Enantioselective Pharmacokinetics of Primaquine in Healthy Human Volunteers


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ABBREVIATIONS: 8-AQ, 8-Aminoquinoline: AUC, Area Under Curve; cPQ, Carboxy primaquine; Cmax, maximum plasma concentration; k_el-Elimination rate constant; LC-MS, Liquid chromatography-Mass spectrometry; PQ, Primaquine; T_{1/2}, Elimination half-life ; T_{max}, Time to maximum concentration; TQ, Tafenoquine.
ABSTRACT

Primaquine (PQ), a racemic drug, is the only treatment available for radical cure of relapsing vivax malaria and blocking transmission of falciparum malaria. Recent studies have shown differential pharmacologic and toxicologic profiles of individual PQ enantiomers in rodents, dog and primate animal models. This study was conducted in six healthy adult human volunteers to determine plasma pharmacokinetic profile of enantiomers of PQ and carboxyprimaquine (cPQ), the major plasma metabolite. The individuals were orally administered with PQ diphosphate, equivalent to 45 mg base, 30 min after a normal breakfast. Blood samples were collected at different time intervals and plasma samples were analyzed for enantiomers of PQ & cPQ. Plasma PQ concentrations were low and variable for both parent enantiomers and peaked around 2-4 hrs. Peak (-)-PQ concentrations ranged from 121-221 ng/mL and peak (+)-PQ concentrations ranged from 168-299 ng/mL. cPQ concentrations were much higher and surprisingly consistent from subject to subject. Essentially all of the cPQ detected in plasma was (-)-cPQ. The peak concentrations of (-)-cPQ were observed at 8 hr (range 1104-1756 ng/mL); however, very high concentrations were sustained through 24 hr. (+)-cPQ was two orders of magnitude lower than (-)-cPQ, and in a few subjects it was detected but only under the limit of quantification. In vitro studies with primary human hepatocytes also suggested more rapid metabolism of (-)-PQ compared to (+)-PQ. The results suggest more rapid metabolism of (-)-PQ to (-) cPQ compared to (+)-PQ. Alternatively, (+)-PQ or (+)-cPQ could be rapidly converted to another metabolite(s) or distributed to tissues. This is the first clinical report on enantioselective pharmacokinetic profiles of PQ and cPQ and supports further clinical evaluation of individual PQ enantiomers.
Introduction

The 8-aminoquinolines (8-AQs) are an important class of anti-infective drugs with promising utility in treatment of infections caused by parasitic protozoa and other emerging infectious disease organisms (Tekwani and Walker, 2006). Primaquine (PQ) is the only 8-AQ drug approved for clinical use for the treatment (radical cure) of relapsing *Plasmodium vivax* malaria (John et al., 2012; Tekwani and Walker, 2006; Vale et al., 2009). It is also used as a prophylactic drug against all major forms of human malaria and in combination with clindamycin for the treatment as well as prophylaxis of *Pneumocystis* pneumonia in HIV/AIDS patients (Kim et al., 2009; Tekwani and Walker, 2006). The utility of PQ has also been shown in prevention and blocking of transmission of *Plasmodium falciparum* malaria (Hill et al., 2006; White et al., 2012), and recently its use has been proposed as a key strategy in malaria control and elimination efforts (Fernando et al., 2011; Galappathy et al., 2013; John et al., 2012; White, 2008). However, the therapeutic utility of PQ has been limited due to severe hemolytic toxicity in individuals with glucose 6-phosphate dehydrogenase deficiency (Baird, 2012; Ganesan et al., 2012; Howes et al., 2013; Youngster et al., 2010)

