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COMPARATIVE PHARMACOKINETICS AND EFFECT KINETICS OF ORALLY ADMINISTERED ARTESUNATE IN HEALTHY VOLUNTEERS AND PATIENTS WITH UNCOMPLICATED FALCIPARUM MALARIA

PAKTIYA TEJA-ISAVADHARM, GEORGE WATT, CHIRAPA EAMSILA, KRISADA JONGSAKUL, QIGUI LI, DUANGSUDA KEERATITHAKUL, NARONGRID SIRISOPANA, LERSAN LUESUTTHIVIBOON, THOMAS G. BREWER, AND DENNIS E. KYLE

Department of Immunology and Medicine, U.S. Army Component, and Department of Retrovirology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Royal Thai Army Component, Armed Forces Research Institute of Medical Sciences, Bangkok Thailand; Department of Pharmacology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C.; Aranyaprathet Army Hospital, Aranyaprathet, Thailand

Abstract. The single-dose pharmacokinetics of 100 mg of orally administered artesunate (AS) were studied in 6 patient volunteers with uncomplicated falciparum malaria and in 6 healthy volunteers. Plasma concentrations of both the parent drug, AS, and its major metabolite, dihydroartemisinin (DHA), were measured simultaneously by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). The antimalarial activity of each plasma sample measured by an *in vitro* bioassay (BA) was used to derive activity concentrations. Artesunate was absorbed rapidly and then almost completely hydrolyzed to DHA in patients, whereas hydrolysis was incomplete in healthy volunteers. The mean ± standard deviation (SD) maximum concentration (C_{max}) of AS was 296 ± 110 nmol/L, the time to peak blood level (t_{max}) was 0.71 ± 0.66 hr, the half-life ($t_{1/2,z}$) was 0.41 ± 0.34 hr, and the bioavailability over 12 hr (area under the curve $[AUC]_{(0–12)}$) was 253 ± 185 nmol hr/L. Measured by HPLC, the C_{max} and $AUC_{(0–12)}$ values of DHA in patients with malaria were significantly greater than in volunteers (1,948 ± 772 and 1,192 ± 315 nmol/L; 4,024 ± 1,585 and 1,763 ± 607 nmol hr/L, respectively; $P \leq 0.05$). These differences were even greater when measured by BA. The C_{max} for patients with malaria was 2,894 ± 2,497 and 795 ± 455 nmol/L for volunteers, and $AUC_{(0–12)}$ was 5,970 ± 3,625 and 1,307 ± 391 nmol hr/L, respectively ($P \leq 0.05$). In contrast, DHA parameter estimates for $t_{1/2,z}$ and t_{max} were similar between patients and healthy volunteers, with values of 0.80 ± 0.30 versus 0.87 ± 0.06 hr and 1.50 ± 0.55 versus 1.13 ± 0.52 hr, respectively ($P > 0.5$). Both drug metabolism and tissue protein binding could contribute to the differences between the antimalarial activity of artemisinin drugs in healthy volunteers and malaria infected patients.

INTRODUCTION

Malaria remains an important cause of morbidity and mortality in the tropical world. An estimated 300–500 million persons are infected annually, and 1.5 to 2.7 million die.[1] Effective treatment has been compromised by increasingly prevalent multidrug-resistant malaria. Artemisinin and its derivatives are rapidly acting antimalarial compounds[2] effective against many resistant strains of falciparum malaria. However, dosage regimens for artemisinin derivatives remain largely empirical. The artemesinin compounds are difficult to measure in body fluids, and there is therefore a paucity of pharmacokinetic data. This is especially true for the water-soluble and most widely used semisynthetic derivative, artesunate (AS). Once absorbed, AS is hydrolyzed to dihydroartemisinin (DHA), which appears to be largely responsible for its antimalarial activity of DHA.[3]

Accurate pharmacokinetic profile data are now possible with the advent of methods capable of accurately measuring both the parent drug, AS, and DHA, its primary metabolite, particularly high-performance liquid chromatography (HPLC) electrochemical detection (ECD).[4] An *in vitro* bioassay (BA) has been developed that allows the quantitative determination of antimalarial activity and provides an ''effect kinetic'' or pharmacodynamic profile of the antimalarial drug.

