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Research article

An increase in urinary primaquine and a reduction in urinary primaquine-5,6-orthoquinone in the Thai population with CYP2D6 reduced enzyme function

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ABSTRACT

Objectives: Primaquine is metabolized by the cytochrome P450-2D6 enzyme (CYP2D6) to an active primaquine-5,6-orthoquinone (POQ). No relationships of *CYP2D6* polymorphisms with the pharmacokinetics of primaquine and POQ were reported in the Thai population.
Methods: We evaluated the genetic distribution of *CYP2D6* in 345 Thai army populations together with the pharmacokinetic profiles of primaquine and POQ in plasma and urine (n = 44, descriptive data are presented in median (range)). All dose-related pharmacokinetic parameters were normalized by primaquine dose per body weight before statistical analysis.
*Results: CYP2D6*10* was the allele observed with the highest frequency (56.62%) corresponding to *CYP2D6*10/*10* (32.94%) and *CYP2D6*1/*10* (27.94%) genotypes. *CYP2D6* intermediate metabolizers (*CYP2D6* IM) were found in 44.41% of the cohort and had an increase in the cumulative amount of primaquine excreted (CAE) in urine compared to normal metabolizers of *CYP2D6* (*CYP2D6* NM); (*CYP2D6* IM vs. *CYP2D6* NM: 2444 (1697–3564) vs. 1757 (1092–2185) μg/mg/kg, p = 0.039), a reduction in urine CAE of POQ (*CYP2D6* IM vs *CYP2D6* NM: 115 (46–297) vs. 318 (92–498) μg/mg/kg, p = 0.047) and a reduction in the POQ/primaquine CAE ratio in urine (*CYP2D6* IM vs. *CYP2D6* NM: 0.06 (0.01–0.11) vs. 0.16 (0.06–0.26), p = 0.009). No significant differences were found in the pharmacokinetic profiles of plasma primaquine and POQ.

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Conclusions: The *CYP2D6* polymorphisms influenced the changes in primaquine and POQ that were noticeable in the urine, supporting the role of the *CYP2D6* gene testing before drug administration.

1. Introduction

Malaria infection remains a major public health problem in tropical and subtropical areas, including South East Asia [1]. *Plasmodium vivax* (*P. vivax*) is the most common type of malaria infection in Thailand (93.97%), with an incidence of 4.44 cases per 1000 population [2]. The population at risk of malaria infection is those who live or work in the endemic area, that is, the border areas of Thailand, including soldiers and police officers.

P. vivax has a vital dormant liver stage known as hypnozoite, which does not cause disease symptoms but can activate and trigger relapse [3]. With effects on the blood and liver stage, primaquine is currently the drug of choice for the treatment and prevention of *P. vivax* infection [4]. Furthermore, low dose primaquine also reduces human malaria transmission through its gametocidal activity [5]. Previous reports have shown variability in primaquine response associated with *P. vivax* resistant protein [6] and other host factors, including health status [7], drug-related problems [7], and genetic variations in primaquine metabolizing enzymes and transporters [8,9].

Primaquine is metabolized by the cytochrome P450-2D6 enzyme (CYP2D6) to an active primaquine-5,6-orthoquinone (POQ) [5,9,10]. Therefore, a reduction in the activity of the CYP2D6 enzyme due to genetic variations in *CYP2D6* can result in ineffective primaquine treatment. *CYP2D6*1* and *CYP2D6*2* are considered functional alleles of *CYP2D6* [11,12]. A previous study in Thai populations reported many genetic variations of *CYP2D6*, including a reduced functional allele (*CYP2D6*10*) and nonfunctional alleles (*CYP2D6*4* and *CYP2D6*5*) [13–19]. In fact, *CYP2D6*10* was observed in 42% and 50% of the East Asian and Thai populations, respectively [19]. Furthermore, *CYP2D6* polymorphisms were associated with clinical relapse of *P. vivax* and variations in primaquine pharmacokinetics [20]. There was also a reduction in plasma concentrations of the active metabolite POQ in individuals with *CYP2D6* reduced enzyme activity phenotypes known as intermediate metabolizers of *CYP2D6* (*CYP2D6* IM) and poor metabolizers of *CYP2D6* (*CYP2D6* PM) [21].

This study aimed to present the distribution of *CYP2D6* variations in the population of the Thai army that live in the endemic area of *P. vivax* infection and to evaluate the pharmacokinetic profiles of primaquine and POQ in plasma and urine.

2. Materials and methods

The study consisted of two parts. The first part was an observational study that presents the distribution of *CYP2D6* single nucleotide polymorphisms (SNP) in Thai army participants. The second part was an intensive pharmacokinetic study of primaquine in subsequent Thai army participants. The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA no. 093/2019) and the Institutional Review Board of the Royal Thai Army Medical Department, Bangkok, Thailand (IRBRTA 471/2562). The study was registered in the Thai Clinical Trials Registry (TCTR20190211002) and was conducted according to the Declaration of Helsinki and the Good Clinical Practice Guidelines. All participants provided their written informed consent before enrolling in the study.

2.1. Participants

In the first part of the study, a total of 345 participants who met the inclusion criteria (aged 18–60 years and deployed from the North-East Military Unit in the Thai-Cambodia border area) were invited for *CYP2D6* genotyping. There were no exclusion criteria for this observational part of the study.

For the subsequent intensive pharmacokinetic study, 44 participants with known predicted *CYP2D6* phenotypes from the first part of the study were consecutively recruited with the proportion of *CYP2D6* IM:*CYP2D6* NM = 1:1. The inclusion criteria for this pharmacokinetic study were participants who were 18–60 years old with a body mass index of 18–29 kg/m^2 and had a normal physical examination. Exclusion criteria for the pharmacokinetic study were participants who regularly took food supplements, herbal substances or medications; participants who, at the time of the study, had malaria infection or signs of malaria infection, or received any antimalarial treatment within 4 weeks before the pharmacokinetic study; participants with a history of gastrointestinal diseases, liver diseases, kidney diseases, glucose-6-phosphate dehydrogenase enzyme deficiency, and any other contraindicated conditions for the use of primaquine; participants who were smokers, alcoholics, or substance addiction.