Synthesis and testing of many different 8-AQs in the 1940s led to discovery of PQ, with its 4-amino-1-methylbutyl side chain (Figure 1), which conferred the best combination of efficacy and tolerability (Edgecomb et al., 1950). This was further demonstrated by extensive structure-activity analysis of 8-AQs (McChesney, 1981; Nodiff et al., 1991; Schmidt, 1983). This side chain contains an asymmetric center at carbon 1, and thus two different configurations (enantiomers) are possible (Figure 1). PQ synthetic methods yield a racemic mixture of these two enantiomers, and when PQ was developed, enantiomeric separation of racemates was not available, and single enantiomers of racemic drugs were not the norm in drug development. Later, when next the generation 8-AQs namely, bulaquine (Krudsood et al., 2006; Rajgor et al., 2003) and tafenoquine...
(TQ) (Crockett and Kain, 2007; Llanos-Cuentas et al., 2013) were developed, the same side chain was retained. Thus, PQ is still available as the racemate, and TQ is also being developed as a racemate (Llanos-Cuentas et al., 2013). Previous as well as more recent findings suggest that the configuration of this side chain can dramatically impact metabolism, toxicity, and efficacy of PQ and these can be differentially affected, depending on species and the test systems employed (Agarwal et al., 1988; Baker and McChesney, 1988; Ward et al., 1987). Actually, more than 37 years ago, Schmidt et al. (1977) provided early evidence about the enantioselective toxic effects of PQ in Rhesus monkeys (Schmidt et al., 1977). He tested hundreds of 8AQs for efficacy in primates under the US Army’s malaria drug development program (Schmidt, 1983). It was observed that though the enantiomers of PQ were equally efficacious in the Rhesus Plasmodium cynomolgi radical cure model, (-)-PQ was at least twice as toxic as (+)-PQ (Schmidt et al., 1977). It should be pointed out that the toxicity tracked by Schmidt et al. (1977) in these studies was likely liver related. He did not report hematological parameters. However, if such a toxicity differential holds for hemolytic potential, as suggested by our other studies (Nanayakkara et al., 2014), it would be a key finding. It is suggested that if such a distinction held true in man, by simply separating the two enantiomers, a doubling of the clinical therapeutic index of PQ could be attained (Schmidt et al., 1977; Tekwani and Walker, 2006). Recent studies at our lab have confirmed differential pharmacologic and toxicologic profiles of individual enantiomers of PQ in different rodent models and beagle dogs, where (+)-PQ was found to be more efficacious as well as hemotoxic compared to (-)-PQ (Nanayakkaya et al., 2014). A more recent study in rhesus macaques (Macaca mulatta) has shown that treatment with (+)-PQ enantiomer caused greater methemoglobin toxicity than that seen for (-)-PQ. (-)-PQ in combination with chloroquine was more effective in preventing P. cynomolgi relapse, a surrogate model for P. vixav relapse, compared to (+)-PQ (Saunders et al., 2014)
From regulatory, scientific and humanitarian perspectives, clinical use of chiral drug with confirmed enantioselective pharmacological and toxicological profiles should not be acceptable in racemic form. De novo development of a drug in enantiomerically pure form or a switch from an existing racemic drug to one of its isomers is the principal scenario in chiral drug development. FDA now requires evaluation of both enantiomers as well racemic mixtures of a chiral drug before its introduction into the clinics (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122883.htm). PQ was approved for clinical use in racemic form more than 60 years ago (Vale et al., 2009), at the time when technologies for chiral chemical synthesis/separation were not well developed and the understanding of enantioselective pharmacology/toxicology of PQ was almost non-existent. We have developed a fractional crystallization method for preparation of individual PQ enantiomers (Nanayakkara et al., 2014) and also analytical method using liquid chromatography/mass spectrometry with electrospary ionization (ESI) for the separation and identification of (-)-(R)- and (+)-(S)-PQ and its major plasma metabolite (-)-(R)- and (+)-(S)-carboxyPQ (cPQ) in plasma samples (Avula et al., 2011). This method quantifies the [M+H]+ ions of PQ, 4-methyl PQ (internal standard) and cPQ at m/z 260.1763, 274.1849 and 275.1396 respectively, in the positive ion mode with extractive ion monitoring (EIM). This method has been useful for investigating enantioselective metabolism of PQ in rodent and primate animal models (Avula et al., 2011; Saunders et al., 2014). This method was further used to explore the pharmacokinetic and metabolic properties of the enantiomers of PQ in healthy human volunteers after administration of the racemic form of the drug.

