

Pharmacokinetic Interactions between Quinine and Lopinavir/Ritonavir in Healthy Thai Adults

Siwalee Rattanapunya, Tim R. Cressey, Ronnatrai Rueangweerayut, Yardpiroon Tawon,
Panida Kongjam, and Kesara Na-Bangchang*

Faculty of Science and Technology, Chiang Mai Rajabhat University, Chiang Mai, Thailand; Program for HIV Prevention and Treatment, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand; Harvard School of Public Health, Boston, Massachusetts; Mae Sot General Hospital, Tak Province, Thailand; Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Thammasat University, Pathumthani, Thailand; Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand

Abstract. This study aimed to investigate the pharmacokinetic interactions between quinine and lopinavir boosted with ritonavir (LPV/r) in healthy Thai adults (8 males and 12 females). Period 1 (day 1): subjects received a single oral dose of 600 mg quinine sulfate. Period 2: subjects received LPV/r (400/100 mg) twice daily. Period 3: subjects received a single quinine sulfate dose plus LPV/r twice a day. Intensive blood sampling was performed during each phase. Quinine AUC_{0-48h} (area under the plasma concentration–time curve from time 0 to 48 hours), $AUC_{0-\infty}$ (area under the plasma concentration–time curve from time 0 to infinity), and C_{max} (maximum concentration over the time-span specified), were 56%, 57%, and 47% lower, respectively, in the presence of LPV/r. 3-Hydroxyquinine AUC_{0-48h} , $AUC_{0-\infty}$, and C_{max} were significantly lower and the metabolite-to-parent ratio was significantly reduced. Lopinavir and ritonavir exposures were not significantly reduced with quinine coadministration, but C_{max} of both drugs were significantly lower. The geometric mean ratio (GMR) and 90% CI of AUC_{0-48h} , $AUC_{0-\infty}$, and C_{max} for quinine, 3-hydroxyquinine, lopinavir, and ritonavir lay outside the bioequivalent range of 0.8–1.25. Drug treatments during all periods were generally well tolerated. The reduction in systemic exposure of quinine and 3-hydroxyquinine with concomitant LPV/r use raises concerns of suboptimal exposure. Studies in HIV/malaria coinfection patients are needed to determine the clinical impact to decide if any change to the quinine dose is warranted.

INTRODUCTION

Malaria and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) are among the top 10 leading causes of death in low-income countries.¹ The geographical areas most affected by these epidemics overlap, particularly, in sub-Saharan Africa, southeast Asia, and Latin America. The incidence of malaria–HIV coinfection is approximately 10% in Africa and southern India.² In Thailand, a recent retrospective analysis in 867 patients with malaria between 2005 and 2013 in Tak province, an area bordering Myanmar, showed an incidence of 1.85% malaria (*Plasmodium falciparum* and *Plasmodium vivax*) and HIV coinfection. Concomitant use of antimalarial and antiretroviral drugs is therefore becoming increasingly frequent in areas where malaria and HIV coexist, and significant interactions between the two diseases and classes of chemotherapeutic drugs have been reported. Malaria infection stimulates immune mechanisms that activate HIV replication causing a transient increase in HIV viral load.^{3–5} HIV infection also increases the risk of malaria frequency and severity.^{6,7} There is accumulating evidence suggesting clinically important drug–drug interactions occur between antimalarial and antiretroviral drugs, which could potentially affect treatment efficacy and/or tolerability.^{8–11}

Quinine is the first-line antimalarial drug recommended by the World Health Organization (WHO) for treatment of pregnant women with uncomplicated *P. falciparum* malaria during the first trimester as 7-day quinine–clindamycin combination. In addition, it is the second-line treatment of severe *P. falciparum* malaria in most endemic areas.¹² Drug-related cardiotoxicity, however, remains a safety concern with qui-

nine use.^{12,13} For HIV therapy, ritonavir-boosted protease inhibitors (PIs) are currently recommended by the WHO as part of second-line antiretroviral therapy for adults. Globally, lopinavir boosted with ritonavir (LPV/r) remains the most commonly used PI due to its availability as a fixed-dose combination and high genetic barrier to resistance.^{14,15} Both quinine and LPV/r are extensively metabolized by the hepatic cytochrome P450 (CYP) 3A4 enzyme.^{16–21} Elimination half-lives of quinine, lopinavir, and ritonavir are in the ranges of 9–15, 6–14, and 3–8 hours, respectively.^{8–11} Ritonavir is a potent inhibitor and/or inducer of CYP3A4 and several membrane transporter proteins.^{16–18,22–24} CYP3A4 inhibition by LPV/r results in a higher concentration of the antimalarial lumefantrine (2- to 3-fold increase in systemic exposure) in healthy subjects,²⁵ and was associated with lower incidence of malaria and longer posttreatment prophylaxis.²⁶ Inhibition of CYP3A4-mediated metabolism of quinine may result in toxic quinine plasma concentrations, leading to risk of toxicity or untoward side effects.