2.2. DNA extraction and CYP2D6 genotyping

Venous blood samples (5 mL) were collected from each participant. Genomic DNA was extracted using a Genomic DNA Mini Kit (Gene Aid Biotech Ltd., Taiwan) according to the manufacturer's instructions. DNA concentration and DNA purity were measured with a NanoDrop-One spectrophotometer (Thermo Fisher Scientific, USA).

*CYP2D6*2, CYP2D6*4, CYP2D6*5,* and *CYP2D6*10* were genotyped according to previous reports in Thai populations [13–19]. The SNPs included 2850C > T (rs16947), 4180G > C (rs1135840), 100C > T (rs1065852), and 1846G > A (rs3892097). Genotyping

was performed with TaqMan® Genotyping Assays (Applied Biosystems, USA; lot numbers 3272427, 3082961, 3128493, and 3252855). *CYP2D6* gene deletion (*CYP2D6*5*) was detected by TaqMan® copy number assay (Applied Biosystem, USA). Two different pairs of primers (Hs00010001_cn and Human RNase P) were used to identify the presence or deletion of the entire *CYP2D6* gene versus baseline.

The translation of *CYP2D6* alleles into predicted *CYP2D6* phenotypes and *CYP2D6* activity scores was carried out according to previous reports [11,12]. For *CYP2D6* activity scores, each allele was scored as follows: 1) null allele (*CYP2D6*4* or *CYP2D6*5*) = score 0.00, 2) reduced activity allele (*CYP2D6*10*) = score 0.25, and 3) functional allele (*CYP2D6*1* or *CYP2D6*2*) = score 1.00. The cumulative activity score was applied to stratify *CYP2D6* activity and the predicted *CYP2D6* phenotypes. *CYP2D6* PM were those with an activity score of 0.00 while *CYP2D6* IM, *CYP2D6* NM, and *CYP2D6* ultrarapid metabolizer (*CYP2D6* UM) were the participants with activity scores of 0.25–1.00, 1.25–2.25, and >2.25, respectively. The classification of the predicted *CYP2D6* phenotypes and the *CYP2D6* activity scores according to the *CYP2D6* genotypes are shown in Supplementary Table S1.

2.3. Intensive pharmacokinetic study and sample collection

Participants in the pharmacokinetic study received a single oral dose of primaquine phosphate (30 mg base). Venous blood samples (3 mL) were collected at each time point, including before dose (0 h) and 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after dose. Urine samples were also collected before dose (0 h) and during 0–4, 4–8, 8–12, 12–18, and 18–24 h after drug administration. Clinical monitoring and adverse events were observed and recorded throughout the study period.

2.4. Instrumentation and chromatographic conditions for bioanalysis

The free form of primaquine and POQ in plasma and urine was quantified by an ultra-high performance liquid chromatography tandem mass spectrometry (LC-MS) system (UltiMate 3000 standard HPLC Systems and TSQ Quantum Access MAX triple stage quadrupole mass spectrometer, Thermo Fisher Scientific, USA) as previously reported by our group [22]. Briefly, separation was performed with a Hypersil GOLD™ aQ C18 column (100 × 2.1 mm, particle size 1.9 μm) with a C18 guard column (4 × 3 mm, Thermo Fisher, USA)). The 25 °C column temperature was maintained with an isocratic mode of mobile phase A (0.1% formic acid in methanol: water (40:60, v/v)) and mobile phase B (0.1% formic acid in acetonitrile) flowed in a ratio of 80:20 at 0.4 mL/min. The injection volume was 1 μL. Mass analysis with an electrospray ionization system was performed with a 4.0 kV spray voltage in positive mode, 40 sheath gas nitrogen pressure, 20 auxiliary nitrogen gas, 350 °C vaporizer temperature, 370 °C ion transfer capillary temperature, and 15 V skimmer offset. The 1.5 mTor collision gas and the collision energy were set at 25 eV for primaquine ($m/z = 260.26 > 187.82$), 33 eV for POQ ($m/z = 260.20 > 147.13$), and 24 eV for an internal standard 8-aminoquinoline (8-AQ), ($m/z = 145.00 > 128.16$). TSQ Tune software version 2.6 SP1 and LC Quan™ software version 3.0 (Thermo Electron Corporation, UK) were used to optimize the tuning parameters, data acquisition, and processing.

2.5. Preparation of standard stock solutions, quality controls, and biological samples

Primaquine, POQ, and 8-AQ stock solutions were prepared separately as 1 mg/mL base in methanol and protected from light at −80 °C. Standard working solutions were prepared from the primary stock at concentrations of 2, 20, and 30 μg/mL for primaquine, POQ, and 8-AQ, respectively. Standard curves (25–1500 ng/mL) for primaquine and POQ were prepared by spiking blank plasma or urine and serially diluting to the desired concentration. 8-AQ (100 ng/mL) was added to all samples. Quality control (QC) samples were prepared in blank plasma or urine at concentrations of 25, 50, 600, and 1245 ng/mL for the lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC) and high quality control (HQC), respectively, and stored at −80 °C before use. Plasma or urine (190 μL) was spiked with 100 ng of 8-AQ (10 μL), briefly mixed, and then added to 0.2 mL of acetonitrile and centrifuged at 10,000 g for 5 min. The supernatant was collected and again added to 0.2 mL of acetonitrile, mixed, and centrifuged at 10,000 g for 5 min to complete protein precipitation. Plasma or urine supernatant was then filtered and transferred to a vial with a glass insert before injection into the LC-MS system.

