Pharmacokinetics and *Ex Vivo* Antimalarial Activity of Artesunate-Azithromycin in Healthy Volunteers[†]

Nguyen Trong Chinh,¹ Nguyen Ngoc Quang,¹ Chu Xuan Anh,¹ Nguyen Xuan Thanh,² Bui Dai,² Geoffrey W. Birrell,³ Marina Chavchich,³ and Michael D. Edstein^{3*}

Department of Infectious Diseases, Military Hospital 108, Hanoi, Vietnam¹; Department of Malaria, Military Institute of Hygiene and Epidemiology, Hanoi, Vietnam²; and Australian Army Malaria Institute, Brisbane, Australia³

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In 18 male healthy subjects, artesunate (200 mg)-azithromycin (1,500 mg) daily for 3 days was found to be well tolerated, with only mild gastrointestinal disturbances reported. The pharmacokinetic properties of artesunate-azithromycin given in combination are comparable to those of the drugs given alone. Artesunate and its major active metabolite, dihydroartemisinin, are responsible for most of the *ex vivo* antimalarial activity, with a delayed contribution by azithromycin.

Artemisinin-based combination therapies (ACTs) are currently recommended for the first-line treatment of uncomplicated *Plasmodium falciparum* malaria, worldwide (25). The two risk groups most vulnerable to malaria are young children and pregnant women, who are immunosuppressed and particularly at risk for severe disease (26). In pregnant women, limited safety and pharmacological data are available on the use of ACTs, particularly during the first trimester. The World Health Organization (WHO) recommends that quinine combined with the antibiotic clindamycin be given for 7 days during the first trimester and, if quinine-clindamycin is either unavailable or fails, an ACT is indicated (25). Historically, 7-day daily regimens are limited by poor compliance in an outpatient setting (9). For the second and third trimesters, WHO recommends artesunate (AS)-clindamycin.

However, a drawback in using clindamycin is its short half-life of 2.4 h (10), which necessitates repeated dosing every 12 h, which may affect compliance and thus effectiveness (20). Unlike clindamycin, the antibiotic azithromycin (AZ) possesses wider therapeutic applications (8) and has pharmacokinetic properties that are characterized by more extensive tissue distribution, prolonged phagocyte concentrations, and a longer elimination half-life of about 50 h (1, 14). These favorable features allow once-daily dosing with AZ compared with multiple daily dosing with clindamycin. Recently, 3-day courses of quinine with either 1,000 or 1,500 mg AZ were shown to be well tolerated and effective in curing multidrug-resistant *P. falciparum* malaria in adults (12). AZ also has a good safety profile in children and pregnant women (6, 21).

Because of AZ's favorable pharmacokinetic properties, artesunate (AS) plus AZ may be a suitable ACT during pregnancy. *In vitro* studies have shown dihydroartemisinin (DHA), the active metabolite of the prodrug AS, combined with AZ to be additive (17) tending to synergistic (16) against multidrug-

resistant *P. falciparum* strains. Recent studies of AS-AZ have shown good efficacy in the treatment of uncomplicated falciparum malaria using 3-day regimens of 200 mg AS daily with either 1,000 mg AZ (cure rate, 89%) (15) or 1,500 mg AZ (cure rates, >92%) (15, 24). The AS-AZ combinations are far more effective than monotherapy, with recrudescence rates of between 10% and 80% with 3- to 5-day courses of AS alone (4) and 67% with 1,000 mg/day AZ for 3 days (7). Similarly, the addition of AZ (1,000 mg daily for 2 days) to 1,500 mg sulfadoxine and 75 mg pyrimethamine for the treatment of malaria during pregnancy increased the cure rate from 71% to 92% compared with sulfadoxine-pyrimethamine alone (11).