Reports on the pharmacokinetic interaction between quinine and ritonavir when given alone or as lopinavir-boosted dose remain controversial. A significant increase in systemic exposure of quinine was reported when quinine was co-administered with ritonavir in healthy subjects,⁸ while a decrease in systemic exposure was found when it was co-administered with LPV/r.¹¹ Our objective was to investigate the pharmacokinetic interactions between quinine and LPV/r at steady state in healthy Thai adults.

MATERIALS AND METHODS

Subjects and study design. This was an open-label, three-way, sequential cross-over pharmacokinetic study in healthy Thai subjects. Inclusion criteria included 1) males and non-pregnant females, 2) aged 15–55 years, 3) body weight 40–65 kg, 4) nonsmokers and non-alcohol drinkers, and 5) residents of

*Address correspondence to Kesara Na-Bangchang, Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Thammasat University, 99 Moo 18 Paholyothin Road, Pathumthani 12121, Thailand. E-mail: kesaratmu@yahoo.com

Mae Sot District, Tak Province. Exclusion criteria included those with 1) hepatic or renal diseases; 2) using any drug or herbal medicine within the past 14 days, except antipyretic or antiemetic drugs; or 3) history of intolerance to quinine, lopinavir, and ritonavir. The minimum requirement of the sample size for the study was 19 subjects based on $\alpha = 0.05$, target power = 80% ($\beta = 0.02$), and coefficients of variation (CV) of clearance = 20%. Consenting adults were screened for eligibility according to the inclusion/exclusion criteria. A physical examination, electrocardiogram, and laboratory safety tests (hematology, biochemistry, urinalysis, and pregnancy status) were performed.

Drug administration. Figure 1 summarizes the study design. The pharmacokinetic investigation was performed sequentially on three occasions (periods 1, 2, and 3). Period 1: starting on day 1, subjects received a single oral dose of 600 mg quinine sulfate (two tablets: 300-mg quinine base per tablet, manufactured by the Government Pharmaceutical Organization, Bangkok, Thailand). There was a 2-week washout period between periods 1 and 2. Period 2: subjects received oral doses of LPV/r (two tablets: 400/100 mg of LPV/r, manufactured by Matrix Laboratories Co. Ltd., India) twice daily for 14 days (27 doses). There was no washout between periods 2 and 3. Period 3: subjects received an oral dose of 600 mg quinine sulfate and LPV/r (400/100 mg) twice daily for 3 days.

All subjects were admitted to Mae Sot General Hospital for observation during the pharmacokinetic sampling period, and drug dosage was taken at least 2 hours before meal with water (standard volume 150 mL). Only analgesic/antipyretic (paracetamol) and antiemetic (dimenhydrinate) were allowed in cases of fever and nausea. Drugs with potential interactions with the study drugs (i.e., CYP3A4 inhibitors such as antiretroviral protease inhibitors, erythromycin, clarithromycin, cyclosporine, verapamil, ketoconazole, itraconazole, and voriconazole and CYP3A4 inducers such as carbamazepine, dexamethasone, efavirenz, nevirapine, phenobarbital, phenytoin, primid-

one, rifampicin, and St. John's wort) were disallowed during the study period.²⁷

Assessments of safety and tolerability. Safety and tolerability of the three-drug regimens were assessed based on clinical and laboratory assessments during follow-up according to National Institute of Health/National Cancer Institute (NIH/NCI) Common Toxicity Criteria Grading System for Adverse Events.²⁸ Clinical assessments included physical examination and monitoring of vital signs and adverse events. Safety laboratory assessments (hematology, biochemistry, and urinalysis) were performed during each period. All female subjects had a pregnancy test (β -human chorionic gonadotropin test) performed during each period. Any abnormal laboratory result was followed up with repeat checks every week until it returned to normal.

Blood sample collection for pharmacokinetic assessment. During periods 1 (quinine alone) and 3 (quinine plus LPV/r), blood samples were drawn before the first dose and at 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours after the first dose. During period 2 (LPV/r), blood samples were drawn before the 27th dose and at 1, 2, 4, 6, 8, and 12 hours after the first dose. Immediately after collection, blood samples were centrifuged ($1,200 \times g$, 10 minutes), and the plasma was stored at -20°C until analysis.

