MAJOR ARTICLE

Dose-Dependent Risk of Neutropenia after 7-Day Courses of Artesunate Monotherapy in Cambodian Patients with Acute *Plasmodium falciparum* Malaria

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Background. Fears of emerging artemisinin resistance in western Cambodia have prompted a series of clinical trials investigating whether slow responses to antimalarial treatment can be overcome by increasing doses of drug.

Methods. Patients with uncomplicated malaria were allocated 1 of 3 oral artesunate monotherapy regimens (2, 4, or 6 mg/kg/day for 7 days) and were observed for 42 days. A series of safety measures, including complete blood count on days 0, 3, 6, and 14, was implemented because of a lack of safety data for these experimental doses.

Results. After 3 doses, geometric mean absolute neutrophil counts were reduced in all groups, and 2 patients required artesunate to be discontinued because of neutropenia (absolute neutrophil count, $<1.0 \times 10^3$ cells/μL). Recipients of the 6 mg/kg/day dosage had significantly lower geometric mean absolute neutrophil counts than did recipients of the 2 and 4 mg/kg/day dosages at 6 and 14 days ($P<.001$ for each). Overall, 5 (19%) of 26 patients who received the 6 mg/kg/day dosage became neutropenic within 14 days, triggering a cohort-halting rule and ending the trial early. Pharmacokinetic data from neutropenic patients showed wide variance, with plasma clearance occurring significantly slower in neutropenic patients than in nonneutropenic patients.

Conclusions. Artesunate remains a crucial drug for the treatment of malaria, and determining optimal dosing regimens is vital to overcome emerging resistant parasite strains along the Thai-Cambodian border. However, future experimental dosing studies must be designed with care, because the safety of such regimens can no longer be assumed. The artemisinin derivatives remain one of the safest classes of antimalarial drugs, but this study demonstrates that the dosing limit may have been reached.

Artemisinin derivatives, such as artesunate (AS), in combination with a longer-acting partner drug, have been adopted as first-line treatment for *Plasmodium falciparum* malaria in almost all malaria-endemic countries [1]. Compared with other commonly used antimalarials, the artemisinin derivatives are considered to have an excellent safety profile, with only infrequent reports of minor adverse events, at the dosages recommended for treatment of a single malaria episode (generally 4 mg/kg/day for 3 days). High doses and/or long courses may be given in certain situations (eg, during treatment of severe malaria or during repeated self-treatment), but there is a lack of high-quality safety data for when the drugs are used in this way. The possibility of significant drug toxicity due to repeated or prolonged exposure has largely been ignored under the assumption that this family of drugs is safe.

Most safety concerns have focused on possible neurological toxicity and have not been substantiated in humans [2, 3]. Other concerns include reduced numbers of reticulocytes and/or red cell mass, rashes, hemoglobinuria, and minor gastrointestinal disturbances. However, most safety analyses have been conducted in the context of malaria infections in which multiple con-

Received 29 April 2010; accepted 16 August 2010; electronically published 11 November 2010.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Army, Department of Defense, or the US Government.

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Clinical Infectious Diseases 2010; 51(12):e105–e114
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1058-4838/2010/5112-00E2$15.00
DOI: 10.1086/657402

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Table 1. Data for Individual Subjects with Neutropenia

Subject	Age, years	Sex	Artesunate dosage	ANC, $\times 10^3$ cells/μL				Clinical course from time of neutropenia
				Baseline	Day 3	Day 6	Day 14	
1	18	Male	6 mg/kg/day	4.7	1.4	2.1	**0.89**	Asymptomatic
2	35	Female	6 mg/kg/day	6.4	**0.95**[a]	1.4	1.4	Suspected coinfection; received antibiotics; recovered well
3	61	Male	6 mg/kg/day	4.4	2.8	3.0	**0.95**	Asymptomatic; ANC was 3.3 $\times$ 10^3 cells/μL on day 28
4	52	Female	6 mg/kg/day	1.7	1.8	2.2	**0.19**	Asymptomatic; ANC was 1.1 $\times$ 10^3 cells/μL on day 16 and 3.8 $\times$ 10^3 cells/μL on day 21
5	19	Male	6 mg/kg/day	2.1	2.0	2.4	**0.74**	Asymptomatic; ANC was 1.9 $\times$ 10^3 cells/μL on day 21
6	19	Female	4 mg/kg/day	4.7	**0.63**[a]	2.6	6.2	Mild cough at time of neutropenia; recovered well

NOTE. Absolute neutrophil count (ANC) values in boldface font indicate the day on which an individual halting rule criterion was met.

[a] Artesunate was discontinued after the third dose. All patients completed 42 days of follow-up.

founding symptoms and signs coexist and in which patients may receive concomitant medications, including partner antimalarial drugs. This has made interpretation of safety data in relation to the artemisinin derivatives very difficult.

Since 2006, studies using experimental 7-day courses of oral AS monotherapy have been conducted along the Thai-Cambodian border to investigate reports of reduced sensitivity of malaria parasites to artemisinin combination therapies (ACTs). Although impractical and inadvisable for routine, unsupervised use, early studies indicate that AS monotherapy regimens yield important scientific information about potentially resistant parasites without the confounding influence of the partner drug and without compromising antimalarial treatment efficacy. Nonetheless, the possibility that resistant or tolerant *P. falciparum* strains may require higher doses of AS to achieve the same pharmacodynamic effect highlights the need to carefully define AS safety profiles in patients with malaria. Here, we report an unexpected finding resulting from the rigorous safety monitoring used during the course of an AS monotherapy trial conducted in western Cambodia.

METHODS

Study Site

Tasanh Health Center is located in Battambang Province in western Cambodia, close to the Thai-Cambodian border and due south of Pailin, an area where growing reports of increasing rates of ACT failure have emerged over recent years [4, 5]. The study was conducted in a purpose-built study ward, staffed by a team of nurses, physicians, and laboratory technicians.