We report here simultaneous pharmacokinetic and pharmacodynamic measurement of orally administered AS determined by HPLC-ECD and by BA. Disposition pharmacokinetics and antimalarial effect kinetics after a single dose of AS in healthy volunteers are compared with those in patients with uncomplicated falciparum malaria.

MATERIALS AND METHODS

Study subjects. Patients with malaria were male Thai rangers stationed near the Thai-Cambodian border. Healthy volunteers of comparable age and weight were recruited from Thai Army soldiers working in Bangkok. After obtaining written informed consent, healthy volunteers were recruited into the study by the Royal Thai Army Component, Armed Forces Research Institute of Medical Sciences. The study was approved by the Ethical Review Subcommittee of the Royal Thai Army Medical Department, Bangkok, Thailand. The screening examination of potential volunteers included a thorough neurological assessment and electrocardiogram. A single 100-mg dose of AS (50-mg tablet, Guilin Pharmaceutical Works, Guangxi, China) was administered orally. Blood samples were collected into sterile lithium heparin 0, 2, 3, 4, 5, 6, 7, 8, and 12 hr later from patients and at 0, 15, 30, 45, 60, 90 min and 2, 3, 4, 5, 6, 9, and 12 hr from volunteers. Plasma was separated by centrifugation (1,000 × *g* for 10 min) immediately and stored at −70°C until analysis.

Drug assays. For HPLC-ECD, the following reagents and equipment were used: Vac Elut SPS24 sample processing station (Analytichem International, Harbour City, CA); Bakerbond spe* Octadecyl (C_{18}), 3 mL, 200 mg per column (J. T. Baker, Phillipsburg, NJ) BAS 200A liquid chromatograph with electrochemical detector (Bioanalytical Systems, West Lafayette, IN); 5414S microcentrifuge (Eppendorf, Brinkmann Instruments, Inc., Westbury, NY); Dri-Block DB-3 sample concentrator (Techne); Nova-Pak C_{18}, 4 μm, 3.9 × 150 mm analytical stainless steel column; and a Maxima 820 chromatography workstation (Waters Chromatography Di-

vision, Millipore Corporation, Milford, MA). All reagents were HPLC grade and included *n*-butyl chloride, ethyl acetate (Burdick and Jackson), acetonitrile, methanol, and glacial acetic acid (J. T. Baker).

We measured AS and DHA by HPLC-ECD[4] with a modified extraction procedure as described previously.[5] Briefly, solid-phase extraction was performed with C_{18} elution cartridges (J. T. Baker) with a Vac Elute SPS24 sample processing station (Analytichem International). The cartridge was preconditioned by sequential washing with acetonitrile, methanol, and water (2.5 mL each). One milliliter of distilled water was then added to the cartridge before adding the plasma sample (0.5 mL). The cartridge was washed with 2.5 mL each of distilled water, 0.05% H_3PO_4 (pH 4.5), and 20% acetonitrile. The compounds were collected by eluting with (0.5 mL each) ethanol 3 times and then with 90:10 *n*-butylchloride : ethylacetate twice. The collections were evaporated to dryness under nitrogen and kept at 5°C until analysis. The residue was reconstituted with 300 μL of 50:50 ethanol : water and analyzed by BAS 200A liquid chromatograph detector (Bioanalytical Systems) with detector oven and dual thin-layer glassy carbon electrode at −1.0 volt versus Ag/AgCl reference electrode. The percentage of recoveries of AS and DHA were 76 and 99%, respectively, from normal spiked plasma.

For the BA, the following reagents and equipment were used: Affi-Gel protein A gel (Bio-Rad Laboratories, Hercules, CA), ^{3}H-hypoxanthine monohydrochloride (Dupont, NEN Research Products), RPMI 1640 powder with L-glutamine, without sodium bicarbonate (Gibco Laboratories), Sterile 96-well cell culture cluster titer plate (Costar), SterilGuard hood (The Baker Company), Pro/Pette Perkin Elmer Cetus Liquid Handling System (Perkin-Elmer), Tomtec Mach II Harvester (Tomtec), Wallac 1205 Betaplate liquid scintillation counter (Pharmacia Biotech), and Eppendorf 5414S microcentrifuge.

