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*CYP2B6**6 Genotype Specific Differences in Artemether-Lumefantrine Disposition in Healthy Volunteers

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Abstract

Cytochrome P450 2B6 (CYP2B6) is involved in the metabolism of the antimalarial drugs artemether and lumefantrine. Here we investigated the effect of *CYP2B6**6 on the plasma pharmacokinetics of artemether, lumefantrine, and their metabolites in healthy volunteers. Thirty healthy and previously genotyped adult volunteers—15 noncarriers (*CYP2B6*1/*1*) and 15 homozygote carriers (*CYP2B6*6/*6*)—selected from a cohort of 150 subjects from the Ilorin metropolitan area were administered the complete 3-day course of artemether and lumefantrine (80 and 480 mg twice daily, respectively). Intensive pharmacokinetic sampling was conducted at different time points before and after the last dose. Plasma concentrations of artemether, lumefantrine, dihydroartemisinin, and desbutyllumefantrine were quantified using validated liquid chromatography–mass spectrometric methods. Pharmacokinetic parameters were evaluated using noncompartmental analysis. Artemether clearance of *CYP2B6*6/*6* volunteers was nonsignificantly lower by 26% (ratios of geometric mean [90% CI]; 0.74 [0.52-1.05]), and total exposure (the area under the plasma concentration-time curve from time 0 to infinity [$AUC_{0-\infty}$]) was greater by 35% (1.35 [0.95-1.93]) when compared with those of **1/*1* volunteers. Similarly, assuming complete bioconversion from artemether, the dihydroartemisinin $AUC_{0-\infty}$ was 22% lower. On the contrary, artemether-to-dihydroartemisinin $AUC_{0-\infty}$ ratio was 73% significantly higher (1.73 [1.27-2.37]). Comparison of lumefantrine exposure and lumefantrine-to-desbutyllumefantrine metabolic ratio of **6/*6* with corresponding data from **1/*1* volunteers showed no differences. The increased artemether-to-dihydroartemisinin metabolic ratio of **6/*6* volunteers is unlikely to result in differences in artemether-lumefantrine efficacy and treatment outcomes. This is the first study in humans to associate *CYP2B6*6* genotype with artemether disposition.

Keywords

*CYP2B6*6*, genotype, malaria, artemether disposition, lumefantrine disposition

Cytochrome P450s (CYPs) are a diverse superfamily of enzymes capable of metabolizing a wide variety of endogenous and xenobiotic substances including drug molecules.[1] CYPs are the predominant phase 1 enzymes and the most important catalysts among all drug-metabolizing enzymes responsible for the oxidative biotransformation of drugs.[2] One of the 15 human CYPs that are commonly involved in the biotransformation of drugs and other xenobiotics is CYP2B6.[3] Initially considered as an insignificant portion of total hepatic CYP content with minor contribution to drug metabolism, the discovery of polymorphisms in its expression and evidence of co-regulation with CYP3A4 have stimulated a renewed interest in the drug-metabolizing enzyme.[4–8] With an estimated 8% relative contribution to CYP-mediated drug metabolism, CYP2B6 is capable of metabolizing 25% to 30% of known clinical drug substrates for CYP3A4.[9,10]

Genetic factors are known to account for an estimated 20% to 95% of variability in drug disposition and effects.[11–13] Most of the observed variations

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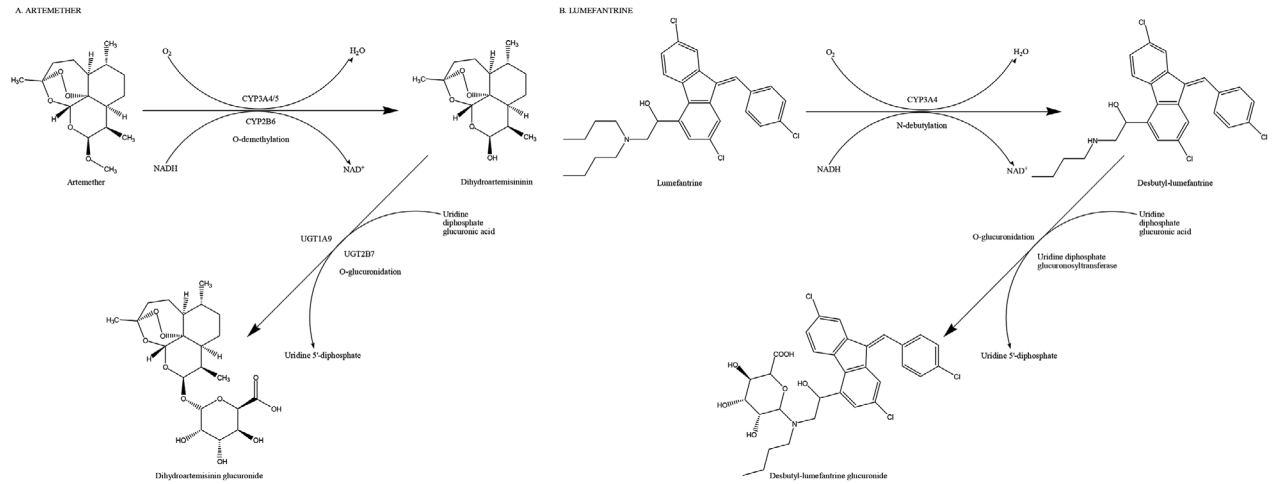