Participants

Consecutive patients were recruited into the study if they fulfilled the following criteria: (1) acute symptomatic *P. falciparum* monoinfection was detected by microscopy, with a parasite density of 1000–200,000 asexual parasites/μL; (2) the patient had a fever or history of fever within the past 48 h; (3) the patient was aged 18–65 years; (4) the patient provided written informed consent to participate; (5) the patient was an otherwise healthy outpatient. Exclusion criteria included the following: (1) pregnancy; (2) mixed malaria infection, as detected by microscopy; (3) history of intolerance or hypersensitivity to AS or to other artemisinin derivatives; (4) history of any malaria drug therapy within the previous 30 days; (5) history of other significant illness; (6) signs or symptoms indicating a requirement for parenteral antimalarial therapy; and (7) signs or symptoms of severe malaria [6].

Procedures

After enrolled patients provided informed consent and underwent screening procedures, they were randomly assigned to 1 of 3 AS treatment regimens: 2 mg/kg/day for 7 days (total dose, 14 mg/kg; the AS2 group), 4 mg/kg/day for 7 days (total dose, 28 mg/kg; the AS4 group), or 6 mg/kg/day for 7 days (total dose, 42 mg/kg; the AS6 group). AS2 was selected as the lower limit of what was considered an "effective dose" to probe for clinical resistance. Recruitment was in a 2:1:2 ratio: the study was powered to compare AS2 and AS6, whereas the AS4 arm was intended primarily to enable comparison with a previous study at the same site. AS doses (using 50-mg tablets; Guilin Pharmaceutical; Guilin, China; WHO pre-qualified) were calculated on the basis of the patient's weight at baseline and rounded up to the nearest quarter-tablet (12.5 mg). Doses were administered with water, and wherever possible, all administration of AS doses was observed directly by study staff. If vomiting occurred within 30 minutes after receipt of the dose, the full dose was repeated; a half-dose was repeated if vomiting occurred within 30–60 minutes.

Blood samples were collected to determine the complete blood count (CBC) and alanine aminotransferase (ALT) level at baseline, before the fourth and seventh AS doses (on days 3 and 6, respectively) and on day 14. Plasma AS and dihydroartemisinin (DHA) concentrations were measured on the first (at 0, 15, 30, and 60 minutes and at 2, 4, 6, and 8 h) and final (at 0, 2, 4, and 6 h) days of treatment. Daily evaluations, including a neurological examination, were performed for all patients before each dose of AS. Patients remained in the study

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Table 2. Laboratory Safety Data for Subjects Treated with 7 Days of Artesunate Monotherapy

Parameter, study day	Geometric mean (95% CI), by artesunate dosage			P[a]
	2 mg/kg/day (n = 40)	4 mg/kg/day (n = 19)	6 mg/kg/day (n = 27)	
WBC count, × 10^3 cells/μL				
0	6.0 (5.3–6.8)	6.9 (6.1–7.8)	5.4 (4.8–6.2)	.13
3	5.6 (5.1–6.1)	5.2 (4.5–6.0)	4.9 (4.4–5.4)	.12
6	6.4 (5.9–7.0)	6.5 (5.8–7.3)	5.2 (4.7–5.8)	.005
14	7.4 (6.7–8.2)	7.6 (6.7–8.6)	5.4 (4.5–6.4)	.004
ANC, ×10^3 cells/μL				
0	3.9 (3.3–4.6)	4.6 (4.0–5.3)	3.5 (2.8–4.4)	.37
3	2.2 (2.0–2.5)	2.1 (1.7–2.5)	2.0 (1.7–2.3)	.51
6	2.7 (2.5–3.0)	2.9 (2.6–3.2)	2.3 (2.0–2.6)	.03
14	3.6 (3.2–4.0)	3.8 (3.3–4.3)	1.9 (1.4–2.6)	<.001
ALC, ×10^3 cells/μL				
0	1.3 (1.1–1.6)	1.5 (1.2–1.9)	1.1 (1.0–1.4)	.15
3	2.3 (2.1–2.6)	2.0 (1.8–2.3)	2.0 (1.8–2.2)	.046
6	2.5 (2.3–2.8)	2.4 (2.1–2.8)	2.0 (1.8–2.3)	.009
14	2.7 (2.4–3.0)	2.8 (2.5–3.1)	2.7 (2.4–3.1)	.93
Hemoglobin concentration,[b] g/dL				
0	12.7 (12.3–13.1)	12.9 (11.9–13.8)	12.2 (11.4–13.1)	.67
3	11.6 (11.1–12.1)	12.0 (11.2–12.7)	11.5 (10.9–12.1)	.41
6	12.0 (11.5–12.5)	12.2 (11.2–13.1)	11.5 (10.7–12.2)	.26
14	11.9 (11.5–12.4)	12.0 (11.5–12.6)	11.1 (10.6–11.7)	.06
Hematocrit,[b] %				
0	38.7 (37.4–40.0)	39.1 (36.2–41.9)	36.2 (33.9–38.5)	.09
3	34.9 (33.3–36.4)	35.8 (33.5–38.0)	34.0 (32.3–35.8)	.22
6	36.1 (34.5–37.8)	36.3 (33.5–39.2)	33.8 (31.7–35.9)	.10
14	35.8 (34.3–37.2)	36.0 (34.4–37.7)	33.3 (31.7–34.9)	.02
Platelet count, platelets/μL				
0	91,813 (74,066–113,812)	115,855 (83,912–159,956)	104,016 (83,244–129,970)	.31
3	131,003 (112,098–153,096)	159,814 (133,340–191,544)	138,517 (119,908–160,013)	.33
6	220,769 (193,153–252,334)	254,949 (229,863–282,773)	253,950 (223,308–288,796)	.41
14	247,686 (218,852–280,318)	274,954 (245,692–307,701)	321,915 (282,185–367,240)	.03
ALT level, U/L				
0	21.5 (17.0–27.2)	17.3 (13.6–22.1)	21.6 (16.3–28.5)	.71
3	21.1 (16.9–26.5)	15.4 (12.0–19.7)	20.1 (15.4–26.3)	.21
6	25.9 (21.5–31.1)	21.8 (15.5–30.8)	33.9 (25.8–44.5)	.06
14	17.6 (14.7–20.9)	17.8 (14.0–20.5)	20.3 (15.8–26.2)	.40

NOTE. ALC, absolute lymphocyte count; ALT, alanine aminotransferase; ANC, absolute neutrophil count; WBC, white blood count.

