of convulsions more than 10 years previously had two tonic clonic seizures on day 4 of treatment with MAM3, 24 hr after receiving mefloquine, and after having made a rapid initial recovery from malaria. She responded to parenteral diazepam and phenobarbitone. A 22-year-old man treated with MAS3, a 36-year-old woman treated with mefloquine alone, and 12-year-old child treated with mefloquine alone became temporarily psychotic with delusions and hallucinations that required hospitalization and sedation. In the first week following treatment with MAS3, two women also developed anxiety and palpitations and two others developed marked disturbance of sleep. These responded to diazepam. All 12 patients with either seizures or neuropsychiatric adverse effects made full recoveries.

Three children (two with initial parasitemias greater than 4% of the red blood cells) treated with mefloquine plus artesunate regimens developed frank hemoglobinuria within 24 hr of the first dose of artesunate. Resolution of hemoglobinuria occurred in all three within 48 hr. Two children < 5 years old developed a generalized urticarial rash 3 hr after their second dose of artesunate. They responded to treatment with chlorpheniramine and did not develop a further episode following treatment with subsequent doses of artesunate.

Two patients treated with a seven-day course of artesunate died. One of these patients, a 17-year-old man, had had a three-month history of swollen ankles, and peripheral paresthesia. He was seen on day 14 of follow-up apparently well, but two days later had a sudden onset of dyspnea and palpitations while digging in the forest. A diagnosis of probable adult beri beri was made. The other patient was a 35-year-old woman who made a rapid and uneventful recovery from her malaria and was followed until day 35 of follow-up with no adverse sequelae. She was reported as having returned to Burma and died two weeks later. No further details were available.

Hematology. Serial white blood cell counts and platelet counts were recorded in 154 patients (132 of whom received artesunate and 22 artemether). Platelet counts were significantly lower on admission than on day 28 (mean [SD] = 169,000/μl [69,000] compared with 247,000/μl [72,000]; $P < 0.001$). Platelet counts decreased below 100,000/μl on day 7 in two patients receiving mefloquine plus artesunate treatment (to 27,000/μl and 92,000/μl), but in both cases these returned to the normal range by day 14. Overall, there was no change in the mean white blood cell or absolute neutrophil counts during follow-up.

Biochemical investigations. Samples for biochemical studies were taken from 180 patients (150 treated with mefloquine plus artesunate and 30 with mefloquine plus artemether). Liver function was assessed by analysis of alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) levels and renal function was assessed by plasma creatinine and blood urea nitrogen levels. On admission, nine (5.5%) patients had increased serum levels of liver enzymes. Of the 156 patients with normal biochemical findings on admission, seven (4.5%) developed increased serum levels of liver enzymes during follow-up. Three adults had small increases in their ALT levels on day 7 (60–70 IU/L) that had returned to normal by day 14. Two children (14 and 10 years old, and treated with MAS3 and MAM3, respectively) developed transient increases in their ALT levels to 97 IU/L and 100 IU/L on days 7 and 21, respectively. These had returned to normal by the subsequent week and were not associated with a concomitant increase in levels of GGT. A 10-year-old boy treated with MAS3 developed increased ALT levels (to 137 IU/L) on day 7 that had decreased to 100 IU/L by day 14, but these was not measured subsequently. A 35-year-old woman treated with MAS3 had a normal GGT level and a high ALT level on admission (104 IU/L) and had an increase in her ALT and GGT levels by day 28 (to 331 and 190 IU/L, respectively). Both patients remained asymptomatic with no clinical evidence of hepatic dysfunction during their 63-day follow-up. There was no evidence of renal dysfunction in any patient tested.

