Pharmacokinetics of Artesunate Alone and in Combination with Sulfadoxine/Pyrimethamine in Healthy Sudanese Volunteers

Kamal M. Matar,* Abdelmoneim I. Awad, and Sakina B. Elamin

Department of Pharmacology and Therapeutics, and Department of Pharmacy Practice, Faculty of Pharmacy, Kuwait University, Kuwait; National Medicines and Poisons Board, Khartoum, Sudan

Abstract. Artesunate (AS) in combination with sulfadoxine/pyrimethamine (SP) is the first-line therapy for management of uncomplicated *Plasmodium falciparum* malaria in Sudan. The objective of this study was to assess the potential impact of SP on the pharmacokinetics of AS and its active metabolite, dihydroartemisinin (DHA), in healthy adults. A single-dose, randomized, open-label, crossover study design with a washout period of three weeks was performed with 16 volunteers. After oral administration of AS alone or in combination with SP, T_{max} values of AS and DHA were significantly prolonged in the combination group ($P < 0.05$). However, there was no significant effect on the other pharmacokinetic parameters ($P > 0.05$). The $t_{1/2}$ values of AS and DHA were significantly higher in females than in males ($P < 0.05$). The present findings suggest that co-administration of SP with AS has no clinically relevant impact on the pharmacokinetics of AS or DHA in healthy persons.

INTRODUCTION

Malaria is one of the deadliest infectious diseases. It results in a high number of deaths because of high prevalence, but in general if treated early is not fatal, unlike some other infections. In 2010, it caused almost 655,000 deaths worldwide, of which 91% were in Africa.¹ Most of the burden is in sub-Saharan Africa, where *Plasmodium falciparum*, in particular, affects young children and pregnant women.² In Sudan, it is estimated that 7.5–10 million cases of malaria and 35,000 deaths occur every year.³

Resistance to most antimalarial therapies is well documented worldwide. In eastern and southern Sudan, there has been a spread of chloroquine and sulfadoxine/pyrimethamine (SP)-resistant *P. falciparum* infections.^{4,5} As older monotherapy regimens become less effective, a consensus has emerged for the use of combination treatments for malaria. Although all artemisinin derivatives are effective against multidrug resistance, but when administered alone have led to high rate of recrudescence. Thus, the World Health Organization recommends the use of artemisinin-based combination therapies (ACTs) to improve antimalarial effectiveness and reduce the selection of drug-resistant parasites to a minimum. The commonly used ACTs were artemether-lumefantrine, artesunate (AS)-amodiaquine, AS-mefloquine, and AS-SP.¹ The ACTs have proved effective against malaria in Asia and Africa, and most countries in Africa, including Sudan, have now changed their national regimens to incorporate ACT regimens as first-line treatment of uncomplicated malaria.^{6,7} In Sudan, the current protocol for managing uncomplicated *P. falciparum* malaria is AS in combination with SP and artemether-lumefantrine as first-line and second-line therapies, respectively.⁸

Artesunate is a semi-synthetic derivative of artemisinin whose water solubility facilitates its absorption and provides an advantage over other derivatives regarding its formulation in various dosage forms including oral, rectal, intramuscular and intravenous preparations. It is rapidly converted

in vivo to its active metabolite dihydroartemisinin (DHA), which is responsible for most of the anti-malarial activity.^{9,10} Because of high relapse rates of short-course AS monotherapy, it is combined with longer acting anti-malarial agents, such as mefloquine or amodiaquine or SP for oral treatment of uncomplicated *falciparum* malaria.^{1,11}

Because of the therapeutic significance of AS and its multiple effective routes of administration, and the need for appropriate dosage regimens to prevent suboptimal efficacy or emerging resistance, research describing the pharmacokinetics of AS and its active metabolite, DHA, is of substantial clinical relevance.¹² Quantitative determination of artemisinin derivatives in biological fluids is a challenging problem because they lack ultraviolet and fluorescent chromophore groups.¹³ Previously, the main methods for their quantification were electrochemical detection with detection limits of approximately 10 ng/mL by using sample volumes of approximately 0.5–1 mL plasma or post-column derivatization with methanolic alkali and subsequent ultraviolet detection with limits of detection of approximately 30 ng/mL.^{14–20} Currently, the suitable method for quantification of these compounds in plasma is liquid chromatography coupled with mass spectrometry detection (LC-MS or LC-MS/MS) with quantification limits of approximately 1 ng/mL using only 50- μ L plasma sample volumes.^{21–25} However, some of the problems encountered the latter bioanalytical methods include lack of appropriate stable isotope-labeled AS and DHA internal standards, in addition to expensive solid phase extraction cartridges that most commonly used for extraction of AS and DHA from biological samples.

Despite the widespread use of the combination of AS and SP, sparse data are available on the pharmacokinetic interactions between these drugs. Two studies have shown that the concomitant administration of AS with SP does not have an impact on the pharmacokinetic parameters of SP.^{26,27} However, the pharmacokinetics of AS when combined with SP is poorly documented. Sulfadoxine is significantly bound to plasma proteins, mainly albumin, and it may potentially compete with other concomitant drugs for protein binding sites. Displacement of plasma protein binding of AS or DHA by sulfadoxine is a potential source for pharmacokinetic drug interactions. The objective of the present study was to investigate the pharmacokinetic profiles of AS

*Address correspondence to Kamal M. Matar, Faculty of Pharmacy, Kuwait University, PO Box 24923, Safat 13110, Kuwait. E-mail: kamal@hsc.edu.kw

and its active metabolite, DHA, after a single oral dose of AS alone and in combination with SP in healthy adult Sudanese volunteers.