The BA procedure was based on the microdilution radioisotope method used for antimalarial drug susceptibility testing.[2,6,7] Concentrations of activity are compared with the equivalent of a known concentration of DHA by means of a standard curve. The BA data are reported as activity equivalent to a known concentration of DHA (expressed in nanomoles per liter).

Standard curve and control standards. The HPLC standard curve validation and control standards were prepared by spiking plasma with 50:50 ethanol : water solutions of AS and DHA in concentrations ranging 5–1,000 ng/mL. The internal standard used was artemisinin at concentrations of 50 or 100 ng/mL. For BA determinations, the standard curve concentrations ranged 2.5–100 ng/mL of DHA.

For comparisons with BA, HPLC drug concentrations of AS and DHA were expressed as molar equivalents (nanomoles per liter) and converted to DHA equivalent by a correction factor of 0.79. This factor was the mean ratio of inhibitory activities of DHA to AS against the W2 parasite clone over the concentrations 2.5–100 ng/mL observed in our laboratory (AS is 0.79 as potent as DHA at inhibiting the growth of W2 clone *in vitro*). The estimated antimalarial activity from both AS (as a DHA equivalent) and DHA measured by HPLC were then compared with the antimalarial activity observed by BA.

Correction for plasma protein binding. On the basis of the report of Li and others[8] of healthy volunteers, plasma protein binding of AS and DHA were 59 and 43%, respectively. The concentrations of AS and DHA measured by HPLC-ECD in healthy volunteers were converted to free drug concentrations (41% for AS and 57% for DHA) before conversion to a DHA equivalent by the correction factor (0.79) mentioned previously.

Pharmacokinetic, effect kinetic, and statistical analyses. Pharmacokinetic and effect kinetic (pharmacodynamic) parameter estimates were derived by model independent (noncompartmental) analysis (WinNonlin Standard, version 2.1, Pharsight, Cary, NC) from HPLC and BA data, respectively. Kinetic parameter estimates for maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were determined. The elimination rate constant (k) was calculated by least-squares regression analysis of the log-linear portion of the plasma drug concentration time and plasma effect time curves. The elimination half-life ($t_{1/2,z}$) was calculated from the ratio of $0.693/k$ (the number of time points ≥ 3). The area under the plasma concentration and effect versus time curves from 0 to 12 hr (AUC_{0-12}) were estimated by the linear trapezoidal rule. As a fraction (F) of dose absorbed, the apparent total body clearance (Cl/F) and volume of distribution (V_z/F) associated with the terminal phase were calculated as dose/AUC_{0-12} and $(Cl/F)/k$, respectively. Groups mean comparison, with significance level of $P \leq 0.05$, were compared by either Students' *t*-test or the Mann-Whitney rank sum test, as appropriate for parametric or nonparametric data, respectively.

RESULTS

Patients. Six male soldiers with uncomplicated falciparum malaria and a median age of 26 years (range, 21–32 years) and weight of 56.5 kg (range, 51–68 kg), were enrolled in the study. Median parasitemia before treatment was 9,284 parasites per microliter of blood (range, 7,920–50,490 parasites/μL). The uninfected status of the cohort of 6 male volunteers was confirmed by microscopic examination of blood smears. The uninfected volunteers had normal temperature, blood pressure, electrocardiograms, physical examination, complete blood count, and biochemistry, and they had no history of previous illness. In addition, the uninfected volunteers were of comparable age (33 years; range, 23–44 years, $P > 0.1$) and weight (61 kg; range, 56–66 kg, $P > 0.1$) to the patients with falciparum malaria and received comparable doses: AS 1.64 mg/kg (4,047 nmol/kg) versus 1.79 mg/kg (4,404 nmol/kg) for the patients with malaria ($P > 0.1$).

Interassay variation and accuracy. The coefficients of variation and accuracy for HPLC measurement of AS and DHA at 3 concentrations (25, 100, and 500 ng/mL) were < 10%, with the exception of AS at 25 ng/mL (14% coefficient of variation and 11% error). The BA coefficient of variation for DHA (50, 12.5, and 5 ng/mL) ranged 4–19% with < 10% error. The quantitative limits of detection for AS, DHA (by HPLC), and DHA equivalent (by BA) were 25, 5, and 2.5 ng/mL, respectively.