Material and Methods

Chemicals and materials. Primaquine phosphate (Sanofi-Aventis, USA) was obtained from a local pharmacy. Each tablet contained 15 mg of PQ base. HPLC-grade acetonitrile and
methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA, USA). PQ diphosphate, ammonium formate and formic acid were purchased from Sigma (St Louis, MO, USA). The individual enantiomers of PQ were prepared by the fractional crystallization method described in Nanayakkara et al. (2014). PQ was resolved to (+)-(S)- and (-)-(R)-forms at NCNPR, their identity and purity were confirmed by spectral data (IR, NMR and High-Resolution MS) and their physical data (mp, [alpha]D) were compared with published values (Carroll et al., 1978). cPQ was prepared using the procedure reported by McChesney et al. (1984). This study was conducted at the Department of Student & Employees Health Services, The University of Mississippi, under the supervision of Dr. Travis W. Yates (MD), following a protocol approved by the IRB (The University of Mississippi IRB file number 2010-0013).

**Subjects, treatment, samples collection and processing.** The study was conducted with six healthy adult human volunteers (age 26-51 years). The information on age, sex and ethnicity of individual human volunteers is given in supplemental data (Supplemental data Table 1). The individuals were orally administered three tablets of primaquine phosphate (equivalent to a total dose of 45 mg primaquine base) (Sanofi-Aventis US) 30 min after a normal breakfast. Blood samples were collected in 9 mL heparin vacutainer® tubes at different time intervals after administration of PQ. The tubes with blood samples were immediately processed for centrifugation under refrigerated conditions (4 °C) and separation of plasma and erythrocytes pellet. The plasma samples from individual volunteers were divided into aliquots (500 μL) in cryovials, kept on dry ice and transferred for storage at -80 °C. The plasma samples were processed further and analyzed using LC-MS for enantiomers of PQ & cPQ as described below.
Liquid chromatography/Mass spectrometry (LC-ESI-TOF). One part of plasma (100 µL) was mixed with four parts of methanol (400 µL). Drug was extracted by vortexing each sample for 30 seconds. After centrifuging at 10,000 rpm for 5 minutes, supernatants were taken out and filtered through 0.2 µm nylon membrane filters. An aliquot of 200 µL was transferred to HPLC vials for analysis (Avula et al., 2011). The details regarding LC-MS analytical method and other conditions have been described in an earlier publication (Avula et al., 2011). The liquid chromatographic system was an Agilent Series 1100 and comprised of the following modular components: quaternary pump, a vacuum solvent micro degasser and an auto sampler with 100-well tray. The mass spectrometric analysis was performed on an LC-ESI-TOF (Model #G1969A, Agilent Technologies, Palo Alto, CA, USA) equipped with an ESI source. The LC-ESI-TOF was calibrated using the Agilent tune mix. All acquisitions were performed under positive ionization mode with a capillary voltage of 3500 V. Nitrogen was used as the nebulizer gas (35 psig) as well as drying gas (11 L/min, 350 ºC). The voltage of PMT, fragmentor and skimmer was set at 850V, 100V and 60V, respectively. Full scan mass spectra were acquired from \( m/z \) 100-900. Data acquisition and processing was done using the Analyst™ QS software (Agilent Technologies, Palo Alto, CA, USA). Separation was achieved on a Chiralcel OD-R; 250 x 4.6 mm I.D.; 10 µm particle size (Chiral Technologies, Inc., West Chester, PA, USA). The column was equipped with a guard column (Chiral Technologies, Inc., West Chester, PA, USA). A gradient LC method was used to separate PQ, cPQ enantiomers and IS from the matrix components, and to avoid ion suppression by the latter during quantification. Linearity of the LC-MS method employed for analysis of human plasma samples ranged from 10-1000 ng/mL for mixture of (±)-PQ and (±)-cPQ concentrations in samples, i.e. 5–500 ng/mL of each enantiomer. The lower limit of quantification (LLOQ) for human plasma samples were, respectively, 5 ng/mL for each enantiomer of PQ and 1 ng/mL for each enantiomer of cPQ. The lower limit of detection (LLOD) for the human plasma samples were 2 ng/mL and 0.5 ng/mL for
each enantiomer of PQ and CPQ, respectively. The extraction recovery varied from 89-90% (with 0.2 mL human plasma containing 10, 250, 500 ng/mL PQ) and 91-92% (with 0.2 mL human plasma containing 10, 250, 500 ng/mL CPQ). On storage at ambient temperature (25 °C) for 12 h, the concentrations of enantiomers of PQ and cPQ in plasma deviated less than ±10% from their calculated concentrations, showing that the samples were stable during preparation and analytical processes. On storage of human plasma samples with individual enantiomers of PQ or cPQ at 4 °C for 24 h, the concentrations of the PQ or cPQ varied no more than ±10% of their calculated concentrations.