Despite the proven efficacy of AS and high-dose AZ in treating malaria, there are no data on the safety and pharmacokinetics of the ACT in healthy subjects to support its use in patients. The present study investigated the tolerability, pharmacokinetic properties, and *ex vivo* antimalarial activity of a 3-day regimen of 200 mg AS plus 1,500 mg AZ in 18 healthy male Vietnamese subjects. Ethical approval for the study was obtained from the Review and Scientific Board of Military Hospital 108 and the Australian Defense Human Research Ethics Committee (ADHREC no. 496/07).

The subjects were coadministered 200 mg AS (Trophaco Joint Stock Company, Hanoi, Vietnam; each tablet contained 50 mg AS) and 1,500 mg AZ (Zitromax; Pfizer Italia, Latina, Italy; each tablet contained 500 mg AZ) daily for 3 days. The ACT was administered to the subjects with 240 ml water after an overnight fast (no food for at least 8 h). Blood chemistry tests (complete blood count, liver function tests, urea, and creatinine) were performed before treatment and repeated at 2 weeks after the last drug administration. Adverse events were assessed daily by a physician with the question "How do you feel since you took the last artesunate-plus-azithromycin tablets?" followed by prespecified questions about specific symptoms, which included loose stools, nausea, abdominal pain, headache, insomnia, tiredness, and dizziness. Electrocardiograms were recorded at 0 h, immediately before and 2 h after the last dose, and at day 7 following the last drug administration.

Venous blood samples (7 ml) were collected with an indwell-

* Corresponding author. Mailing address: Australian Army Malaria Institute, Enoggera, Brisbane QLD 4051, Australia. Phone: 61-7-33324801. Fax: 61-7-33324800. E-mail: mike.edstein@defence.gov.au.

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ing cannula within 0.5 h (baseline) before the last dose and at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 12 h after drug administration. Subsequent blood samples were collected by venipuncture at days 1, 2, 3, 5, 7, 10, and 14 after the last dose. For measurement of AS and DHA in plasma, blood samples were drawn into sodium fluoride-potassium oxalate blood collection tubes to prevent hydrolysis of AS by plasma esterases (2). For serum AZ analysis, blood samples were drawn into plain tubes. Blood samples were centrifuged at $1,400 \times g$ for 10 min, and the separated plasma or serum samples were stored at -80°C until transported on dry ice to Australia for drug analysis, which was carried out within 12 months of collection.

Plasma concentrations of AS and DHA and serum concentrations of AZ were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS) (see the supplemental material). The lower limit of quantification (LLOQ) of AS was 1 ng/ml, with an interassay precision of analysis (percent coefficient of variation [CV]) for AS of 6.6% at 1 ng/ml, 4.3% at 100 ng/ml, and 3.8% at 500 ng/ml ($n = 18$). The LLOQ for DHA was 1 ng/ml, with an interassay precision for DHA of 5.8% at 1 ng/ml, 3.8% at 100 ng/ml, and 4.2% at 2,000 ng/ml ($n = 18$). For AZ, the LLOQ was 5 ng/ml, with an interassay precision for AZ of 1.9% at 5 ng/ml, 0.9% at 500 ng/ml, and 2.3% at 2,000 ng/ml ($n = 3$). Pharmacokinetic parameters (peak concentration [$C_{\max}$], time to reach maximum concentration [$T_{\max}$], area under the concentration-time curve from day 2 to last data point [$\text{AUC}_{\text{day } 2 \rightarrow \text{last}}$] and day 2 to infinity [$\text{AUC}_{\text{day } 2 \rightarrow \infty}$], and terminal half-life [$t_{1/2}$]) were determined from the concentration-time data using noncompartmental methods. The *ex vivo* antimalarial activity of AS-AZ was assessed by bioassay, which involves culturing malaria parasites *in vitro* in the presence of subjects' serum samples collected after the last administration of the ACT using the method described by Nagelschmitz et al. (13), with minor modifications. They were as follows: (i) the *in vitro* drug susceptibilities of the multidrug (atovaquone, chloroquine, quinine, and pyrimethamine)-resistant TM90-C2B line of *P. falciparum* to AS, DHA, and AZ and the subjects' serum samples were determined in parallel; (ii) 50 μl of human serum was used, so the final concentration of serum/plasma in the well was 50%; and (iii) an incubation period of 96 h of drug exposure was applied due to AZ's slower parasitocidal action (22, 27). Fifty percent inhibitory concentration (IC_{50}) and 50% inhibitory dilution (ID_{50}) values were defined as the drug concentrations and dilutions that produced a 50% inhibition of uptake of tritiated hypoxanthine by intraerythrocytic malaria parasites compared to drug-free serum samples (controls). The bioassay data are reported as activities equivalent to a known concentration of DHA, which were estimated by multiplying the ID_{50} of the subject's serum samples after AS-AZ administration by the IC_{50} of DHA (expressed as ng/ml).