[a] Determined using analysis of variance of log-transformed values.

[b] Expressed as mean (95% confidence interval) because data were normally distributed.

ward as inpatients throughout the dosing period, then returned for weekly follow-up until day 42. Adverse event reporting, in accordance with standard Common Terminology Criteria for Adverse Events (CTCAE) classification [7], began from the time informed consent was given until the final follow-up visit.

Because of the previously untested oral dose of 42 mg/kg (AS6), the following safety measures were built into the protocol both to elicit symptoms or signs of possible AS toxicity and to prevent further subjects being exposed to this dose in the event clinically significant toxicity was demonstrated.

Safety monitoring committee (SMC). An SMC, independent of the protocol team, was established.

Treatment-emergent adverse events. These were defined as adverse events that arose during the course of AS treatment that were absent before treatment or worsened during treatment. Before administration of each dose of AS, the patient was assessed by a study physician for treatment-emergent adverse events occurring any time since the previous dose. Particular emphasis was placed on eliciting symptoms and/or signs representing possible AS toxicity [8, 9].

Individual halting rules. These were developed based on preclinical toxicity data (R. Scott Miller; unpublished observations), and were considered by the protocol team to represent potential AS toxicity. If a halting rule was met, the case was reported to the SMC for determination of causality and to the institutional review board before AS treatment could recom-

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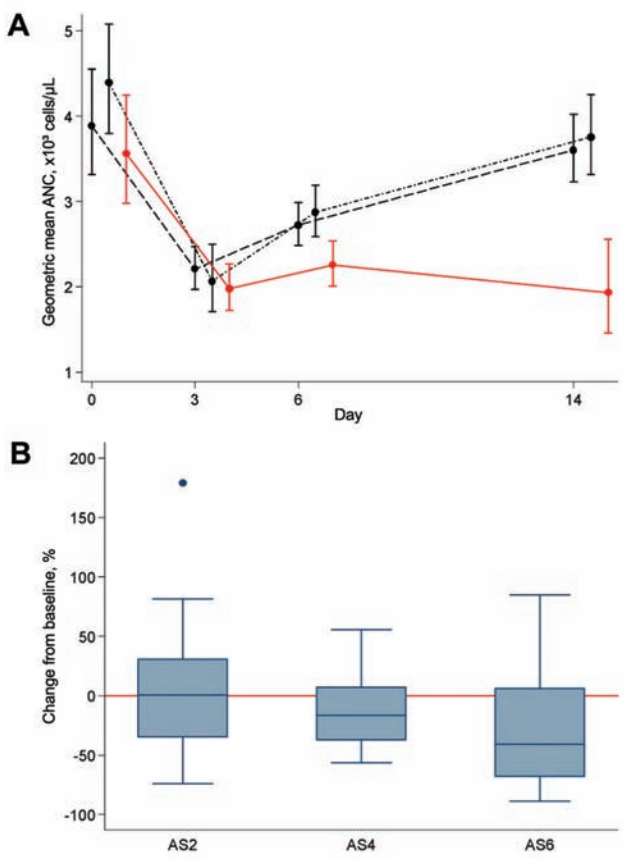

Figure 1. *A*, Geometric mean (95% confidence interval) absolute neutrophil count (ANC) in 87 consecutive patients with malaria receiving 1 of 3 seven-day artesunate (AS) monotherapy regimens: 2 mg/kg/day (the AS2 group; *dashed line*), 4 mg/kg/day (the AS4 group; *dotted line*), or 6 mg/kg/day (the AS6 group; *red line*). Differences between regimens are significant on day 6 ($P = .03$) and day 14 ($P < .001$. *B*, Box plot showing the median change (interquartile range and upper and lower adjacent values) in ANC from baseline to day 14 ($P = .003$ for differences between groups).

mence. The criteria were as follows: hemoglobin concentration, <7 mg/dL; decrease in hemoglobin concentration >3.6 mg/dL from baseline; absolute neutrophil count (ANC), $<1.0 \times 10^3$ cells/μL; obtundation; new or worsening ataxia; >1 seizure; visibly bloody stools not due to another etiology; and visible hematuria or hemoglobinuria not due to another etiology.

Cohort halting rules. If any of the following safety criteria were met, the treatment group was suspended pending review by the SMC and institutional review board: >4 individual halts in the same arm for the same adverse event; >1 serious adverse event in any enrollment arm; and termination or suspension at any time at the discretion of the primary investigator or SMC.

Enrollment pause in AS6 arm for SMC safety review. This occurred after enrollment of the first 5 subjects.

Laboratory Methods

CBCs were analyzed using a Beckman Coulter AcT5diff (Beckman Coulter). The ALT level was measured in plasma samples a Reflotron Plus analyzer (Roche Diagnostics). AS and DHA concentrations were analyzed by liquid chomatopgraphy–mass spectrometry, as follows: plasma samples and standards (100 μL) were treated with 2 volumes of ice-cold acetonitrile for protein precipitation; 5 μL of the supernatant was injected directly into the LC-MS system. A reversed-phase column (3.5 μm; 2.1×50 mm; XTerra MS C18; Waters Corporation) and precolumn (2.1×10 mm) were connected to an Alliance 2695 Liquid Chromatography system equipped with a single quadruple Micromass ZQ (MM1) Mass Spectrometry detector (Waters Corporation) in the positive electrospray ionization mode and single ion recording. Analytes were eluted from the column with 6.25 mM ammonium acetate buffer (pH, 4.5) and acetonitrile gradient from 20% to 40% in 9 minutes at flow rate 0.4 mL/min. Ammonium-adducts of DHA and AS were detected at the m/z ratios of 302 and 402, respectively, and eluted near 5 and 6 min, respectively, with a total analysis time of 12 min. The assay was linear over the quantification range between 10 and 3000 nM for AS and DHA. Intra-assay and interassay accuracy and precision were within $\pm 11\%$ error; coefficient of variation values were <15%.

