Title: Age, weight, and *CYP2D6* genotype are major determinants of primaquine pharmacokinetics in African children

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Short running title: Primaquine pharmacokinetics in African children

Abstract

Low dose primaquine is recommended to prevent *Plasmodium falciparum* malaria transmission in areas threatened by artemisinin resistance and areas aiming for malaria elimination. Community treatment campaigns with artemisinin-based combination therapy in combination with the gametocytocidal primaquine dose target all age groups but no studies thus far have assessed the pharmacokinetics of this gametocytocidal drug in African children. We recruited forty children participating in a primaquine efficacy trial in Burkina Faso to study primaquine pharmacokinetics. These children received artemether-lumefantrine and either a 0.25 or a 0.40 mg/kg primaquine dose. Seven blood samples were collected from each participant for primaquine and carboxy-primaquine plasma levels determinations: one sample was collected before primaquine administration and six, after, according to partially overlapping sampling schedules. Physiological population pharmacokinetic modelling was used to assess the impact of weight, age and *CYP2D6* genotype on primaquine and carboxy-primaquine pharmacokinetics. Despite linear weight normalized dosing, the areas under the plasma concentration – time curves and the peak concentrations for both primaquine and carboxy-primaquine increased with age and body weight. Children who were CYP2D6 poor metabolizers had higher levels of the parent compound, indicating lower PQ CYP2D6-mediated metabolism. Our data indicate that primaquine and carboxy-primaquine pharmacokinetics are influenced by age, weight and *CYP2D6* genotype and suggest that dosing strategies may have to be re-considered to maximize the transmission-blocking properties of primaquine.

Keywords: pharmacokinetics, primaquine, *Plasmodium falciparum*, CYP2D6

Introduction

Since 2012, the World Health Organisation (WHO) recommends the use of a single 0.25 mg/kg dose of primaquine (PQ) in combination with standard artemisinin-based combination therapy (ACT) for the treatment of *Plasmodium falciparum* malaria in elimination and resistance containment settings (1). The rationale for using PQ is to prevent transmission of malaria to mosquitoes as it is the only currently available antimalarial that accelerates the clearance of mature gametocytes post-ACT (2). Several recent trials assessed the efficacy of the WHO-recommended dose and concluded that it reduces gametocyte carriage compared to ACT alone and effectively prevents transmission in mosquito infection experiments (3-6). In addition to its use to prevent *P. falciparum* transmission as single dose treatment, PQ has been used for decades in multiple-dose regimens for clearance of *Plasmodium vivax* hypnozoites (7, 8).

The parent compound is not responsible for PQ effects on *P. vivax* hypnozoites (9) and *P. falciparum* gametocytes (10), and the drug metabolising cytochrome P450 2D6 (CYP2D6) enzyme has been implicated in the formation of unknown active metabolites that are responsible for the pharmacological effect of PQ (9, 11-13). In mice, knocking out the *CYP2D* locus can reduce the metabolism of PQ into its active metabolite against *P. berghei* (11), and increase the area under the plasma concentration – time curve for PQ (14). The gene coding for this enzyme (*CYP2D6*) is hypervariable in humans and there is limited knowledge on the effects of the variation at this locus on the pharmacokinetics of PQ in humans (9). Early pharmacokinetic studies of PQ in adults (15-19) identified the main PQ metabolite, carboxy-PQ (C-PQ), which is slowly eliminated and is present at plasma concentrations up to 10 times higher than those of its parent compound (16). C-PQ is produced by monoamine

oxidase (MAO)-A (12), an enzyme involved in drug metabolism in the liver (20), and indirect evidence (9, 21) suggests that it is not the active metabolite against malaria parasites or one of its precursors.