The subjects' mean ($\pm$ standard deviation) age was 20.7 (1.6) years, with a mean weight of 58.8 (2.6) kg. AS-AZ did not appear to cause any clinically significant changes in hematological and biochemical indices, with values within the normal range before and after drug administration (see the supplemental material). Electrocardiograms did not change as a result of AS-AZ administration (data not shown). After the first dose of AS-AZ, 11% (2/18) of subjects had abdominal pain and 61% (11/18) had loose stools. These adverse events were

FIG. 1. Mean (standard deviation) concentrations of AS, DHA, and AZ. (A) Profiles of plasma concentrations of AS (●) and DHA (▲) and serum concentrations of azithromycin (■) versus time after the last dose of a 3-day course of daily 200-mg AS and 1,500-mg AZ in 18 healthy Vietnamese subjects. The *ex vivo* pharmacodynamic antimalarial activity profile of subjects' serum DHA equivalent concentrations (△) was estimated by bioassay using the TM90-C2B line of *P. falciparum*. (B) Profile of serum concentrations of AZ (■) versus time from day 2 to day 16 after commencement of treatment.

again seen after the second dose, with the frequency of abdominal pain and loose stools increasing to 22% and 67%, respectively. However, no gastrointestinal (GI) disturbances were reported by the subjects after the third dose of AS-AZ. On each of the first 2 days of drug administration, GI disturbances were mild (discomfort not affecting daily activities and requiring no medical intervention) in severity and transient, with most adverse events occurring between 1 and 6 h after dosing. In the subjects with abdominal pain, the discomfort lasted between 0.5 and 1 h. It is assumed that the high dose of AZ was responsible for the GI adverse events, as this class of compound is associated with causing GI disturbances and those disturbances appear to be dose related (18), which is not the case for AS (5, 19). The high frequency of GI disturbances in the healthy subjects was unexpected, as a lower frequency of abdominal pain (7.7%) and loose stools (3.8%) was reported by malaria patients treated with the same AS-AZ regimen (24). With the exception of GI disturbances, no other adverse events were reported by the subjects in the present study.

TABLE 1. Pharmacokinetic parameters of AS, DHA, and AZ in 18 healthy Vietnamese subjects after the last dose of a 3-day course of daily 200-mg AS and 1,500-mg AZ

Pharmacokinetic parameter	Mean $\pm$ SD for drug:		
	AS (plasma concn)	DHA (plasma concn)	AZ (serum concn)
$C_{\max}$ (ng/ml)	197 $\pm$ 105	644 $\pm$ 221	1,624 $\pm$ 626
$T_{\max}$ (h)	0.38 $\pm$ 0.30	0.75 $\pm$ 0.45	2.5 $\pm$ 1.0
AUC _{day 2$\rightarrow$last} (ng $\cdot$ h/ml)	90 $\pm$ 45	990 $\pm$ 211	32,300 $\pm$ 6,600
AUC _{day 2$\rightarrow$$\infty$} (ng $\cdot$ h/ml)	96 $\pm$ 43	993 $\pm$ 212	34,200 $\pm$ 7,100
$t_{1/2}$ (h)	0.26 $\pm$ 0.17	0.83 $\pm$ 0.12	87.4 $\pm$ 5.5