Pharmacokinetic Analysis

The maximum concentration was estimated by inspection of data. The area under the curve (AUC), plasma half-life, mean residence time, volume of distribution, and clearance rate were calculated using noncompartmental analysis with PK Solutions Software (Summit Research Services). To correct for the limited sampling on day 6 and to allow for comparison of AUC values between day 0 and day 6, a "limited AUC" using data at 0, 2, 4, and 6 h was calculated [10].

Table 3. Common Terminology Criteria for Adverse Events (CTCAE) Grade for Lowest Measured Absolute Neutrophil Count

		No. (%) of patients, by artesunate dosage		
CTCAE grade	ANC level, $\times 10^3$ cells/μL	2 mg/kg/day (n = 40)	4 mg/kg/day (n = 18)	6 mg/kg/day (n = 26)
0	⩾2.0	20 (50)	9 (50)	9 (35)
1	1.5 to <2.0	14 (35)	7 (38)	4 (15)
2	1.0 to <1.5	6 (15)	1 (6)	8 (31)
3	0.5 to <1.0	0 (0)	1 (6)	4 (15)
4	<0.5	0 (0)	0 (0)	1 (4)
5	…[a]	0 (0)	0 (0)	0 (0)

NOTE. The table presents the maximum CTCAE grade reached for absolute neutrophil counts (ANCs) measured on days 3, 6, and 14, by dosing group [7].

[a] The criterion for CTCAE grade 5 was death.

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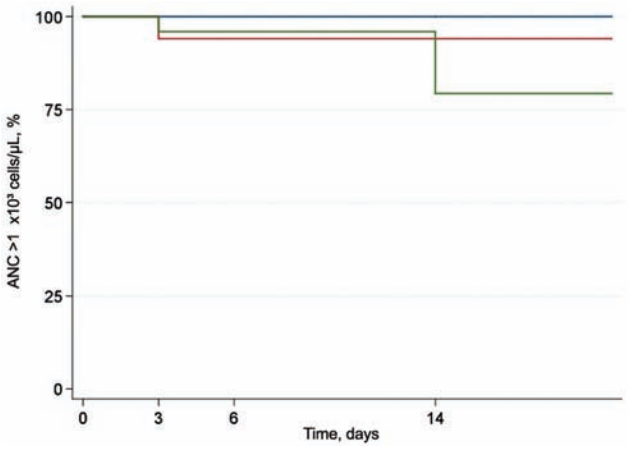

Figure 2. Survival curve showing development of neutropenia in 86 patients with uncomplicated malaria treated with 1 of 3 artesunate (AS) monotherapy regimens: 2 mg/kg/day (the AS2 group; *blue line*), 4 mg/kg/day (the AS4 group; *red line*), or 6 mg/kg/day (the AS6 group; *green line*). $P = .015$ for equality of survivor functions to day 14, by Wilcoxon (Breslow) test. One failure occurred in the AS4 group and 5 occurred in the AS6 group.

Statistical Analysis

Data were analyzed using Stata software, version 10.0 (Stata Corp). Proportions were compared between groups using the χ^2 test. Normally distributed data were expressed as means (with 95% confidence intervals), and nonnormally distributed data were expressed as geometric means (with 95% confidence intervals) or medians (interquartile ranges with 95% confidence intervals), as appropriate. To adjust for differences at baseline the percentage change was calculated (ie, [day 14 − day 0]/day 0) [11]. Means were compared using analysis of variance; otherwise, the nonparametric Kruskall-Wallis test was used. Time to an ANC of $<1.0 \times 10^3$ cells/μL was assessed using Kaplan-Meier methods, and differences between groups were compared using the Wilcoxon-Breslow-Gehan test of equality.

RESULTS

In April 2009, 8 months after the first patient was recruited, enrollment into the high-dose arm of the study was halted because a cohort halting rule had been met; a fifth patient had become neutropenic (Table 1). Neutropenia was detected on day 14 in 4 of these patients; none had fever or other symptoms or signs associated with infection, and all subsequently remained healthy without intervention until day 42. The fifth patient was neutropenic after 3 AS doses when she also developed fever, nausea, and vomiting; she was treated empirically for possible bacterial/rickettsial coinfection and AS was discontinued. Her ANC increased to $>1.0 \times 10^3$ cells/μL on day 6 but remained at this level for an additional 6 weeks before rebounding vigorously. A sixth patient, in the AS4 group, was also neutropenic at day 3; although this patient was otherwise healthy, AS was discontinued and quinine-tetracycline was administered. The subject made an uneventful recovery.

As a result of the cohort halt, analysis of safety data from all enrolled subjects was performed. In total 95 patients had been screened, of whom 87 were randomized to one of the treatment groups (AS2, 40 patients; AS4, 19 patients; and AS6, 28 patients). One patient in AS6 was withdrawn on day 1 because of clinical deterioration requiring transfer to another hospital; 2 others (one each in the AS4 and AS6 groups) withdrew consent on day 3. Overall 40, 17, and 25 patients completed 7 days of monotherapy in the AS2, AS4, and AS6, respectively. The pause in recruitment in the AS6 arm for safe-