Electrocardiographic findings. Electrocardiograms were recorded in 216 patients during the course of treatment (88 were treated with mefloquine plus artesunate, 56 with mefloquine plus artemether, 53 with artesunate monotherapy, and 19 with artemether monotherapy). Of these patients 113 patients also had ECGs recorded immediately prior to receiving each dose of artesunate (n = 94) or artemether (n = 19) and again 1 hr later. There were no significant changes in the serial measures of the RR, QRS, PR, QT, or QTc intervals between day 28 and day 63. Day 63 values were therefore taken as being representative of the healthy population. Apart from changes in heart rate associated with illness and fever, there were no significant differences in any of the other ECG parameters on days 3 or 7 compared with day 63. One hour after taking an artemisinin derivative (when peak blood concentrations would be anticipated), there was a small but significant decrease in the heart rate (3.5%, 95% CI = 2.9–4.1, $P < 0.001$), a small increase in the PR interval (5.3%, 95% CI = 4.4–6.2, $P < 0.001$), and a very small decrease in the rate corrected QT interval (QTc) (0.7%, 95% CI = 0.3–1.1, $P < 0.001$). Overall, 2% (5 of 216) of the cases had borderline first-degree heart block (PR = 0.21–0.22 sec) on admission that was unchanged by treatment. Neither the small increase in QTc or PR was influ-

enced by the coadministration of mefloquine, the type of artemisinin derivative, or the dose given. The QRS interval did not change significantly during the study. Although the rate-corrected QT interval or QTc is considered to be a measure of the QT interval independent of heart rate, both the QTc and the PR interval were in fact significantly correlated with the RR interval ($r_s = -0.25$ and 0.27, respectively; $P < 0.001$). Therefore, the changes observed after day 2 may be attributable to changes in the heart rate alone.

DISCUSSION

Artemisinin and its derivatives have become the treatment of choice for multidrug-resistant falciparum malaria. Use of these drugs in the treatment of both severe and uncomplicated malaria is increasing in many tropical areas. The artemisinin derivatives appear to be remarkably safe in clinical practice, and have been used to treat several million patients without reported serious adverse effects.[16–18] Worldwide, artesunate is the most widely used of these antimalarials, but in some countries the cheaper but 5–10 times less active parent compound artemisinin is used. *In vivo* artesunate and artemether are both biotransformed rapidly to the active metabolite dihydroartemisinin, which is eliminated with a half-life of approximately 1 hr. Artemisinin is also eliminated rapidly, although it is not biotransformed to dihydroartemisinin. When these drugs are used alone, seven-day treatment regimens are required for optimum cure rates.[19,20] There is now increasing acceptance that they should be deployed in combination with a second antimalarial to protect both compounds against resistance and to ensure high cure rates.[19] This large study is an overview of data that were gathered prospectively during a series of large antimalarial drug trials involving oral artesunate and artemether individually, and in combination with mefloquine. The individual treatment regimens of 10–12 mg/kg total dose of artesunate or artemether used in this series are now standard practice with these compounds in this area. The median total cumulative dose of artemisinin derivative given to each patient was 384 mg with a maximum exposure of 115 mg/kg. Artesunate and artemether were remarkably well tolerated with no evidence of attributable neurotoxicity, cardiotoxicity, or allergic reactions. Since acute malaria is associated with symptoms of lassitude, nausea, vomiting, abdominal pain, dizziness, headache, muscle pain, and sometimes diarrhea, it is often difficult in the acute phase of the disease to distinguish disease effects from drug effects. In this large series, these symptoms and signs all resolved with recovery from malaria, and in those patients who received treatment with the artemisinin derivatives for longer periods, there was no relationship between duration of treatment and duration of symptoms. This suggested that the symptoms resulted from malaria and not its treatment.