Pharmacokinetic analysis

The pharmacokinetic parameters for PQ and cPQ were computed as described by Cuong et al. (2006). Maximum plasma drug concentration (Cmax) and time to maximum concentration (T\textsubscript{max}) were obtained from the plasma drug concentration–time curve (Binh et. al., 2009). The elimination rate constant (k\textsubscript{el}) was estimated by least-squares regression analysis of the post-absorption and distribution log plasma drug concentration–time data using at least 4 points. The elimination half-life (T\textsubscript{1/2}) was calculated from the ratio ln2/k\textsubscript{el}. The area under the drug concentration–time curve from zero to 24h (the last data point) (AUC\textsubscript{0–24h}) was calculated by the linear trapezoidal rule from the beginning of PQ administration to the last data point. Apparent oral clearance (CL/F) was computed by (dose)/(AUC\textsubscript{0–24h}). The results were statistically analyzed for significance by Student’s t test using GraphPad Prism.

In vitro PQ-primary human hepatocyte incubation

Freshly isolated primary human hepatocytes (BD Biosciences) were used in these experiments. The hepatocytes used for in vitro PQ metabolism studies were from a 61 years old Caucasian female donor with no history of any liver disease, tested negative for HIV, hepatitis B and C.
BD-BioCoat®, a fully defined serum free hepatocyte culture medium without EGF was used. The cells received in suspension were immediately centrifuged at 1000g for 15 min, washed with BioCoat and re-suspended in BioCoat® at a cell density of 1X10^6 cells/mL. The metabolic reactions were set up in a clear cell culture grade 24 well polystyrene plate. The in vitro incubation mixtures were consisted of 480 µL cell suspension and 20 µL medium or primaquine (1mM) (to achieve final PQ concentration of 20 µM). The plate was incubated in a CO2 incubator at 37 °C with 5% CO2. The contents from individual wells (three replicates for each time point) were withdrawn at different time intervals, transferred to micro-centrifuge tubes, 500 µL pre-chilled HPLC grade methanol added to each tube, vortexed and stored over night at -80 °C. The samples were centrifuged at 10,000 g. The clear supernatants were filtered through 0.2 µm nylon membrane filters, 100 µL aliquots were transferred to HPLC sample vials and analyzed for enantiomers of PQ and cPQ by LC-MS method as described above.

**Results**

PQ (45 mg) was administered as three tablets of PQ diphosphate with a glass of water to each individual after a regular breakfast. This dose was well tolerated by all the subjects and no adverse reactions were observed in any of the six individuals who participated in this study. The plasma concentrations of enantiomers of PQ and cPQ were analyzed by the chiral LC-MS method and quantitated against a calibration curve prepared with authentic standards. The concentrations of enantiomers of PQ and cPQ in individual subjects at the different time intervals are presented in the supplement data (Supplemental data Tables 2, 3 and 4). The computed pharmacokinetic parameters for plasma PQ and cPQ enantiomers for each individual subject are presented in supplemental data (Supplemental data Tables 5 and 6) and average pharmacokinetics data are presented in Table 1.
In five subjects, the concentrations of (+)-PQ as well as (-)-PQ enantiomers were first detected at 60 minutes after administration of the drug. In one subject, (+)-PQ and (-)-PQ were detected as early as 30 min after administration of racemic PQ (Supplemental data Table 2). The plasma concentrations of (-)-PQ ranged from 121.3 to 221.6 ng/mL and peaked between 2-4 hours. The plasma concentrations of (+)-PQ, ranged between 168.8 to 299.2 ng/mL. The (+)-PQ concentrations also peaked during 2-4 hours. The plasma half–life (T1/2) for (-)-PQ and (+)-PQ was not significantly different. However, the Cmax for (+)-PQ was significantly higher than for (-)-PQ (P=0.017). The AUC value, computed for the period 0-24 hr, was about 1.5 fold higher for (+)-PQ than for (-)-PQ. This difference in AUC values for (-)-PQ and (+)-PQ was statistically significant (P= 0.022). The higher AUC value to (+)-PQ compared to (-)-PQ resulted into significantly lower clearance (CL/F) for (+)-PQ than for (-)-PQ.