2.6. Pharmacokinetic parameters

Noncompartmental pharmacokinetic analyses were performed with STATA software version 15.1 (StataCorp LLC, Texas, USA). The reported pharmacokinetic parameters included the maximum plasma concentration (Cmax), the time to Cmax (Tmax), the area under the concentration time curve to the last sample collection time point of the sample (AUC_{0-t}), the area under the concentration time curve to infinity ($AUC_{0\text{-}inf}$), elimination half-life (t1/2) and elimination rate constant (Ke). The apparent clearance (CL/F) and the apparent volume of distribution (Vd/F) were also calculated with the bioavailability (F) of 0.96 applied [23].

For urine data, the amount excreted in urine (AE), the cumulative amount excreted (CAE), and renal clearance (CLr) were calculated for primaquine and POQ. CLr was calculated from CAE versus time. The POQ/primaquine CAE ratio was calculated to represent the proportion of an active POQ in the body. Dose-related pharmacokinetic parameters, for example, CAE and CAE ratio of POQ/primaquine, were normalized by the dose of primaquine per body weight.

2.7. Statistical analyses

Statistical analyses were determined using SPSS version 22 (IBM Corporation, Armonk, NY, USA). Continuous data were presented as mean ± standard deviation (mean ± SD), median (range), or median ± median absolute deviation (mean ± MAD), where appropriate. MAD was calculated as median ($|Xi–\overline{X}|$) where $\overline{X}$ = median (Xi). Differences in plasma primaquine and POQ concentrations between predicted *CYP2D6* phenotypes were analyzed by the independent *t*-test or the Mann-Whitney *U* test, where appropriate. Furthermore, differences in pharmacokinetic parameters between *CYP2D6* activity scores were analyzed with one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, followed by Turkey post hoc test, where appropriate. The significant level was a p value < 0.05.

3. Results

3.1. Characteristics of the participants

A total of 345 participants (aged 41.36 ± 13.8 years, all men) were recruited for *CYP2D6* genotyping. Five samples were excluded due to insufficient blood volumes (n = 3) and low DNA quality DNA (n = 2), resulting in a total of 340 samples available for the distribution of allele frequencies and genotypes of *CYP2D6*. The baseline characteristics of the participants enrolled in the pharmacokinetic study (n = 44) are presented in Table 1. There were no differences in baseline clinical data when participants were classified according to predicted *CYP2D6* phenotypes or *CYP2D6* activity scores (Table 1). No adverse events were observed in all pharmacokinetic participants.

3.2. CYP2D6 allele frequency, genotypes, predicted phenotypes, and activity scores

The highest frequency of *CYP2D6* alleles observed in this cohort was *CYP2D6*10* (56.62%), (Table 2). For genotypes, *CYP2D6*10/*10* and **1/*10* represented 60% of the cohort (*CYP2D6*10/*10* = 32.94% and *CYP2D6*1/*10* = 27.94%). Regarding the *CYP2D6* activity score, approximately 16% of the participants had a *CYP2D6* activity score of 2, 40% of them had a *CYP2D6* activity score of 1.25, while 45% of them had a *CYP2D6* activity score of <1 (Table 2). When participants were classified according to their predicted phenotypes, 55.59% and 44.41% were *CYP2D6* NM and *CYP2D6* IM, respectively (Table 2). *CYP2D6* PM was not found.

Table 1
Baseline characteristics of the participants in the pharmacokinetic study (n = 44) according to the predicted *CYP2D6*phenotypes and *CYP2D6* activity scores.

Groups	*CYP2D6* predicted phenotypes			*CYP2D6* activity scores					
	NM (n = 22)	IM (n = 22)	p value	0.25 (n = 7)	0.50 (n = 11)	1.00 (n = 4)	1.25 (n = 11)	2.00 (n = 11)	p value
Age (years)	50 (24–59)	41 (27–58)	0.702	43 (29–53)	56 (27–58)	46 (34–58)	49 (24–59)	51 (28–59)	0.979
Body mass index (kg/m^2)	24.8 (17.7–29.0)	24.6 (19.4–38.1)	0.171	22.2 (20.3–36.2)	22.2 (8.6–38.1)	25.1 (24.6–28.1)	23.0 (20.2–25.7)	26.3 (17.7–29.0)	0.153
Primaquine dose (mg/kg)	0.44 (0.34–0.57)	0.44 (0.29–0.61)	0.282	0.45 (0.32–0.54)	0.54 (0.29–0.61)	0.42 (0.36–0.45)	0.43 (0.39–0.57)	0.45 (0.35–0.54)	0.561
Creatinine (mg/dL)	0.99 (0.79–1.18)	0.98 (0.87–1.36)	0.368	0.97 (0.87–1.15)	1.13 (0.87–1.36)	0.97 (0.90–1.25)	0.96 (0.79–1.18)	1.03 (0.84–1.13)	0.778
Total protein (g/dL)	7.5 (6.6–8.3)	7.1 (6.6–7.9)	0.117	7.0 (6.9–7.9)	7.0 (3.8–7.9)	7.2 (6.7–7.6)	7.5 (6.6–7.8)	7.2 (6.9–8.3)	0.489
Albumin (g/dL)	4.5 (4.1–5.2)	4.4 (3.6–4.7)	0.083	4.4 (3.6–4.7)	3.8 (3.0–4.7)	4.5 (4.1–4.6)	4.5 (4.1–4.9)	4.5 (4.1–5.2)	0.426
Total bilirubin (mg/dL)	0.59 (0.24–1.27)	0.61 (0.20–1.24)	0.717	0.80 (0.43–1.16)	0.29 (0.11–0.69)	0.69 (0.30–1.24)	0.64 (0.24–0.88)	0.53 (0.33–1.27)	0.286
Direct bilirubin (mg/dL)	0.19 (0.09–0.33)	0.21 (0.07–0.42)	0.428	0.22 (0.11–0.42)	0.27 (0.11–0.29)	0.20 (0.07–0.39)	0.21 (0.09–0.30)	0.17 (0.10–0.33)	0.797
Aspartate Aminotransferase (U/L)	26 (13–52)	30 (13–72)	0.299	31 (21–47)	27 (13–72)	31 (20–55)	26 (14–51)	26 (13–52)	0.882
Alanine aminotransferase (U/L)	26 (9–74)	28 (14–70)	0.959	26 (17–46)	50 (14–71)	37 (20–46)	24 (16–63)	27 (9–74)	0.941
Alkaline phosphatase (U/L)	63 (39–124)	66 (47–106)	0.765	79 (58–96)	57 (14–78)	71.5 (51–106)	61 (39–109)	71 (41–124)	0.447
Gamma-glutamyl transpeptidase (U/L)	60 (17–265)	55 (14–253)	0.464	36 (19–253)	47 (8–121)	105 (62–116)	68 (30–265)	59 (17–136)	0.196