To date, there are only limited PQ pharmacokinetic data (16-18, 22). This is particularly evident for single low dose PQ and for pharmacokinetic data in children: only one study, undertaken in Papua New Guinea and using single PQ doses of 0.5 or 1.0 mg/kg, recruited children (22). The difficulty to accurately dose children by extrapolating dosing schemes from adults (23-26) was previously illustrated for the antimalarials sulphadoxine-pyrimethamine and dihydroartemisinin-piperaquine (27). Since children are frequently infectious to mosquitoes (28) and comprise an important part of the human infectious reservoir for malaria (29), data on single low dose PQ pharmacokinetics in children are highly needed for the planning of community treatment campaigns with PQ to reduce *P. falciparum* transmission.

To identify factors that impact PQ pharmacokinetics in children, we have performed a pharmacokinetic study of PQ in the largest paediatric population thus far.

Materials and Methods

Study site, approvals and patients

A randomised placebo-controlled trial to assess the effect of low dose PQ on malaria transmission was undertaken in Balonghin, a village with endemic malaria transmission in Burkina Faso. Study procedures and results were described in detail elsewhere (4). Briefly,

parasitaemic children aged between 2 and 15 years without malaria symptoms (no measured fever, reported fever or anaemia) and with normal glucose-6-phosphate dehydrogenase (G6PD) enzyme activity were recruited and treated with artemether-lumefantrine (AL) alone, AL and a 0.25 mg/kg PQ dose or AL and a 0.40 mg/kg PQ dose. PQ dosing was achieved by crushing a 15 mg PQ tablet and preparing 1 mg/mL solution by dissolving the crushed tablet in 15 mL of water. This allowed precise dosing: the mean difference between the actual dose given and the assigned dose was -0.004 (95% confidence interval -0.006 – -0.001) mg of PQ/kg of body weight for participants included in this pharmacokinetic study. AL was given twice daily over three days, and PQ or placebo was administered with the fifth AL dose. A subset of study subjects not included in mosquito membrane feeding experiments was invited to participate in the pharmacokinetic study. To minimise the number of blood samples taken per participant whilst maximizing the number of time-points with information on PQ and C-PQ levels, partially overlapping sampling schedules were designed and sampling times were sequentially allocated to participants. The exact time when each blood sample was collected was recorded and used in pharmacokinetic analyses. A total of seven 1.5 – 2 mL venous blood samples were collected for each study subject: one sample before PQ or placebo administration, four in the first 12 hours following this dose, and two between 24 and 72 hours. All samples were centrifuged within two hours of collection and plasma was subsequently stored at -80°C. Forty participants, 20 from each PQ study arm, had PQ and C-PQ plasma levels quantified and were included in this analysis.

The study was registered at ClinicalTrials.gov (reference number NCT01935882). Written informed consent was obtained for participation in the pharmacokinetics sampling. The clinical trial received ethics approval from the London School of Hygiene and Tropical Medicine ethics committee (reference number 6274), and the Comité d'Ethique pour la Recherche en Santé (Ministère de la Santé du Burkina Faso; reference number 2012-10-78).

Quantification of PQ and C-PQ plasma levels

PQ and C-PQ levels were determined by liquid chromatography/mass spectrometry (LC-MS) as previously described (30). The system consisted of a Shimadzu LCMS-2010A mass spectrometer operated using electrospray ionization (ESI) in positive ion detection mode. Data were collected in the selected ion monitoring mode at 325.35 m/z for quinine (internal standard, retention time 3.7 minutes), 260.30 m/z for PQ (retention time 5 minutes) and 275.25 m/z for C-PQ (retention time 8 minutes). The analytical column was a Phenomenex Synergi Polar RP (150 x 2 mm, 4u), preceded by a Phenomenex Polar RP security guard column (2 x 4mm, Torrance, CA). The standard curve ranged from 4 to 1,000 ng/mL, with a lower limit of quantitation of 4 ng/mL and a lower level of detection of 1 ng/mL. All control values were within 15% of their nominal value.