MATERIALS AND METHODS

Chemicals and reagents. Artesunate, DHA and internal standard (IS), artemisinin, were kindly supplied by Dafra Pharma (Turnhout, Belgium). Water was purified using a Milli-Q water device (Millipore, Bedford, MA). The Oasis[®] HLB solid-phase extraction (SPE) cartridges were obtained from Waters Corporation (Milford, MA). All other chemicals and reagents were analytical grade and solvents were high-performance liquid chromatography grade.

Volunteers. The sample size was calculated on the basis of a cross-over design, considering an intra-individual coefficient of variation of 20%, a power of 80% and a significance level of 5%.²⁸ The calculated sample size was 18 volunteers; thus, taking into consideration potential withdrawals and dropouts, 24 volunteers were enrolled. Of these volunteers, six were excluded because of significant deviations from the parameters of clinical chemistry, renal and liver dysfunction, and a known history of allergy to sulfa drugs. In addition, two women failed to complete the study because of personal reasons were also excluded. Sixteen healthy, non-obese, adult volunteers (nine men and seven women) participated in this study. The women were not breast-feeding or pregnant.

The volunteers were considered healthy and included in the study if they had no history of chronic disease states (normal physical examination and laboratory tests results for complete blood count, serum creatinine, blood urea nitrogen, total protein, serum bilirubin, liver enzymes), were free of infectious diseases (e.g., malaria, infection with human immunodeficiency virus, hepatitis), non-smokers and no known history of allergy to AS, pyrimethamine, or sulfa drugs. The volunteers had not taken SP for four weeks preceding the study period, and were instructed to abstain from taking any medication at least one week before and during the study. Written informed consent was obtained from each volunteer before enrolling in the study. Ethical approval was obtained from the Ethical Board of Federal Ministry of Health-Research Directorate, Sudan.

Procedure. The study was conducted in Khartoum, Sudan, during March-May (warm and dry season), and was in accordance with the Helsinki Declaration (as revised in 2004, Tokyo, Japan). The study design was a randomized, open, crossover with a wash-out period of three weeks. Simple random sampling was used for volunteer allocation into two groups. After a 12-hour overnight fasting, the first group received a single AS oral dose of 200 mg and the second group received a single oral dose of AS (200 mg) in combination with Fansidar[®] (Roche, Basel, Switzerland) (3 tablets: 500 mg of sulfadoxine/25 mg of pyrimethamine per tablet). The first and second groups received the opposite treatment after a washout period of three weeks.

The doses were swallowed with 240 mL of water. Food and water were not allowed for four hours post-drug administration. Blood (5 mL) samples were withdrawn into heparinized tubes before dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5, 5, 6, 9, and 12 hours after drug administration. Plasma samples were immediately separated

by centrifugation at $2,000 \times g$ and kept frozen at -80°C pending analysis.

Instrumentation. The chromatographic system, Waters Alliance 2695, consisted of a solvent delivery system, and an autosampler (Waters Corporation). Separation of the analytes was performed on a Symmetry[®] C₁₈ column (5 μm , 3.9×50 mm) and a guard column of the same material. The mobile phase used consisted of methanol-ammonium acetate (10 mM), (85:15, v/v) and delivered at a flow rate of 0.2 mL/min to a positive electrospray ionization interface of a tandem triple quadrupole mass spectrometer (Quattro LC; Micromass, Manchester, United Kingdom). Tuning parameters of MS and MS-MS were optimized by direct infusion of solutions of AS, DHA, and the IS in the mobile phase into the ionization probe at a flow rate of 10 $\mu\text{L}/\text{min}$ by using a syringe. The ion source and desolvation temperatures were set at 100°C and 275°C , respectively. The capillary voltage was adjusted at 3.5 kV. The cone voltage was adjusted at 15, 20, and 25 V for AS, DHA and IS, respectively. The collision energy was adjusted at 10 eV and collision gas pressure at $< 1.0 \times 10^{-4}$ mbar. The MRM transitions at m/z 402.2 $>$ 267.2, 302.3 $>$ 267.3, and 300.3 $>$ 209.2 were selected for quantification of AS, DHA and IS, respectively. The data were processed by employing MassLynx NT Software Version 4.1 (Micromass).

Standard solutions, calibration standards, and quality control samples. Stock solutions (1.0 mg/mL) of AS and DHA were prepared separately by dissolving 10 mg of each compound powder in 10 mL of acetonitrile. A 1.0-mL aliquot of AS or DHA stock solution (1.0 mg/mL) was further diluted with methanol to yield a working standard solution of 1.0 $\mu\text{g}/\text{mL}$. Conversely, a stock solution of the internal standard, IS, was prepared by dissolving 10 mg of the IS powder in 10 mL of acetonitrile and then further diluted it in methanol to yield a working standard solution of 10 $\mu\text{g}/\text{mL}$. All solutions were stored at -20°C pending analysis. The calibration curves of AS and DHA were prepared by spiking drug-free human plasma with concentrations of 1, 5, 20, 40, 100, 200, 500, 1,000, and 2,000 ng/mL. The spiked plasma samples (10 mL) were aliquoted (600 μL) into Eppendorff (Hamburg, Germany) polypropylene tubes and kept frozen at -80°C pending analysis.