In all subjects except one, cPQ first appeared at 60 min after administration of racemic PQ. The key finding in this study was that essentially almost all of the cPQ detected in plasma was (-)-cPQ, yielding a peak plasma concentration 60 times higher than that observed for (+)-cPQ; and consistent with the kinetics of the parent enantiomers, having a Tmax 4 h longer than the (+)-PQ. The peak plasma concentrations of (-)-cPQ were observed at 8-24 hr in the range of 1104-1756, with average peak plasma cPQ concentration of 1399 ng/mL. However, very high concentrations of plasma (-)-cPQ were still present at 24 hr. The concentrations of (-)-cPQ only marginally declined during 8-24 hours, and in two subjects, peak (-)-cPQ concentrations were detected at 24 hours. The observations also indicate a long plasma half-life of (+)-cPQ. The AUC values computed for the period of 0-24 hours for (-)-cPQ were in the range 23573 to 33827 ng.hr/mL. The PQ treated individuals were therefore exposed to more than 15 fold higher exposure to plasma cPQ concentrations than the PQ concentrations. The concentrations of (+)-cPQ were two orders of magnitude lower than (-)-cPQ. In one subject (-)-cPQ was
only detected under the limit of quantification throughout the period of the study. The peak concentrations of (+)-cPQ ranged between below the limit of quantification to 36.8 ng/mL.

The in vitro studies on metabolism of PQ with primary human hepatocytes also showed results similar to that observed with human pharmacokinetics studies. Incubation of racemic PQ with human hepatocytes showed more rapid depletion of (-)-PQ (78% in 4 hours) compared to (+)-PQ (22% in 4 hours) (Figure 4A). Concomitant formation of cPQ was observed on in vitro incubation of PQ with primary human hepatocytes (Figure 4B). During initial 2 hours of incubation 96.1% of the total (±)-cPQ formed with human hepatocytes was (-)-cPQ. A very slow formation of (+)-cPQ was observed after 4 hours. Still, (+)-cPQ represented only 13% of the total (±)-cPQ formed at 16 hours (Figure 4B).

**Discussion**

Most of the antimalarials namely, chloroquine, PQ, mefloquine, halofantrine, tafenoquine and lumefantrine are chiral but are used as racemates (Brocks and Mehvar, 2003). For antimalarials, stereoselectivity has been mainly noted in their ability to cause adverse effects. Development of analytical methods capable of measuring the individual enantiomers of these antimalarials has shown that almost all antimalarial drugs display stereoselectivity in their pharmacokinetics (Brocks and Mehvar, 2003).

Our group has reported recently on enantioselective pharmacologic, pharmacokinetic and toxicologic properties for PQ in mice, dogs, and primates (Nanayakkara et al., 2014; Saunders et al., 2014). These studies added to a body of literature that suggested important differences in these biological activities for PQ enantiomers. Although no stereoselectivity was noticed in the metabolism of (+)-(S) and (-)-(R) isomers of PQ in vitro by isolated perfused liver (Nicholl et al., 1987) and rat liver microsomes (Ward et al., 1987), when racemic PQ was administered to rats
the majority of residual PQ excreted in urine was found to be the (+)-isomer (Baker and McChesney, 1988). The (+)-isomer of PQ caused significantly higher generation of methemoglobin in erythrocytes from a glucose 6-phosphate deficient individual than the (-) isomer (Agarwal et al., 1988 and 1991). Schmidt et al. (1977) examined the relative curative and toxic activities of PQ and its (+)- and (-)-isomers in mice and rhesus monkeys. They confirmed their earlier report that (+)-PQ was approximately 4 times more toxic as compared to the (-)-form and at least twice more toxic than racemic PQ in mice; they also indicated that the opposite is true in the Rhesus monkey, in which the (-)-PQ was 3 to 5 times more hepatotoxic as compared to (+)-PQ and at least twice more toxic than racemic PQ. More importantly, all three forms of PQ, the (+)-PQ and (-)-PQ and (±)-PQ showed similar curative potencies against sporozoite-induced *P. cynomolgi* infections. In several studies on the metabolism of (+)- and (-)-isomers, it was shown that the metabolic rates for these isomers were different under most conditions (Baker and McChesney 1977, Nicholl et al., 1987). However, this is the first ever study in humans, which provide conclusive evidences regarding differential pharmacokinetic profiles of PQ enantiomers. The results obtained with human pharmacokinetic studies are further supported by enantioselective metabolism of PQ *in vitro* by primary human hepatocytes.