Measurement of drug concentrations. Measurement of plasma concentrations of quinine (both free and bound forms) and its metabolite, 3-hydroxyquinine, was performed using high-performance liquid chromatography (HPLC) with fluorescence detection, according to the methods of Karbwang and others²⁷ at the Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Thammasat University. Unbound concentration of quinine in plasma at 2 hours after dosing was measured in 0.5-mL plasma samples after ultrafiltration at 25°C using an Amicon YMT system (Amicon Corporation, Bedford, MA). The assay limit of quantification (LOQ) for quinine and 3-hydroxyquinine

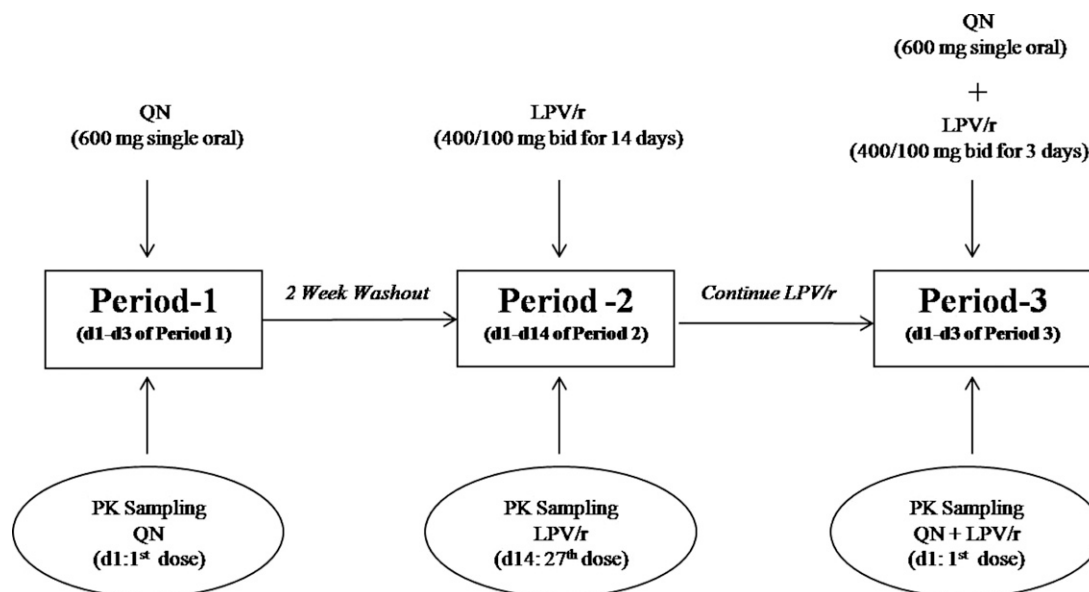


FIGURE 1. Schematic diagram depicting the study design for investigation of pharmacokinetic interaction between quinine (QN) and lopinavir boosted with ritonavir (LPV/r) in healthy Thai subjects.

was 25 and 50 ng/mL, respectively. Recoveries for both compounds were between 96% and 112% and between 94% and 101%, respectively. The intra- and inter-day %CV of quinine and 3-hydroxyquinine ranged from 0.9% to 2.1% and 0.2% to 10.4% and from 1.1% to 2.4% and 0.7% to 9.2%, respectively.

Lopinavir and ritonavir plasma drug concentrations were measured using a validated HPLC ultraviolet assay at the Faculty of Associated Medical Sciences, Chiang Mai University.²⁹ The Program for HIV Prevention and Treatment Laboratory participates in the U.S. AIDS Clinical Trial Group Pharmacology Quality Control (precision testing) program.³¹ The assay LOQ was 100 ng/mL for lopinavir and 50 ng/mL for ritonavir. The recoveries of lopinavir and ritonavir were between 96% and 112% and between 90% and 94%, respectively. Intra- and inter-assay precisions were less than 4% of the CV.

Quality control (QC) samples were run in duplicate in each analytical batch at low, medium, and high concentrations. Criteria for acceptability were four out of six of the QC analyses to lie inside $100 \pm 15\%$ of the nominal values.

Pharmacokinetic analysis and criteria for pharmacokinetic drug interaction. Pharmacokinetic parameters were determined by a non-compartmental analysis³¹ using WinNonLin software (version 6.3, Pharsight, Certara, St. Louis, MO). Concentrations of drugs lower than the LOQ levels were expressed as zero (undetectable). The C_{\max} (maximum concentration over the time-span specified) and t_{\max} (time of maximum concentration) were determined by direct inspection of the plasma concentration–time data. AUC_{0-48h} (area under the plasma concentration–time curve from time 0 to 48 hours) and $AUC_{0-\infty}$ (area under the plasma concentration–time curve from time 0 to infinity) were calculated using the trapezoidal rule. Other pharmacokinetic parameters analyzed included elimination rate constant (λ_z) calculated from at least five concentration–time points of elimination phase, apparent oral clearance (CL/F) calculated as dose/ $AUC_{0-\infty}$, volume of distribution (V_z/F) calculated as CL/F/ λ_z , and the terminal half-life ($t_{1/2z}$) calculated as $0.693/\lambda_z$. Metabolic ratio (MR) was defined as the ratio between $AUC_{0-\infty}$ of 3-hydroxyquinine and quinine. The geometric mean ratio (GMR: the ratio of the value of the parameter for the drug when used in combination versus the corresponding value for the drug used alone) and its 90% confidence interval (CI) were determined for each parameter. A clinically significant pharmacokinetic drug interaction occurred whenever the 90% CI for systemic exposure ratio fell entirely outside the equivalence range of 0.8–1.25.³²

Statistical analysis. Statistical analysis of the data was performed using SPSS version 16.0 (SPSS Inc., Gorinchem, The Netherlands). Pharmacokinetic parameters are presented as median and 95% CI. Comparison of all pharmacokinetic parameters of quinine, 3-hydroxyquinine, lopinavir, and ritonavir obtained during the two periods (period 1 versus period 3; period 2 versus period 3) were performed using Wilcoxon signed-rank test. Comparison of the frequency of subjects with adverse events between the two groups was performed using a χ^2 test. Statistical significance level was set at $\alpha = 0.05$ for all tests.