Data are presented in median (range).
IM: intermediate metabolizers; NM: normal metabolizers.

Table 2
Frequency of the *CYP2D6* alleles, genotypes, phenotypes and activity scores.

CYP2D6	Total participants (n = 340)	Pharmacokinetic study (n = 44)
Alleles (n (%))		
**1*	173 (25.44)	30 (34.09)
**2*	83 (12.21)	7 (7.95)
**4*	4 (0.59)	1 (1.14)
**5*	35 (5.15)	10 (11.36)
**10*	385 (56.62)	40 (45.45)
Genotypes (n (%))		
**1/*1*	25 (7.35)	7 (15.91)
**1/*2*	18 (5.29)	1 (2.27)
**1/*4*	0 (0.00)	0 (0.00)
**1/*5*	10 (2.94)	4 (9.10)
**1/*10*	95 (27.94)	9 (20.45)
**2/*2*	10 (2.94)	3 (6.82)
**2/*4*	1 (0.29)	0 (0.00)
**2/*5*	3 (0.88)	0 (0.00)
**2/*10*	41 (12.06)	2 (4.54)
**4/*10*	3 (0.88)	1 (2.27)
**5/*10*	22 (6.47)	6 (13.64)
**10/*10*	112 (32.94)	11 (25.00)
Predicted phenotypes (n (%))		
Normal metabolizers	189 (55.59)	22 (50.00)
Intermediate metabolizers	151 (44.41)	22 (50.00)
Poor metabolizers	0 (0.00)	0 (0.00)
Activity scores (n (%))		
0.25	25 (7.35)	7 (15.90)
0.50	112 (33.94)	11 (25.00)
1.00	14 (4.12)	4 (9.10)
1.25	136 (40.00)	11 (25.00)
2.00	53 (15.59)	11 (25.00)

Table 3
Pharmacokinetic parameters of primaquine and 5,6-orthoquinone primaquine in plasma and urine according to the predicted *CYP2D6* phenotypes.

Parameters	Normal metabolizers (n = 22)	Intermediate metabolizers (n = 22)	p value
Primaquine in plasma			
Tmax (hr)	2.00 (1.25–3.00)	2.00 (1.00–3.00)	0.410
Cmax (ng/mL)	126.72 (87.04–153.11)	127.78 (97.63–154.65)	0.869
$AUC_{0\text{-}t}$ (ng.hr/mL)	1136 (783–1306)	1256 (1068–1520)	0.425
$AUC_{0\text{-}inf}$ (ng.hr/mL)	1160 (855–1355)	1315 (1079–1547)	0.336
Half-life (hr)	9.22 (8.20–12.03)	9.59 (8.20–10.91)	0.690
Elimination rate constant (1/hr)	0.08 (0.06–0.08)	0.07 (0.06–0.08)	0.690
CL/F (mL/hr.kg)	381.85 (347.87–550.96)	336.91 (288.56–453.41)	0.189
Vd/F (mL/kg)	30.17 (25.84–33.79)	23.84 (20.93–30.94)	0.105
Dose/weight normalized Cmax (ng/mL/mg/kg)	279.11 (201.32–352.71)	294.11 (246.44–325.07)	0.526
Dose/weight normalized $AUC_{0\text{-}t}$ (ng.hr/mL/mg/kg)	2651 (1758–2945)	3033 (2274–3509)	0.386
Dose/weight normalized $AUC_{0\text{-}inf}$ (ng.hr/mL/mg/kg)	2728 (1904–2994)	3093 (2298–3611)	0.387
Dose/weight normalized CL/F (mL/hr.mg)	897.94 (768.87–1219.03)	792.70 (673.93–965.27)	0.417
Dose/weight normalized Vd/F (mL/mg)	70.19 (52.62–78.28)	54.72 (50.78–71.25)	0.222
Primaquine in urine			
Total CAE (μg)	851 (549–974)	1006 (720–1260)	0.125
CLr (mL/hr/kg)	9.47 (7.18–14.14)	10.96 (8.53–15.81)	0.442
Dose/weight normalized total CAE (μg/mg/kg)	1757 (1092–2185)	2444 (1697–3564)	0.039
Dose/weight normalized CLr (mL/hr.mg)	21.01 (16.57–30.06)	29.76 (18.64–37.74)	0.303
POQ in urine			
Total CAE (ng)	134 (46–226)	51 (30–142)	0.069
Dose/weight normalized total CAE (μg/mg/kg)	318 (92–498)	115 (46–297)	0.047
CAE ratio			
POQ/primaquine CAE ratio	0.16 (0.06–0.26)	0.06 (0.01–0.11)	0.009

Data are presented in median (range).
POQ: primaquine-5,6-orthoquinone; $AUC_{0\text{-}t}$: area under the concentration-time curve from time 0 to t; $AUC_{0\text{-}inf}$: area under the concentration-time curve from time 0 to infinity; CAE: cumulative amount excreted in urine; Cmax: maximum plasma concentration; CL/F: apparent clearance; CLr: renal clearance; F: bioavailability; PQ: primaquine; Tmax: time to Cmax; Vd/F: apparent volume of distribution.