Similarly, quality control samples were prepared in drug-free human plasma at concentrations of 10, 50, 300, and 1,500 ng/mL. The spiked plasma samples were aliquoted (600 μL) into Eppendorff polypropylene tubes and kept frozen at -80°C pending analysis.

Assay procedure. Before assay, frozen human plasma samples, including calibrators, QC samples or volunteer samples, were thawed at ambient temperature and then vortex-mixed for 30 seconds before extraction. The extraction procedure was carried out using Oasis[®] HLB SPE cartridges. For each sample, an SPE cartridge was conditioned with 1 mL of methanol and then equilibrated with 1 mL of 1.0 M acetic acid. A 500- μL aliquot of each plasma sample, followed by 25 μL of the IS (10 $\mu\text{g}/\text{mL}$), were loaded onto the activated cartridges and mixed gently for 30 seconds. After loading, the cartridges were then washed with 2 mL of acetic acid and 20% methanol in 1.0 M acetic acid. The AS, DHA, and the IS were then eluted with 2 mL of 40% ethyl acetate in chlorobutane under a vacuum of 5 mm of Hg into a clean glass test tube. The eluent was evaporated under stream of nitrogen gas and then reconstituted with 200 μL of mobile phase. A 100- μL aliquot of this solution was transferred

into the autosampler and 10 μ L was then injected into the LC-MS/MS system. The tandem mass assay method for simultaneous quantification of AS and DHA was developed for human plasma and fully validated. The assay method was validated for linearity, accuracy, precision, selectivity, stability, and matrix effect according to the standard methods.^{29,30}

Pharmacokinetic analysis. The pharmacokinetic parameters for AS and DHA were estimated by standard non-compartmental models. The maximum plasma concentration (C_{max}) and time needed to attain this concentration (T_{max}) were directly obtained from the drug plasma profiles; the drug plasma elimination half-life ($t_{1/2}$) values were calculated as $\ln 2/k_{el}$. Areas under the plasma concentration-time curves ($AUC_{0-\infty}$) were calculated by using the linear trapezoidal rule. The oral clearance (CL/F) was calculated as $(\text{dose}/AUC_{0-\infty})$, and the volume of distribution (Vd/F) was calculated as $(\text{Dose}/k_{el} \cdot AUC_{0-\infty})$. The pharmacokinetic computations were performed by using WinNonLin version 5.10 software (Pharsight, St. Louis, MO). Data were entered into SPSS version 17 (IBM, Armonk, NY), and a descriptive analysis was performed. The comparison of pharmacokinetic data between both groups (AS alone and AS in combination with SP) was carried out by using the Wilcoxon signed rank test. The Student's t test was used to compare demographic characteristics between men and women. These characteristics are normally distributed. The Mann-Whitney test was used to ascertain significant differences between pharmacokinetic parameters of men and women. $P < 0.05$ was considered the cut-off value for significance.

RESULTS

The mean $\pm$ SD age of the volunteers was 23.9 ± 4.1 (range = 20–35 years), the mean $\pm$ SD body weight was 67.4 ± 9.1 kg (range = 57–78 kg), and the mean $\pm$ SD height was 169.9 ± 6.5 cm (range = 158–180 cm). The mean values of age, weight, and height were not significantly different between men and women ($P > 0.05$). There were no significant side effects observed in any volunteer.

The described tandem mass spectrometric method was fully validated and proved to be sensitive, selective, and

FIGURE 1. Mean ($\pm$ SEM) plasma concentration-time profile of artesunate (AS) after a single oral dose of AS (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/tablet) to 16 healthy volunteers, Sudan.

FIGURE 2. Mean ($\pm$ SEM) plasma concentration-time profile of dihydroartemisinin after a single oral dose of artesunate (AS) (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/tablet) to 16 healthy volunteers, Sudan.

accurate for quantification of AS and its active metabolite, DHA, in human plasma samples. The linear range of the method was 1–2000 ng/mL and the lower limit of quantification for both drugs was 1 ng/mL (relative SD $< 20\%$). The precision study for AS and DHA was conducted by using quality control samples of 10, 50, 300, and 1,500 ng/mL for both drugs. The inter-run precisions were in the range of 4.4–12.6% for AS and 4.3–12.9% for DHA. The intra-run precisions were ranged between 4.2% and 9.2% for AS and 5.2% and 10.0% for DHA. The assay method exhibited lack of ion suppression/enhancement when assessed for matrix effect.