Figure 1. Major metabolic pathways of (A) artemether and (B) lumefantrine depicting the phase 1 and phase 2 metabolic reactions catalyzed by cytochrome P450 (CYP) and uridine diphosphate-α-glucuronosyltransferase (UGT) enzymes, respectively.

in DNA sequence (genetic polymorphisms) are due to single base-pair substitutions/mutations known as single-nucleotide polymorphisms.[12,14] Genetic polymorphisms can cause alterations in the amount, activity, structure, binding, and/or function of drug-metabolizing enzymes and transporters, thus potentially affecting their efficacy and toxicity.[15] *CYP2B6* polymorphisms are among the major contributors to interindividual variability in CYP2B6 expression, activity, and pharmacokinetics of drug substrates including the antimalarial artemisinin.[5,7]

Artemisinin-based combination therapies are recommended by the World Health Organization as the first- and second-line treatment for uncomplicated *Plasmodium falciparum* malaria and chloroquine-resistant *Plasmodium vivax* malaria.[16] Artemether-lumefantrine is a first-line choice, with 3-day regimens for the treatment of children and adults (except pregnant women in their first trimester).[16] It combines an artemisinin derivative artemether with a partner drug, lumefantrine. Artemether reduces the number of parasites during the first 3 days of treatment (reduction of parasite biomass), and lumefantrine eliminates the remaining parasites (cure).[17]

Artemether is rapidly and extensively demethylated (both in vitro and in humans) to the biologically active main metabolite dihydroartemisinin, predominantly through CYP3A4/5.[18–20] However, a previous study has shown that CYP2B6 has a more important role in the demethylation of artemether than CYP3A4/5 (Figure 1A).[21] Dihydroartemisinin is converted to inactive metabolites via glucuronidation catalyzed by UDP-glucuronosyltransferases (UGTs), mainly UGT1A9 and 2B7.[22] Lumefantrine is N-debutylated, mainly by CYP3A4, to desbutyllumefantrine followed by glucuronidation in human liver microsomes (Figure 1B).[23]

To date, clinical studies that have investigated the influence of host genetics on the pharmacokinetics of artemether-lumefantrine are sparse. Apart from the study of the influence *CYP2B6*6* genotype on artemether-lumefantrine disposition in Cambodian and Tanzanian malaria patients, which reported nonsignificant association in both drugs, subsequent studies have only reported the impact of host genetics on day-7 plasma lumefantrine levels.[24–27] A nonsignificant correlation of *CYP2B6*6* genotype with day-7 plasma lumefantrine concentration in HIV-malaria–coinfected patients was reported by Maganda et al.[25] Furthermore, Mutagonda et al investigated the impact of pharmacogenetics on day-7 plasma lumefantrine concentration in pregnant Tanzanian malaria patients.[26] Composite *CYP2B6*6/*18* genotype was nonsignificantly related to day-7 plasma lumefantrine concentrations.[26] However, in another recent study *ABCC2*14* genotype was found to significantly influence day-7 lumefantrine level.[27] Therefore, the present study investigated the effect of the common *CYP2B6*6* (c.516G>T, Q172H, and c.785A>G, K262R) variant, which is found mainly in populations of African descent, on the plasma artemether and lumefantrine pharmacokinetics in retroviral-negative Nigerian volunteers without clinical malaria.

Methods

Approval for the study was granted by Obafemi Awolowo University Teaching Hospitals Complex Health Research Ethics Committee, and the study was conducted in accordance with the guidelines of the Helsinki Declaration of 2013.[28] Participants were enrolled in the study only after obtaining written informed consent. Demographic information of each subject was obtained and recorded anonymously.

Sample Population and Study Design

Thirty healthy volunteers without clinical malaria from a cohort of 150 retrovirus-negative subjects previously recruited from Ilorin metropolis (North Central Nigeria) and genotyped for their *CYP2B6*6* status were divided into 2 groups of 15 each with *CYP2B6*1/*1* and **6/*6* genotypes.[29] Participants were eligible for the study if they were at least 18 years old and able to understand the study information and excluded from the study for any of the following reasons: (1) those who took artemether-lumefantrine or other artemisinin-based combination therapies 30 days or less before the start of the pharmacokinetic study; (2) hypersensitivity to artemether derivatives or lumefantrine; (3) inability to comply with the dosing regimen and scheduled follow-up plan; (4) pregnancy; (5) breastfeeding; (6) use of substances or drugs known to inhibit or induce or known to be substrates of CYP enzymes (eg, tobacco, alcohol, antihypertensive or antidiabetic agents). Other reasons for exclusion were a history of acute or chronic illnesses including hypertension, diabetes mellitus, psychiatric illness, and renal or hepatic impairment.

Drug Treatment, Sample Collection, and Storage

Each participant received 6 doses of artemether (80 mg) and lumefantrine (480 mg) as per standard recommendations (ie, at 0, 8, 24, 36, 48, and 60 hours). The sixth dose was administered with a high-fat meal. Approximately 5-mL blood samples were collected in lithium heparinized plasma separating tubes just before and at 0.5, 1, 2, 3, 4, 6, 8, 24, 72, 96, 168, and 336 hours after the last dose. The blood samples from each of the timepoints were centrifuged at 3000 *g* for 10 minutes, and the plasma was stored at −80°C until analysis. Plasma samples were shipped in dry ice to the Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand for drug quantification.