3.3. Pharmacokinetic profiles of primaquine and POQ in plasma and urine

No statistically significant changes were observed in plasma primaquine pharmacokinetic profiles when participants were classified according to predicted *CYP2D6* phenotypes (Table 3, Fig. 1A) or *CYP2D6* activity scores (Supplementary Table S2, Fig. 1B). POQ was undetected in plasma. For urine data, *CYP2D6* IM showed a significant increase in normalized total CAE of primaquine compared to *CYP2D6* NM (2444 (1697–3594) vs. 1757 (1092–2185) μg/mg/kg, respectively, $p = 0.039$, Table 3, Figs. 2A and 3A), but not for CLr. The normalized total CAE of POQ was significantly lower in *CYP2D6* IM than in *CYP2D6* NM (115 (46–297) vs. 318 (92–498) μg/mg/kg, respectively, $p = 0.047$, Table 3, Figs. 2B and 3B). Furthermore, the POQ/primaquine CAE ratio was significantly reduced in *CYP2D6* IM compared to *CYP2D6* NM (0.06 (0.01–0.11) vs 0.16 (0.06–0.26), respectively, $p = 0.009$, Table 3, Fig. 3C).

When participants were classified according to their *CYP2D6* activity score, there were significant differences in the normalized total CAE of POQ and the urine CAE ratio between the activity score groups ($p = 0.039$ and $p = 0.034$, respectively, Supplementary Table S2, Supplementary Figs. S1 and S2).

4. Discussion

This study reported the distribution of *CYP2D6* polymorphisms, as well as the variability in plasma and urine pharmacokinetic profiles of primaquine and its active metabolite POQ in Thai army population. Although no differences were found in plasma pharmacokinetic parameters, urine data showed a significant increase in primaquine and a reduction in POQ in *CYP2D6* IM, supporting the alteration of primaquine metabolism with reduced *CYP2D6* activity.

The frequency of *CYP2D6* alleles in our study was similar to previous reports in the Thai population [13–19]. Furthermore, the proportions of *CYP2D6* IM and *CYP2D6* NM were consistent with those reported in Asians [21]. It is important to note that, in 2020, the consensus guideline for translating *CYP2D6* genotypes into predicted *CYP2D6* phenotypes was revised with an activity score of 0.25 applied for *CYP2D6*10* [11]. Although in this study, the revised classification of the *CYP2D6* activity scores was used, the *CYP2D6* genotypes and predicted *CYP2D6* phenotypes were still in line with previous reports in the Thai population [13,18].

The overall pharmacokinetic parameters of plasma primaquine in our cohort were similar to others [20,21,23,24]. However, when participants were classified according to predicted *CYP2D6* phenotypes, our findings were different from reports in other ethnic groups [20,21]. A study by Bennett et al. showed that *CYP2D6* IM and *CYP2D6* PM had a decreased clearance of primaquine and an increased residual plasma primaquine concentration after 24 h represented by an increase in AUC and half-life and a reduction in CL/F and Vd/F compared to *CYP2D6* NM [20]. A study in 44 participants that included *CYP2D6* NM, *CYP2D6* IM, and *CYP2D6* PM also reported that primaquine Cmax, AUC, and half-life were increased in *CYP2D6* IM and *CYP2D6* PM compared to *CYP2D6* NM [21]. For both previous studies [20,21], all changes were highly prominent in *CYP2D6* PM. Although our data showed the same trend of decrease primaquine clearance in *CYP2D6* IM compared to *CYP2D6* NM (Table 3, Fig. 1), the differences did not reach statistical significance. This may be explained in part by differences in ethnicity and the fact that there is no *CYP2D6* PM in our cohort.

Despite the use of an ultra high-sensitivity quadrupole time-of-flight mass spectrometer, our plasma POQ was undetectable. This finding is consistent with data from several studies regardless of primaquine doses (30 mg, 45 mg, and 15 mg) [21,25,26]. This may be explained by the accumulation of POQ in other compartments of the body, for example, the liver and erythrocytes and therefore, was undetected in plasma [5,21,27].

In this study, independent of CLr, different amounts of primaquine and POQ were excreted in urine and significant differences were observed in the CAE ratios between *CYP2D6* IM and *CYP2D6* NM (Table 3, Figs. 2 and 3) [5,9,10]. These findings are crucial because