Ethical considerations. Ethical approval was obtained for all of the studies from which data were obtained in this analysis, and the investigators adhered to the Declaration of Helsinki and Good Clinical Practice. The study protocol was approved by the Institute for Development of Human Research Protection at the Ministry of Public Health in

TABLE 1
Demographic, clinical, and laboratory data of 19 healthy Thai subjects (8 males and 11 females) at baseline

	Median (interquartile range)
Age (years)	
Male	32 (22–41)
Female	29 (21–38)
Body weight (kg)	
Male	59 (55–62)
Female	50 (47–55)
White blood cell count ($\times 10^{-3}/\mu\text{L}$)	7.8 (7.1–9.5)
Red blood cell count ($\times 10^{-6}/\mu\text{L}$)	5.1 (4.6–5.5)
Hematocrit (%)	39.4 (36.6–41.2)
Hemoglobin (g/dL)	13.2 (12–14.4)
Platelet count ($\times 10^{-3}/\mu\text{L}$)	2.63 (2.21–3.12)
BUN (mg/dL)	8.8 (7.4–10.4)
Creatinine (mg/dL)	0.80 (0.70–1.00)
AST (U/L)	24.00 (18.00–28.00)
ALT (U/L)	23 (18.00–33.00)
Total protein (g/dL)	7.00 (6.80–7.20)
Albumin (g/dL)	4.40 (4.20–4.50)
Triglyceride (mg/dL)	87 (77–118)
Fasted blood sugar (mg/dL)	84 (76–94)
Blood pressure (mmHg)	121 (114–138), 76 (65–95)
PR interval (ms)	150 (134–158)
QRS duration (ms)	88 (86–96)
QT interval (ms)	382 (374–398)
The corrected QT interval (msec)	407 (400–427)

ALT = alanine transaminase; AST = aspartate transaminase; BUN = blood urea nitrogen. Data are presented as median (interquartile range).

Thailand. Approval was also obtained from each independent ethics committee and local institutional review board. All participants provided written informed consents for study participation.

RESULTS

Subject characteristics. Twenty healthy subjects (8 males and 12 females) were enrolled in the study. The demographic, clinical, and laboratory information at baseline are summarized in Table 1. All were healthy as verified by results of clinical and laboratory investigations. One female subject discontinued from the study on day 11 because of skin rash during period 2 (LPV/r alone).

Pharmacokinetics of quinine and 3-hydroxyquinine. Pharmacokinetic analysis of plasma concentration–time profiles of quinine and 3-hydroxyquinine was performed using data from 190 samples collected from subjects during each period. For quinine, plasma concentrations in 21 (11.05%) and 29 (16.20%) samples were below the LOQ levels of samples collected during phases 1 and 3, respectively. The corresponding values for 3-hydroxyquinine were 46 (24.21%) and 77 (40.52%), respectively. The median plasma concentration–time profiles of quinine and 3-hydroxyquinine after administration of 600 mg quinine sulfate alone (period 1) and 600 mg quinine sulfate in combination with LPV/r (period 3) are shown in Figure 2. The pharmacokinetic parameters of quinine and 3-hydroxyquinine are summarized in Table 2. Relatively high interindividual variation was observed for most pharmacokinetic parameters of quinine (31% CV) and 3-hydroxyquinine (45% CV). In the presence of steady-state LPV/r, statistically significant differences in quinine pharmacokinetic parameters were observed. C_{\max} , AUC_{0-48h} , and $AUC_{0-\infty}$ of quinine including free quinine concentrations at 2 hours were significantly decreased ($P < 0.005$ for all), while V_z/F and CL/F were significantly increased