The mean $\pm$ SEM plasma concentration-time profile of AS after oral administration of AS alone and in combination with SP is shown in Figure 1. The mean $\pm$ SEM plasma concentration time profile of the active metabolite, DHA, under the same conditions described above, is shown in Figure 2. The computed pharmacokinetic parameters of AS and DHA for all volunteers are shown in Tables 1 and 2, respectively. The pharmacokinetic parameters derived for DHA assumed complete conversion of AS.³¹ As shown in Tables 1 and 2, the pharmacokinetic data for AS and DHA after AS alone or in combination with SP were similar except for T_{max} , which was significantly increased ($P < 0.05$) for AS and DHA when AS was combined with SP. The median (interquartile range) of T_{max} for AS after

TABLE 1

Pharmacokinetic parameters of artesunate (AS) after a single oral dose of AS (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/tablet), Sudan*

Parameter	AS alone, median (IQR) n = 16	AS + SP, median (IQR) n = 16	P
$t_{1/2}$ (h)	0.36, (0.24–1.18)	0.34 (0.23–0.72)	0.61
T_{max} (h)	1.0 (0.5–1.5)	1.5 (1.1–2.0)	0.02†
C_{max} (ng/mL)	65.5 (48.6–143.2)	78.0 (32.5–177.6)	0.68
$AUC_{0-\infty}$ (ng.h/mL)	94.5 (63.7–163.7)	89.7 (54.7–149.0)	0.84
Vd/F (L/kg)	24.6 (9.4–40.9)	24.2 (8.8–44.8)	0.64
CL/F (L/h/kg)	33.8 (21.6–46.5)	32.1 (19.2–54.0)	0.76

*IQR = interquartile range; $t_{1/2}$ = elimination half-life; T_{max} = time needed to attain maximum concentration; C_{max} = maximum plasma concentration; $AUC_{0-\infty}$ = areas under the plasma concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance. †Statistically significant ($P < 0.05$) by Wilcoxon matched-pair signed-rank test.

TABLE 2

Pharmacokinetic parameters of dihydroartemisinin (DHA) after a single oral dose of artesunate (AS) (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/Tablet), Sudan*

Parameter	DHA after administration of AS, median (IQR) n = 16	DHA after administration of AS + SP, median (IQR) n = 16	P
$t_{1/2}$ (h)	1.18 (0.74–2.04)	1.55 (0.84–2.0)	0.11
T_{max} (h)	1.5 (1.0–1.5)	2.0 (1.6–3.8)	0.03†
C_{max} (ng/mL)	900.1 (365.7–1,116.5)	951.5 (257.0–1,671.2)	0.30
$AUC_{0-\infty}$ (ng.h/mL)	1,364.5 (1,047.2–1,802.9)	1,738.4 (923.0–2,066.2)	0.18
Vd/F (L/kg)	4.9 (2.5–7.3)	4.8 (2.5–7.0)	0.80
CL/F (L/h/kg)	2.2 (1.7–3.3)	2.1 (1.4–2.9)	0.82

*IQR = interquartile range; $t_{1/2}$ = elimination half-life; T_{max} = time needed to attain maximum concentration; C_{max} = maximum plasma concentration; $AUC_{0-\infty}$ = areas under the plasma concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance.

†Statistically significant ($P < 0.05$) by Wilcoxon matched-pair signed-rank test.

AS alone or in combination with SP was 1.0 (0.5–1.5) hours and 1.5 (1.1–2.0) hours, respectively (Table 1). Similarly, the median (interquartile range) of T_{max} for DHA after AS alone or in combination with SP was 1.5 (1.0–1.5) hours and 2.0 (1.6–3.8) hours, respectively (Table 2).

The pharmacokinetic parameters of AS and DHA for men and women are shown in Tables 3 and 4, respectively. As shown in Table 3, the $t_{1/2}$ values of AS and DHA were significantly ($P < 0.05$) increased in women compared with those in men after AS alone or in combination with SP. In addition, after combination therapy, the AS oral clearance (CL/F) value was substantially decreased ($P < 0.05$) in women than in men. However, for AS monotherapy, the oral clearance was similar ($P > 0.05$) in men and women (Table 3). Moreover, the other pharmacokinetic parameters of AS were similar in both groups (Table 3). After AS alone, the Vd of DHA was appreciably ($P < 0.05$) higher in women than in men. However, for combination therapy, the Vd of DHA was not significantly ($P > 0.05$) different in men and women (Table 4). In addition, the other pharmacokinetic parameters of DHA were similar in both groups after AS alone or in combination with SP.

DISCUSSION

Artesunate in combination with SP is currently considered the first-line therapy for treatment of uncomplicated *P. falciparum* malaria in Sudan and worldwide. However, limited data are available on the impact of SP on the pharmacokinetics of AS and its active metabolite, DHA. The main objective of the present study was to investigate the pharmacokinetic parameters of AS and DHA in Sudanese healthy adult volunteers when an orally administered AS dose was co-administered with oral doses of SP.

After oral administration, high inter-individual variability was observed in plasma levels, as well as the pharmacokinetic parameters of AS and DHA (Figures 1 and 2 and Tables 1 and 2), which is consistent with results of previous studies.^{32,33} This finding may be caused by several factors, the most important of which is related to drug metabolism. Artesunate is presumed to undergo marked first-pass effect; thus, the variability might be caused by differences in the extent of the enzyme activity among persons.¹⁵ After AS alone or in combination with SP, it has also been observed that the C_{max} and AUC values of DHA, in the present study, exceeded that of AS (Tables 1 and 2).