Generally, there was rapid absorption of PQ following the single oral administration of (±)-PQ with mean peak blood concentrations attained within less than 3 h (160 min). This was followed by a less rapid but steady fall in plasma concentration with a mean elimination half-life of 9.39 h. The extent of absorption of individual PQ enantiomers was not captured in this study, but as earlier pharmacokinetic studies with (±)-PQ have reported that PQ is almost completely absorbed in man following oral administration (96% bioavailability) (Mihaly et al., 1985).
The key finding in this study was that essentially all of the cPQ detected in plasma was (-)-cPQ. (+)-cPQ was two orders of magnitude lower than that of (-)-cPQ, and in most samples it was only detected under the limit of quantification. Very high concentrations of cPQ, mostly (-)-cPQ, were still present at 24 hr. Therefore the half-life for cPQ enantiomers could not be computed from this experiment. However, the persistence of the (-)-cPQ is consistent with earlier published studies on pharmacokinetics of racemic PQ (Bangchang et al., 1994; Bhatia et al., 1986; Kim et al., 2004; Ward et al., 1985) and PQ in combination with chloroquine (Pukrittayakamee et al., 2014) and artemisinin combinations (ACT) therapies (Hanboonkunupakarn et al., 2014; Jittamala et al., 2015). The computed elimination half-lives of (±)-PQ, (-)-PQ and (+)-PQ were similar. Thus, in the event of future enantioselective utility of PQ, dosing frequency with enantiomers may not be different for the racemic mixture.

The attainment of peak plasma concentration of a drug often reflects the net balance between the rate of absorption, tissue distribution and elimination. Several factors can be attributed to the observed significantly higher peak plasma concentration of (+)-PQ in comparison to (-)-PQ. Variation in enantioselective susceptibility to presystemic metabolism including intestinal efflux activities may result in varying blood concentrations. These factors are more likely to be responsible in this case, as supported by the delayed $T_{\text{max}}$ observed with (-)-PQ in addition to its lower $C_{\text{max}}$. Alternatively, discrepancies in the enantiomers’ affinity for tissue penetration and protein binding might affect the measurable concentrations in the blood. Thus, if (-)-PQ is more distributed in the tissues, less blood concentration will be expected. Analysis of PQ and cPQ in tissues is beyond the scope of this study, as extravascular concentrations of the enantiomers are not captured. However, in vitro studies also showed more rapid metabolism of (-)-PQ compared to (+)-PQ by primary human hepatocytes, which was further reflected in terms of more rapid
formation of (-)cPQ compared to (+)-PQ. Another possible reason for varying Cmax of the enantiomers may be the variation in systemic clearance of individual PQ enantiomers.

In conclusion, the results presented here suggest a markedly more rapid metabolism of (-)-PQ to (-)-cPQ than (+)-PQ to (+)-cPQ. Alternatively, the (+)-PQ or (+)-cPQ could be rapidly converted to another metabolite(s) or distributed to the tissues. This study confirms enantioselective pharmacokinetic and metabolic profiles of PQ and suggests the need for further clinical evaluation of the efficacy and safety of PQ enantiomers in humans. The computed elimination half-lives of (±)-PQ, (-)-PQ and (+)-PQ were similar. Thus, in the event of future clinical evaluation of individual PQ enantiomers to determine enantioselective therapeutic advantage of PQ, dosing frequency with enantiomers may not be different from current dose regimens evaluated for racemic PQ. Comparative clinical pharmacokinetic profiles of (-)-PQ vs (+)-PQ and (-)-cPQ vs (+)-PQ strongly support further clinical evaluation of individual enantiomers of PQ regarding safety and other therapeutic advantages. Initial analyses of these results suggest a better therapeutic value of (+)-PQ over (-)-PQ. A lower clearance (CL/F) and higher exposure (AUC) for (+)-PQ compared to (-)-PQ suggest that a lower dose of (+)-PQ may be required for the malaria patients. Further, any potential adverse effects of exposure to cPQ would be eliminated since (+)-PQ would generate very low levels of (+)-cPQ. Additionally, it would be interesting to investigate enantioselective profile for other PQ metabolites, which may be directly implicated to relative efficacy and toxicity of PQ. The analytical methods for phenotyping the potentially reactive phenolic metabolites of PQ generated with human hepatocytes and recombinant human CYP2D6 have been reported recently and may be applied to clinical studies with individual PQ enantiomers.