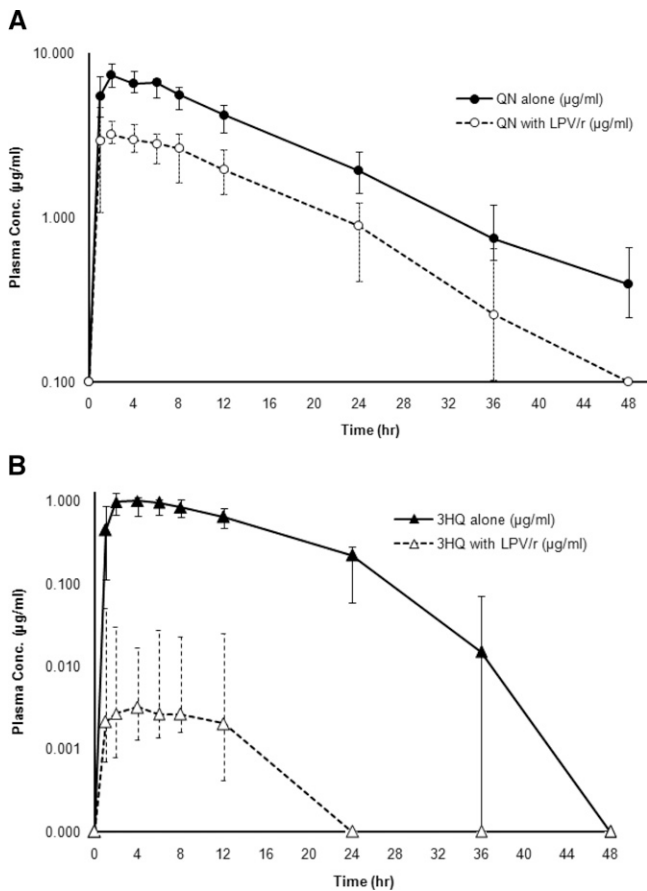


FIGURE 2. Plasma concentration–time profiles of (A) quinine (QN) and (B) 3-hydroxyquinine after administrations of a single 600-mg oral dose of quinine sulfate with (QN with lopinavir boosted with ritonavir [LPV/r]) or without (QN alone) steady-state oral doses of LPV/r.

($P < 0.005$ for both). The $t_{1/2z}$ of quinine was also significantly shorter ($P < 0.01$). The C_{max} , AUC_{0-48h} , and $AUC_{0-\infty}$ of 3-hydroxyquinine were also significantly decreased ($P < 0.005$ for all). The MR of 3-hydroxyquinine to quinine was significantly reduced from 0.12 to 0.0016 ($P < 0.005$).

Pharmacokinetics of LPV/r. Pharmacokinetic analysis of plasma concentration–time profiles of lopinavir and ritonavir was performed using data from 133 samples collected from sub-

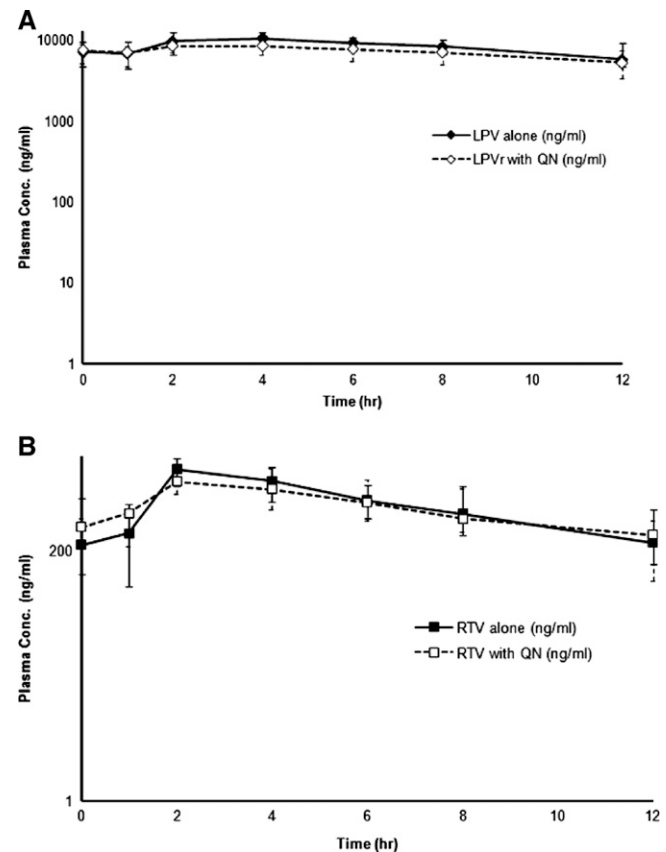


FIGURE 3. Plasma concentration–time profiles of (A) lopinavir (LPV) and (B) ritonavir (RTV) following oral doses of 400 mg LPV plus 100 mg RTV twice a day with (RTV with quinine [QN]) or without (RTV alone) a single oral dose of 600 mg quinine sulfate.

jects during each period. For lopinavir, plasma concentrations in 2 (1.51%) and 0 (0%) samples were below the LOQ levels of samples collected during phases 2 and 3, respectively. The corresponding numbers for ritonavir were 1 (0.77%) and 0 (0%), respectively. The median plasma concentration–time profiles of LPV/r after the administration during periods 2 and 3 are shown in Figure 3. One subject had undetectable plasma lopinavir and ritonavir concentrations until 1 hour after the first dose in period 3. The pharmacokinetics of lopinavir (400 mg) given as ritonavir-boosted dose alone

TABLE 2

Pharmacokinetic parameters of quinine and 3-hydroxyquinine when quinine was administered alone and in combination with LPV/r ($n = 19$)