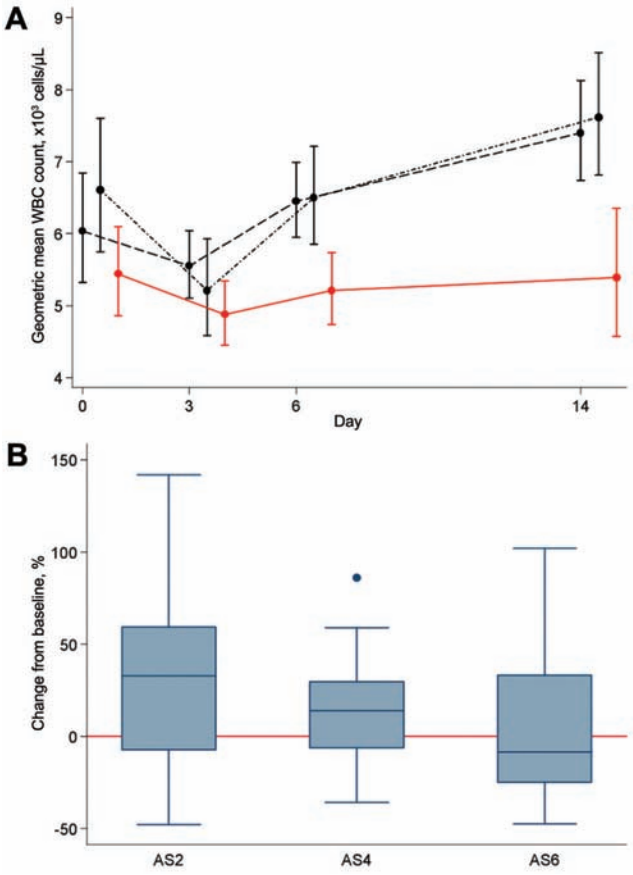

Figure 3. *A*, Geometric mean (95% confidence interval) white blood cell (WBC) count in 87 patients with malaria who completed 1 of 3 seven-day artesunate (AS) monotherapy regimens: 2 mg/kg/day (the AS2 group; *dashed line*), 4 mg/kg/day (the AS4 group; *dotted line*), or 6 mg/kg/day (the AS6 group; *red line*). Differences between regimens are statistically significant on day 6 ($P = .005$) and on day 14 ($P = .004$). *B*, Box plot showing the median change (interquartile range and upper and lower adjacent values) in WBC count from baseline to day 14 ($P = .04$ for differences between groups).

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Table 4. Concomitant Medications: Number of Patients Taking ⩾1 Dose of Medicines Other Than Artesunate before and during the First 3 Weeks of the Study

	Patients with neutropenia (n = 6)				Patients without neutropenia (n = 80)			
Drug class, drug	Before the study	Week 1	Week 2	Week 3	Before the study	Week 1	Week 2	Week 3
Antipyretics								
Acetominophen	6	6	0	0	65	65	7	3
Ibuprofen	0	0	0	0	0	2	0	0
Anti-infectives								
Ampicillin, amoxicillin, or penicillin	0	0	0	0	6	0	3	4
Ceftriaxone	0	1	0	0	0	1	0	0
Tetracycline[a]	0	1	0	0	0	0	0	0
Chloramphenicol[b]	0	1	0	0	2	0	0	0
Ciprofloxacin	0	1	0	0	0	0	0	0
Albendazole or mebendazole	0	1	1	1	0	2	3	3
Quinine[a]	0	1	0	0	0	0	0	0
Doxycycline	0	1	0	0	0	0	0	0
Mefloquine	0	0	0	0	0	1	0	0
Chloroquine	0	0	0	0	0	0	0	3
Clotrimazole	0	0	0	0	0	0	1	0
Antiemetics								
Dimenhydrinate	0	4	0	0	0	31	0	0
Domperidone	0	1	1	0	0	0	0	0
Metoclopramide	0	1	0	0	0	0	0	0
Gastrointestinal agents								
Ranitidine or cimetidine	0	1	1	1	0	7	4	2
Aluminium hydroxide	0	1	0	0	0	11	7	2
Calcium carbonate	0	1	1	1	0	0	0	0
Simeticone	0	0	0	0	0	3	0	0
Supplementary agents								
Ferrous sulphate	0	1	1	3	0	5	5	11
Calcium sandoz or gluconate	0	1	0	0	0	2	0	0
Multivitamins	0	5	3	3	0	8	8	14
Folic acid	0	0	0	0	0	3	2	4
Other								
Chlorpheniramine	0	1	1	3	0	8	4	2
Dextromethorphan	0	0	0	0	0	2	0	0

NOTE. Artesunate was administered daily during week 1.

[a] Quinine-tetracycline used in place of artesunate to treat 1 patient who became neutropenic on day 3.

[b] Intravenous chloramphenicol was used for 24 h to treat suspected rickettsial infection after neutropenia was detected, and doxycycline was substituted once vomiting had settled.

ty analysis by the SMC resulted in fewer patients in this group than the AS2 group.

CBC

ANC. At day 3, geometric mean ANC values were reduced from baseline in all 3 arms (Table 2 and Figure 1*A*). By day 6, geometric mean ANC values were recovering in the AS2 and AS4 groups, and this continued to day 14 with no difference between these 2 groups ($P = .50$ and $P = .66$ for days 6 and 14, respectively). However geometric mean ANCs in the AS6 group remained significantly lower than in the AS2 or AS4 groups on both day 6 ($P = .02$ and $P = .008$, respectively) and day 14 (both $P<.001$). The CTCAE grades of the lowest recorded ANC for individual patients are shown in Table 3. On day 14, the median ANC value was still reduced by 41% (interquartile range, −68 to +6%) from the baseline level in the AS6 group and by 17% (interquartile range, −37 to +7%) in the AS4 group; however, in the AS2 group, it had returned to the baseline level ($P = .003$) (Figure 1*B*). Survival analysis confirmed a statistically significant difference in development of neutropenia over 14 days between treatment groups ($P = .015$) (Figure 2). Logistic regression modeling did not identify baseline parameters (such as white blood cell count, age, sex, or parasitemia) that predicted neutropenia at day 14. Omission

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Table 5. Geometric Mean Plasma Pharmacokinetic Values in Patients Receiving Artesunate (AS) at 2, 4, or 6 mg/kg/day for 7 Days (AS2, AS4, and AS6, Respectively) Who Did Not Develop Neutropenia and in 6 Patients Who Subsequently Became Neutropenic