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Authorship contributions

Participated in research design: Tekwani, Stanford and Walker

Conducted experiments: Tekwani, Avula, Sahu, Chaurasiya, Fasinu, S Khan, Jain, Stanford and Yates (clinical oversight)

Contributed new reagents or analytical tools: Bandara-Herath, Nanayakkara and I Khan

Performed data analysis: Tekwani, Avula, Sahu, Nanayakkara McChesney. ElSohly and Walker

Wrote or contributed to the writing of the manuscript: Tekwani, Avula, Sahu, Nanayakkara, S Khan, McChesney. ElSohly and Walker
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Footnotes

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Figure legends

Figure 1- Chemical structures of enantiomers of primaquine and carboxyprimaquine

Figures 2- Plasma concentrations of enantiomers of primaquine (PQ) in healthy human volunteers treated with a single 45 mg dose of racemic primaquine. The data are mean ± S.D. from six volunteers. The data on individual human volunteers are presented in table S1 (supplemental data).

Figure 3 A&B- Plasma concentrations of enantiomers of carboxyprimaquine (cPQ) in healthy human volunteers treated with a single 45 mg dose of racemic primaquine. The data are mean ± S.D. from six volunteers. The data on individual human volunteers are presented in table S1 (supplemental data). (3A) The concentrations of cPQ are presented in linear scale to show the difference in the concentrations of (-) cPQ and (+) cPQ (3B) the concentrations of cPQ are presented in logarithmic scale to show low concentrations of (+) cPQ.

Figure 4- In vitro metabolism of primaquine (PQ) and formation of carboxyprimaquine (cPQ) with primary human hepatocytes. (A) Enantioselective depletion/metabolism of PQ measured as depletion of (-)-PQ and (+)-PQ. (B) Enantioselective formation of cPQ measured as formation of (-)cPQ and (+)-PQ. Each data point represents values mean±S.D. of atleast triplicate observations.
Table 1- Pharmacokinetic parameters of enantiomers of primaquine (PQ) and carboxyprimaquine (cPQ) after a single oral dose of 45 mg primaquine (base, administered as diphosphate) racemate in healthy human volunteers