Pharmacokinetic parameter	Quinine			3-Hydroxyquinine		
	Alone	With LPV/r	GMR (90% CI)	Alone	With LPV/r	GMR (90% CI)
AUC_{0-48h} ($\mu\text{g}\cdot\text{h/mL}$)	132.04 (108.96–155.12)	57.04 (40.70–73.38)†	0.44 (0.33–0.59)	15.56 (11.57–19.55)	0.07 (0.04–0.53)†	0.01 (0.00–0.02)
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/mL}$)	136.83 (112.71–160.95)	57.06 (38.17–75.96)†	0.43 (0.32–0.59)	16.95 (13.02–20.87)	0.09 (–0.80 to 0.97)†	0.02 (0.01–0.04)
C_{max} ($\mu\text{g/mL}$)	7.45 (6.50–8.39)	3.78 (2.86–4.70)†	0.53 (0.41–0.68)	1.05 (0.76–1.34)	0.0 (–0.03 to 0.03)†	0.15 (0.01–0.04)
$C_{max\ free}$ ($\mu\text{g/mL}$)	0.11 (0.05–0.82)	0.37 (0.23–0.50)†	NA	NA	NA	NA
t_{max} (hour)	2 (2–2)	2 (1.47–2.53)	0.85 (0.64–1.20)	2 (0.94–3.06)	2 (–1.18 to 5.18)	1.10 (0.67–1.80)
$t_{1/2z}$ (hour)	10.95 (9.26–12.64)	8.44 (5.72–11.16)*	0.79 (0.63–0.98)	5.49 (3.96–7.01)	13.26 (7.51–19.00)	1.95 (1.17–3.25)
CL/F (L/h)	4.39 (3.58–5.20)	10.51 (6.92–14.10)†	2.31 (1.70–3.15)	NA	NA	NA
V_z/F (L/kg)	1.06 (0.86–1.26)	2.18 (1.75–2.61)†	1.82 (1.40–2.37)	NA	NA	NA

AUC = area under the plasma concentration–time curve; CI = confidence interval; CL = oral clearance; GMR = geometric mean ratio; LPV/r = lopinavir boosted with ritonavir; NA = not applicable (no data).

Significantly different from quinine alone with P value * < 0.01 and † < 0.005 , respectively.

TABLE 3
Pharmacokinetic parameters of LPV/r when administered alone and in combination with quinine ($n = 19$)

Pharmacokinetic parameter	Lopinavir			Ritonavir		
	Alone	With quinine	GMR (90% CI)	Alone	With quinine	GMR (90% CI)
AUC _{0-12h} (μg·h/mL)	100.51 (80.16–120.86)	78.68 (58.41–98.95)	0.94 (0.75–1.18)	6.85 (4.21–9.49)	5.97 (3.64–8.30)	0.93 (0.67–1.29)
AUC _{0-∞} (μg·h/mL)	181.44 (92.42–270.46)	127.96 (59.83–196.09)	0.78 (0.53–1.16)	8.58 (5.20–11.96)	7.11 (2.70–11.51)	0.81 (0.55–1.19)
C _{max} (μg/mL)	10.72 (8.84–12.60)	8.54 (6.86–10.22)*	0.87 (0.71–1.05)	1.23 (0.61–1.85)	0.99 (0.65–1.33)*	0.78 (0.54–1.11)
t _{max} (hour)	2 (0.94–3.01)	2 (0.94–3.06)	1.20 (0.90–1.60)	2 (0.94–3.06)	2 (0.94–3.06)	1.36 (0.97–1.90)
t _{1/2z} (hour)	11.04 (8.17–13.91)	8.40 (5.33–11.47)	0.89 (0.65–1.22)	4.42 (3.70–5.13)	4.14 (2.53–5.74)	0.99 (0.77–1.28)
CL/F (L/h)	2.21 (1.39–3.02)	3.13 (1.94–4.32)	1.28 (0.86–1.89)	11.83 (6.99–16.66)	14.07 (7.18–20.96)	1.24 (0.84–1.82)
V _z /F (L/kg)	0.48 (0.37–0.59)	0.64 (0.49–0.79)	1.14 (0.92–1.42)	1.07 (0.66–1.47)	1.52 (1.04–1.99)	1.24 (0.86–1.79)

AUC = area under the plasma concentration–time curve; CI = confidence interval; CL = oral clearance; GMR = geometric mean ratio; LPV/r = lopinavir boosted with ritonavir.
*Significantly different from quinine alone with P value < 0.01.

(period 2) and in combination with quinine (period 3) are summarized in Table 3. In the presence of quinine, C_{max} of both lopinavir and ritonavir were significantly decreased ($P < 0.01$ for both).

Pharmacokinetic interaction between quinine and LPV/r. On the basis of the criteria set,³² clinically relevant drug interaction is likely to occur when quinine and LPV/r were co-administered. The 90% CI of the GMR of AUC_{0-48h}, AUC_{0-∞}, and C_{max} for quinine, 3-hydroxyquinine, lopinavir, and ritonavir were all outside the acceptable bioequivalent range of 0.8–1.25 (Tables 2 and 3).

