HHS Public Access

Author manuscript

*Drug Metab Pharmacokinet*. Author manuscript; available in PMC 2022 December 24.

Published in final edited form as:

*Drug Metab Pharmacokinet*. 2022 August ; 45: 100463. doi:10.1016/j.dmpk.2022.100463.

# Comparative single dose pharmacokinetics and metabolism of racemic primaquine and its enantiomers in human volunteers

**Washim Khan**[a,1], **Yan-Hong Wang**[a,1], **Narayan D. Chaurasiya**[b,1], **NP Dhammika Nanayakkara**[a], **HM Bandara Herath**[a], **Kerri A. Harrison**[a], **Gray Dale**[a], **Donald A. Stanford**[a], **Eric P. Dahl**[a], **James D. McChesney**[f], **Waseem Gul**[e], **Mahmoud A. ElSohly**[a,d,e], **Shabana I. Khan**[a], **Pius S. Fasinu**[g], **Ikhlas A. Khan**[a,c], **Babu L. Tekwani**[b,**], **Larry A. Walker**[a,*]

[a]National Center for Natural Products Research, The University of Mississippi, University, MS, 38677, USA

[b]Department of Infectious Diseases, Division of Drug Discovery, Southern Research Institute, Birmingham, AL, 35205, USA

[c]Departments of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA

[d]Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA

[e]ElSohly Laboratories, Inc., Oxford, MS, 38655, USA

[f]Ironstone Separations, Inc., Etta, MS, 38627, USA

[g]Department of Pharmacology & Toxicology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

## Abstract

Primaquine (PQ) is a racemic drug used in treatment of malaria for six decades. Recent studies suggest that the two enantiomers of PQ are differentially metabolized in animals, and this results in different pharmacological and toxicological profiles. The current study characterizes the pharmacokinetic (PK) properties, metabolism and tolerability of the individual enantiomers of PQ in healthy human volunteers with normal glucose-6-phosphate dehydrogenase (G6PD)



*Corresponding author. lwalker@olemiss.edu (L.A. Walker). **Corresponding author. btekwani@southernresearch.org (B.L. Tekwani).

[1]These authors have contributed equally.

Author contributions

BLT, PSF and LAW conceptualized and planed the study; KAH, GD, BLT, PSF and LAW prepared the clinical protocols for IND and IRB approvals and submission to clinicaltrials.gov; DS reviewed the SOPs for quality control and GLP; NDC, KAH, GD, BLT and LAW performed on-site clinical study; WK, YW and NDC processed the samples for bioanalytical LC-MS/MS methods and performed the LC-MS/MS analysis; NPDN and HMBH prepared the metabolites' standards; WG, DS and MAE prepared the material for clinical dosing based on Chemistry Manufacturing and Controls (CMC) Guidance; JDM, SIJ and IAK supported critical analysis of the results; WK, YW, NDC, BLT and LAW prepared the manuscript draft; All the authors contributed to the manuscript editing and read the final draft.

Declaration of competing interest

The authors have declared no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dmpk.2022.100463.

activity. Two cohorts (at two dose levels), each with 18 subjects, participated in three study arms in a crossover fashion: a single dose of the (−)-*R* enantiomer (RPQ), a single dose of the (+)-*S* enantiomer (SPQ), and a single dose of racemic PQ (RSPQ). PQ and its key metabolites carboxyprimaquine (cPQ) and PQ-N-carbamoyl glucuronide (PQ-N-CG) were analyzed. Clear differences were observed in PK and metabolism of the two enantiomers. Relative PQ exposure was higher with SPQ as compared to RPQ. PQ maximum plasma concentration ($C_{max}$) and area under the plasma concentration-time curve were higher for SPQ, while the apparent volume of distribution and total body clearance were higher for RPQ. Metabolism of the two enantiomers showed dramatic differences: plasma PQ-N-CG was derived solely from SPQ, while RPQ was much more efficiently converted to cPQ than was SPQ. $C_{max}$ of cPQ and PQ-N-CG were 10 and 2 times higher, respectively, than the parent drugs. The study demonstrates that the PK properties of PQ enantiomers show clear differences, and metabolism is highly enantioselective. Such differences in metabolism suggest potentially distinct toxicity profiles in multi-dose regimens, especially in G6PD-deficient subjects.

**Keywords**

Primaquine; Racemate; Enantiomers; Carboxyprimaquine; Primaquine-N-Carbamoyl-glucuronide; Pharmacokinetics; UHPLC-MS/MS

## 1. Introduction

Primaquine (PQ) is the prototype 8-aminoquinoline (8-AQ) antimalarial drug, and has been the standard regimen for the radical cure of *Plasmodium vivax* and *Plasmodium ovale* malaria since the early 1950s [1]. It has significant asexual-stage activity against these parasites. The 8-AQs are the only approved drug class with hypnozoiticidal properties; i.e., it kills the dormant liver stage parasites and prevents relapse (has radical curative activity) [2]. PQ is also an effective gametocytocidal drug, eliminating mature *Plasmodium falciparum* gametocytes, and thereby reducing transmission of malaria from humans to mosquitoes [3]. The WHO recommends 0.25–0.5 mg of PQ base/kg body weight once daily for 14 days for the treatment of *P. vivax* and *P. ovale* malaria in children and adults [1]. The major limitation of PQ is that it causes dose-dependent hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency [4], which has a prevalence in tropical areas between 3% and 30% [5]. To mitigate the hemolytic risk in individuals with milder variants of G6PD deficiency, once-weekly PQ (0.75 mg base/kg) for 8 weeks is recommended [6]. PQ is currently used clinically as a racemic (50:50) mixture of (*S*)- and (*R*)-enantiomers.

Early studies of 8-AQs and their structure-activity relationships demonstrated empirically the optimized activity of the 4-amino, 1-methylbutyl side chain on the amine at the 8-position [7]. This side chain includes a chiral center, and later studies showed that the stereochemistry of the side chain in PQ makes a marked difference in the metabolism, toxicity, and effectiveness of PQ, though this may vary somewhat depending on the species and test systems used. PQ was introduced and approved well ahead of our appreciation of the impact of chirality on drug metabolism and drug action, and before required regulatory assessment of stereochemical configurations. However, the toxicity of PQ was shown in

the 1970s to be enantioselective in Rhesus monkeys and in mice, although the enantiomers were equally effective in the Rhesus *Plasmodium cynomolgi* radical cure model [8]. Our group has done extensive characterization of PQ enantiomers in animal models in terms of metabolism, kinetics, and toxicity [9–13]. We also demonstrated the marked divergence of activity controlled by this same stereocenter in other 8-AQs, and could show reduced toxicity and enhanced efficacy, as exemplified in the enantiomer analogs NPC1161A and NPC1161B [14,15].

PQ is very well absorbed after oral administration [16] and is rapidly metabolized in the liver. For a number of years, cPQ has been recognized as the major circulating metabolite [17], and it is likely formed principally by hepatic monoamine oxidase A (MAO-A) [18]. The metabolite cPQ has been reported as pharmacologically inactive [19]. Our initial studies of different pharmacokinetic profiles of the individual PQ enantiomers in humans after racemic PQ administration showed higher SPQ in plasma than RPQ, and cPQ detected in plasma was predominantly formed from RPQ [20].