Drug Quantification and Pharmacokinetic Analysis

Quantification of artemether, dihydroartemisinin, lumefantrine and desbutyllumefantrine in plasma samples was performed using liquid chromatography tandem mass spectrometry methods previously validated in line with US FDA guidelines.[30–32] Calibration ranges of 1.14-575 ng mL^{-1} for artemether, dihydroartemisinin, and desbutyllumefantrine and 7.77-23 000 ng mL^{-1} for lumefantrine were reported. The lower limits of quantification were 1.43 ng mL^{-1} for artemether and dihydroartemisinin, 1.01 ng mL^{-1} for desbutyllumefantrine, and 9.71 ng mL^{-1} for lumefantrine.

Pharmacokinetic analysis was performed with Kinetica (InnaPhase Corporation, Philadelphia, Pennsylvania) version 4.1 using a standard noncompartmental approach. Pharmacokinetic parameters such as peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the area under the concentration-time curve from time 0 over the time span of the dosing interval ($AUC_{0\text{-}8}$ for artemether and dihydroartemisinin and $AUC_{0\text{-}336}$ for lumefantrine and desbutyllumefantrine), total AUC from time 0 to infinity ($AUC_{0\text{-}\infty}$), half-life of the terminal disposition phase ($t_{½}$), ratio of dose administered to AUC, which is oral clearance (CL/F), apparent oral volume of distribution, and the metabolic ratio of parent drug-to-metabolite were calculated from the plasma concentration-time data of 0-8 hours for artemether and dihydroartemisinin and from 0 to 336 hours for lumefantrine and desbutyllumefantrine. Artemether and lumefantrine were assumed to be fully transformed into dihydroartemisinin and desbutyllumefantrine in vivo, and the relative difference of their respective molecular weights (ie, dihydroartemisinin/artemether or desbutyllumefantrine/lumefantrine) was used to calculate the putative dose of administered dihydroartemisinin (76.24 mg) and desbutyllumefantrine (429.08 mg).[18]

Statistical Analyses

Mean (SD) range was used to describe participants' characteristics. Pharmacokinetic parameters are presented as geometric mean (SD). Comparison of pharmacokinetic parameters between *CYP2B6*6/*6* and **1/*1* volunteers was evaluated using independent-sample t-test and according to EMA guidelines.[33] Differences between parameters were evaluated by ratios of geometric mean and 90% CI using log-transformed data expressed as linear values. Differences were considered significant if the 90% CI did not include 1. All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM, Armonk, New York), and GraphPad Prism version 6.01 (GraphPad Software Inc, San Diego, California) was used to produce figures.

Results

Baseline Characteristics of Sampled Participants

Briefly, the average age (SD) of the full cohort of 150 retroviral negative volunteers was 30.6 years (11.8) with a body mass index (standard deviation) of 23.1 kg/m2 (4.6). The genotype number (frequency) of the *CYP2B6*1/*1* volunteers was 58 (38.7%) while that of **6/*6* volunteers was 17 (11.3%) with majority of the volunteers being male (number [%]: 94 [62.7%]).[29] The mean age, weight, body mass index (SD), and sex distribution of *CYP2B6*1/*1* versus **6/*6* groups were not significantly different, as presented in Table 1 for all the 30 volunteers recruited from the full cohort.

Table 1. Baseline Characteristics of the Volunteers Based on *CYP2B6*6* Metabolic Status at the Time of Intensive Pharmacokinetic Sampling

Characteristics	*CYP2B6*1/*1*	*CYP2B6*6/*6*	*P*-Value
Age (y)	25.9 (9.0) 26.0	27.1 (10.2) 37.0	.823
Weight (kg)	61.1 (9.2) 32.1	59.9 (8.2) 34.1	.871
BMI (kg/m^2)	21.7 (3.7) 11.7	21.8 (1.8) 7.4	.969
Sex [male n (%)]	9 (60)	11 (73)	.700

n indicates sample size.
Age, weight, and body mass index (BMI) are presented as mean (SD) range; sex as number (%) of volunteers.

Table 2. Comparison of Artemether and Dihydroartemisinin Pharmacokinetics Between *CYP2B6*1/*1* and **6/*6* Healthy Volunteers