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (hours)</th>
<th>AUC(0-24h) (ng.hr/mL)</th>
<th>Ke</th>
<th>t1/2 (hr)</th>
<th>CL/F (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(-)-PQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>(-)-PQ vs (+)-PQ</strong></td>
</tr>
<tr>
<td>(-)-PQ Cmax (ng/mL)</td>
<td>163.35</td>
<td>221.60</td>
<td>121.30</td>
<td>37.64</td>
<td>0.017 (S)</td>
<td></td>
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<tr>
<td>(-)-PQ Tmax (hours)</td>
<td>3.00</td>
<td>4.00</td>
<td>2.00</td>
<td>1.09</td>
<td>0.500 (NS)</td>
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<tr>
<td>(-)-PQ AUC(0-24h) (ng.hr/mL)</td>
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<td>1768.70</td>
<td>374.64</td>
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<td>(-)-PQ Ke</td>
<td>0.085</td>
<td>0.043</td>
<td>0.114</td>
<td>0.025</td>
<td>0.782 (NS)</td>
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<td>(-)-PQ T1/2 (hr)</td>
<td>9.00</td>
<td>16.00</td>
<td>6.05</td>
<td>3.65</td>
<td>0.991 (NS)</td>
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<tr>
<td>(-)-PQ CL/F (L/h)</td>
<td>168.1</td>
<td>212.0</td>
<td>135.0</td>
<td>28.3</td>
<td>0.010 (S)</td>
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<tr>
<td><strong>(+)-PQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>(+)-PQ vs (-)-PQ</strong></td>
</tr>
<tr>
<td>(+)-PQ Cmax (ng/mL)</td>
<td>234.66</td>
<td>299.2</td>
<td>168.8</td>
<td>48.9</td>
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<tr>
<td>(+)-PQ Tmax (hours)</td>
<td>2.67</td>
<td>4.00</td>
<td>2.00</td>
<td>1.03</td>
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<tr>
<td>(+)-PQ AUC(0-24h) (ng.hr/mL)</td>
<td>3221.55</td>
<td>4546</td>
<td>2359.5</td>
<td>813.9</td>
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<tr>
<td>(+)-PQ Ke</td>
<td>0.081</td>
<td>0.056</td>
<td>0.102</td>
<td>0.019</td>
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<tr>
<td>(+)-PQ t1/2 (hr)</td>
<td>8.98</td>
<td>12.32</td>
<td>6.82</td>
<td>2.36</td>
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<tr>
<td>(+)-PQ CL/F (L/h)</td>
<td>116.4</td>
<td>158.9</td>
<td>82.5</td>
<td>28.6</td>
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<tr>
<td><strong>Total PQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>Total PQ vs (-)-PQ</strong></td>
</tr>
<tr>
<td>Total PQ Cmax (ng/mL)</td>
<td>394.93</td>
<td>520.8</td>
<td>292</td>
<td>85.14</td>
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</tr>
<tr>
<td>Total PQ Tmax (hours)</td>
<td>2.67</td>
<td>4.00</td>
<td>2.00</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total PQ AUC(0-24h) (ng.hr/mL)</td>
<td>5402.55</td>
<td>7324.6</td>
<td>4021.2</td>
<td>1233.12</td>
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<tr>
<td>Total PQ Ke</td>
<td>0.079</td>
<td>0.043</td>
<td>0.099</td>
<td>0.0201</td>
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<tr>
<td>Total PQ t1/2 (hr)</td>
<td>9.39</td>
<td>15.90</td>
<td>7.00</td>
<td>3.31</td>
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<tr>
<td><strong>(-) cPQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>(-) cPQ vs (+) cPQ</strong></td>
</tr>
<tr>
<td>(-) cPQ Cmax (ng/mL)</td>
<td>1398.66</td>
<td>1756</td>
<td>1104</td>
<td>277.97</td>
<td>&lt;0.001 (S)</td>
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<tr>
<td>(-) cPQ Tmax (hours)</td>
<td>13.33</td>
<td>1440</td>
<td>480</td>
<td>495.74</td>
<td>0.406 (NS)</td>
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<tr>
<td>(-) cPQ AUC(0-24h) (ng.hr/mL)</td>
<td>28033.96</td>
<td>33827.7</td>
<td>23573.1</td>
<td>4502.43</td>
<td>&lt;0.001 (S)</td>
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<tr>
<td><strong>(+)+ cPQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>(+)+ cPQ vs (-) cPQ</strong></td>
</tr>
<tr>
<td>(+)+ cPQ Cmax (ng/mL)</td>
<td>23.416</td>
<td>36.88</td>
<td>15.8</td>
<td>8.41</td>
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<tr>
<td>(+)+ cPQ Tmax (minutes)</td>
<td>1056</td>
<td>1440</td>
<td>480</td>
<td>525.81</td>
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<tr>
<td>(+)+ cPQ AUC(0-24h) (ng.hr/mL)</td>
<td>312.2</td>
<td>489.4</td>
<td>198.4</td>
<td>115.86</td>
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<tr>
<td><strong>Total cPQ</strong></td>
<td><strong>Mean</strong></td>
<td><strong>MAX</strong></td>
<td><strong>MIN</strong></td>
<td><strong>SD</strong></td>
<td><strong>P value</strong></td>
<td><strong>Total cPQ vs (+)+ cPQ</strong></td>
</tr>
<tr>
<td>Total cPQ Cmax (ng/mL)</td>
<td>1396.16</td>
<td>1771.8</td>
<td>1069.2</td>
<td>302.47</td>
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<tr>
<td>Total cPQ Tmax (minutes)</td>
<td>800</td>
<td>1440</td>
<td>480</td>
<td>495.74</td>
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<td>Total cPQ AUC(0-24h) (ng.hr/mL)</td>
<td>28283.76</td>
<td>34026.1</td>
<td>23755.1</td>
<td>4461.47</td>
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</tr>
</tbody>
</table>
Figure 1

(-)-(R)-Primaquine (1)  (++)-(S)-Primaquine (2)

(-)-(R)-Carboxyprimaquine (3)  (++)-(S)-Carboxyprimaquine (4)