It is recognized that additional pathways are important for PQ metabolism [21,22], namely a quinoline ring hydroxylation pathway, in which several hydroxylated metabolites are formed, and a pathway of carbamoylation and glucuronidation of PQ [13]. All of these show some stereochemical preferences for RPQ or SPQ. With human cytochrome P450 2D6 (CYP 2D6), ring hydroxylation showed differences in enantiomer preferences [23], and in human hepatocytes, an apparent direct phase II conjugation pathway, carbamoylation of the terminal amine and glucuronidation, was specific to SPQ [13].

Given the risk of hemolysis by PQ in G6PD-deficient populations, the importance of PQ metabolism to its hemolytic effects, and the divergent metabolism, pharmacology and toxicology of the two enantiomers of PQ in animals, there is a need to understand clearly the impact of these enantioselective pathways on PQ's efficacy and toxicity in humans. The quantitative contribution of other pathways for each enantiomer, the pharmacokinetic profiles and potential interactions between the enantiomers are unknown; importantly, how the distinct patterns of metabolism or kinetics may impact efficacy and toxicity are unknown. The current study constitutes the first step toward evaluating in humans of the kinetics, metabolism and tolerability of the two enantiomers separately. Here we have addressed the pharmacokinetics of the two enantiomers of PQ, considered individually, along with the racemic mixture, at two different dose levels in human subjects with normal G6PD activity. Formation and disposition of the major circulating metabolites of PQ, which have shown enantioselective profile for generation in previous *in vitro* and *in vivo* animal studies [12,21-23] were also investigated. It is hypothesized that the individual enantiomers will have different metabolic, kinetic, and tolerability profiles; if one has lower toxicity in G6PD deficient subjects but retained efficacy compared to racemic PQ this would represent an important advance. To determine this will likely require multiple day dosing regimens, because a single dose generally does not cause hemolytic toxicity clinically. This study represents the first-in-human evaluation of individual PQ enantiomers and provides the groundwork for their comparative PK and tolerability in multi-dose regimens in G6PD-deficient subjects.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Reference standards for PQ, cPQ cPQ lactam, PQ-5,6-orthoquinone (POQ), PQ-N-carbamoyl glucuronide (PQ-N-CG), and deuterated PQ (d3-PQ, internal standard) were synthesized at National Center for Natural Products Research (NCNPR), University of Mississippi. MS/MS fragmentation profiles of PQ and these metabolites are reported in our earlier study [21]. HPLC grade methanol, acetonitrile, and formic acid (FA) used for chromatographic separation and extraction were procured from Fisher Scientific USA. Water used in analysis, was purified using a Millipore Synergy UV Water Purification System (Millipore SAS, Molsheim, France). Methanol (70% v/v in HLPC grade water) containing 2% FA was used as a resuspending solution.

### 2.2. UHPLC-UV-MS/MS system and analytical conditions

Chromatographic analysis of metabolites in human was achieved by ultra-high-performance liquid chromatography (Waters Acquity UPLC™ system, Waters Corp., Milford, MA, USA) coupled with a photodiode array (PDA) detector and a Xevo G2-S QToF mass spectrometer (UHPLC-UV-MS) using gradient elution and detected by electrospray ionization (ESI) in positive mode. For the separation, an injection volume of 5.0 μL was introduced to a reversed-phase column (Acquity UPLC HSS T3, 1.8 μm, 2.1 × 100 mm, Waters, USA) kept at 35 °C. The system was operated at 0.4 mL/min flow rate consisting of solvent A (acetonitrile with 0.05% formic acid, v/v), and solvent B (water with 0.05% formic acid) as mobile phases. Gradient started with 5% A (0 min), a linear gradient from 5% to 45% A (0–9 min), later up to 100% A (9–10 min). Between the injections, 2 min washing procedure with 100% B and 3 min re-equilibration with the initial condition were included. The total analysis time for each sample was 17 min. Two wash solvents, i.e., weak needle wash (10/90; acetonitrile/water, v/v) and strong needle wash (90/10; acetonitrile/water, v/v) were used. The PDA detection wavelength was 265 nm for quantitative determination of cPQ content above 1000 ng/mL in the extracted samples. Peaks were assigned by spiking the samples with standard compounds, followed by comparing retention time along with mass spectra of the respective compounds.

Tandem mass (MS/MS) experiments were carried on Waters Xevo G2-S QToF mass spectrometer that was connected to the UHPLC system via an ESI interface. The ESI source was operated in the positive ionization mode with the capillary voltage and cone voltage at 0.3 kV and 30 V, respectively. The source and desolvation temperatures were set at 80 and 400 °C, respectively. The cone and desolvation gas flows were 50 and 800 L/h, respectively. All data collected in centroid mode were acquired using MassLynx™ NT 4.1 software. Accurate mass tolerance of molecular ion and major fragments was limited to 5 ppm, while minor fragments of parent ions were tolerated up to 10 ppm. For mass accuracy, leucine-enkephalin as lock mass was used at a concentration of 1 ng/mL and flow rate of 5 μL/min. During the whole analysis, molecular ion of leucine-enkephalin $[M+H]^+$ (*m/z* 556.2771 Da) and fragment ion (*m/z* 278.1141 Da) were employed to ensure mass accuracy. The system was set at the lock spray interval of 30 s, and average data of three scans were recorded. The mass spectrometer under MS/MS tandem mode was programmed and scanned

in a range of 50–600 *m/z* at 19 ms scan time to each precursor. The precursor ions of POQ, PQ, cPQ, PQ-N-CG, cPQ lactam, and d3-PQ were *m/z* 260.1, 260.1, 275.1, 480.2, 257.1, and 263.2, respectively. Collision energy applied to targeted ions and the corresponding tandem masses were recorded. The quantification of POQ, PQ, cPQ, PQ-N-CG, cPQ lactam, and d3-PQ, respectively, were selected MS/MS transitions corresponding to *m/z* 260.1 > 175.0 (collision energy (CV), 20 eV), 260.1 > 175.1 (CV, 18 eV), 275.1 > 175.1 (CV, 22 eV), 480.2 > 243.1 (CV, 22 eV), 257.1 > 175.1 (CV, 22 eV), and 263.2 > 178.1 (CV, 18 eV). cPQ lactam was not detected as a metabolite in plasma. However, cPQ in 70% methanol aqueous containing 2% formic acid can be converted spontaneously into cPQ lactam during analysis. Therefore, quantification of cPQ included the sum of cPQ and cPQ lactam in each sample. The developed UHPLC-UV-MS/MS method for the quantification of PQ and its metabolites was validated and details are provided in supplementary data.