PK Parameters	*CYP2B6*1/*1* (n = 10)	*CYP2B6*6/*6* (n = 12)	RoGM (90% CI)	*P*-Value
Artemether				
C_{max} (ng/mL)	27.4 (14.6)	40.7 (13.1)	1.49 (1.10-2.00)	.055
T_{max} (h)	1.7 (0.4)	1.4 (0.9)	0.77 (0.54-1.10)	.483
AUC_{0-8} (ng·h/mL)	71 (26)	97 (53)	1.37 (0.96-1.92)	.074
$AUC_{0-\infty}$ (ng·h/mL)	75 (27)	102 (57)	1.36 (0.95-1.93)	.078
$t_{½}$ (h)	1.6 (0.5)	1.4 (0.6)	0.90 (0.68-1.19)	.618
CL/F (L/h)	1064 (391)	787 (584)	0.74 (0.52-1.05)	.355
V_z/F (L)	2406 (1 242)	1604 (980)	0.67 (0.48-0.93)	.092
Dihydroartemisinin				
C_{max} (ng/mL)	56.3 (30.5)	51.2 (21.5)	0.91 (0.64-1.29)	.463
T_{max} (h)	1.9 (0.9)	1.7 (1.1)	0.85 (0.55-1.32)	.819
AUC_{0-8} (ng·h/mL)	156 (76)	120 (62)	0.77 (0.54-1.10)	.187
$AUC_{0-\infty}$ (ng·h/mL)	160 (79)	125 (67)	0.78 (0.54-1.12)	.221
$t_{½}$ (h)	1.2 (0.3)	1.3 (0.7)	1.07 (0.82-1.39)	.508
Metabolic ratio[a]	0.5 (0.2)	0.8 (0.4)	1.73 (1.27-2.37)	.011

AUC indicates area under the concentration-time curve; CL/F, oral clearance; C_{max}, peak concentration; PK, pharmacokinetics; RoGM, ratio of geometric means; T_{max}, time to reach C_{max}; $t_{½}$, half-time of terminal elimination; V_z/F, apparent volume of distribution.
[a]Metabolic ratio of parent drug to metabolite.

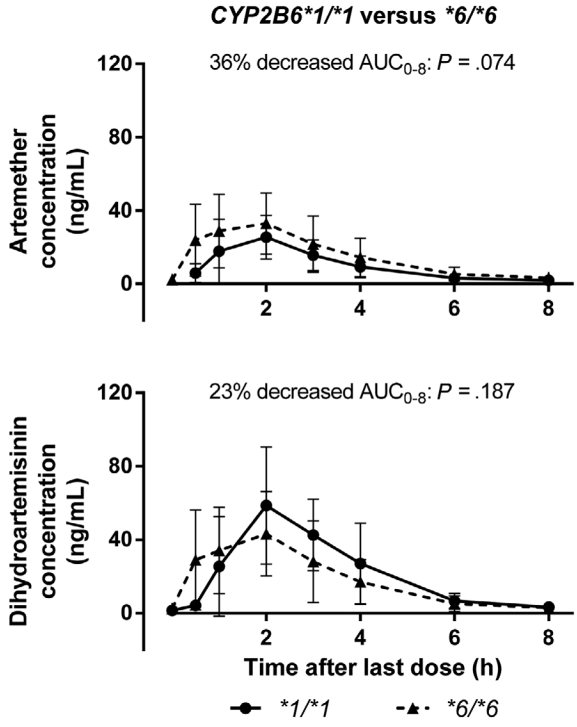

Figure 2. Mean (SD) plasma artemether and dihydroartemisinin concentration-time profiles of *CYP2B6*1/*1* vs **6/*6* volunteers.

Influence of *CYP2B6*6* Genotype on Artemether and Dihydroartemisinin Disposition

A total of 303 plasma samples from the participants were analyzed for artemether and its main active metabolite (dihydroartemisinin), out of which 86 (28.4%) artemether and 74 (24.4%) dihydroartemisinin samples from **6/*6* volunteers were quantified to be below the assay lower limit of quantification (LLOQ). To avoid potential bias from this unbalanced data censoring between groups, the first individual LLOQ sample in the terminal elimination phase was replaced with LLOQ/2, and the rest of the LLOQ data were omitted. Both artemether and dihydroartemisinin showed large interindividual variability in pharmacokinetic parameters. A 36% and 49% nonsignificant higher plasma artemether total exposure ($AUC_{0-\infty}$) and maximum level (C_{max}), respectively, were observed when values of *CYP2B6*6/*6* volunteers were compared with those of **1/*1* volunteers as demonstrated by the 26% reduction in CL/F (Table 2). Similarly, dihydroartemisinin $AUC_{0-\infty}$ was nonsignificantly lower by 22%. The plasma concentration-time profiles as depicted in Figure 2 summarized the effects of *CYP2B6*6* genotype on artemether and dihydroartemisinin exposures. However, the metabolic ratio of artemether to dihydroartemisinin using $AUC_{0-\infty}$ was significantly greater by 73% (ratio of geometric mean [90% CI] 1.73 [1.27-2.37]). In addition, although artemether and dihydroartemisinin oral clearances correlated to a high degree in **1/*1* volunteers ($r = -0.783$; $P = .022$), the reverse was the case in **6/*6* ($r = -0.370$; $P = .237$) volunteers. In the same vein, Person correlation coefficients between artemether and dihydroartemisinin total exposures showed a strong positive correlation in **1/*1* ($r = 0.658$; $P = .002$) but a weak positive correlation in **6/*6* ($r = 0.185$; $P = .386$) genotype group.