CYP2D6 *genotyping*

EDTA-anticoagulated venous blood and/or saliva samples collected with Oragene kit (OG-500 or OG-575) were used as sources of human genomic DNA for *CYP2D6* genotyping. DNA was extracted using a MagNAPure LC automated extractor and extraction kits for large volume samples according to manufacturer's instructions. DNA concentration was measured fluorometrically using a Qubit fluorometer and accompanying high sensitivity (HS) kit. Samples were diluted according to manufacturer's instructions for assays determining copy number variation (CNV) and for preamplification as well as sequence variant determination with QuantStudio 12K Flex OpenArrays with TaqMan assays. OpenArray analysis was repeated for five samples without preamplification, due to undetermined genotype. In total,

two CNV assays (hs00010001_cn targeting exon 9 and hs04083572_cn targeting intron 2 of *CYP2D6*) and 19 sequence variants in *CYP2D6* were analysed (see Supplemental Material **Table S1** for assay details) and genotype was determined according to the cytochrome P450 allele nomenclature website (31). Inferred phenotype was determined using the Activity Score (AS) (32).

Pharmacokinetic modelling

A physiological population pharmacokinetic model was developed to allow better extrapolation and enable identification of pharmacokinetic parameters that cannot be identified in classical empirical models (33). Pharmacokinetic analysis was performed by means of non-linear mixed effects modelling with the software NONMEM V.7.3.0, and Piraña as an interface for NONMEM, R-statistics and Perl Speaks Nonmem V4.6.0 (34). The covariance step in NONMEM was used to calculate parameter precision. To account *a priori* for changes in pharmacokinetics related to growth, liver volume (V_L) was calculated from total body weight and height (35). All other volumes and flow parameters were allometrically scaled to a total body weight of 70 kg, as previously proposed. The exponents of the allometric models were fixed at 0.75 and 1 for flow and volume parameters, respectively (36). Due to the high co-linearity of age and weight, this enabled to separately assess the impact of age and weight on PQ pharmacokinetics. The PQ dose and measured plasma concentrations of PQ and C-PQ were converted to their molar equivalents for this analysis. Parameter shrinkage, with a shrinkage of >25% indicating uninformative data to estimate the parameter (37), was derived from the NONMEM results file.

As PQ is thought to be mainly metabolised by MAO and cytochrome P450s in the liver (12), a well-stirred liver model, a well-established model to describe hepatic metabolism of drugs,

was implemented to describe the physiologically plausible relationship between first-pass and central metabolism (38, 39). Apparent intrinsic hepatic clearances for MAO- and CYP2D6-mediated metabolism ($CL_{int,MAO}$ and $CL_{int,CYP2D6}$, respectively) were estimated. C-PQ was assumed to originate from the MAO-mediated metabolism of PQ. The individual CYP2D6 intrinsic clearance ($CL_{int,CYP2D6,i}$) was calculated from the population intrinsic clearance ($CL_{int,CYP2D6,pop}$) and CYP2D6 AS with the formula

$$CL_{int,CYP2D6,i} = AS \times CL_{int,CYP2D6,pop}$$

We assumed a liver plasma flow (Q_H) of 49.5 L/h, derived from an adult total blood flow of 90 L/h and a plasma fraction of 55% in whole blood (haematocrit level of 45%). The hepatic extraction (E_H) was defined as

$$E_H = CL_{int}/(Q_{HP} + CL_{int})$$

and the apparent MAO- and CYP2D6-mediated hepatic clearances ($CL_{H,MAO}$ and $CL_{H,CYP2D6}$) were calculated using the formula

$$CL_H = E_H \times Q_{HP}$$

Gradual onset of oral drug absorption was described with a chain of transition compartments, as described earlier (40). In short, the mean absorption time (MAT) was estimated and the rate constant (k_{tr}) for these transition compartments was calculated using

$$k_{tr} = (n + 1)/MAT$$

where n equals the number of transition compartments. The inter-individual variability was modelled by means of an exponential variance model. Throughout model building, basic goodness-of-fit plots and prediction-corrected visual predictive checks (41) were explored. Concentrations that were below the limit of quantification were retained in the analysis