### 2.3. Clinical study details

The study was conducted in two cohorts of 18 healthy adult human volunteers each (ages 18–55 years) (ClinicalTrials.gov Identifier: NCT02898779). Eight subjects participated in both cohorts, so that a total of 28 individual subjects were recruited. Informed consent was obtained from each subject prior to inclusion in the study. All subjects were screened for deficiency of glucose-6-phosphate dehydrogenase (G6PD) activity, and deficient subjects were excluded from enrollment. The demographic information on the study subjects included for these studies is summarized in Table 1. The PQ enantiomer APIs, and the clinical formulations (capsules with microcrystalline cellulose diluent) were prepared specifically for this study, as the phosphate salts, with full characterization, purity and stability, by ElSohly Laboratories, Inc. (ELI), Oxford, MS. Enantiomers were prepared at 7.5 mg of drug base per capsule, and racemic PQ was prepared at 15 mg base per capsule. The individuals received, 30 min after a normal breakfast, a single dose of two capsules of either (R)-primaquine (RPQ), (S)-primaquine (SPQ), or the racemate, (RS)-primaquine (RSPQ). In cohort I, the doses of RPQ, SPQ, and RSPQ were 15 mg, 15 mg, and 30 mg, respectively. In cohort II, the doses of RPQ, SPQ, and RSPQ were 22.5 mg, 22.5 mg, and 45 mg, respectively. Participant crossover between treatment arms (within each cohort) followed at least a one-week wash-out period. After the administration of PQ, blood samples for PK and clinical evaluations were collected in 9 mL heparinized Vacutainer® tubes at pre-dose, then at 30 min, 1, 2, 4, 8 and 24 h after the study drug administration. The tubes were immediately processed for centrifugation under refrigerated conditions (4 °C) to separate plasma and erythrocyte pellets. The buffy coat layer containing white blood cells was drawn off by pipette. The plasma and erythrocyte samples from individual volunteers were divided into aliquots, kept on dry ice, and transferred for storage at −80 °C till further analysis. The aliquots of plasma samples were processed and analyzed using UHPLC-UV-MS/MS for quantification of parent drug and its metabolites.

Subjects' general health and clinical laboratory parameters were evaluated at screening, and again at 24 h, and on day 4 after test article administration. These included the following laboratory parameters: Complete Blood Count (CBC: red/white blood cell count, hemoglobin, methemoglobin, MCV, platelet count); Metabolic Panel (Sodium, potassium, blood urea nitrogen (BUN), HCO3, creatinine, alkaline phosphatase,

aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, haptoglobin, glutathione and total bilirubin).

### 2.4. Pharmacokinetic analysis

Noncompartmental pharmacokinetic parameters were derived from each subject's data using the actual sampling times with Phoenix 64 Ver 8.2.0.4383 (CERTARA, USA). Maximum concentration ($C_{max}$) of the drug in plasma and time taken to reach $C_{max}$ ($T_{max}$) was recorded from the observed data. The elimination rate constant ($k_{el}$) was derived from a linear regression of the terminal log-linear disposition phase of the concentration-time curve for each subject. The elimination half-life ($t_{1/2}$) was calculated as ln (2)/$k_{el}$. The area under the time-plasma concentration curve from the time of dosing to the last measurable concentration ($AUC_{0\text{-}t}$) for the single-dose was calculated using the linear trapezoidal rule applied up to $T_{max}$. The area under the time-concentration curve from the time of dosing to infinity ($AUC_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}t}C(t)/k_{el}$, where extrapolation is based on the predicted concentration at the last quantifiable time point [C(t)]. The predicted value was based on the linear regression performed to estimate the terminal elimination rate constant and was dependent on three or more data points. For the purpose of the pharmacokinetic analysis of PQ metabolites, it was assumed that PQ had been completely converted into its respective metabolites. Statistical comparison was made to assess the potential pharmacokinetic interactions between two enantiomers of PQ in terms of total drug exposure. An analysis of variance (ANOVA) was carried out on the log-transformed pharmacokinetic exposure parameters ($C_{max}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, Vd, CL, and MRT) to assess the bioequivalence of the enantiomer administrations (alone or in combination). Bioequivalence was assumed if the confidence intervals of the geometric mean ratio (combination/alone) of these pharmacokinetic parameters fell within 80%–125% [24] and to visualize the data, results were presented in Forest plots [25].

### 2.5. Statistics

All pharmacokinetic parameter data were expressed as mean ± standard error mean (mean ± SEM). Comparison of individual data between the groups were performed through one-way ANOVA and non-parametric test for statistically significant analysis using GraphPad Prism version 9.2.0 (332) (GraphPad Software, San Diego, CA, USA). P values < 0.05 were considered as significant differences.

## 3. Results

### 3.1. Pharmacokinetic analysis

In both cohorts of subjects completing the crossover studies, there were no serious adverse events that could be attributed to administration of the drug. None of the subjects reported any gastrointestinal disturbance or other issues at 24 h and 96 h follow-up examinations. Two subjects showed mild and transient elevations in serum AST, ALT, at 24 h after administration of RPQ or the racemate RSPQ, but not SPQ. Another subject showed a mild increase in serum LDH at 96 h after administration of RPQ and RSPQ but not SPQ. These subjects did not report any complaints and the physician observed no clinical signs or symptoms.

The primary safety concern reported with PQ administration is its effects on hematological parameters. Given that these volunteers show normal G6PD activity, it was not expected that these would be affected with a single dose of PQ. Still, hematological parameters, including hemoglobin, hematocrit, a standard CBC parameter, methemoglobin, and haptoglobin were carefully monitored in all the subjects. No significant changes were noted in any of the subjects in any of these parameters throughout the course of this study. Hematocrit values trended marginally downward from the baseline at days 1 and 4, but the magnitude was very small (on average 2 points, from 43 to 41%), and was not different for the enantiomers and racemate. Even for methemoglobin, usually a sensitive endpoint with multiple PQ doses, recorded values were less than 1% in all the subjects, and did not change significantly with administration of RPQ, SPQ, or RSPQ, over 8 h postdosing, and at 24 and 96 h. This lack of change is not surprising, since subjects received only a single dose. Clinically, PQ is administered in 7- or 14-day regimens and the hematological effects generally appear after a few days of dosing.

The derived PK parameters for single oral doses of different PQ enantiomers are summarized in Table 2. Plasma concentration-time curves for PQ in both cohorts are shown in Fig. 1. Significant differences in PK were observed between the two enantiomers of PQ, in that exposure to RPQ was significantly lower than for SPQ, as indicated by the reduced $C_{max}$ and AUC. Forest plots of the geometric mean ratios of PK parameters for both cohorts are depicted in Fig. 2A. The geometric mean ratios for exposure parameters of RPQ vs. SPQ were: $C_{max}$(0.55 ± 0.13), $AUC_{0\text{-}t}$ (0.52 ± 0.13), and $AUC_{0\text{-}\infty}$ (0.56 ± 0.09). By contrast, apparent volume of distribution ($V_D$) and total body clearance (CL) were significantly higher in the subjects receiving RPQ as compared to the subjects receiving SPQ. The geometric mean ratio for Vd, CL, and mean residence time (MRT) for RPQ vs. SPQ were 1.84 ± 0.11, 1.72 ± 0.09, 1.06 ± 0.10, respectively (Fig. 2A). These results are consistent with an increased “first-pass” metabolism of RPQ, as compared to SPQ. As might be expected, with RSPQ (15 mg each of R and S), the $C_{max}$ and AUC of PQ reflected essentially the sum of the individual values with RPQ and SPQ, and apparent volume of distribution and total body clearance showed intermediate values between RPQ and SPQ. For both the enantiomers and the racemate, terminal half-life (9–10 h), $T_{max}$ (~2 h), mean residence time (13–15 h), and rate constant for elimination (~0.08) were similar.