Influence of *CYP2B6*6* Genotype on Lumefantrine and Desbutyllumefantrine Disposition

Comparison of lumefantrine pharmacokinetic parameters indicated no changes with the exception of the 20% higher T_{max} of *CYP2B6*6/*6* over **1/*1* volunteers as illustrated in Table 3. Similarly, there were no changes in desbutyllumefantrine parameters as presented in Table 3. Plasma concentration-time profiles of lumefantrine and desbutyllumefantrine of **6//*6* versus **1/*1* volunteers are presented in Figure 3. Lumefantrine-to-desbutyllumefantrine metabolic ratio

Table 3. Comparison of Lumefantrine and Desbutyllumefantrine Baseline Pharmacokinetics Between *CYP2B6*1/*1* and **6/*6* Healthy Volunteers

PK Parameters	*CYP2B6*1/*1* (n = 15)	*CYP2B6*6/*6* (n = 14)	RoGM (90% CI)	*P*-Value
Lumefantrine				
C_{max}(ng/mL)	10 023 (2685)	11 001 (3689)	1.10 (0.91-1.32)	.321
T_{max}(h)	5.9 (1.2)	4.7 (1.0)	0.80 (0.68-0.93)	.022
$AUC_{0\text{-}336}$ (ng·h/mL)	397 477 (114 661)	417 748 (141 396)	1.05 (0.86-1.28)	.563
$AUC_{0\text{-}\infty}$ (ng·h/mL)	431 403 (122 776)	451 892 (155 957)	1.05 (0.86-1.27)	.569
$t_{½}$ (h)	104 (30)	107 (20)	1.03 (0.90-1.20)	.863
CL/F (L/h)	1.1 (0.3)	1.1 (0.4)	0.95 (0.78-1.16)	.840
V_z/F (L)	161 (59)	165 (63)	1.02 (0.83-1.26)	.852
$C_{d\text{-}6}$ (ng/mL)	1203 (348)	1216 (464)	1.01 (0.82-1.25)	.785
$C_{d\text{-}10}$ (ng/mL)	511 (156)	536 (168)	1.05 (0.85-1.29)	.614
Desbutyllumefantrine				
C_{max} (ng/mL)	96 (112)	93 (164)	0.97 (0.60-1.54)	.909
T_{max} (h)	5.1 (2.8)	6.4 (5.2)	1.25 (0.78-2.00)	.359
$AUC_{0\text{-}336}$ (ng·h/mL)	9710 (4525)	10 147 (7451)	1.04 (0.76-1.43)	.562
$AUC_{0\text{-}\infty}$ (ng·h/mL)	12 112 (5534)	11 947 (8739)	0.99 (0.72-1.35)	.739
$t_{½}$ (h)	145 (42)	124 (22)	0.86 (0.73-1.01)	.051
$C_{d\text{-}6}$ (ng/mL)	36.9 (15.7)	40.5 (27.0)	1.10 (0.81-1.48)	.405
$C_{d\text{-}10}$ (ng/mL)	21.2 (8.3)	22.4 (14.2)	1.05 (0.79-1.41)	.542
Metabolic ratio[a]	35.6 (15.1)	37.8 (17.6)	1.06 (0.80-1.41)	.683

$AUC_{0\text{-}8}$ indicates area under the concentration time curve from 0 to 8 hour; $AUC_{0\text{-}\infty}$, AUC curve extrapolated to infinity; $C_{d\text{-}6}$ and $C_{d\text{-}10}$, plasma concentrations on days 6 and 10; CL/F, oral clearance; C_{max}, maximum concentration; PK, pharmacokinetics; RoGM, ratio of geometric means; T_{max}, time to reach C_{max}; $t_{½}$, half-time of terminal elimination; V_z/F, apparent volume of distribution.

All data are presented as geometric mean (SD). Differences in parameters were assessed by ratios of geometric means (RoGM), that is, **6/*6/*1/*1* and 90% CI.

[a]Metabolic ratio of parent drug to metabolite.