Safety and tolerability. Drug treatments during all investigation periods were generally well tolerated. Only mild-to-moderate (NIH/NCI Grade 1 and 2) severity grade adverse events possibly related to the study drugs were observed. The frequency of adverse events occurred during the three periods were similar. The adverse events during period 1 (quinine alone) included vertigo (five cases, 26.3%), tinnitus (three cases, 21.1%), and nausea/vomiting (two cases, 10.5%). The adverse events observed during period 2 (LPV/r) were diarrhea (five cases, 26.3%) and skin rash, stomach ache, and vertigo (one case 5.3%). One case (5.3%) reported nausea/vomiting and syncope during drug administration during period 3 (quinine plus LPV/r). One female subject discontinued from the study on day 11 because of skin rash during period 2 (LPV/r alone). QTc prolongation (from 460 to 481 ms) was observed in one female during period 3 without any sign and symptom of cardiac dysrhythmia. A markedly high proportion of subjects (16/19) with increased serum triglyceride (about 2–7.7 times of baseline) was observed during period 2 after 34 doses of LPV/r. However, the levels in almost all subjects returned to normal within 8 weeks after the first dose, except in one male subject, whose level returned to normal at 12 weeks of the first dose.

DISCUSSION

This study is the first reporting the pharmacokinetic interactions between quinine and LPV/r in Asian subjects. We found marked changes in the pharmacokinetics of quinine and its active plasma metabolite, 3-hydroxyquinine. The impact of quinine on LPV/r pharmacokinetics was less pronounced. A greater systemic exposure of both quinine and 3-hydroxyquinine was observed in Thai compared with Caucasian subjects, while the pharmacokinetics of lopinavir and ritonavir were similar with those previously reported.¹¹ This could be explained by the higher dose per kilogram body weight in Thai population. A relatively lower frequency of

adverse events was found in this study compared with previous studies using a similar dose regimen and design.^{11,33,34} The adverse events after quinine and LPV/r administration included gastrointestinal-related symptoms, that is, vertigo, nausea/vomiting, and diarrhea. These symptoms are the most common adverse effects of lopinavir and quinine.^{35,36} Skin rash and stomach ache were found only after LPV/r dosing. Increase in serum triglyceride level was commonly observed after LPV/r treatment.³⁷

Almost all of the key pharmacokinetic parameters of quinine were modified when quinine was administered in combination with LPV/r. The systemic exposure of quinine represented by C_{max}, AUC_{0-48h}, and AUC_{0-∞} was significantly reduced (49.3%, 56.8%, and 58.0%, respectively). The decrease in systemic exposure is unlikely to be due to impaired drug absorption since there was no delay in t_{max} of either quinine or 3-hydroxyquinine. A significant increase (13%) in free (unbound) quinine concentration at 2 hours (C_{max}) resulted in an expansion of apparent volume of distribution (106%). A parallel significant reduction in the systemic exposure of its active plasma metabolite, 3-hydroxyquinine, as well as the metabolic ratio was unexpected. The 3-hydroxyquinine metabolite exhibits about 10–15% antimalarial activity of quinine. If the mechanism by which the reduced systemic exposure and increased clearance of quinine was due to induction of CYP3A4-mediated hepatic metabolism of quinine, the systemic exposure of 3-hydroxyquinine should have increased. Our observation is in agreement with the report by Nyunt and others¹¹ who assessed the effect of LPV/r on quinine/3-hydroxyquinine pharmacokinetics in healthy Caucasians, where the exposure of both quinine and 3-hydroxyquinine were significantly reduced in the presence of steady-state LPV/r concentrations. One difference we observed was an increase instead of a decrease in free quinine concentration at 2 hours post-dose. The total oral clearance of quinine in healthy Thai subjects was about half of that observed in Caucasian subjects, resulting in about 2- to 3-fold higher quinine and 3-hydroxyquinine concentrations. Another study conducted in healthy Nigerian subjects⁸ found a 4-fold increase in systemic exposure (C_{max} and AUC_{0-∞}) and a 20% increase in t_{1/2z} when quinine (600 mg) was coadministered with ritonavir (200 mg every 12 hours for nine doses), although the metabolic ratio was shown to be significantly reduced. The discrepancy of results obtained in this study and that reported by Soyinka and others⁸ could be explained by the difference in ethnicity and study design. In the study in Nigerian subjects,⁸ only ritonavir was given at the dose of 200 mg every 12 hours for 13 doses alone, and 200 mg every 12 hours for five doses

together with quinine. In addition, quinine dose was given on day 8 during the 15th ritonavir dose. The systemic exposure and half-life of ritonavir was significantly increased, and the observed reduction of systemic exposure of 3-hydroxyquinine was similar. The authors concluded that the mechanism underlying this change was likely to be due to inhibition of hepatic metabolism of quinine, and a reduction in quinine dosing was recommended. Although the study design was similar to our study, a discrepancy between the two studies could be due difference in dose administration (ritonavir was given alone) and possibly, ethnic difference in hepatic metabolism and disposition of quinine.