Pharmacokinetic parameter	Mean (95% CI)			Neutropenic patients (n = 6)[a]	P[b]
	AS2 group (n = 21)	AS4 group (n = 16)	AS6 group (n = 22)		
Day 0					
AS					
C_{max}, μg/L	111 (68–180)	282 (179–443)	321 (225–457)	631 (244–1635)	<.001
AUC_{0-8}, μg.h/L	123 (97–157)	284 (211–381)	391 (319–480)	623 (388–999)	<.001
Vd, L	10.3 (7.2–14.7)	10.3 (6.2–17.0)	9.7 (6.5–14.5)	4.0 (1.5–10.3)	.11
Mrt, h	0.99 (0.73–1.35)	0.95 (0.74–1.23)	1.21 (0.87–1.68)	0.95 (0.59–1.53)	.65
Cl, L/h/kg	15.4 (12.1–19.7)	13.7 (10.4–18.1)	14.8 (12.1–18.3)	8.3 (5.8–11.9)	.06
$t_{1/2}$, h	0.46 (0.34–0.65)	0.52 (0.37–0.73)	0.46 (0.34–0.63)	0.32 (0.16–0.66)	.57
DHA					
C_{max}, μg/L	587 (442–779)	1506 (1148–1976)	2198 (1751–2759)	2651 (1640–4287)	<.001
AUC_{0-8}, μg.h/L	1328 (1090–1618)	3332 (2716–4090)	5824 (4873–6962)	7381 (5271–10335)	<.001
AUC_{0-6L}, μg.h/L	904 (788–1038)	2460 (1936–3126)	4036 (3174–5131)	5903 (4925–7075)	<.001
Vd, L	1.68 (1.32–2.15)	1.64 (1.14–2.37)	1.32 (0.96–1.81)	1.25 (0.74–2.11)	.40
Mrt, h	1.99 (1.70–2.32)	2.00 (1.75–2.28)	2.44 (2.06–2.89)	2.53 (2.08–3.07)	.09
Cl, L/h/kg	1.50 (1.23–1.82)	1.19 (0.97–1.45)	1.00 (0.84–1.20)	0.74 (0.58–0.95)	<.001
$t_{1/2}$, h	0.78 (0.61–0.98)	0.97 (0.75–1.24)	0.93 (0.69–1.25)	1.18 (0.82–1.71)	.21
Day 6					
DHA					
C_{max}, μg/L	200 (160–250)	441 (346–561)	825 (684–995)	1189 (761–1858)	<.001
AUC_{0-6L}, μg.h/L	498 (399–620)	1102 (884–1373)	2098 (1777–2477)	2967 (1594–5522)	<.001
Vd, L	4.35 (3.38–5.60)	4.12 (3.30–5.16)	3.33 (2.89–3.84)	2.43 (1.34–4.41)	.03
Mrt, h	2.61 (2.41–2.83)	2.54 (2.37–2.72)	2.66 (2.44–2.90)	2.54 (1.86–3.46)	.87
Cl, L/h/kg	3.93 (3.15–4.91)	3.54 (2.83–4.44)	2.76 (2.32–3.27)	1.96 (1.00–3.84)	.007
$t_{1/2}$, h	0.77 (0.64–1.06)	0.81 (0.67–0.98)	0.84 (0.72–0.99)	0.86 (0.44–1.34)	.77

NOTE. AUC_{0-8}, area under the plasma concentration-time curve using all time points; AUC_{0-6L}, area under the plasma concentration-time curve using limited sampling at 0, 2, 4, and 6 h (to allow comparison between day 0 and day 6 values); CI, confidence interval; Cl, clearance from plasma; C_{max}, maximum plasma concentration; MRT, mean residual time; $t_{1/2}$, plasma half-life; Vd, volume of distribution.

[a] Determined using analysis of variance of log-transformed or 1/square root–transformed values.

[b] Two of 6 neutropenic patients did not have pharmacokinetic parameters measured on day 6 because AS treatment had been discontinued. One neutropenic patient was receiving the 4 mg/kg/day dosease, and the rest were receiving the 6 mg/kg/day dosage.

from analysis of the 2 patients who were neutropenic on day 3 did not alter the findings.

White blood cell count. As with the ANC value, the geometric mean white blood cell count was depressed in all groups on day 3 (Figure 3*A*). Geometric mean white blood cell counts were significantly different between treatment groups on day 6 ($P = .005$) and on day 14 ($P = .004$). Recovery of median white blood cell count to levels greater than at baseline occurred by day 14 in the AS2 and AS4 groups but was still reduced by −8% (interquartile range, −25 to +33) in the AS6 group ($P = .04$) (Figure 3*B*).

Other CBC parameters. Mean hemoglobin values decreased by ~1.0 mg/dL over 14 days, regardless of AS dose (Table 2). One patient in the AS2 group fulfilled a halting rule criterion on day 14 for a decrease in the hemoglobin concentration >3.6 mg/dL from baseline, but the decrease was attributed to hemoconcentration secondary to fever and poor fluid intake before enrollment. Recovery of baseline platelet counts was not influenced by AS dose.

ALT Level

No subjects recorded an ALT value outside of the normal range at any of the time points (Table 2). However, there was wide variance in ALT values on days 6 in the AS6 group, and geometric mean values were higher compared with the other 2 groups ($P = .06$); this effect had disappeared by day 14.

Other Adverse Events

There were no differences in adverse event reports among the 3 arms. Assessment of treatment-emergent adverse events showed no emergent neurological symptoms or signs that could be attributed to AS therapy. There was no relationship between development of neutropenia and use of concomitant medications (Table 4).