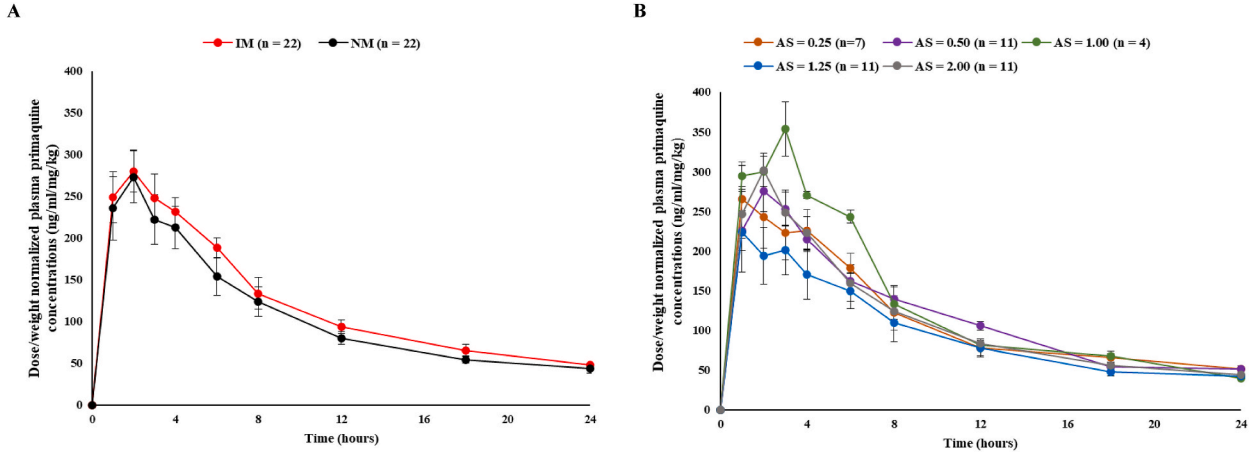

Fig. 1. Plasma primaquine concentration time profiles according to the predicted *CYP2D6* phenotypes (A) and the *CYP2D6* activity scores (B) Data are presented in median ± median absolute deviation (MAD). MAD was calculated as median ($|Xi\text{-}\overline{X}|$) where $\overline{X}$ = median (Xi). AS: activity score; IM: intermediate metabolizers; NM: normal metabolizers.

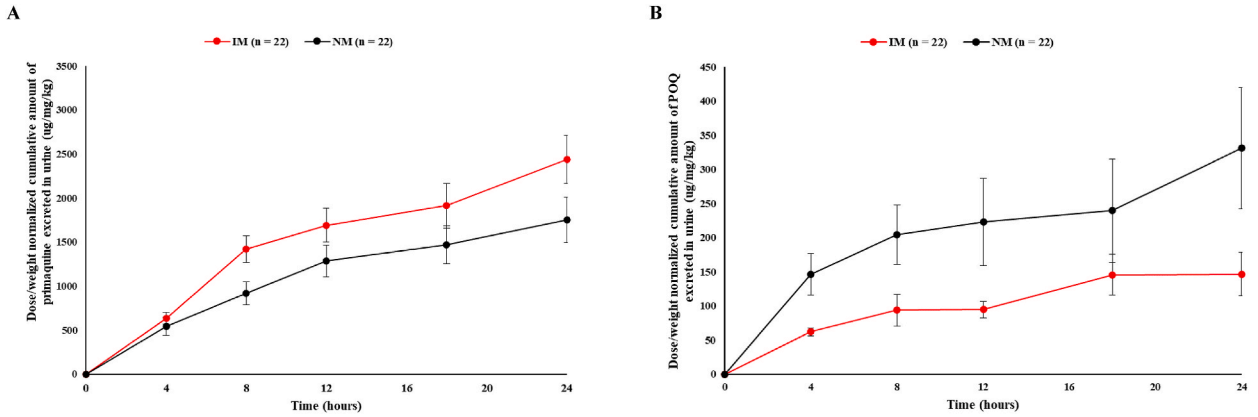

Fig. 2. The cumulative amount-time profile of primaquine (A) and primaquine 5,6-orthoquinone (B) in urine according to the predicted *CYP2D6* phenotypes.

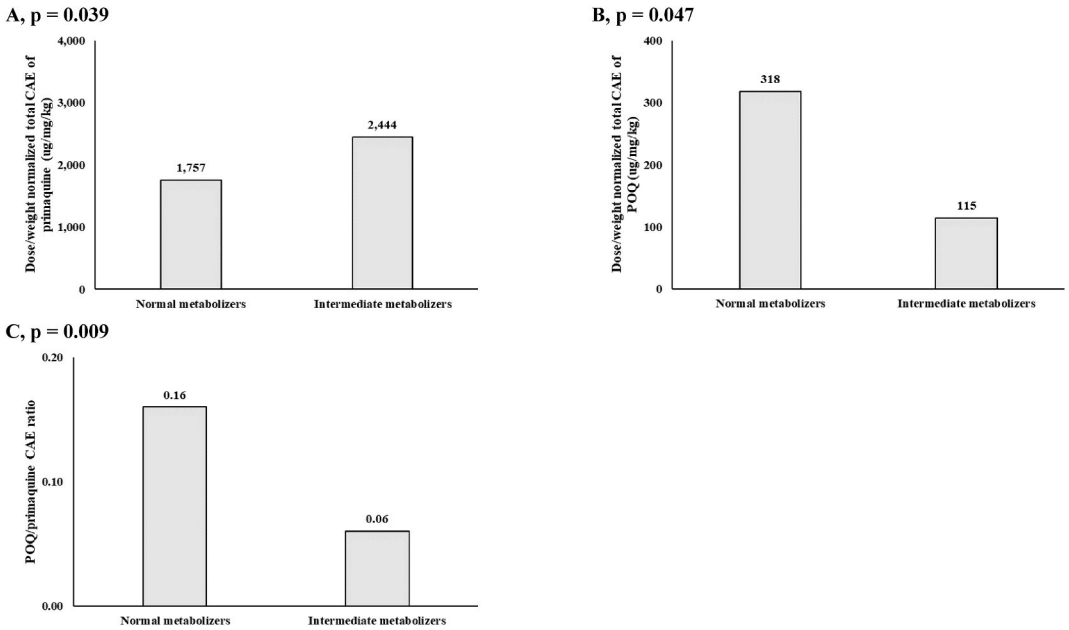

Fig. 3. The cumulative amount excreted (CAE) in urine of primaquine (A) and primaquine-5,6-orthoquinone (B) and CAE ratio (C) according to the predicted CYP2D6 phenotypes Data are presented as median. p value for the Mann-Whitney *U* test between normal metabolizers and intermediate metabolizers. CAE: cumulative amount excreted (CAE) in urine; POQ: primaquine-5,6-orthoquinone.

they represent the metabolic function of the drug-metabolizing enzyme in different predicted *CYP2D6* phenotypes and support the hypothesis that primaquine is less metabolized to POQ in *CYP2D6* IM, leading to an insufficient POQ at the site of action.