Pharmacokinetics. The log linear concentration time and antimalarial activity time profiles of AS and DHA are shown in Figure 1. Pharmacokinetic and effect kinetic parameter estimates are summarized in Table 1. Artesunate was almost completely biotransformed to DHA in patients within 3 hr after

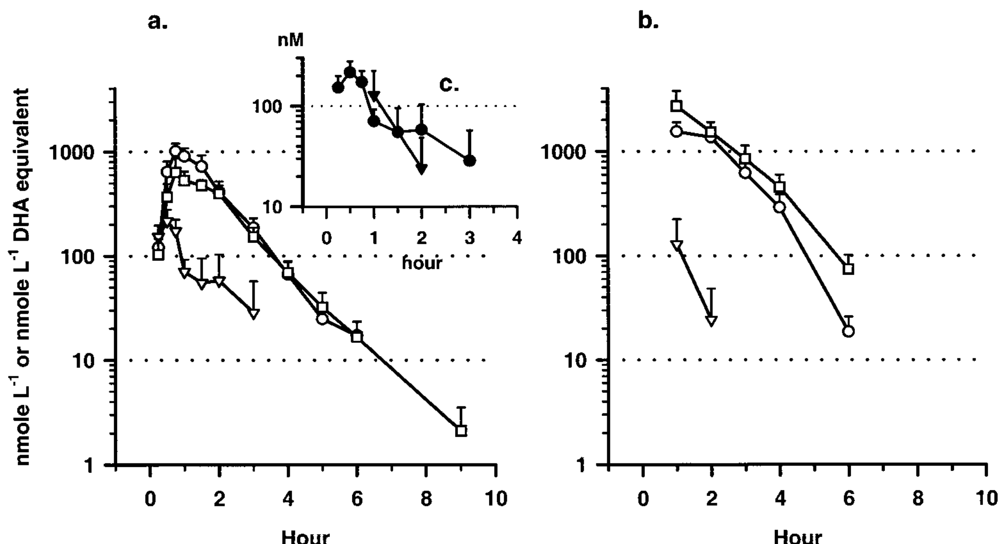

FIGURE 1. Artesunate (AS), dihydroartemisinin (DHA), and total antimalarial activity after oral administration of 100 mg of AS to **(a)** healthy volunteers ($n = 6$) and **(b)** patients with uncomplicated falciparum malaria ($n = 6$). The concentrations of AS (▽) and DHA (○) were measured by high-performance liquid chromatography with electrochemical detection, whereas the bioassay (□) results were calculated from antimalarial activity equivalent to known concentration of DHA. The inset **(c)** compares the disposition of AS in healthy volunteers (●) with that of patients with malaria (▼).

dosing, but only partial conversion of AS was seen until 4 hr in healthy volunteers. In healthy volunteers, the mean ± standard deviation pharmacokinetic parameter estimates of AS were C_{max} of 296 ± 110 nmol/L, t_{max} of 0.71 ± 0.66 hr, $t_{1/2,z}$ of 0.41 ± 0.34 hr, and $AUC_{(0-12)}$ of 253 ± 185 nmol hr/L, V_z/F of 14.8 ± 17.2 L/kg, and Cl/F of 20.6 ± 10.6 L/hr/kg.

All the above 4 pharmacokinetic parameter estimates of DHA in healthy volunteers were higher than for parent drug except the V_z/F and Cl/F values. The C_{max} and $AUC_{(0-12)}$ values of DHA in patients with uncomplicated falciparum malaria were significantly ($P \leq 0.05$) greater than healthy volunteers. On the other hand, the V_z/F and Cl/F values of DHA in patients were significantly smaller ($P < 0.05$) than in healthy counterparts (Table 1). However, there were no significant differences for either the t_{max} or $t_{1/2,z}$ of DHA in patients compared with healthy volunteers ($P > 0. 5$).