The median increase in the AUC of DHA was more than 10 fold after AS alone or in combination with SP, and this

TABLE 3

Pharmacokinetic parameters of artesunate (AS) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets in healthy volunteers, Sudan*

Parameter	AS alone, median (IQR) (n = 9 men, 7 women)	P	AS + SP, median (IQR) (n = 9 men, 7 women)	P
$t_{1/2}$ (h)		0.04†		0.02†
Male	0.29 (0.18–0.46)		0.28 (0.20–0.41)	
Female	1.21 (0.29–2.29)		0.73 (0.32–2.81)	
T_{max} (h)		0.47		0.26
Male	1.0 (0.5–1.5)		1.5 (0.5–2.0)	
Female	1.0 (1.0–1.5)		2.0 (1.5–2.0)	
C_{max} (ng/mL)		0.79		0.60
Male	88.0 (60.2–223.2)		61.2 (51.9–142.5)	
Female	61.3 (47.5–141.2)		97.5 (76.9–189.0)	
$AUC_{0-\infty}$ (ng.h/mL)		0.50		0.27
Male	76.8 (53.2–139.8)		88.2 (71.7–109.1)	
Female	105.4 (76.8–181.1)		144.8 (92.0–149.6)	
Vd/F (L/kg)		0.27		0.22
Male	12.4 (7.8–41.5)		20.1 (10.3–36.0)	
Female	32.1 (21.5–59.6)		42.5 (8.2–85.9)	
CL/F (L/h/kg)		0.19		0.04†
Male	37.6 (29.8–48.2)		53.8 (35.6–58.3)	
Female	25.3 (18.7–34.9)		23.4 (18.1–28.6)	

*IQR = interquartile range; $t_{1/2}$ = elimination half-life; T_{max} = time needed to attain maximum concentration; C_{max} = maximum plasma concentration; $AUC_{0-\infty}$ = areas under the plasma concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance.

†Statistically significant ($P < 0.05$) by Mann-Whitney test.

TABLE 4

Pharmacokinetic parameters of dihydroartemisinin (DHA) after oral administration of artesunate (AS) alone and combined with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets in healthy volunteers, Sudan*

Parameter	DHA after administration of AS, median (IQR) (n = 9 men, 7 women)	P	DHA after administration of AS + SP, median (IQR) (n = 9 men, 7 women)	P
$t_{1/2}$ (h)		0.002†		0.001†
Male	0.74 (0.46–0.86)		1.07 (0.49–1.74)	
Female	2.06 (1.46–2.71)		2.22 (2.10–2.49)	
T_{max} (h)		0.86		0.87
Male	1.5 (0.5–1.5)		2.0 (1.5–4.5)	
Female	1.5 (1.0–1.5)		2.0 (2.0–4.0)	
C_{max} (ng/mL)		0.71		0.71
Male	989.7 (359.4–1,280.0)		396.3 (166.0–1,731.9)	
Female	876.2 (384.4–1,076.1)		1,096.6 (313.6–1,489.0)	
$AUC_{0-\infty}$ (ng.h/mL)		0.43		0.63
Male	1,247.1 (763.4–1,913.8)		955.8 (716.4–1,967.7)	
Female	1,475.0 (1,217.3–1,824.3)		1,881.6 (1,250.5–1,972.3)	
Vd/F (L/kg)		0.04†		0.19
Male	2.3 (0.9–3.7)		2.7 (2.0–7.1)	
Female	6.1 (4.4–8.5)		4.9 (4.8–9.0)	
CL/F (L/h/kg)		0.32		0.48
Male	2.2 (1.5–3.4)		2.8 (1.3–3.8)	
Female	2.1 (1.6–2.4)		1.7 (1.3–2.8)	

*IQR = interquartile range; $t_{1/2}$ = elimination half-life; T_{max} = time needed to attain maximum concentration; C_{max} = maximum plasma concentration; $AUC_{0-\infty}$ = areas under the plasma concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance.

†Statistically significant ($P < 0.05$) by Mann-Whitney test.

result is consistent with previously reported findings.²⁸ This finding could be caused by rapid and complete biotransformation of AS to DHA. Furthermore, the present study demonstrated that AS was rapidly absorbed and reached its peak plasma concentration within 1.0 hour and 1.5 hours when administered alone and in combination with SP, respectively. Similarly, DHA reached its peak plasma concentration 1.5 hours and 2.0 hours after AS monotherapy and in combination with SP, respectively. As demonstrated by the marked changes in T_{max} , co-administration of SP with AS substantially delayed the rate of AS oral absorption ($P = 0.02$), as well the rate of appearance of the active metabolite, DHA, ($P = 0.03$). However, the C_{max} values of the two drugs were not significantly different during AS monotherapy or in combination with SP (Tables 1 and 2). This finding was probably caused by decreased dissolution of AS when combined with SP in the gastrointestinal tract, which led to a decreased absorption rate of AS. Furthermore, the decrease in AS dissolution, as a consequence of SP combination, would probably also delay the rate of appearance of DHA. In this regard, a previous study reported that the catalytic hydroxylation of AS to DHA is pH dependent and this factor may influence the rates at which either compound are absorbed into the gastrointestinal tract.³⁴

The other pharmacokinetic parameters of AS and DHA ($t_{1/2}$, AUC, CL/F, and Vd/F) were not significantly different when AS was administered alone or in combination with SP in healthy volunteers (Tables 1 and 2). Although T_{max} median values of AS and DHA were significantly increased, there was no impact on C_{max} or AUC; the increase is thus considered as clinically irrelevant.