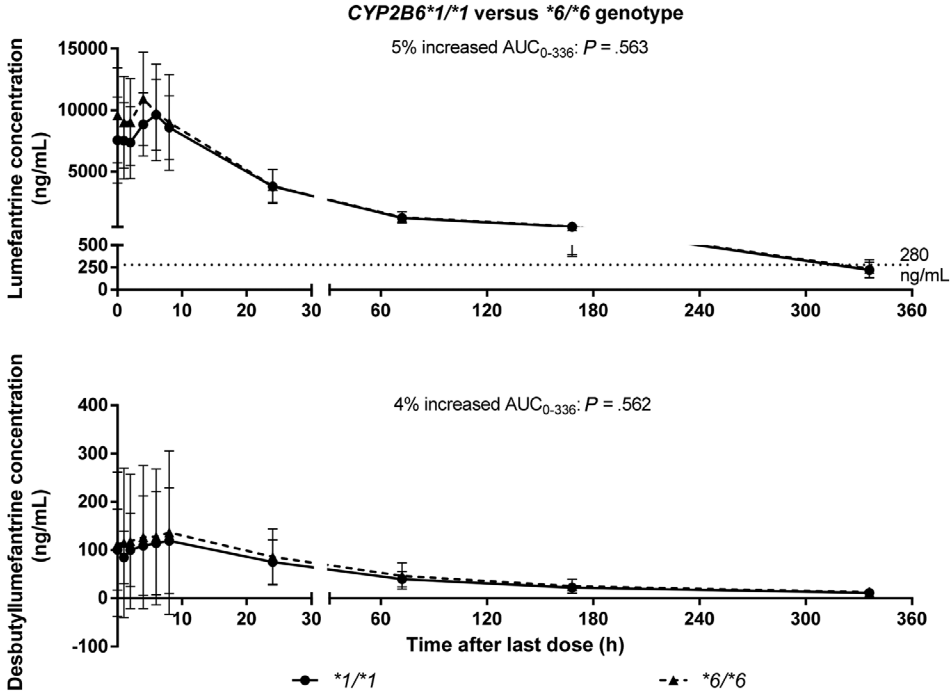

Figure 3. Mean (SD) plasma lumefantrine and desbutyllumefantrine concentration-time profiles of *CYP2B6*1/*1* vs **6/*6* volunteers: 280 ng/mL is lumefantrine threshold value as depicted by the dashed straight line.

of *CYP2B6*6/*6* volunteers compared with **1/*1* volunteers also showed no change. Additionally, lumefantrine CL/F was strongly correlated with desbutyllumefantrine CL/F in both **1/*1* (r = 0.630; P = .0016) and **6/*6* (r = 0.731; P = .005) volunteers. Correlation coefficients also indicated strong negative correlations between lumefantrine and desbutyllumefantrine total exposures in both genotype groups (ie, r = –0.932, $P < .001$ for **1/*1*, and r = –0.908; $P < .001$ for **6/*6*).

An overall summary of the comparisons by genotypes is depicted by the scatter plots of $AUC_{0\text{-}\infty}$ as well

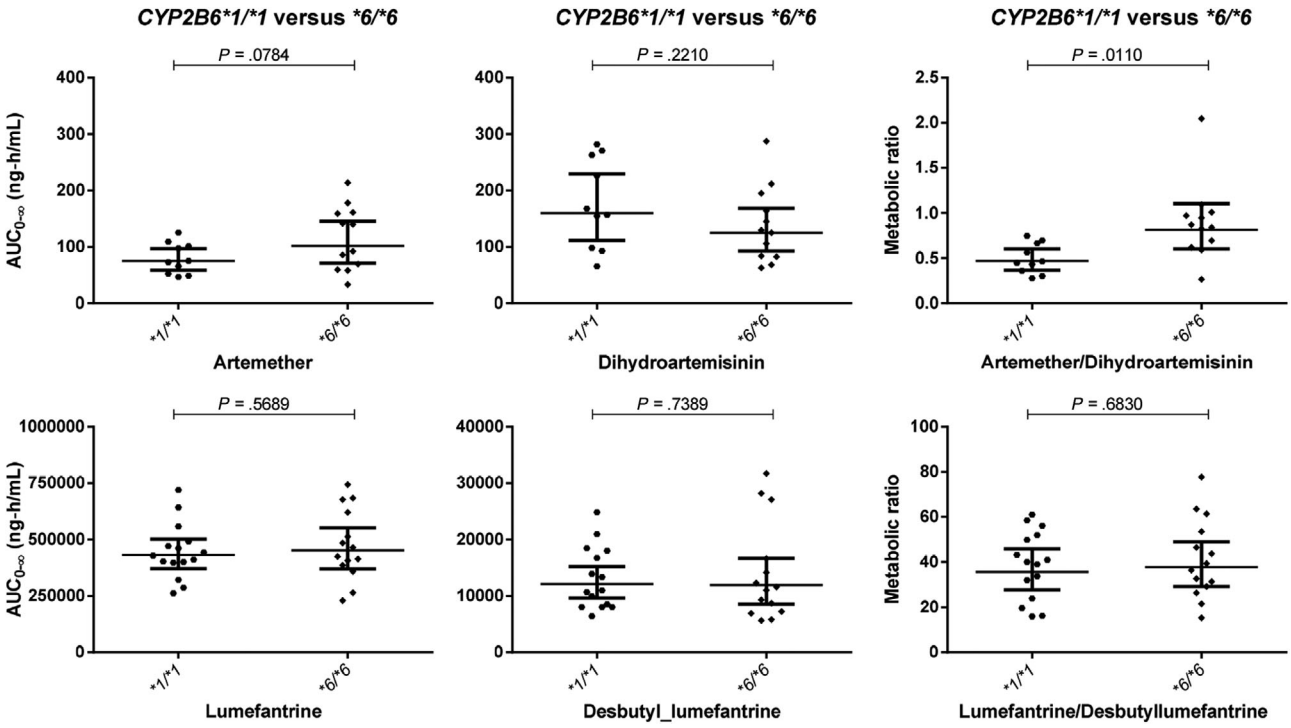

Figure 4. Geometric mean (95% CI) scatter plots of area under the plasma concentration-time curves from time 0 to infinity ($AUC_{0-\infty}$) of artemether, lumefantrine, and their respective metabolites, as well as metabolic ratios of parent drug to metabolites of *CYP2B6*1/*1* vs **6/*6* genotype.

as ratio of parent drug to metabolite of both drugs (Figure 4). Artemether-lumefantrine was well tolerated, and there were no reports of any serious adverse events from the participants.