Effect kinetics. The antimalarial effect kinetics were similar to the pharmacokinetics of DHA in all aspects, both for patients and healthy volunteers. Both C_{max} and $AUC_{(0-12)}$ of the *in vitro* antimalarial activity derived from BA measurement were significantly greater ($P \leq 0.05$) in plasma from patients with malaria than from healthy volunteers. Conversely, both the V_z/F and Cl/F of antimalarial activity in patients were significantly smaller than healthy volunteers. The t_{max} and $t_{1/2,z}$ of antimalarial activity were not significantly different between patients and healthy volunteers ($P > 0.05$).

The effect kinetics derived from antimalarial activity in the BA also was compared with the estimated antimalarial activity derived from the HPLC data. In patients, both estimated and observed effect kinetic parameter estimates were not significantly different. In healthy volunteers, both C_{max} and $AUC_{(0-12)}$ of estimated activity with mean ± SD of 1,284 ± 234 nmol/L and 1,964 ± 586 nmol hr/L, respectively, were also significantly ($P \leq 0.05$) greater than the observed

TABLE 1
Pharmacokinetic parameters following a single (100 mg) orally administered dose of artesunate*

Parameter	Administered to:	AS	DHA	Bioassay
Maximum concentration, C_{max} (nmol/L)	HC	296 ± 110 (105–428)	1,192 ± 315 (979–1,456)	795 ± 455 (368–1,682)
	PWM		1,948 ± 772† (880–2,841)	2,894 ± 2,497† (818–6,535)
Peak blood level, t_{max} (hr)	HC	0.71 ± 0.66 (0.25–2.0)	1.13 ± 0.52 (0.75–2.0)	1.29 ± 0.62 (0.75–2.0)
	PWM		1.50 ± 0.55 (1.0–2.0)	1.67 ± 1.21 (1.0–4.0)
Elimination half-life, $t_{1/2,z}$ (hr)	HC	0.41 ± 0.34 (0.13–0.99)	0.87 ± 0.06 (0.79–0.95)	0.85 ± 0.25 (0.57–1.18)
	PWM		0.80 ± 0.30‡ (0.68–1.38)	1.06 ± 0.30 (0.86–1.48)
$AUC_{(0-12)}$ (nmol hr/L)	HC	253 ± 185 (81–604)	1,763 ± 607 (1,117–2,664)	1,307 ± 391 (803–1,734)
	PWM		4,024 ± 1,585† (1,816–6,506)	5,970 ± 3,625† (2,275–11,300)
Volume of distribution, V_z/F (L/kg)	HC	14.8 ± 17.2 (4.20–49.60)	3.02 ± 0.93 (1.99–4.45)	4.14 ± 1.99 (2.05–7.95)
	PWM		1.33 ± 0.47† (0.70–2.70)	1.55 ± 1.06† (0.71–3.16)
Apparent total body clearance Cl/F (L/hr/kg)	HC	20.6 ± 10.6 (4.69–29.0)	2.42 ± 0.65 (1.66–3.26)	3.35 ± 1.03 (2.28–4.65)
	PWM		1.22 ± 0.47† (0.72–1.48)	1.01 ± 0.58† (0.36–1.59)

* AS = artesunate; AUC = area under the curve; DHA = dihydroartemisinin; HC = healthy control; PWM = patient with malaria. Values of AS and DHA are expressed as molar concentration derived from high-performance liquid chromatography. Bioassay measured plasma total antimalarial activities and expressed as the DHA concentration that produced equivalent activities. Values are mean ± standard deviation (range) from 6 healthy volunteers and 6 patients with uncomplicated falciparum malaria.
† Significant difference ($P \leq 0.05$) between patients and healthy volunteers.
‡ Significant difference ($P \leq 0.05$) between high-performance liquid chromatography with electrochemical detection and bioassay.