The mean plasma concentration-time profiles of AS and DHA and serum concentration-time profile of AZ after a 3-day course of AS-AZ are shown in Fig. 1. The pharmacokinetic properties of the drugs are summarized in Table 1. The mean $C_{\max}$ and AUC values of AS were markedly higher in our subjects than in a previous study in Vietnamese subjects by Diem Thuy et al. (5) ($C_{\max}$, 197 versus 67 ng/ml; AUC, 96 versus 67 ng $\cdot$ h/ml) given the same daily dose of AS. The prodrug was rapidly eliminated, with a mean $t_{1/2}$ of 0.26 h, and by 2 h after drug administration, 33% (6/18) of subjects had AS concentrations below the LLOQ. AS was rapidly and extensively hydrolyzed to DHA, with measurable concentrations (>1 ng/ml) in all subjects at 0.25 h after dosing. The $C_{\max}$, AUC, and $t_{1/2}$ values of DHA in our participants were similar to those reported by Diem Thuy et al. (5) ($C_{\max}$, 644 versus 632 ng/ml; AUC, 933 versus 1,158 ng $\cdot$ h/ml; $t_{1/2}$, 0.83 versus 0.81 h). After adjusting for dose differences, the pharmacokinetics of AS and DHA were also comparable with values obtained in healthy Thai subjects administered 100 mg AS (23).

AZ was rapidly absorbed, with maximum serum drug concentrations of 1,624 ng/ml attained at about 2.5 h after the last dose. Thereafter, serum concentrations of AZ fell rapidly and biphasically, with a mean $t_{1/2}$ of 87.4 h. The $C_{\max}$, AUC, and $t_{1/2}$ values of AZ obtained in our subjects are in accord with those reported by Cook et al. (3) following a 3-day regimen of daily 1,000-mg AZ alone in healthy subjects, after adjusting for dose and body weight differences between the 2 studies.

Based on IC_{50} s (geometric mean [95% confidence interval]), AS (0.47 ng/ml [0.35 to 0.63, $n = 14$]) and DHA (0.30 ng/ml [0.23 to 0.38, $n = 18$]) were markedly more active than AZ (2,971 ng/ml [2,162 to 4,082, $n = 14$]) in inhibiting the TM90-C2B line. The IC_{50} s of DHA (0.30 ng/ml versus 0.53 ng/ml) and AZ (2,971 ng/ml versus 2,570 ng/ml) were comparable to that obtained by Noedl et al. (16) against fresh clinical *P. falciparum* isolates from Thailand. The selection to estimate DHA equivalent concentrations by bioassay was based on AS and DHA having similar IC_{50} s and on the finding that AZ is markedly less active than the artemisinins, *in vitro*.

The mean DHA equivalent concentrations obtained by bioassay were higher than the composite AS and DHA concentrations measured by LC/MS/MS from 1 h to 10 h after the last dose of the ACT (Fig. 1). At 8 h, DHA concentrations were below the *in vitro* MIC for *P. falciparum* of <2 ng/ml, with physiological concentrations of AZ remaining to exert its parasitocidal effect. The extra antimalarial activity as shown by the

higher DHA equivalent concentrations is suggestive of AZ's delayed death effect in inhibiting the next generation of parasites (22). This observation is further supported by a marked reduction in inhibitory concentrations of AZ, ranging between 28-fold and 522-fold in 5 *P. falciparum* lines, with different levels of chloroquine sensitivity, between the first and second asexual erythrocytic cycles (22, 27).

In conclusion, the 3-day regimen of 200 mg AS daily plus 1,500 mg AZ used in clinical studies (15, 24) was well tolerated in healthy Vietnamese subjects, with the exception of only mild and transient GI disturbances. The *ex vivo* antimalarial activity of the subjects' serum concentrations after AS-AZ treatment revealed that most of the potency in killing a multidrug-resistant *P. falciparum* line was due to the artemisinins, with a noticeable contribution by AZ. The findings of this study suggest that AS-AZ is worthy of further investigations in pregnant women and young children to assess its potential as an alternative ACT for the treatment of malaria in these special risk groups.

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