194 employing the M6 method, as proposed by Beal (42) and, therefore, the first concentrations
195 below the limit of quantification (BLOQ) were fixed to ½ LOQ and a fixed residual additive
196 error of ½ LOQ was introduced in the model. Individuals with missing or inconclusive
197 *CYP2D6* genotype data (N=4) were retained in the model by imputing the individual activity
198 scores using mixture modelling, based on the frequencies observed in the study population, as
199 proposed earlier (43). The Bayesian imputed activity score was estimated to be 1.5 for all
200 four children. As fixing the individual activity scores manually resulted in better model
201 stability and no significant change of model goodness-of-fit, in the final model these missing
202 activity scores were manually set to 1.5. The final model was used to obtain the empirical
203 Bayes estimates for the area under the concentration – time curve (*AUC*) to infinity, the
204 maximum concentration (C_{max}), and the time of C_{max} (T_{max}) for both PQ and C-PQ.

205

206 **Results**

207

208 *Study population*

209 40 afebrile children aged 2 to 14 years who received a single low dose (0.25 or 0.40 mg/kg)
210 of PQ on the final day of the 6-dose AL regimen were included in this study. 37/40 had
211 patent asexual stage *P. falciparum* parasites at enrolment (median and interquartile range
212 [IQR] 1,252 [578-2,503] parasites per μL). Median (IQR) haemoglobin level at enrolment
213 was 11.6 (10.8 – 12.5) g/dL and similar in the two study arms. **Table 1** summarises
214 demographics and baseline laboratory results for these 40 children.

215

216 CYP2D6 *genotyping*

217 *CYP2D6* genotyping was successful for 36/40 children. For 3/40 participants, no samples
218 were available for genotyping, and for one, genotyping was inconclusive. Allele frequencies

are presented in the Supplemental Material (**Table S2).** 1/36 (2.8 %), 10/36 (27.8 %), 22/36 (61.1 %) and 3/36 (8.3 %) study subjects were classified as poor metabolizer (PM, AS of 0), intermediate metabolizer (IM, AS of 0.5 or 1.0), extensive or normal metabolizer (EM, AS of 1.5 or 2.0) and ultrarapid metabolizer (UM, AS of 3.0), respectively.(44, 45)

Pharmacokinetics

A total of 274 plasma samples were collected. Two of the 40 children were excluded from the pharmacokinetic analysis because it was not possible to determine PQ and C-PQ levels in their samples due to inadequate sample volume.

The raw pharmacokinetic data of PQ and C-PQ per dose group are depicted in **Figure 1**. As observed, PQ was rapidly absorbed and plasma C-PQ concentrations were generally higher than plasma PQ concentrations. Overall, the pharmacokinetics of the parent compound and of its main plasma metabolite presented substantial inter-individual variation.

A physiological pharmacokinetic model was developed to estimate pharmacokinetic parameters for both PQ and C-PQ (**Figure 2**). First order kinetics with two absorption transit compartments and one compartment disposition for PQ and C-PQ fit the observed plasma levels well. PQ was rapidly absorbed with a mean absorption time of 0.706 h (relative standard error [coefficient of variation] 12%). Although allometric clearance appeared to explain most weight-related variability in pharmacokinetics, over-prediction of plasma PQ and C-PQ concentrations was observed in the youngest children of our study population. This resulted in higher than expected estimates for apparent volume of distribution and apparent clearance in these children. This phenomenon may be explained by a reduced relative

bioavailability at younger age. Therefore, maturation of relative bioavailability (F) with age (i.e., the increase in bioavailability with age) was described by an E_{max} model with the formula

$$F = Age\ /(Age + F_{50})$$

where F_{50} is the age in years at which the relative bioavailability is 50% that of the mature value. F_{50} was estimated to be 4.27 years (relative standard error 44%), explained all observed inter-individual variability in relative bioavailability, significantly (P<0.001) improved model fit and was, therefore, retained in the model. In addition to apparent age-dependent bioavailability, the inclusion of CYP2D6-mediated clearance of PQ, assumed to be linearly related to the CYP2D6 activity score, significantly improved model fit (p<0.001) and was also retained in the final model. Diagnostic prediction-corrected visual checks of the model are shown in **Figure 3** and additional goodness-of-fit assessments are presented in the Supplemental Material (**Figures S1** and **S2**). Estimated model parameters and their variability are presented in **Table 2**.