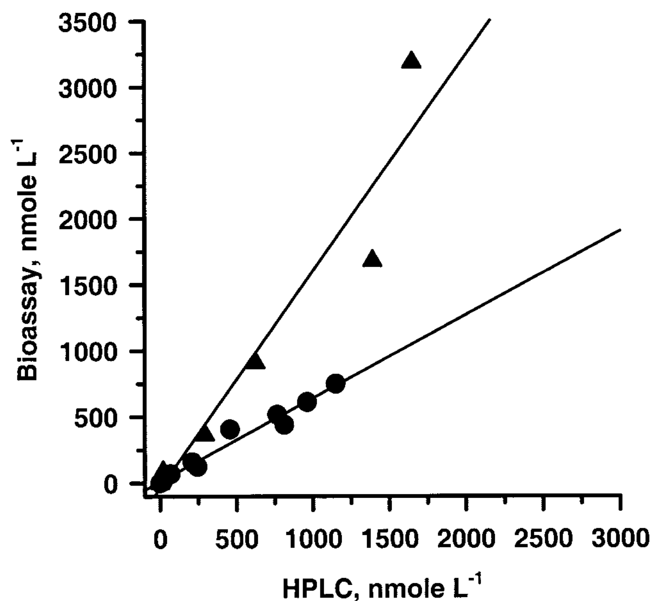

FIGURE 2. The mean total antimalarial activities in plasma observed by bioassay were plotted against the mean estimated activities derived from high-performance liquid chromatography for healthy volunteers (●, slope = 0.63, r^2 = 0.96) and patients with malaria (▲, slope = 1.56, r^2 = 0.95).

activity from BA measurements with mean ± SD of 795 ± 455 nmol/L and 1,307 ± 391 nmol hr/L, respectively. However, after correction for protein binding and converting AS concentration to DHA equivalents, C_{max} and $AUC_{(0-12)}$ of the estimated total bioactivity were comparable to that observed by BA with the mean ± SD of 717 ± 146 nmol/L and 1,087 ± 335 nmol hr/L, respectively ($P > 0.1$).

Simultaneous HPLC drug determination versus BA effect measurement in plasma samples. The total antimalarial activity (x-axis) estimated from HPLC-ECD (AS + DHA) correlated with the *in vitro* antimalarial activity (y-axis) measured by BA in 5 out of the 6 healthy volunteers, with a mean ± SD r^2 of 0.96 ± 0.04. No correlation was observed for the sixth volunteer (r^2 = 0.05, slope = 0.05, intercept = 103 nmol/L). In this subject, the t_{max} estimated from BA data was 2.6-fold greater than that estimated from HPLC data (2.0 versus 0.75 hr), and in contrast, the C_{max} estimated from BA was 4-fold lower than that estimated from HPLC (368 versus 1,421 nmol/L). Therefore, this healthy volunteer was excluded from subsequent analyses. The mean ± SD slope of the effect-concentration plot for healthy volunteers was 0.63 ± 0.18 (Figure 2).

The 2 methods gave comparable results in 5 of 6 patients with a mean ± SD r^2 of 0.95 ± 0.05. The sixth patient showed poor correlation (r^2 = 0.64) and was omitted from the analyses (inclusion of his data did not change statistical test results). The mean ± SD slope of the effect-concentration plot was 1.56 ± 1.04 (Figure 2). However, there was no statistically significant difference in the slopes of the effect-concentration plots of patients with malaria and those of healthy volunteers ($P = 0.08$).

DISCUSSION

Orally administered AS (1.6 mg/kg) was absorbed rapidly and hydrolyzed to DHA. Indeed, parent AS was undetectable in 2 patients and was measurable at 2 hr after the first dose, but not by 3 hr in others ($n = 4$). These patients showed detectable drug concentrations in samples at 1–2 time points, and maximum concentrations ranged 61–569 nmol/L. The interindividual variability in pharmacokinetics in patients with malaria was greater than in healthy volunteers in this study. This finding has also been observed in the treatment of uncomplicated *P. falciparum* with orally administered artemisinin.[9]

The pharmacokinetic parameters of AS and DHA in healthy volunteers observed in this study are comparable to those estimated by Benakis and others[10] in healthy Vietnamese volunteers given 2.5 mg/kg AS orally. De Vries and others[11] showed that the pharmacokinetics of artemisinin were not altered in patients with uncomplicated falciparum malaria, although the conversion of AS to DHA was slightly faster in patients with uncomplicated malaria than in healthy subjects. The DHA pharmacokinetic parameters estimates were significantly different in patients with malaria than in healthy volunteers. Both the C_{max} and $AUC_{(0-12)}$ values were 1.6- and 2.3-fold greater (P = 0.05 and 0.009) in patients with malaria than in healthy volunteers, respectively. In contrast, the V_z/F and Cl/F in healthy volunteers were 2.3- and 2.0-fold greater (P = 0.003 and 0.004) than those of patients, respectively. The C_{max} of DHA after orally administered artemether tended to be higher in patients with malaria than in healthy volunteers, but not significantly so.[12]