Discussion

Data from this study implicate CYP2B6 in the biotransformation of artemether to dihydroartemisinin as demonstrated by (1) the significant increase in artemether-to-dihydroartemisinin metabolic ratio of *CYP2B6*6/*6* volunteers over their **1/*1* counterparts and (2) the weak correlations of artemether elimination clearances as well as total exposures with dihydroartemisinin clearances and exposures in volunteers with **6/*6* genotype, in contrast to the good correlations seen in **1/*1* volunteers. It is also in contrast to the strong correlations of lumefantrine clearances and exposures with desbutyllumefantrine clearances and exposures observed in both **1/*1* and **6/*6* volunteers, an indication of a lack of association of CYP2B6 with lumefantrine disposition. This is the first study in humans to establish association of the *CYP2B6*6* genotype with artemether pharmacokinetics following Honda et al's in vitro screening of 14 recombinant CYPs and identification of CYP2B6 as the primary enzyme responsible for the metabolism of artemether to dihydroartemisinin.[21] The present study was not adequately powered to detect significant differences in the other pharmacokinetic parameters due to the smaller sample sizes available for the final artemether and dihydroartemisinin statistical analysis (**1/*1* [n = 10] and **6/*6* [n =12]) compared with lumefantrine and desbutyllumefantrine analysis (**1/*1* [n = 15] and **6/*6* [n =14]). Nevertheless, metabolic ratios are good sensitive markers to detect the effects of the *CYP2B6* genotype on artemether and lumefantrine pharmacokinetics.[34]

Furthermore, apart from the small sample size, the nonsignificant differences in the plasma artemether clearance and total exposure of **6/*6* compared with **1/*1* volunteers may imply a lesser extent of CYP2B6 involvement in artemether biotransformation compared with CYP3A. Artemisinin derivatives are known to be metabolized through different pathways. Artemisinin itself is primarily metabolized by CYP2B6, followed by CYP2A6 and 3A4,[35] whereas artemether, like arteether, is primarily metabolized by CYP3A4/5 with a secondary contribution of CYP2B6.[36–38] Unlike the Honda et al study on human liver microsomes, van Agtmael et al's clinical studies, which identified CYP3A4 as the main enzyme of artemether demethylation, as in the present study, were carried out in healthy male volunteers.[36,37] Thus, the biotransformation of artemether to dihydroartemisinin through the major CYP3A pathways may have masked any detection of significant relationship of the other pharmacokinetic parameters with the secondary CYP2B6 pathway.

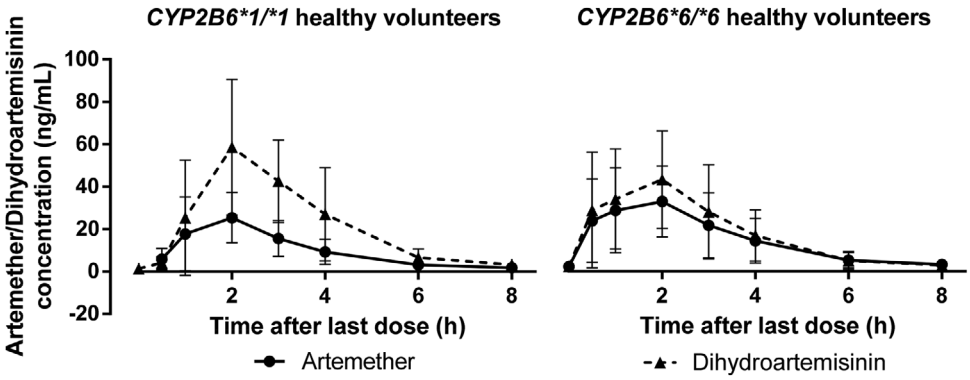

Figure 5. Mean (SD) plasma artemether vs dihydroartemisinin concentration-time profiles of *CYP2B6*1/*1* and **6/*6* healthy volunteers.

The artemether and dihydroartemisinin pharmacokinetic parameters obtained in the wild-type (*CYP2B6*1/*1*) volunteers are comparable to those reported by Djimdé and Lefèvre for healthy volunteers.[39] Artemether C_{max} (27.4 versus 30.8 ng/mL), $AUC_{0\text{-}8}$ (71.0 versus 61.4 ng·h mL^{-1}) and $t_{½}$ (1.6 versus 2.0 hours), and dihydroartemisinin C_{max} (59.3 versus 84.5 ng mL^{-1}), $AUC_{0\text{-}336}$ (156 versus 178 ng·h mL^{-1}) and $t_{½}$ (1.2 versus 1.2 hours) were relatively comparable. Moreover, the observed relationships between artemether and dihydroartemisinin exposures in both **1/*1* and **6/*6* volunteers agree with previous data that dihydroartemisinin paralleled the pharmacokinetics of artemether and reached a higher C_{max} and $AUC_{0\text{-}8}$,[37] as demonstrated in plasma concentration-time profiles of artemether/dihydroartemisinin (Figure 5).