In experimental studies in mice, rats, dogs, and monkeys, artemether, arteether, artesunate, and the common metabolite dihydroartemisinin have all produced an unusual selective pattern of damage to certain brain-stem nuclei (Brewer T, unpublished data).[21] Two patterns of damage have been observed: irreversible neuronal loss leading to permanent neurologic deficit or death, and transient neurologic abnormalities with full recovery of function. The brain stem nuclei most affected include those involved in the auditory and vestibular relays. There is some interspecies variability in susceptibility; for example, mice appear to be more resistant than rats, but in all species tested this unusual anatomic pattern of damage is observed. Neurotoxicity in experimental animals has been associated particularly with the use of intramuscular injections of artemether or arteether.[21] These two oil-based compounds, which are released relatively slowly from the intramuscular injection site, are more toxic than the rapidly absorbed water-soluble artesunate.[22] Intramuscular administration of artemether or arteether is also more neurotoxic than oral administration of the same drugs. Neurotoxicity has been observed with parenteral doses close to those used in the treatment of malaria and has given rise to concern that similar effects could occur in humans.[23] The principal objective of this large study was to identify whether there was any evidence of clinically apparent neurotoxicity with the routine use of these oral artemisinin derivatives in the treatment of malaria. None was found. Although five patients had seizures and an five additional patients had neuropsychiatric reactions, these could be attributed to malaria and mefloquine exposure.

There was no difference in reported side effects between artesunate or artemether nor any observed dose-related phenomenon. Artemisinin and its derivatives are known to suppress reticulocyte production. There was no difference in mean hematocrit or the prevalence of anemia in patients who received antimalarial treatment with artemisinin derivative and those who did not. In the early Chinese studies, neutropenia was reported,[17] but no significant changes in leukocyte counts were noted this study. Hemoglobinuria had been reported in previous studies with artemether and artesunate[24,25] and occurred in three patients in this study. All had high parasite counts (104,000–790,000/μl). The relative contributions of the drug, deficiency of glucose-6-phosphate dehydrogenase, and the infection cannot be assessed. Although two children had generalized urticarial reactions, subsequent treatment with artesunate did not cause the reactions to recur, and their relationship to the drug is again uncertain.

This largely negative study in more than 3,500 treated patients is reassuring. If clinically detectable neurologic deficit follows administration of oral artemether or oral artesunate, there is a less than a 5% chance that it occurs with a frequency greater than one per 1,000 treatments, if it occurs at all. These drugs are safe and remarkably well tolerated in the treatment of malaria. Since mid 1994, the three-day artesunate plus mefloquine treatment regimen has been the treatment of choice for uncomplicated falciparum malaria in the study area (with a population of more than 100,000). No other untoward or unusual reactions to artesunate have been documented. Animal studies suggest that the therapeutic ratio for the orally administered compounds is greater than that with intramuscular injection of the oil-based derivatives (arteether and artemether) (Brewer TG, unpublished data and Nontprasert A, Purkittayakamee S, White NJ, unpublished data). Neurotoxicity appears to result from continuous exposure of the central nervous system to a toxic level of drug. The transiently high concentrations following oral administration contrast with the sustained levels that follow depot intramuscular injection. Nevertheless, further studies on this important subject are needed and in particular

histopathologic studies from malaria fatalities who received these drugs will be necessary to exclude subclinical but potentially important neuronal damage. In the mean time, the physician may be reassured that neurotoxicity from the artemisinin derivatives with short course oral treatment is unlikely.

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Authors' addresses: Ric N. Price, Christine Luxemburger, Rose McReady, and François Nosten, Shoklo Malaria Research Unit, PO Box 46, Mae Sod 63110, Tak Province, Thailand, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom. Michele van Vugt, Shoklo Malaria Research Unit, PO Box 46, Mae Sod 63110, Tak Province, Thailand, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and Department of Infectious Diseases and AIDS, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. Lucy Phaipun and Am Khan, Shoklo Malaria Research Unit, PO Box 46, Mae Sod 63110, Tak Province, Thailand. Julie Simpson and Nicholas J. White, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand and Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom. Feiko ter Kuile, Shoklo Malaria Research Unit, PO Box 46, Mae Sod 63110, Tak Province, Thailand and Department of Infectious Diseases and AIDS, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. Tan Chongsuphajaisiddhi, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

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