Measured by BA, the antimalarial effects of orally administered AS varied more between malaria-infected and uninfected patients than did those of DHA. A far greater plasma antimalarial effect occurred in patients with malaria than in healthy volunteers, and this effect would be underestimated by measuring drug levels alone. A crossover study by Newton and others[13] demonstrated that the antimalarial effect of orally administered AS (2 mg/kg) during acute uncomplicated falciparum malaria was greater than that at convalescence. It appeared that acute disease was associated with reduced AS clearance.

The apparent volume of distribution of protein-bound basic drugs such as quinine was reduced during acute malaria because of binding to α-1-acid glycoprotein.[14,15] Data are lacking on whether reduced artemisinin clearance is related to α-1-acid glycoprotein binding or to decreased metabolic conversion. Decreased metabolic conversion of arteether to DHA in rat liver microsomes was seen in malaria-infected rodents, but hepatic conversion to DHA was not affected by disease in the isolated perfused rat liver model.[16]

Peak DHA concentrations after orally administered AS in healthy Thais were 2-fold greater than after orally administered DHA in Vietnamese volunteers, but AUC values were comparable.[17] C_{max} varied greatly between individuals, consistent with other reports.[2,18] These study results confirm the close relationship between measurement of discrete drug concentration and BA equivalents.[7,19–21] Simultaneous HPLC and BA data provide more information than do drug levels alone.

Simultaneous pharmacokinetic and BA data demonstrate that DHA is the main contributor to *in vitro* antimalarial activity and that the contribution is proportionately greater in patients in whom little parent drug is found. In volunteers in whom some AS was still present, the estimated antima-

larial activity from both AS and DHA concentrations measured by HPLC-ECD (1,284 nmol/L DHA equivalent) was significantly ($P = 0.04$) higher than the observed bioactivity measured by BA (795 nmol/L DHA equivalent). This observed bioactivity was even lower than expected from plasma DHA concentration as measured by HPLC-ECD. A previous report showed plasma protein binding of AS and DHA of 59 and 43%, respectively, in healthy volunteers.[8] One possible likely explanation is the effect of acute phase protein binding on the free drug fraction in plasma. If these data are used to convert AS concentration to DHA equivalents, the mean $\pm$ SD estimated total bioactivity in this study was 717 $\pm$ 146 compared with 795 $\pm$ 455 nmol/L DHA equivalents observed by BA.

Simultaneous HPLC-ECD and BA measurements showed that the DHA metabolite is largely responsible for antimalarial activity. The parent compound, AS, is almost completely hydrolyzed to DHA in patients with malaria, whereas hydrolysis takes place more slowly in healthy volunteers. Bioassay results demonstrate clearly that the overall antimalarial activity of AS is greater in patients with malaria than in healthy subjects.

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Authors' addresses: Paktiya Teja-Isavadharm and Duangsuda Keeratithakul, Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Bangkok 10400, Thailand. George Watt and Krisada Jongsakul, Department of Retrovirology, AFRIMS, 315/6 Rajvithi Road, Bangkok 10400, Thailand. Chirapa Eamsila and Narongrid Sirisopana, Royal Thai Army Component, AFRIMS, 315/6 Rajvithi Road, Bangkok 10400, Thailand. Lersan Luesutthiviboon, Department of Medicine, Phramongkutklao Hospital, 315 Rajvithi Road, Bangkok 10200, Thailand. Dennis E. Kyle and Qigui Li, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500. Thomas G. Brewer, Deputy for Medical Research, Deputy Assistant Secretary of the Army for Logistics and Technology, Attn.: SAAL-TM, 2511 Jefferson Davis Highway, Suite 9000, Arlington, VA 22202-3911.

Reprint requests: Paktiya Teja-Isavadharm, Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Bangkok, Thailand.

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