Altogether, results from this study support the supposition of a complex pharmacokinetic interaction between quinine and LPV/r involving hepatic/gastrointestinal metabolism, drug transportation across membranes, and plasma protein binding. Quinine is primarily eliminated through hepatic metabolism.^{18,20} Lopinavir and ritonavir are substrates or potent inhibitors of CYP3A4, CYP2B6, and CYP2D6, as well as inducers of CYP1A2, 2B6, 2C9, 2C19 and uridine 5'-diphospho-glucuronosyltransferase (UGTs).³⁸⁻⁴³ All protease inhibitors are both substrates and inhibitors of P-glycoprotein (P-gp), with ritonavir being the most potent inhibitor,⁴⁴⁻⁴⁷ while quinine is a substrate for P-gp.^{48,49} The significant changes in the pharmacokinetics of quinine and 3-hydroxyquinine observed with LPV/r coadministration suggests the involvement of multiple drug metabolizing enzymes and drug transporters in the interactions. The relatively high magnitude of the decrease in systemic exposure of 3-hydroxyquinine compared with the parent drug quinine suggests sequential inhibition of CYP3A4-mediated quinine metabolism, followed by the induction of UGT-mediated 3-hydroxyquinine metabolism. The reduction of systemic exposure of quinine could also be due to decrease in its oral bioavailability as a result of induction of pre-systemic metabolism of quinine by ritonavir. The decrease in metabolic ratio was a consequence of the more pronounced reduction of 3-hydroxyquinine exposure compared with quinine. Apart from the metabolic and transporter interactions and plasma protein binding displacement of quinine by ritonavir are also possible. Ritonavir binds extensively to α 1-acidglycoprotein, while the extent of the binding of quinine is moderate.^{49,50} This assumption is supported by the increase in free quinine concentrations observed when quinine was coadministered with LPV/r. However, since quinine was administered orally and has a low hepatic extraction ratio 54, the contribution of a change in plasma protein binding would be likely minimal.

The large reduction in systemic exposure of quinine and its active plasma metabolite, 3-hydroxyquinine, raises concern regarding the higher risk of treatment failure rate when quinine is prescribed to treat malaria in patients with HIV coinfection receiving LPV/r. However, this effect could be counterbalanced by a 3-fold increase in the pharmacologically active free quinine when given with LPV/r. Moreover, the pharmacokinetics of the quinine and LPV/r interaction would be even more complex in patients with malaria considering the increase in α 1-acid glycoprotein with disease severity.^{49,50} Furthermore, multiple doses of quinine are used to treat malaria (600 mg three times a day for 7 days),¹² and not a single dose was used in this study.

In vitro studies suggest that antiretroviral protease inhibitors may have activity against *Plasmodium* parasite,⁵¹ and

could potentiate the efficacy of antimalarial drugs.⁵² A recent randomized study comparing treatment with nevirapine versus LPV/r in HIV-infected children in sub-Saharan Africa found that patients in the nevirapine arm had a significantly higher risk of developing malaria.²⁴ High plasma protein binding of both lopinavir and ritonavir and lack of information on their penetration into red cells suggests that further work is required to establish the clinical relevance of these findings. The more favorable drug interaction profile of the LPV/r arm and potential antimalarial activity of this protease inhibitor may have contributed to this effect. The limitations of this study design are that the study was conducted in healthy subjects (quinine exposure is known to be increased during acute-phase malaria infection). In addition, quinine was given as a single dose (standard treatment is multiple dose of 600 mg, every 7 hours for 7 days), and the unbound concentrations were measured only at a single point (2 hours). Further investigation in patients with malaria and HIV coinfection is required to clarify the magnitude and clinical significance of these potential interactions before any decision on quinine dose optimization can be recommended.

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Authors' addresses: Siwalee Rattanapunya, Faculty of Science and Technology, Chiang Mai Rajabhat University, Chiang Mai, Thailand, E-mail: sirk2012@gmail.com. Tim R. Cressey and Yardpiroon Tawon, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand, and Program for HIV Prevention and Treatment (PHPT/IRD URI 174), Department of Medical Technology, Chiang Mai University, Chiang Mai, Thailand, E-mails: tim.cressey@phpt.org and yardpiroon.tawon@phpt.org. Ronnatrai Ruengweerayut, Department of Medicine, Mae Sot Hospital, Tak Province, Thailand, E-mail: ronnatrai@hotmail.com. Panida Kongjam, Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand, and Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Pathumthani, Thailand, E-mail: panida210@hotmail.com. Kesara Na-Bangchang, Faculty of Allied Health Sciences, Thammasat University (Rangsit Campus), Pathumthani, Thailand, E-mail: kesaratmu@yahoo.com.

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