The findings of the present study demonstrated that after AS alone, the median value of DHA oral clearance (CL/F) was 2.2 L/kg/hour, which is consistent with that of a previous report of healthy adult volunteers, in which the result was 3.35 L/kg/hour.³⁵ Moreover, after AS alone, the median value of the Vd of DHA in our study was 4.9 L/kg, which is also consistent with the value of 4.14 L/kg in the previous

report.³⁵ Furthermore, after AS alone, the median value of $t_{1/2}$ for DHA in our study was 1.6 hours and was comparable to the values reported in previous studies with healthy volunteers (1.5–1.68 hours).^{35–37}

The lack of effect of SP on the pharmacokinetic parameters of AS and DHA could be caused by the difference in metabolic pathways of AS and SP. In this regard, AS is known to be completely hydrolyzed in the blood by tissue cholinesterases to yield its active metabolite, DHA,³⁸ and DHA is further conjugated by the UDP-glucuronosyltransferase system.³⁹ In addition, AS has been reported to be metabolized by CYP450. In this regard, CYP2A6 has been found to be the major metabolizing enzyme for AS.⁴⁰ The genetic polymorphisms of CYP2A6 may play a clinically relevant role and presumably be responsible for the inter-individual variability seen in the present study. Conversely, sulfadoxine is metabolized in the liver by conjugation, acetylation, and glucuronidation, and only 3% of the parent drug is excreted unchanged in the urine, whereas pyrimethamine is metabolized to various unidentified metabolites and finally excreted primarily in the urine.⁴¹ Thus, the prediction of drug–drug interaction involving AS in combination with SP cannot easily be predicted.

The present findings also showed that sex has an impact on the pharmacokinetic parameters of AS and DHA when AS was administered alone or in combination with SP. In this regard, our findings demonstrated that sex has a substantial effect on the $t_{1/2}$ of DHA and AS in men and women for both therapies (Tables 3 and 4). The $t_{1/2}$ values of AS and DHA were considerably high in women than in men ($P < 0.05$) and this finding could be caused by the higher values of the volume of distribution (Vd) in women than in men (Tables 3 and 4). In addition, a difference in $t_{1/2}$ could also be caused by a change in CL/F because there was a trend of higher values of Vd and lower CL/F for women than for men for both drugs (although not all of these values reached statistical significance). The other pharmacokinetic parameters were similar among the two groups (Tables 3

and 4). The Vd of DHA was significantly higher in women than in men, and this finding could be caused by the lower bioavailability or protein binding of DHA in women than in men. Also, the protein binding of AS and DHA is relatively high (75% and 93% for AS and DHA, respectively).^{24,42} Because AS and DHA are non-restrictive clearance drugs,⁴³ any change in the level of protein binding is unlikely to produce significant changes in the clearance of AS or DHA because the clearance of these drugs is dependent on hepatic blood flow and independent on the protein binding capacity of such drugs. This finding could presumably explain the unaltered clearance levels of AS (after AS alone) and DHA (after AS alone and combined therapy) in healthy men and women in the present study. However, the significant increase in CL/F values for AS in men in contrast to that in women (after combination therapy) could be caused by substantial inter-individual and intra-individual variability in the plasma levels of AS or low bioavailability (F) values of AS in men. However, the effect of low bioavailability values needs further investigation.

The main limitations of the present study were the relatively small sample size (n = 16) and the fact that the study was conducted only with healthy volunteers and did not involve patients with uncomplicated *falciparum* malaria. However, these limitations would be taken into consideration in further studies.

In conclusion, the lack of statistically significant effect on the pharmacokinetic parameters of AS and DHA in the present study may suggest effectiveness of AS combined with SP therapy. Sex had an impact on the $t_{1/2}$ of AS and DHA in both groups when AS was administered alone or in combination with SP in healthy Sudanese persons. Further investigations with a larger number of persons are warranted to assess the pharmacokinetic profiles of AS and DHA when AS is combined with SP in Sudanese patients with uncomplicated *falciparum* malaria.

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Authors' addresses: Kamal M. Matar, Department of Pharmacology and Therapeutics, Kuwait University, Kuwait, E-mail: kamal@hsc.edu.kw. Abdelmoneim I. Awad, Department of Pharmacy Practice, Kuwait University, Kuwait, E-mail: amoneim@hsc.edu.kw. Sakina B. Elamin, Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia, E-mail: sakinaba@hotmail.com.