In Cohort II, conditions were identical, except that the dose was increased by 50% (to 22.5 mg for RPQ and SPQ, and 45 mg for RSPQ. The findings were similar to those in Cohort I, with respect to comparative PK of RPQ and SPQ (Fig. 2B). but the $C_{max}$ and AUC values, while rising somewhat, were not elevated in proportion to dose (Table 2). Higher variability was observed in several parameters, including terminal half-life. Apparent $T_{max}$ was slightly increased for SPQ and RSPQ, but clearly RPQ exposure was lower than for SPQ.

cPQ and PQ-N-CG are the major PQ metabolites observed in plasma of human subjects after PQ administration. These display a marked stereochemical selectivity, in that cPQ is primarily derived from RPQ, while PQ-N-CG is derived solely from SPQ. Plasma concentration – time curves of cPQ and PQ-N-CG are shown in Fig. 3A-D. Plasma concentrations of cPQ were rapidly and sharply elevated, 30 to 36-fold compared to PQ in the subjects administered with RPQ (Fig. 3A and B). But for SPQ, much less cPQ is

formed, and the $T_{max}$ was significantly prolonged. With RSPQ administration, the cPQ concentrations are similar to those observed with RPQ alone. In Cohort II, in spite of the increase in dose of the drugs, cPQ concentrations did not show a commensurate increase compared to cohort I. As illustrated in Fig. 3C and D, PQ-N-CG is formed from SPQ, and not detectable in the subjects administered RPQ. However, with RSPQ, it appears that somewhat more of the PQ-N-CG is formed, though the amount of substrate SPQ is the same. This suggests that RPQ may enhance the metabolism of the other through this conjugation pathway.

PK parameters of the metabolites cPQ and PQ-N-CG are presented in Table 3. As expected, the $C_{max}$ and AUC for cPQ are many fold higher than for PQ, and the $T_{max}$ and terminal half-life are prolonged compared to PQ. The PK profiles of cPQ were not significantly different for RPQ and RSPQ groups, as SPQ contributes insignificantly to the cPQ PK profile. PQ-N-CG also showed, compared to PQ, similar elevations in $C_{max}$, AUC, and prolonged $T_{max}$ and half-life in both cohorts.

The relative PQ exposure with respect to dose (D) was plotted as $AUC_{0\text{-}t}/D$ and $C_{max}/D$ in Fig. 4. In both cohorts, the PQ exposure parameters were found to be almost twice as high for SPQ than for RPQ. In the case of RSPQ, the relative exposure with respect to dose is intermediate between that of the two enantiomers.

## 4. Discussion

Until recently, for more than 65 years, PQ was the only approved drug for the treatment of relapsing malaria, by virtue of its activity against hypnozoites of *P. vivax* [1]. In terms of human pharmacokinetic studies, Greaves et al. (1980) conducted a PQ pharmacokinetic analysis and concluded that oral PQ at a dose of 45 mg is rapidly absorbed and reached the maximum level in plasma within 2 h [26]. It was reported that PQ is well absorbed in humans after oral administration of racemic PQ [16]. PQ has long been known to be metabolized via oxidative deamination of its side chain terminal amine by monoamine oxidases [18,27], resulting in formation of cPQ. Circulating concentrations of cPQ are 10–100 times higher than parent drug, with a much longer half-life [17,20].

The differential metabolism and disposal of SPQ and RPQ were reported in previous studies in rat with the greater conversion of RPQ to the carboxy metabolite in the perfused liver, as well as in the liver microsomes [9,28], and similar results were reported from our group, using human hepatocytes [12] and recombinant human MAO-A [13]. Clinical studies from our laboratories also demonstrated, using chiral HPLC-MS, the differential metabolism of PQ enantiomers to cPQ after administration of racemic PQ in normal human volunteers. Almost all of the circulating cPQ was due to metabolism of the RPQ [20].

The aim of the present study was to examine the clinical pharmacokinetics of specific enantiomers of PQ after their individual administration of single oral doses. These studies are intended as part of a more comprehensive evaluation of the comparative tolerability, safety, and efficacy of the two PQ enantiomers in typical anti-relapse regimens, and in particular to the safety profile in G6PD deficient subjects. Thus, these studies constitute the

initial characterization of the two enantiomers in G6PD normal individuals after single dose administration.

PQ was rapidly absorbed following oral administration and peak plasma concentrations were reached at 2 h of dosing, regardless of which enantiomer was administered. However, the $C_{max}$ for RPQ was much lower than for SPQ (by about half) and administration of the two enantiomers together as RSPQ was additive. AUCs for RPQ were also quite low compared to SPQ, and the two enantiomers behaved essentially in additive fashion when administered as RSPQ. PQ exposure parameters, low $T_{max}$ and high $C_{max}$ were recorded for SPQ as compared to RPQ. It may be due to enantioselective absorption, tissue distribution, metabolism and elimination. Another possible explanation may be protein binding, and tissue distribution may be higher for RPQ compared to SPQ. This assumption was supported by higher volume of distribution for RPQ compared to SPQ.

The fact that RPQ is rapidly converted into its carboxylic acid metabolite (cPQ) appears to account for the lower exposure with RPQ compared to SPQ. These observations reflect greater conversion of RPQ to cPQ in liver [12,28]. At 24-h after the oral administration of RPQ or RSPQ, the concentrations of cPQ are still sustained, as much as 100 times above the level of parent drug PQ. Monoamine oxidase A activity is enriched in the liver. This enzyme oxidizes the terminal amine of the PQ side chain to its aldehyde, which is further oxidized, mainly to the carboxylic acid by aldehyde dehydrogenase [18]. Differential interaction of PQ enantiomers *in vitro* with human MAO enzymes was recently reported. RSPQ and RPQ both showed marginally greater inhibition of MAO-A as compared to MAO-B. However, SPQ showed a reverse selectivity with greater inhibition of MAO-B than MAO-A [29]. However, implication of these observations in more rapid metabolism RPQ than SPQ through MAO-A mediated pathway is not clear. Because cPQ has been regarded as a pharmacologically inactive metabolite, lacking antimalarial efficacy, this would imply that RPQ is much less effective as an antimalarial in humans. Certainly, this has been confirmed to be true by our group in mice [10]; however, in the Rhesus monkey, the two enantiomers show similar efficacy [8,11], and the differential in the enantiomeric conversion to cPQ, while certainly present, is not as dramatic in primates [11], compared to that seen in the present study.

In contrast to RPQ, SPQ shows very little conversion to cPQ, but instead is metabolized directly through phase 2 conjugation pathway to N-carbamoyl glucuronide (PQ-N-CG). This metabolite was previously identified as a minor PQ metabolite in human hepatocytes *in vitro* by our collaborative 8-AQ working group [22], and then in human plasma and urine [21]. This represents the first report of the enantio-specificity of generation of this metabolite in human plasma *in vivo*, and the present studies show it is exclusively formed from SPQ. The significance of this metabolite is unknown, but it is of interest in that other glucuronides of PQ, which are reflected in urinary excretion in animals (unpublished findings) and humans [21], are not detected in plasma. But PQ-N-CG persists in plasma with a higher $C_{max}$ and AUC and longer terminal half-life as compared to PQ. An additional observation on this metabolite is that, although it is only formed from SPQ, when the racemic version RSPQ is administered, relatively more PQ-N-CG metabolite is formed. This suggests that when RPQ

is present, it favors shunting of SPQ into the carbamoylation/glucuronidation pathway, as compared to other metabolic routes or binding sites.