Lumefantrine has a longer elimination half-life, which helps to eradicate residual parasites and protect the host against recurrent infection.[40] However, it has a relatively short half-life compared with desbutyllumefantrine,[39] as observed in the present study (ie, 104 hours versus 145 hours for **1/*1* volunteers). Although desbutyllumefantrine has substantial antimalarial activity and is more potent, the predominant antimalarial effect is provided by lumefantrine.[41] Day-7 plasma lumefantrine concentration provides a simple measure of lumefantrine exposure as a surrogate marker and is associated with treatment response.[40] Decreased day-7 plasma lumefantrine concentration is likely to increase the risk of treatment failure and emergence of drug resistance.[40,42] Threshold values of 280 ng mL^{-1} and 175 ng mL^{-1} after first dose have been set.[43–45] Although data on day-7 plasma lumefantrine concentrations after the first dose were not available to permit direct comparison to threshold values, the available study day-6 ($C_{day\text{-}6}$) and day-10 ($C_{day\text{-}10}$) lumefantrine concentrations post first dose were far above the cut-off value of 280 ng mL^{-1} in both genotype groups (Figure 3). Moreover, the observed lumefantrine pharmacokinetic parameters of **1/*1* volunteers are also comparable to the parameters reported by Djimdé and Lefèvre for healthy volunteers.[39] Lumefantrine C_{max} (10 023 versus 10 000 ng mL^{-1}), T_{max} (5.9 versus 6.0 hours), and $AUC_{0\text{-}336}$ (397 477 versus 383 000 ng·h mL^{-1}) are relatively comparable.

Large interindividual variabilities in the pharmacokinetic profiles, response, and toxicity of the artemisinin drugs have been reported, and polymorphisms in drug-metabolizing enzymes and transport proteins are among the major contributors.[46] However, genetic variability on its own may be complex due to the contribution of various host genetic factors. For instance, both CYP2B6 and UGT2B7, which are involved in the phase 1 and phase 2 biotransformation pathways of artemether, are highly polymorphic and commonly observed in malaria-endemic regions, particularly in African countries where *CYP2B6*6* and *UGT2B7*2* are highly prevalent. Approximately 33% of West Africans are carriers of both variant alleles, and polymorphisms in their enzymes may contribute to the variability in the metabolism and efficacy of artemether.[47] Food intake, sex, and disease state are the other nongenetic factors that could affect artemether pharmacokinetic variability.[18]

Dihydroartemisinin and lumefantrine are the 2 major determinants of antimalarial treatment outcome. Decreased artemether-to-dihydroartemisinin metabolic ratio and increased lumefantrine-to-desbutyllumefantrine ratio are expected to favor clinical outcome in malaria patients receiving treatment. Unlike lumefantrine, the cutoff value for dihydroartemisinin exposure or minimum effective concentration has not been reported, but the nonsignificant difference in the lumefantrine-to-desbutyllumefantrine metabolic ratio of **6/*6* compared with **1/*1* volunteers precludes any possibility of treatment failure as a result of the higher artemether-to-dihydroartemisinin ratio in this group of malaria patients. Moreover, the artemether-lumefantrine wide

therapeutic index may also be responsible for the lack of clinical significance of the higher artemether-to-dihydroartemisinin $AUC_{0-\infty}$ ratio. Nonetheless, this study underlines the clinical relevance of pharmacogenetics in the optimization of antimalarial treatment regimens. It suggests the need for wider application of pharmacogenetics in antimalarial drug metabolism and in pharmacokinetics studies where genetics could influence efficacy or determine toxicity. Genotyping of patients from malaria-endemic regions where genetic variations in drug-metabolizing enzymes are prevalent will help to optimize drug regimens and to reduce the chances of undesired clinical outcomes (antimalarial failures and toxicities) and resistance development.

Among the limitations of the present study are the use of healthy volunteers instead of malaria patients and the small sample sizes available for the final statistical analysis. Previous studies have reported higher exposure to artemether and dihydroartemisinin in malaria patients compared with healthy volunteers.[39] In addition, artemether-lumefantrine was self-administered at home by the participants, and only the last dose was observed. Therefore, strict adherence to the dosage regimen could not be guaranteed. Moreover, this study only investigated the influence of *CYP2B6*6* genotype on artemether-lumefantrine pharmacokinetics based on previously available genetic data of the subjects. Composite *CYP2B6* genotype, particularly based on *CYP2B6*6* and **18*, could have provided more information on the contribution of CYP2B6 to artemether disposition and also increased the power to detect significant differences in the pharmacokinetic parameters between composite carriers and noncarriers of the variant alleles. Thus, future research on malaria patients should aim at determining (1) the population groups most at risk of artemisinin-based combination therapy failures and/or adverse effects, and (2) the impact of genetic influence (incorporating other potential host genetic variations) on the efficacy and safety of first-line artemisinin-based therapy regimens, especially for drugs with narrow therapeutic indices, is warranted. This is necessary to sustain the long-term efficacy and delay the development of resistance to artemisinin-based combination therapies.

Conclusions

*CYP2B6*6/*6* was associated with higher artemether-to-dihydroartemisinin metabolic ratio compared with the wild-type genotype in heathy volunteers, whereas the lumefantrine-to-desbutyllumefantrine ratio remain unchanged. Given the wide therapeutic range of artemether-lumefantrine, the relatively higher artemether-to-dihydroartemisinin exposure is unlikely to impact on artemether-lumefantrine efficacy or clinical outcomes in malaria patients of this genotype group.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Data Sharing

All data requests should be submitted to the corresponding author for consideration. Access to anonymized data may be granted following review.

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