REFERENCES

- World Health Organization, 2011. *Guidelines for the Treatment of Malaria, 2011*. Available at: http://www.who.int/malaria/world_malaria_report_2011/WMR2011_noprofiles_lowres.pdf.
- Olhario PL, Taylor WRJ, 2003. Review article: antimalarial compound from bench to bedside. *J Exp Biol* 206: 35753–35759.
- Malik EM, Khalfalla OM, 2004. Malaria in Sudan: past, present and the future. *Gezira J Health Sci I (Suppl)*: 47–51.
- Salah MT, Mohammed MM, Himeidan YE, Malik EM, Elbasher MI, Adam I, 2005. A randomized comparison of sulphadoxinepyrimethamine and combination of sulphadoxine pyrimethamine with chloroquine in the treatment of uncomplicated *falciparum* malaria in eastern Sudan. *Saudi Med J* 26: 147–148.
- van den Broek IV, Gatkoi T, Lowoko B, Nzila A, Ochong E, Keus K, 2003. Chloroquine, sulfadoxine-pyrimethamine and amodiaquine efficacy for the treatment of uncomplicated *Plasmodium falciparum* malaria in Upper Nile, south Sudan. *Trans R Soc Trop Med Hyg* 97: 229–235.
- World Health Organization, 2006. *Global Antimalarial Drug Policies database-AFRO: 2006. Antimalarial Treatment Policies for P. falciparum and P. vivax by country in WHO Africa Region*. Available Via DIALOG. Available at: http://www.who.int/malaria/amdp/amdp_afro.htm.
- Adjuik M, Babiker A, Garner P, Olhario P, Taylor W, White N, 2004. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 363: 9–17.
- Elamin SB, Awad AI, Eltayeb IB, Elmardi KA, Al Hassan AH, Mohamed AO, Malik EM, Mohamad TA, 2010. Descriptive study on the efficacy of artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in Sudan. *Eur J Clin Pharmacol* 66: 231–237.
- Haynes RK, Chan HW, Lung CM, Ng NC, Wong HN, Shek LY, Williams ID, Cartwright A, Gomes MF, 2007. Artesunate and dihydroartemisinin (DHA): unusual decomposition products formed under mild conditions and comments on the fitness of DHA as an antimalarial drug. *ChemMedChem* 2: 1448–1463.
- Davis TM, Karunajeewa HA, Ilett KF, 2005. Artemisinin-based combination therapies for uncomplicated malaria. *Med J Aust* 182: 181–185.
- Nosten F, White NJ, 2007. Artemisinin-based combination treatment of *falciparum* malaria. *Am J Trop Med Hyg* 77 (Suppl): 181–192.
- Morris CA, Duparc S, Borghini-Fuhrer I, Jung D, Shin CS, Fleckenstein L, 2011. Review of the clinical pharmacokinetics of artesunate and its active metabolite dihydroartemisinin following intravenous, intramuscular, oral or rectal administration. *Malar J* 10: 263.
- Sabchareon A, Attanath P, Chanthavanich P, Phanusaksook P, Prarinyanupharb V, Poonpanich Y, Mookmanee D, Teja-Isavadharm P, Heppner DG, Brewer TG, Chongsuphajaisiddhi T, 1998. Comparative clinical trial of artesunate in combination with mefloquine in the treatment of children with acute *falciparum* malaria. *Am J Trop Med Hyg* 58: 11–16.
- Lai CS, Nair NK, Mansor SM, Olhario PL, Navaratnam V, 2007. An analytical method with a single extraction procedure and two separate high performance liquid chromatographic systems for the determination of artesunate, dihydroartemisinin and mefloquine in human plasma for application in clinical pharmacological studies of the drug combination. *J Chromatogr B Analyt Technol Biomed Life Sci* 857: 308–314.
- Awad MI, Eltayeb IB, Baraka OZ, Behrens RH, Alkadru AM, 2004. Pharmacokinetics of artesunate following oral and rectal administration in healthy Sudanese volunteers. *Trop Doct* 34: 132–135.
- Gordi T, Nielsen E, Yu Z, Westerlund D, Ashton M, 2000. Direct analysis of artemisinin in plasma and saliva using coupled-column high-performance liquid chromatography with a restricted-access material pre-column. *J Chromatogr B Biomed Sci Appl* 742: 155–162.
- Na-Bangchang K, Congpuong K, Hung LN, Molunto P, Karbwang J, 1998. Simple high-performance liquid chromatographic method with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in biological fluids. *J Chromatogr B Biomed Sci Appl* 708: 201–207.
- Benakis A, Paris M, Loutan L, Plessas CT, Plessas ST, 1997. Pharmacokinetics of artemisinin and artesunate after oral administration in healthy volunteers. *Am J Trop Med Hyg* 56: 17–23.
- Navaratnam V, Mordi MN, Mansor SM, 1997. Simultaneous determination of artesunate and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J Chromatogr B Biomed Sci Appl* 692: 157–162.
- Batty KT, Davis TM, Thu LT, Binh TQ, Anh TK, Ilett KF, 1996. Selective high-performance liquid chromatographic determination of artesunate and alpha- and beta-dihydroartemisinin in patients with *falciparum* malaria. *J Chromatogr B Biomed Sci Appl* 677: 345–350.