Mammalian processes use glucuronidation as an essential mechanism for clearing and removing both endogenous and foreign chemicals. Many functional groups such as amines, carboxyl, thiols, and hydroxyl are susceptible to conjugation with glucuronic acid. Through a reversible reaction, primary and secondary amines can also react to carbon dioxide ($CO_2$) by forming a carbamic acid. The carbamic acid is a glucuronidation substrate and forms a stable carbamate glucuronide metabolite [30]. In the literature, a few drugs such as tocainide, amosulalol, sitagliptin, mofegiline have been reported to undergo N-carbamoyl glucuronidation, which under high $CO_2$ conditions can be facilitated for *in vitro* formation with glucuronyl transferases [31]. Thus, glucuronide conjugation of PQ is apparently facilitated by the spontaneous addition of $CO_2$ at the terminal amine group. At physiological pH of 7.4, the carbamic acid of PQ would be formed only if the pKa of the amino group is low.

Our findings are consistent with those observed previously by our group [20] after administration of racemic PQ, and analysis of PQ and metabolites by chiral HPLC-MS. Chairat et al. (2018) also obtained similar findings with racemic PQ, in their studies of interactions of PQ with other antimalarial drugs [32]. It was pointed out by the latter that SPQ would be seem likely a more important substrate for CYP2D6-mediated metabolism than RPQ, which is consistent with our findings of the preferential hydroxylation of SPQ by human recombinant CYP2D6 [23]. Hydroxylated PQ and PQ ortho-quinone metabolites were not observed in circulation after PQ administration in any of these human studies. Presumably, these are further metabolized and/or rapidly disposed directly via phase II conjugation pathways.

This study included a fairly diverse set of subjects with respect to race/ethnicity, though small numbers overall. The pharmacokinetic parameters were analyzed using a one-way ANOVA, and no statistically significant differences in pharmacokinetic parameters ($AUC_{0\text{-}t}$ and $C_{max}$) for PQ, cPQ and PQ-N-CG could be identified. However, Asian subjects did appear to show a tendency toward higher $C_{max}$ and AUC for PQ, and higher AUC for cPQ, especially in Cohort II. Details are shown in the supplementary data.

Comparative clinical pharmacokinetic profiles of RPQ vs. SPQ vs RSPQ as well as PQ's enantioselective and enantiospecific metabolites strongly support further clinical evaluation of individual enantiomers of PQ in terms of their therapeutic advantages. The calculated elimination half-lives of RSPQ, RPQ, and SPQ were similar. To determine a potential therapeutic advantage, clinical assessment of safety and efficacy of multiple doses are needed. The tolerability concern with PQ is predominantly the hemolytic responses in G6PD deficiency. A single dose of PQ at 0.25 mg/kg is well tolerated in G6PD deficient subjects infected with *P. falciparum* [33,34]. However, with multiple day dosing, in deficient individuals, 0.25–0.5 mg/kg/d (15–30 mg/day in a 60 kg subject) will cause hemolysis within 3–4 days in most deficient subjects [4]. This dose of PQ represents 7.5–15 mg/day of each enantiomer. The question is whether these enantiomers given individually will cause the same toxic response, and whether they will still be effective as antimalarials.

Our current study confirms the tolerability of a single dose of the enantiomers in G6PD normal individuals but understanding the hematological risk will require studies in deficient subjects and likely multi-day dosing. Multi-dose pharmacokinetic studies of the enantiomers of PQ in G6PD normal and deficient subjects are continuing in our group. Additionally, it would be interesting to investigate the enantioselective and enantiospecific PQ metabolites in erythrocytes. Analytical methods for quantitative estimation of PQ-5,6-orthquinone and cPQ-5,6-orthoquine in erythrocytes have been developed [35] and may be applied in future clinical studies.

## 5. Conclusion

PQ enantiomers are well tolerated by the healthy subjects at single 15 mg and 22.5 mg doses. The major circulating metabolites are cPQ and PQ-N-CG. The pharmacokinetic properties of the two enantiomers of PQ showed substantial differences, with significantly lower exposure for RPQ, as compared to SPQ, likely because RPQ is extensively and rapidly metabolized to cPQ. In contrast, while SPQ exposure is higher, much less is converted to cPQ. The PQ-N-CG is associated only with SPQ. The elevated levels of PQ metabolites are sustained for a long time in plasma, much longer than the parent drugs. The pharmacokinetic profile for RSPQ was additive (for both parent drug as well as major metabolites) when compared with individual enantiomers. A comparison between PK profiles for low and high doses did not show a corresponding increase in pharmacokinetic parameters for parent drug and metabolites. These observations indicate a dose-saturation of PK profile, which may have potential pharmacological and toxicological implications. These studies should be extended to multiple dose studies and to evaluation in G6PD-deficient individuals.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This study was supported by the US Army Medical Research & Materiel Command Award (No. W81XWH-15-1-0704 and W81XWH-18-2-0029) to the University of Mississippi (LAW, NPDN, and BLT). BLT is also supported by NIAID grant (1R01AI130134-01A1) (PI- Peter A Zimmerman CWRU).

## References

[1]. Milligan R, Daher A, Villanueva G, Bergman H, Graves PM. Primaquine alternative dosing schedules for preventing malaria relapse in people with *Plasmodium vivax*. Cochrane Database Syst Rev 2020;(8):1–74. 10.1002/14651858.CD012656.pub3. Art. No.: CD012656.

[2]. World Health Organization. Guidelines for the treatment of malaria, 3rd ed. World Health Organization 2015. https://apps.who.int/iris/handle/10665/162441.

[3]. Graves PM, Choi L, Gelband H, Garner P. Primaquine or other 8-aminoquinolines for reducing *Plasmodium falciparum* transmission. Cochrane Database Syst Rev 2018;(2):1–118. 10.1002/14651858.CD008152.pub5. Art. No.: CD008152.

[4]. Recht J, Ashley EA, White NJ. Use of primaquine and glucose-6-phosphate dehydrogenase deficiency testing: divergent policies and practices in malaria endemic countries. PLoS Neglected Trop Dis 2018;12:1–27. 10.1371/journal.pntd.0006230.

[5]. World Health Organization. Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale*: policy brief. World Heal Organ; 2016. 10.1016/B978-012134645-4/50031-7.

[6]. World Health Organization. Guidelines for the treatment of malaria. third ed. 2015. p. 316. 10.1016/0035-9203(91)90261-V.