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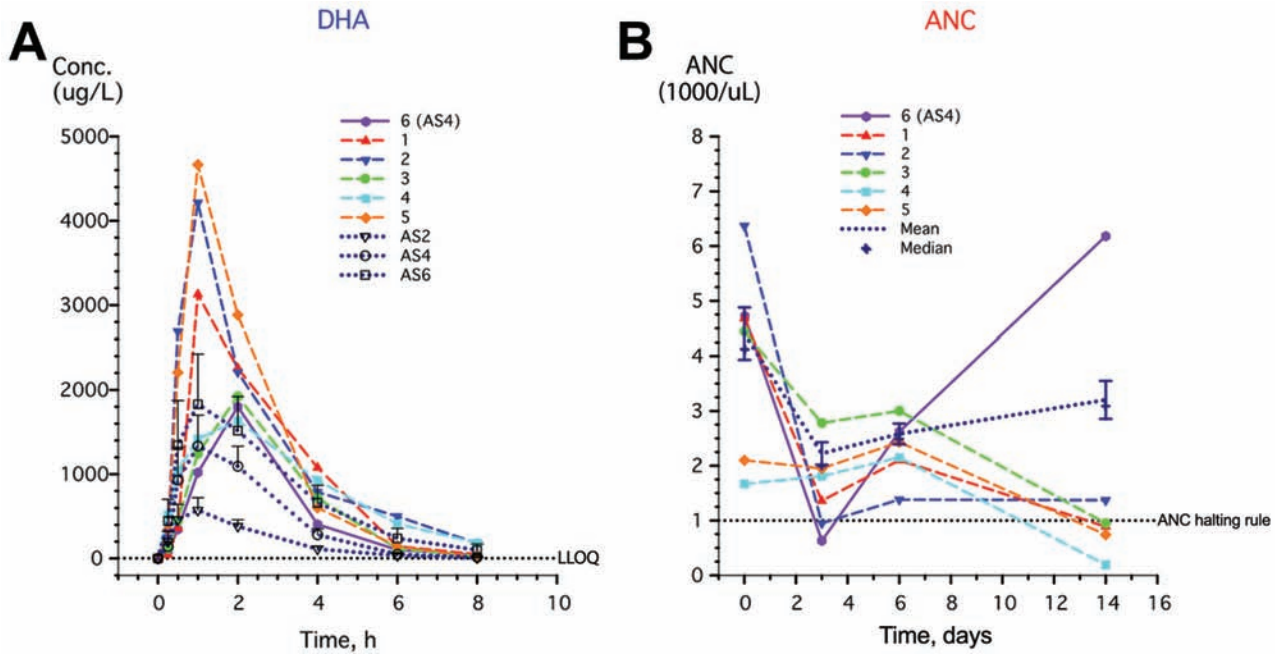

Figure 4. *A,* Mean and individual plasma concentration–time graphs for DHA on the first day of dosing for patients receiving 1 of 3 seven-day artesunate (AS) monotherapy regimens (2 mg/kg/day [the AS2 group], 4 mg/kg/day [the AS4 group], or 6 mg/kg/day [the AS6 group]) and in individual patients who subsequently developed neutropenia. *B,* Individual absolute neutrophil counts (ANCs) for neutropenic patients during the first 14 days of the study.

Pharmacokinetics

As expected, there were dose-dependent differences in calculated pharmacokinetic parameters between groups (Table 5). AS was almost undetectable on day 6, probably because sampling was more limited. Compared with nonneutropenic subjects, neutropenic patients had greater drug exposure, although there was considerable variance in individual values; these differences persisted to day 6 (Figures 4 and 5).

DISCUSSION

This is the first published study to demonstrate dose-dependent toxicity of AS in patients with malaria. Geometric mean ANC values were significantly lower at 6 and 14 days among patients who received high-dose treatment than in the other 2 groups, and 19% patients in this arm developed neutropenia—the majority after AS treatment had finished. Adjusting for differences between groups at baseline did not alter the differences seen at days 6 or 14. Although a dose-limiting toxicity occurred in the AS6 group, it remains unclear whether this was purely a concentration-dependent effect or whether there was a time-dependent component as well.

Despite the suppression of ANC values, none of the subjects who were neutropenic on day 14 had symptoms or signs of infection. All remained well, and follow-up ANC values rebounded very quickly. In the patient with the lowest recorded ANC (0.19×10^3 cells/μL), the value recorded 48 h later was 1.1×10^3 cells/μL, suggesting the nadir ANC following 7 days of AS administration occurs 7–14 days after commencement of treatment, with a rapid recovery thereafter. This is supported by preclinical safety data from healthy adult rhesus monkeys receiving 7-day regimens of intravenous AS, although higher doses than those used here were required before an effect on neutrophils was seen (R. Scott Miller, personal communication).

The pharmacokinetic profiles confirm that exposure to both AS and DHA were dose dependent. Lower plasma concentrations were seen on day 6, compared with day 0, in all patients, at least partly attributable to autoinduction of liver enzymes, which has been well described previously [12, 13], as well as to a disease effect. Thus, the dose-dependent differences in observed geometric mean ANC values can be explained by higher geometric mean plasma concentrations of AS and/or DHA. Moreover, the trend for individuals subsequently developing neutropenia to have higher drug exposure raises the possibility that there may be a sub-group of individuals at increased risk of the myelosuppressive effects of AS. In our study population, there are no data on genetic polymorphisms of enzymes involved in the metabolic pathways of AS, such as esterases, UGT or CYP3A4, which might explain the pharmacokinetic differences observed.

Malaria infections themselves produce a variety of effects on the blood and bone marrow. Despite many potential confounding factors in patients with malaria, it is generally accepted that