21. Hanpithakpong H, Kamanikom B, Singhasivanon P, White NJ, Day NPJ, Lindegardh N, 2009. A liquid chromatographic-tandem mass spectrometric method for determination of artemether and its metabolite dihydroartemisinin in human plasma. *Bioanalysis* 1: 37–46.
22. Lindegardh N, Tarning J, Toi PV, Hien TT, Farrar J, Singhasivanon P, White NJ, Ashton M, Day NP, 2009. Quantification of artemisinin in human plasma using liquid chromatography coupled to tandem mass spectrometry. *J Pharm Biomed Anal* 49: 768–773.
23. Hanpithakpong W, Kamanikom B, Dondorp AM, Singhasivanon P, White NJ, Day NP, Lindegardh N, 2008. A liquid chromatographic-tandem mass spectrometric method for determination of artesunate and its metabolite dihydroartemisinin in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 876: 61–68.
24. Lindegardh N, Hanpithakpong W, Kamanikom B, Singhasivanon P, Socheat D, Yi P, Dondorp AM, McGready R, Nosten F, White NJ, Day NP, 2008. Major pitfalls in the measurement of artemisinin derivatives in plasma in clinical studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 876: 54–60.
25. Xing J, Yan H, Zhang S, Ren G, Gao Y, 2006. A high-performance liquid chromatography/ tandem mass spectrometry method for the determination of artemisinin in rat plasma. *Rapid Commun Mass Spectrom* 20: 1463–1468.
26. Tekete MM, Toure S, Fredericks A, Beavogui AH, Sangare CP, Evans A, Smith P, Maiga H, Traore ZI, Doumbo OK, Barnes KI, Djimde AA, 2011. Effects of amodiaquine and artesunate on sulphadoxine-pyrimethamine pharmacokinetic parameters in children under five in Mali. *Malar J* 10: 275.
27. Minzi OM, Gupta A, Haule AF, Kagashe GA, Massele AY, Gustafsson LL, 2007. Lack of impact of artesunate on the disposition kinetics of sulfadoxine/pyrimethamine when the two drugs are concomitantly administered. *Eur J Clin Pharmacol* 63: 457–462.
28. Chow SC, Wang H, 2001. On sample size calculation in bioequivalence trials. *J Pharmacokinet Pharmacodyn* 28: 155–169.
29. Peters F, Drummer O, Musshoff F, 2007. Validation of new methods. *Forensic Sci Int* 165: 216–224.
30. Matuszewski B, Constanzer M, Chavez-Eng C, 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem* 75: 3019–3030.
31. Lee IS, Hufford CD, 1990. Metabolism of antimalarial sesquiterpene lactones. *Pharmacol Ther* 48: 345–355.
32. Simpson JA, Agbenyega T, Barnes KI, Di Perri G, Folb P, Gomes M, Krishna S, Krudsood S, Looareesuwan S, Mansor S, McIlerron H, Miller R, Molyneux M, Mwenechanya J, Navaratnam V, Nosten F, Olhario P, Pang L, Ribeiro I, Tembo M, van Vugt M, Ward S, Weerasuriya K, Win K, White NJ, 2006. Population pharmacokinetics of artesunate and dihydroartemisinin following intra-rectal dosing of artesunate in malaria patients. *PLoS Med* 3: e444.
33. Bethell DB, Teja-Isavadharm P, Cao XT, Pham TT, Ta TT, Tran TN, Nguyen TT, Pham TP, Kyle D, Day NP, White NJ, 1997. Pharmacokinetics of oral artesunate in children with moderately severe *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 91: 195–198.
34. Olhario PL, Nair NK, Sathasivam K, Mansor SM, Navaratnam V, 2001. Pharmacokinetics of artesunate after single oral administration to rats. *BMC Pharmacol* 1: 1–4.
35. Teja-Isavadharm P, Watt G, Eamsila C, Jongsakul K, Li Q, Keeratithakul G, Sirisopana N, Luesutthiviboon L, Brewer TG, Kyle DE, 2001. Comparative pharmacokinetics and effect kinetics of orally administered artesunate in healthy volunteers and patients with uncomplicated falciparum malaria. *Am J Trop Med Hyg* 65: 717–721.
36. Navaratnam V, Ramanathan S, Wahab MS, Siew Hua G, Mansor SM, Kiechel JR, Vaillant M, Taylor WR, Olhario P, 2009. Tolerability and pharmacokinetics of non-fixed and fixed combinations of artesunate and amodiaquine in Malaysian healthy normal volunteers. *Eur J Clin Pharmacol* 65: 809–821.
37. Zhang SQ, Hai TN, Ilett KF, Huong DX, Davis TM, Ashton M, 2001. Multiple dose study of interactions between artesunate and artemisinin in healthy volunteers. *Br J Clin Pharmacol* 52: 377–385.
38. Gautam A, Ahmed T, Batra V, Paliwal J, 2009. Pharmacokinetics and pharmacodynamics of endoperoxide antimalarials. *Curr Drug Metab* 10: 289–306.
39. Ilett KF, Ethell BT, Maggs JL, Davis TM, Batty KT, Burchell B, Binh TQ, Thu le TA, Hung NC, Pirmohamed M, Park BK, Edwards G, 2002. Glucuronidation of dihydroartemisinin *in vivo* and by human liver microsomes and expressed UDP-glucuronidation-syltransferases. *Drug Metab Dispos* 30: 1005–1012.
40. Li XQ, Björkman A, Andersson TB, Gustafsson LL, Masimirembwa CM, 2003. Identification of human cytochrome P(450)s that metabolise anti-parasitic drugs and predictions of *in vivo* drug hepatic clearance from *in vitro* data. *Eur J Clin Pharmacol* 59: 429–442.
41. Gutman J, Kachur S, Slutsker L, Nzila A, Mutabingwa T, 2012. Combination of probenecid-sulphadoxine-pyrimethamine for intermittent preventive treatment in pregnancy. *Malar J* 11: 39.
42. Li Q, Xie LH, Haeberle A, Zhang J, Weina P, 2006. The evaluation of radiolabeled artesunate on tissue distribution in rats and protein binding in humans. *Am J Trop Med Hyg* 75: 817–826.
43. Batty KT, Ilett KE, Powell SM, Martin J, Davis TM, 2002. Relative bioavailability of artesunate and dihydroartemisinin: investigations in the isolated perfused rat liver and in healthy Caucasian volunteers. *Am J Trop Med Hyg* 66: 130–136.