[7]. Schmidt LH. Relationships between chemical structures of 8-aminoquinolines and their capacities for radical cure of infections with *Plasmodium cynomolgi* in rhesus monkeys. Antimicrob Agents Chemother 1983;24:615–52. 10.1128/AAC.24.5.615. [PubMed: 6660845]

[8]. Schmidt LH, Alexander S, Allen L, Rasco J. Comparison of the curative anti-malarial activities and toxicities of primaquine and its d and l isomers. Antimicrob Agents Chemother 1977;12:51–60. 10.1128/AAC.12.1.51. [PubMed: 407841]

[9]. Baker JK, McChesney JD. Differential metabolism of the enantiomers of primaquine. J Pharmacol Sci 1988;77:380–2. 10.1002/jps.2600770503.

[10]. Nanayakkara NPD, Tekwani BL, Herath HMTB, Sahu R, Gettayacamin M, Tungtaeng A, et al. Scalable preparation and differential pharmacologic and toxicologic profiles of primaquine enantiomers. Antimicrob Agents Chemother 2014;58:4737–44. 10.1128/AAC.02674-13. [PubMed: 24913163]

[11]. Saunders D, Vanachayangkul P, Imerbsin R, Khemawoot P, Siripokasupkul R, Tekwani BL, et al. Pharmacokinetics and pharmacodynamics of (+)-Primaquine and (−)-Primaquine enantiomers in rhesus macaques (*Macaca mulatta*). Antimicrob Agents Chemother 2014;58:7283–91. 10.1128/aac.02576-13. [PubMed: 25267666]

[12]. Fasinu PS, Avula B, Tekwani BL, Dhammika Nanayakkara NP, Wang YH, Bandara Herath HMT, et al. Differential kinetic profiles and metabolism of primaquine enantiomers by human hepatocytes. Malar J 2016;15:224. 10.1186/s12936-016-1270-1. [PubMed: 27093859]

[13]. Fasinu PS, Tekwani BL, Avula B, Chaurasiya ND, Nanayakkara NPD, Wang YH, et al. Pathway-specific inhibition of primaquine metabolism by chloroquine/quinine. Malar J 2016;15:1–12. 10.1186/s12936-016-1509-x. [PubMed: 26729363]

[14]. Nanayakkara NPD, Ager AL, Bartlett MS, Yardley V, Croft SL, Khan IA, et al. Antiparasitic activities and toxicities of individual enantiomers of the 8-aminoquinoline 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4- dichlorophenoxy]quinoline succinate. Antimicrob Agents Chemother 2008;52:2130–7. 10.1128/AAC.00645-07. [PubMed: 18378716]

[15]. Tekwani BL, Walker LA. 8-Aminoquinolines: future role as antiprotozoal drugs. Curr Opin Infect Dis 2006;19:623–31. 10.1097/QCO.0b013e328010b848. [PubMed: 17075340]

[16]. Mihaly G, Ward S, Edwards G, Nicholl D, Orme M, Breckenridge A. Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size. Br J Clin Pharmacol 1985;19:745–50. 10.1111/j.1365-2125.1985.tb02709.x. [PubMed: 4027117]

[17]. Mihaly G, Ward S, Edwards G, Orme M, Breckenridge A. Pharmacokinetics of primaquine in man: identification of the carboxylic acid derivative as a major plasma metabolite. Br J Clin Pharmacol 1984;17:441–6. 10.1111/j.1365-2125.1984.tb02369.x. [PubMed: 6721990]

[18]. Frischer H, Mellovitz RL, Ahmad T, Nora MV. The conversion of primaquine into primaquine-aldehyde, primaquine-alcohol, and carboxyprimaquine, a major plasma metabolite. J Lab Clin Med 1991;117:468–76. [PubMed: 2045714]

[19]. Bates MD, Meshnick SR, Sigler CI, Leland P, Hollingdale MR. In vitro effects of primaquine and primaquine metabolites on exoerythrocytic stages of *Plasmodium berghei*. Am J Trop Med Hyg 1990;42:532–7. 10.4269/ajtmh.1990.42.532. [PubMed: 2164790]

[20]. Tekwani BL, Avula B, Sahu R, Chaurasiya ND, Khan SI, Jain S, et al. Enantioselective pharmacokinetics of primaquine in healthy human volunteers. Drug Metab Dispos 2015;43:571–7. 10.1124/dmd.114.061127. [PubMed: 25637634]

[21]. Avula B, Tekwani BL, Chaurasiya ND, Fasinu P, Dhammika Nanayakkara NP, Bhandara Herath HMT, 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 2018;17(1):294. 10.1186/s12936-018-2433-z. [PubMed: 30103751]

[22]. Pybus BS, Sousa JC, Jin X, Ferguson JA, Christian RE, Barnhart R, et al. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, anti-malarial drug primaquine. Malar J 2012;11:259. 10.1186/1475-2875-11-259. [PubMed: 22856549]

[23]. Fasinu PS, Tekwani BL, Nanayakkara NPD, Avula B, Herath HMTB, Wang YH, et al. Enantioselective metabolism of primaquine by human CYP2D6. Malar J 2014;13:507. 10.1186/1475-2875-13-507. [PubMed: 25518709]

[24]. US Food and Drug Administration. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products — general guidance for industry bioavailability and bioequivalence. 2002. https://www.gmp-compliance.org/files/guidemgr/ucm154838.pdf.

[25]. Bogaty P, Brophy J. Forest plots and the interpretation of subgroups. Lancet 2005;365:1308. [PubMed: 15823379]

[26]. Greaves J, Evans D, Gilles H, Fletcher K, Bunnag D, Harinasuta T. Plasma kinetics and urinary excretion of primaquine in man. Br J Clin Pharmacol 1980;10:399–404. 10.1111/j.1365-2125.1980.tb01777.x. [PubMed: 6934796]

[27]. Constantino L, Paixão P, Moreira R, Portela MJ, Do Rosario VE, Iley J. Metabolism of primaquine by liver homogenate fractions. Evidence for monoamine oxidase and cytochrome P450 involvement in the oxidative deamination of primaquine to carboxyprimaquine. Exp Toxicol Pathol 1999;51:299–303. [PubMed: 10445386]

[28]. Nicholl DD, Edwards G, Ward SA, Orme MLE, Breckenridge AM. The disposition of primaquine in the isolated perfused rat liver. Stereoselective formation of the carboxylic acid metabolite. Biochem Pharmacol 1987;36:3365–9. 10.1016/0006-2952(87)90312-1. [PubMed: 3675598]

[29]. Chaurasiya ND, Liu H, Doerksen RJ, Dhammika Nanayakkara NP, Walker LA, Tekwani BL. Enantioselective interactions of anti-infective 8-aminoquinoline therapeutics with human monoamine oxidases A and B. Pharmaceuticals 2021;14:398. 10.3390/ph14050398.

[30]. Schaefer W. Reaction of primary and secondary amines to form carbamic acid glucuronides. Curr Drug Metabol 2006;7:873–81. 10.2174/138920006779010629.