Model-derived median (range) AUC, C_{max} and T_{max} for PQ were 600.26 (259.87 – 3,315.40) h x ng/mL, 68.42 (20.21 – 391.16) ng/mL and 1.59 (0.96 – 2.12) h, respectively, and are in agreement with previous studies (18); for C-PQ these values were 3,468.35 (962.05 – 10,506) h x ng/mL, 147.23 (25.61 – 403.95) ng/mL and 6.80 (2.73 – 16.03) h, respectively. The 0.40 mg/kg PQ dose was associated with higher AUC and C_{max} compared to the 0.25 mg/kg dose (**Table 3**), although there was substantial variation within each study arm: C_{max} estimates included values that were 6 – 10 times higher than the lowest model-derived C_{max} in each study arm; a similar pattern was observed for AUC.

Host characteristics influencing PQ pharmacokinetics

PQ doses are linearly scaled with body weight. To assess whether this approach is appropriate, we analysed plasma PQ and C-PQ concentrations by weight. In **Figure 4**, the distribution of model-derived *AUC* values by weight and PQ dose is presented. For each dose, despite linear dose extrapolation based on weight, *AUC* estimates of PQ and C-PQ were positively correlated with body weight. A similar pattern was observed when analysing the relationship between *AUC* values and age (Supplemental Material, **Figure S3**).

CYP2D6 AS was an important determinant of PQ and C-PQ concentrations. In **Figure 5**, typical plasma PQ and C-PQ concentration – time curves for children aged 2 (body weight 12 kg) and 14 (body weight 40 kg) years old and with different CYP2D6 metabolizer status are presented. Toddlers who are IM have particularly low plasma levels of PQ compared to schoolchildren with PM status.

Discussion

The use of PQ to prevent *P. falciparum* transmission may support malaria elimination activities and efforts to contain artemisinin resistance. Understanding PQ pharmacokinetics is important to optimise dosing regimen aimed at clearing gametocytes and reducing infectivity to mosquitoes. We performed the first PQ pharmacokinetic study in African children and observed that age, body weight and *CYP2D6* genotype influenced PQ and C-PQ plasma levels: younger children and children with lower body weight have lower levels of PQ and C-PQ, while poor CYP2D6 metabolizers have higher levels of PQ. These findings indicate that linear weight-based dosing may be sub-optimal to achieve efficacious PQ concentrations.

Since the 1980s several PQ pharmacokinetic studies have been performed; however only one of these studies enrolled children (22). This is the first PQ pharmacokinetic study recruiting African children, a group that may represent 28.4 – 51.8% of all individuals capable of infecting mosquitoes in some endemic areas (28) and thus are an important target population for PQ treatment. Our analysis generated several insights into PQ pharmacokinetics in this age group. First, both age and weight explain variability in PQ pharmacokinetics in children and show a non-linear relationship with PQ exposure. This is illustrated by the clear relationship between age and PQ exposure despite linear dose normalization based on weight. Therefore, linear dosing based on total body weight is inappropriate to obtain similar drug exposure in children as in adults, confirming earlier findings that (non-linear) allometric scaling of pharmacokinetics of PQ accounted for the observed variability in Papua New Guinean children (22). However, this study did not report an age effect on bioavailability as observed by us. This might be explained by the fact that in our study PQ was administered using 1 mg/mL solutions, which allows for more accurate dosing than tablet-based regimens, and that our study population covered a wider age range (2 – 14 years) compared to the previous study in Papua New Guinea (6 – 10 years). The physiological cause of the observed maturation of bioavailability remains unclear. This phenomenon is not uncommon in paediatric pharmacokinetics: for example, the bioavailability of the liquid formulation of the antiretroviral drug efavirenz also matured with age in young children (46). Potential causes could be age-related changes in gastrointestinal motility, pH or pre-hepatic expression of metabolic enzymes or transporters, possibly resulting in increased absorptive capacity with age (47, 48). These findings should be confirmed prospectively with additional PQ pharmacokinetic and pharmacodynamic studies in paediatric as well as adult populations to assess the effect of weight and age on PQ-related pharmacokinetics as well as gametocyte clearance, transmission reduction and toxicity.