Our findings also support previous data that the effects of primaquine on hypnozoites and gametocytes were associated with the concentrations of primaquine active metabolite [10]. The efficacy of primaquine for radical cure of *P. vivax* infection depends more on the total dose of primaquine administered to patients than on the duration of treatment and the CAE assessment was applied to predict an appropriate dose of primaquine [28]. Our findings are consistent with this as our results showed an increase in the total CAE of POQ in *CYP2D6* NM (Table 3, Figs. 2 and 3). Therefore, *CYP2D6* NM would benefits more from primaquine treatment compared to *CYP2D6* IM when the same dose of primaquine was administered. Our data also suggest that, for the treatment of malaria infection, an increase in the first primaquine dose may be beneficial and the *CYP2D6* genetic-guided dose of primaquine should be further investigated. Studies also noted that if the first treatment failed without adjusting the first dose of primaquine, increasing the total dose of primaquine would be suggestive as a second-line treatment [10,29]. However, the acute hemolytic adverse effect related to primaquine must be closely monitored, especially if CYP2D6-metabolized drugs, such as codeine, dextromethorphan, metoprolol, nebivolol, and risperidone or CYP2D6 enzyme inhibitors, such as bupropion, fluoxetine, quinidine, and terbinafine, are co-administrated with primaquine [10,29].

Currently, tafenoquine is the only alternative drug approved for the prevention of *P. vivax* relapsed infection. However, activation of tafenoquine still requires metabolism by the CYP2D6 enzyme [30]. The development of new antimalarial drugs that do not depend on the metabolism of the CYP2D6 enzyme would help improve the efficacy and safety of treatment in patients with low activity of the CYP2D6 enzyme.

This study has several limitations. The population of various ethnicities lives in the border area of the country. Our studied cohort was male Thai military personnel, not the general population living in the border area; therefore, it can be assumed to be Thai. However, extrapolating the distribution of the CYP2D6 allele to the total Thai population or to specific groups of subjects, for example children or women, must be done with caution. Only five candidate *CYP2D6* alleles (*CYP2D6*1, CYP2D6*2, CYP2D6*4, CYP2D6*5,* and *CYP2D6*10*) were reported, while other undetected variants were assumed and reported as *CYP2D6*1* [31]. Therefore, the proportion with the default *CYP2D6*1* allele may have been slightly overestimated. Some rare alleles, such as *CYP2D6*36* and *CYP2D6*41*, can be missed, as well as new variants, such as *CYP2D6*143*, *CYP2D6*142*, and *CYP2D6*144* [12,19,32]. Furthermore, the undetermined *CYP2D6* UM might slightly affect the classification of the predicted *CYP2D6* phenotypes. In addition, comparisons between *CYP2D6* activity score groups lacked the power to detect differences due to the small number of participants in some groups (for example, *CYP2D6* activity score = 1.00; n = 4). Future pharmacokinetic studies are required in a larger cohort size to assess the effects of the *CYP2D6* activity score on primaquine pharmacokinetics and its metabolites. Furthermore, primaquine metabolism is very complex, involving several other drug-metabolizing enzymes and transporters [8,9]. The monoamine oxidase A (MAO-A) enzyme is a major enzyme that transforms primaquine into the most abundant inactive metabolite, carboxy-primaquine [9]. Slow primaquine metabolism was reported in a group of Malaysian and Chinese when genetic variations of *MAO-A* (rs6323, 891G > T), *CYP2D6* (rs1065852, 100C > T), and *CYP2C19* (rs4244285, 19154G > A) were considered in combination [33]. Genetic variations of these drug-metabolizing enzymes and transporters were not evaluated in our study and warrant further investigation.

In conclusion, *CYP2D6* IM had an increase in primaquine urine excretion and a reduction in POQ urine excretion compared to *CYP2D6* NM. It can be implied that primaquine was less metabolized by the CYP2D6 enzyme to POQ, suggesting a lower POQ at the site of action. *CYP2D6* genetic-guided dose of primaquine warrants further investigation in larger population.

Ethical approval

The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA no. 093/2019) and the Royal Thai Army Medical Department, Bangkok, Thailand (IRBRTA 471/2562).

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CRediT authorship contribution statement

Waritda Pookmanee: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing - original draft. **Siriwan Thongthip:** Data curation. **Mathirut Mungthin:** Conceptualization, Formal analysis, Supervision. **Chonlaphat Sukasem:** Formal analysis, Resources. **Jeeranut Tankanitlert:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision. **Pajaree Chariyavilaskul:** Formal analysis, Resources, Supervision, Writing - review & editing. **Supeecha Wittayalertpanya:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24351.