[31]. Gunduz M, Argikar UA, Baeschlin D, Ferreira S, Hosagrahara V, Harriman S. Identification of a novel N-carbamoyl glucuronide: *In vitro, in vivo*, and mechanistic studies. Drug Metab Dispos 2010;38:361–7. 10.1124/dmd.109.030650. [PubMed: 20008038]

[32]. Chairat K, Jittamala P, Hanboonkunupakarn B, Pukrittayakamee S, Hanpithakpong W, Blessborn D, et al. Enantiospecific pharmacokinetics and drug–drug interactions of primaquine and blood-stage antimalarial drugs. J Antimicrob Chemother 2018;73:3102–13. 10.1093/jac/dky297. [PubMed: 30085149]

[33]. Bancone G, Chowwiwat N, Somsakchaicharoen R, Poodpanya L, Moo PK, Gornsawun G, et al. Single low dose primaquine (0.25mg/kg) does not cause clinically significant haemolysis in G6PD deficient subjects. PLoS One 2016;11(3):e0151898. 10.1371/journal.pone.0151898. [PubMed: 27010542]

[34]. Dysoley L, Kim S, Lopes S, Khim N, Bjorges S, Top S, et al. The tolerability of single low dose primaquine in glucose-6-phosphate deficient and normal falciparum-infected Cambodians. BMC Infect Dis 2019;19:1–11. 10.1186/s12879-019-3862-1. [PubMed: 30606108]

[35]. Khan W, Wang YH, Nanayakkara NPD, Herath HMTB, Catchings Z, Khan S, 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 2021;1163:122510. 10.1016/j.jchromb.2020.122510.

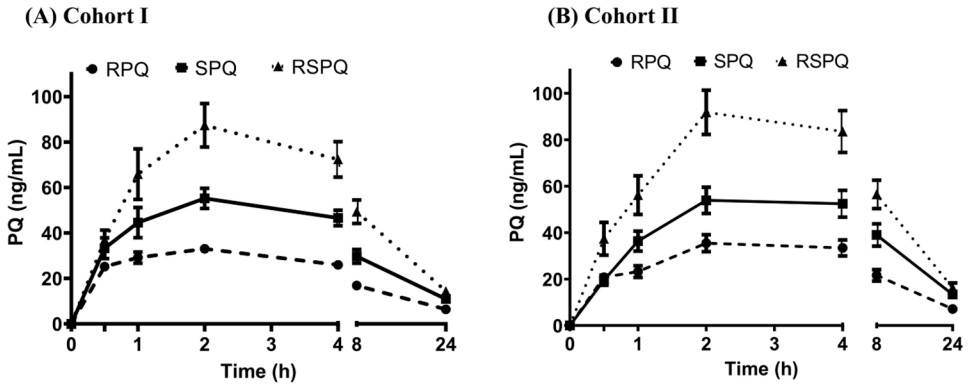


**Fig. 1.**

Mean plasma concentrations of PQ in (A) Cohort I and (B) Cohort II.

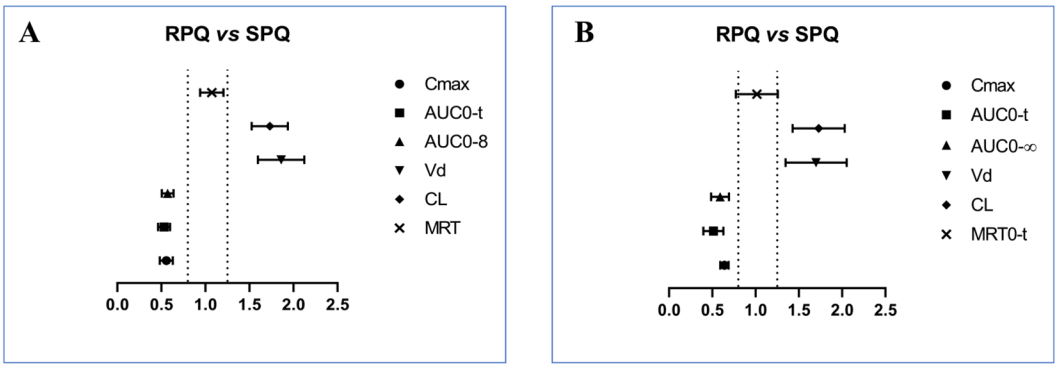


**Fig. 2.**

Forest plots of the geometric mean ratios (at 90% confidence intervals) of different enantiomer of PQ in (A) Cohort I and (B) Cohort II. Pharmacokinetic parameters of PQ were logarithmically transformed. The vertical dashed lines represent the US FDA criteria of 80–125% for assuming bioequivalence.

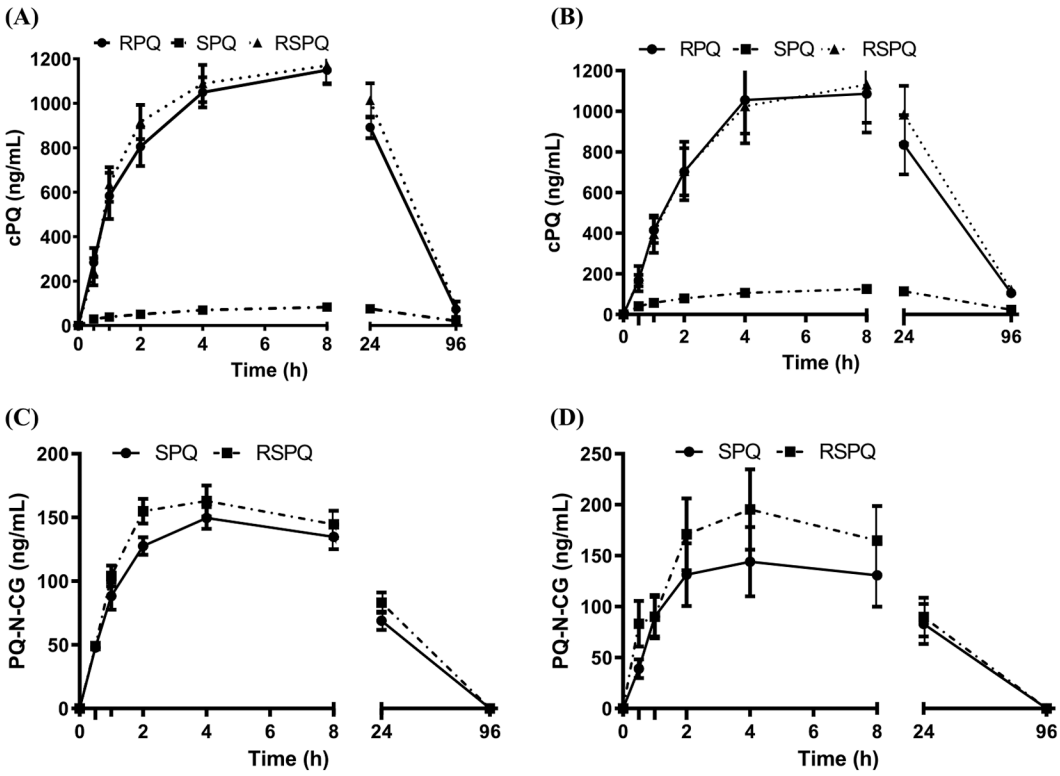


**Fig. 3.**

Mean plasma concentrations of cPQ and PQ-N-CG. cPQ in (A) Cohort I and (B) Cohort II. PQ-N-CG in (C) Cohort I and (D) Cohort II.