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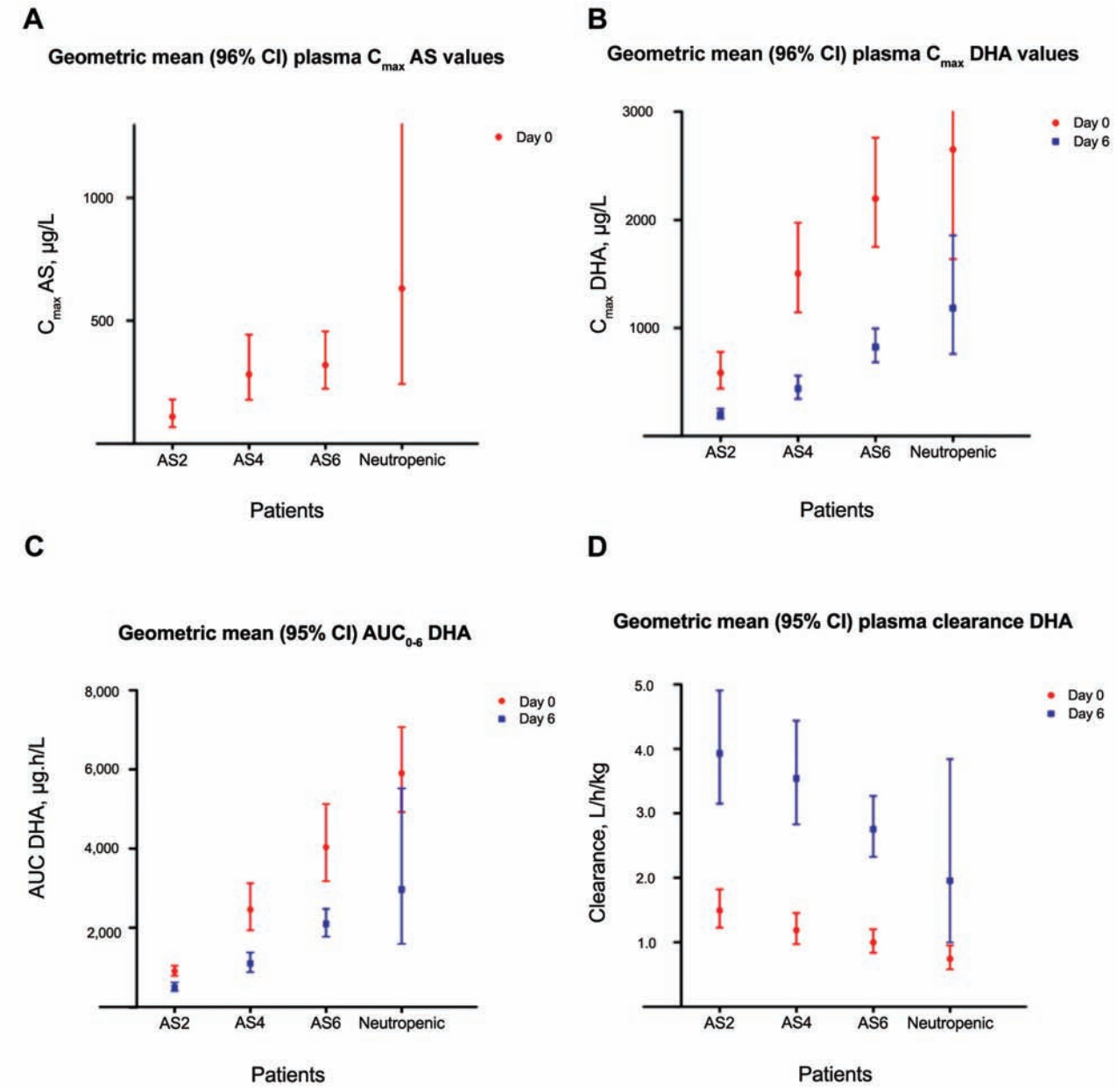

Figure 5. Pharmacokinetic data in nonneutropenic and neutropenic patients. Geometric mean maximum concentration (C_{max}) of artesunate (AS; *A*) and DHA (*B*), area under the curve values for 0–6 h (AUC_{0-6}) DHA (*C*), and plasma clearance DHA (*D*) graphs are for patients who received AS at 2, 4, or 6 mg/kg/day who did not develop neutropenia (AS2, AS4, and AS6, respectively) and for patients who subsequently became neutropenic. AS was discontinued on day 3 in 2 of the 6 neutropenic patients, so these patients have no pharmacokinetic data for day 6. AUC_{0-6} values on both days 0 and 6 and are calculated using the same time points of 0, 2, 4, and 6 h. Patients who subsequently developed neutropenia had significantly lower plasma clearance of DHA than other patients in the 3 dosing groups ($P = .008$).

the peripheral ANC may be increased during the first 2 days of a malaria infection, with subsequent reduction thereafter [14, 15]. Studies of experimental human malaria infections demonstrated that white blood cell counts generally reached a nadir 2 days after initiation of antimalarial treatment and then increased [16]; this mirrors the finding of reduced white blood cell counts in all groups on day 3 of our study.

Although the results for the AS2 and AS4 groups confirm the safety of AS given at standard daily doses and for standard (3-day) duration, there are a number of clinical scenarios in which larger doses of AS could be administered: patients with severe malaria receiving parenteral AS, with or without ACT follow-up treatment, will have prolonged duration of treatment; overestimation of a patient's weight, or failure to weigh at all,

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could lead to excessive drug administration; finally, repeated self-administration, particularly where non coformulated ACTs are available, could suppress the peripheral neutrophil count in some patients with malaria.

These concerns become more relevant when potential interactions between the drug, the disease, and other myelosuppressive factors occur. Gasasira et al [17] demonstrated significant neutropenia (ANC, $<1.0 \times 10^3$ cells/μL) in human immunodeficiency virus (HIV)–infected children with malaria taking AS-amodiaquine compared with non-HIV infected children treated with the same regimen (45% vs 6%). In this study, the observed neutropenia was attributed to the combination of AS-amodiaquine and concomitant antiretroviral drugs (risk of neutropenia at day 14 was 75% if antiretrovirals were taken), but a contributory role for AS was not considered. Even though the total dose of AS used (12 mg/kg over 3 days) was lower than in any of the regimens used in our study, there will be an additive effect on myelosuppression if several medications with similar potential toxicity are used concurrently. This is particularly likely in HIV-infected patients with malaria. HIV testing was not performed in our study, but prevalence of HIV infection in this rural population is believed to be low, and none of our patients were taking antiretroviral drugs.

Given the global emphasis on increasing patient access to ACTs, it is hard to ignore this finding of a dose-related effect of AS on delayed recovery of neutrophil counts in patients with uncomplicated malaria and without other confounding factors. However, although the potential for hematologic adverse events exists, the lack of clinical manifestations in our population together with the lack of alternative antimalarial drugs both argue for defining the effects on the neutrophil more carefully, particularly in vulnerable populations and severe disease. Future experimental artemisinin regimens should be designed with careful attention to safety monitoring. Despite these concerns, the artemisinin derivatives retain a highly favorable risk-benefit ratio and remain the safest and best tolerated class of antimalarial drugs, but this study demonstrates that the dosing limit may have been reached.

Acknowledgments

We thank the patients and staff at Tasanh Health Center. We are grateful to Dr Stephen Thomas, SMC Chair, and colleagues, as well as Dr Pascal Ringwald of the World Health Organization, for their advice and support during the course of the study.

Financial support. This study was funded by a Bill and Melinda Gates Foundation Program Grant through the World Health Organization, and the US Department of Defense Global Emerging Infections System (GEIS) Program. The Sponsor was the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. The main study is registered at ClinicalTrials.gov: study number NCT00722150.

Potential conflicts of interest. All authors: no conflicts.

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