References

[1] World Health Organization, World Malaria Report 2021, World Health Organization, Geneva, 2021.
[2] Department of Disease Control and Ministry of Public Health, Thailand malaria elimination program [cited 2021 3 July 2022]; Available from: http://malaria.ddc.moph.go.th/malariaR10/index_newversion.php, 2021.
[3] Centers for Disease Control and Prevention, Biology [cited 2022 31 July]; Available from: https://www.cdc.gov/malaria/about/biology/, 2020.
[4] World Health Organization, WHO Guidelines for Malaria, World Health Organization, Geneva, 2022.
[5] B.M. Potter, et al., Differential CYP 2D6 metabolism alters primaquine pharmacokinetics, Antimicrob. Agents Chemother. 59 (4) (2015) 2380–2387.
[6] L.E. Buyon, B. Elsworth, M.T. Duraisingh, The molecular basis of antimalarial drug resistance in Plasmodium vivax, Int. J. Parasitol. Drugs Drug Resist. 16 (2021) 23–37.
[7] J.K. Baird, Resistance to therapies for infection by Plasmodium vivax, Clin. Microbiol. Rev. 22 (3) (2009) 508–534.
[8] X. Jin, et al., Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations, J. Pharmacol. Toxicol. Methods 70 (2) (2014) 188–194.
[9] S.R. Marcsisin, G. Reichard, B.S. Pybus, Primaquine pharmacology in the context of CYP 2D6 pharmacogenomics: current state of the art, Pharmacol. Ther. 161 (2016) 1–10.
[10] G. Camarda, et al., Antimalarial activity of primaquine operates via a two-step biochemical relay, Nat. Commun. 10 (1) (2019) 3226.
[11] K.E. Caudle, et al., Standardizing CYP2D6 genotype to phenotype translation: consensus recommendations from the clinical pharmacogenetics implementation consortium and Dutch pharmacogenetics working group, Clin. Transl. Sci. 13 (1) (2020) 116–124.
[12] Pharmacogene Variation Consortium, CYP2D6, Available from: https://www.pharmvar.org/gene/CYP2D6, 2022.
[13] M. Chamnanphon, et al., CYP2D6 genotype analysis of a Thai population: platform comparison, Pharmacogenomics 19 (12) (2018) 947–960.
[14] M. Chamnanphon, et al., Pharmacogene variation in Thai plasmodium vivax relapse patients treated with a combination of primaquine and chloroquine, Pharmgenomics Pers. Med. 13 (2020) 1–12.
[15] M. Chamnanphon, et al., CYP2D6 predicts plasma donepezil concentrations in a cohort of Thai patients with mild to moderate dementia, Pharmgenomics Pers. Med. 13 (2020) 543–551.
[16] J. Mauleekoonphairoj, et al., Phenotype prediction and characterization of 25 pharmacogenes in Thais from whole genome sequencing for clinical implementation, Sci. Rep. 10 (1) (2020) 18969.
[17] N. Wankaew, et al., Genotypic and phenotypic landscapes of 51 pharmacogenes derived from whole-genome sequencing in a Thai population, PLoS One 17 (2) (2022) e0263621.
[18] Y. Hongkaew, et al., Relationship between CYP2D6 genotype, activity score and phenotype in a pediatric Thai population treated with risperidone, Sci. Rep. 11 (1) (2021) 4158.
[19] A. Puangpetch, et al., CYP2D6 polymorphisms and their influence on risperidone treatment, Pharmgenomics Pers. Med. 9 (2016) 131–147.
[20] J.W. Bennett, et al., Primaquine failure and cytochrome P-450 2D6 in Plasmodium vivax malaria, N. Engl. J. Med. 369 (14) (2013) 1381–1382.
[21] M.D. Spring, et al., Determination of cytochrome P450 isoenzyme 2D6 (CYP2D6) genotypes and pharmacogenomic impact on primaquine metabolism in an active-duty US military population, J. Infect. Dis. 220 (11) (2019) 1761–1770.
[22] W. Pookmanee, et al., Simplified and rapid determination of primaquine and 5,6-orthoquinone primaquine by UHPLC-MS/MS: its application to a pharmacokinetic study, Molecules 26 (14) (2021).
[23] G.W. Mihaly, et al., Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size, Br. J. Clin. Pharmacol. 19 (6) (1985) 745–750.
[24] S. Pukrittayakamee, et al., Pharmacokinetic interactions between primaquine and chloroquine, Antimicrob. Agents Chemother. 58 (6) (2014) 3354–3359.
[25] B. Avula, et al., Metabolism of primaquine in normal human volunteers: investigation of phase I and phase II metabolites from plasma and urine using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry, Malar. J. 17 (1) (2018) 294.
[26] P. Vanachayangkul, et al., Measurements of 5,6-orthoquinone, a surrogate for the presumed active primaquine metabolite 5-hydroxyprimaquine, in the urine of Cambodian adults, Antimicrob. Agents Chemother. 66 (3) (2022) e0182121.
[27] W. Khan, et al., Quantitative determination of primaquine-5,6-ortho-quinone and carboxyprimaquine-5,6-ortho-quinone in human erythrocytes by UHPLC-MS/MS, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1163 (2021) 122510.
[28] W.R.J. Taylor, et al., Short-course primaquine for the radical cure of Plasmodium vivax malaria: a multicentre, randomised, placebo-controlled non-inferiority trial, Lancet 394 (10202) (2019) 929–938.
[29] A. de Pina-Costa, et al., Increased primaquine total dose prevents Plasmodium vivax relapses in patients with impaired CYP2D6 activity: report of three cases, Malar. J. 20 (1) (2021) 341.
[30] Y.A. Ebstie, et al., Tafenoquine and its potential in the treatment and relapse prevention of Plasmodium vivax malaria: the evidence to date, Drug Des. Dev. Ther. 10 (2016) 2387–2399.
[31] E. Lyon, et al., Laboratory testing of CYP2D6 alleles in relation to tamoxifen therapy, Genet. Med. 14 (12) (2012) 990–1000.
[32] Y. Hongkaew, et al., Resolving discordant CYP2D6 genotyping results in Thai subjects: platform limitations and novel haplotypes, Pharmacogenomics 22 (9) (2021) 529–541.
[33] N.M. Ariffin, et al., Effects of MAO-A and CYP450 on primaquine metabolism in healthy volunteers, Parasitol. Res. 118 (3) (2019) 1011–1018.