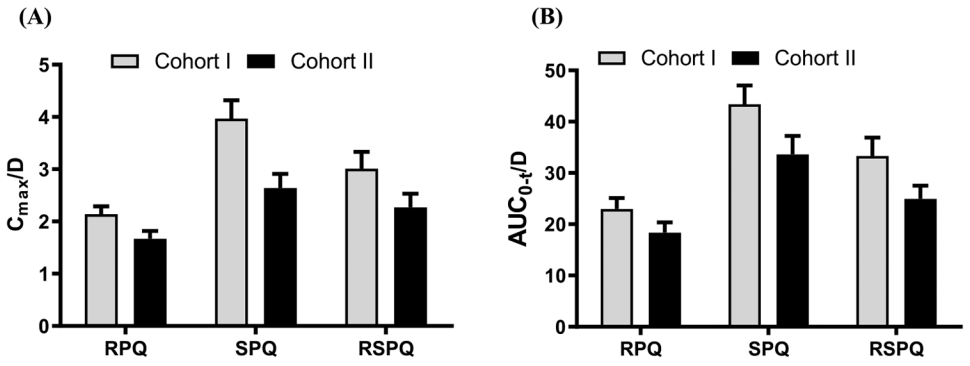


**Fig. 4.**

The relative PQ exposure, expressed as (A) $C_{max}$/D and (B) $AUC_{0-t}$/D in the two cohorts.

**Table 1**

Subject demographics in the two cohorts. There were 18 subjects that participated in both cohorts.

| Parameters | | Gender | | Age | | | |
|---|---|---|---|---|---|---|---|
| | Total | Male | Female | 18–25 | 26–35 | 36–45 | 46–55 |
| | | | Cohort I | | | | |
| | 18 | 12 | 6 | 12 | 4 | 1 | 1 |
| African/Afr. American | 4 | 4 | 0 | 4 | 0 | 0 | 0 |
| Caucasian | 9 | 6 | 3 | 7 | 0 | 1 | 1 |
| Asian | 4 | 2 | 2 | 0 | 4 | 0 | 0 |
| Hispanic | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| | | | Cohort II | | | | |
| | 18 | 13 | 5 | 11 | 6 | 0 | 1 |
| African/Afr. American | 4 | 3 | 1 | 3 | 1 | 0 | 0 |
| Caucasian | 9 | 7 | 2 | 8 | 0 | 0 | 1 |
| Asian | 5 | 3 | 2 | 0 | 5 | 0 | 0 |

**Table 2**

Pharmacokinetic parameters for PQ in single dose for Cohort I [RPQ (15 mg), SPQ (15 mg) and RSPQ (30 mg)] and Cohort II [RPQ (22.5 mg), SPQ (22.5 mg) and RSPQ (45 mg)]. Data are expressed as mean ± SEM.

| Sub | Rate constant ($K_{el}$) (1/h) | Terminal Half-life (h) | $T_{max}$(h) | $C_{max}$ (ng/mL) | $AUC_{0-t}$ (h*ng/mL) | $AUC_{0-\infty}$ (h*ng/mL) | Volume of distribution (L) | Total Body Clearance (L/h) | Mean residence time (h) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **Cohort I** | | | | | |
| **RPQ** | 0.07 ± 0.01 | 10.5 ± 1.1 | 2.3 ± 0.2 | 32.1 ± 2.2 | 344.4 ± 31.8 | 486.9 ± 42.9 | 475.4 ± 31.4 | 34.2 ± 2.7 | 15.1 ± 1.7 |
| **SPQ** | 0.07 ± 0.01 | 9.5 ± 0.8 | 2.0 ± 0.1 | 59.6 ± 5.2 | 650.8 ± 55.3 | 776.7 ± 69.7 | 300.3 ± 35.9 | 22.1 ± 3.7 | 13.5 ± 1.2 |
| **RSPQ** | 0.08 ± 0.01 | 9.4 ± 1.0 | 2.3 ± 0.3 | 90.4 ± 9.6 | 999.8 ± 106.6 | 1245.3 ± 123.9 | 382.5 ± 59.6 | 25.2 ± 3.8 | 13.3 ± 1.4 |
| | | | | **Cohort II** | | | | | |
| **RPQ** | 0.07 ± 0.01 | 13.2 ± 3.3 | 2.2 ± 0.2 | 37.5 ± 3.4 | 415.1 ± 43.7 | 593.6 ± 105.3 | 711.1 ± 66.4 | 45.2 ± 5.2 | 19.1 ± 5.1 |
| **SPQ** | 0.07 ± 0.01 | 9.7 ± 0.8 | 3.1 ± 0.4 | 59.6 ± 5.9 | 756.2 ± 81.1 | 904.8 ± 114.2 | 403.9 ± 48.1 | 29.5 ± 2.5 | 13.8 ± 1.2 |
| **RSPQ** | 0.07 ± 0.01 | 10.1 ± 0.9 | 2.9 ± 0.4 | 102.2 ± 12.4 | 1121.2 ± 117.4 | 1374.1 ± 128.1 | 515.1 ± 53.5 | 36.8 ± 2.8 | 14.3 ± 1.4 |

## Table 3

Pharmacokinetic parameters for cPQ and PQ-N-CG, metabolites of PQ, after single dose oral administration of RPQ, SPQ, and RSPQ. Data are expressed as mean ± SEM.

| Drug | Terminal Half-life (h) | $T_{max}$ (h) | $C_{max}$ (ng/mL) | $AUC_{0-t}$ (h*ng/mL) |
|---|---|---|---|---|
| **cPQ in Cohort I** | | | | |
| RPQ | 20.1 ± 0.6 | 8.0 ± 1.1 | 1167.6 ± 66.6 | 58670.8 ± 3257.5 |
| **SPQ** | – | 11.7 ± 1.6 | 84.7 ± 5.7 | 1745.1 ± 115.9 |
| **RSPQ** | 18.3 ± 2.3 | 8.8 ± 1.4 | 1267.3 ± 62.6 | 63609.0 ± 4590.3 |
| **cPQ Cohort II** | | | | |
| **RPQ** | 31.1 ± 3.1 | 7.2 ± 1.1 | 1162.2 ± 187.7 | 53595.1 ± 9161.3 |
| **SPQ** | – | 12.4 ± 2.0 | 134.9 ± 12.1 | 7198.8 ± 675.5 |
| **RSPQ** | 31.8 ± 4.7 | 12.2 ± 2.1 | 1207.2 ± 182.6 | 62755.7 ± 8968.6 |
| **PQ-N-CG in Cohort I** | | | | |
| **SPQ** | 14.3 ± 0.1 | 4.5 ± 0.5 | 157.7 ± 9.8 | 2583.5 ± 180.9 |
| **RSPQ** | 16.5 ± 2.1 | 4.2 ± 0.4 | 179.1 ± 10.9 | 2845.2 ± 218.1 |
| **PQ-N-CG in Cohort II** | | | | |
| **SPQ** | 13.9 ± 1.6 | 4.3 ± 0.5 | 176.3 ± 24.1 | 3164.1 ± 505.1 |
| **RSPQ** | 13.6 ± 1.7 | 4.6 ± 0.6 | 208.1 ± 33.5 | 2578.1 ± 400.0 |