Another important finding of our study is that C*YP2D6* genotype influences PQ pharmacokinetics (14). Initial PQ concentrations are affected by *CYP2D6* genotype since CYP2D6-mediated metabolism occurs both systemically and pre-systemically. The influence of CYP2D6 metabolizer status on PQ efficacy was previously shown by an increased *P. vivax* relapse rate in individuals with *CYP2D6* PM and IM genotypes (9). Individuals with higher relapse rates, presumably because of an inability to clear *P. vivax* hypnozoites, had a significantly higher PQ *AUC* than extensive metabolizers but no differences in C-PQ pharmacokinetics parameters, which suggests that CYP2D6 activity is a rate-limiting step in the formation of active metabolite(s) against *P. vivax* hypnozoites (9, 11). It has been hypothesized that unknown metabolites formed through CYP2D6 are also responsible for the effect of PQ on *P. falciparum* transmission, however direct evidence for this is lacking and available field studies with comprehensive CYP2D6 data are too small to assess the effect of CYP2D6 metabolizer status on gametocytaemia and transmission potential after single low dose PQ. CYP2D6-related differences in PQ transmission-blocking efficacy would be particularly relevant in malaria elimination settings, where mass drug administration are used to accelerate transmission interruption: CYP2D6 poor metabolizers might remain infectious for longer periods of time after PQ administration compared to individuals with other *CYP2D6* genotypes and could represent a source of residual malaria transmission in these areas, depending on the frequency of alleles linked to this phenotype in the population.

Since PQ metabolism is considered essential to its effect on falciparum transmission (10), one may argue that exposure to the parent drug (PQ) is not important. However, as formation of active metabolites depends on the presence of parent drug, one should aim for adequate initial exposure of PQ. Our findings suggest that age, body weight and *CYP2D6* genotype can all

influence PQ levels and consequently may determine the levels of active metabolites generated. These observations and the fact that currently available PQ tablet sizes are not optimal for paediatric dosing, posing further challenges in achieving the target PQ dose in children, indicate that PQ dosing strategies may have to be re-considered. The therapeutic range over which PQ prevents malaria transmission is currently not well established but may include doses lower than the WHO-recommended PQ dose of 0.25 mg/kg (5). PQ efficacy and added value over ACT alone may also depend on the ACT used (4, 5, 49). The gametocytocidal and transmission blocking effects of AL are superior to that of dihydroartemisinin-piperaquine but there are concerns for drug-drug interactions between AL and PQ. Indeed, although artemether does not influence PQ metabolism (50), lumefantrine inhibits CYP2D6 *in vitro* (51, 52). In our study, although participants were treated with lumefantrine, we found that CYP2D6 Activity Score explained variability in PQ pharmacokinetics, indicating that if CYP2D6 was indeed inhibited by lumefantrine, this inhibition was incomplete at the time of PQ administration. Since AL alone is by far the most widely used ACT, our CYP2D6-related findings are of immediate relevance for ACT-PQ policies and our findings of age-dependent exposure to PQ and C-PQ are likely to be independent of the type of ACT-PQ combination.

PQ is considered a valuable tool to support malaria elimination efforts. Whether it is deployed during mass drug administration campaigns, when all individuals in a community receive treatment, or during treatment of symptomatic falciparum malaria episodes, a substantial proportion of PQ doses are likely to be given to young children. Age-related changes in PQ pharmacokinetics, like maturation of bioavailability or non-linear change in clearance with weight, indicate that dose extrapolation from adult regimens based solely and linearly on weight may not be an optimal approach. A limitation of our study is that the

studied population was relatively small and did not include subjects outside the 2 – 14 years range. Extrapolation of our findings outside this range should, therefore, be performed with caution. Future studies relating plasma concentrations of PQ and its (active) metabolites are needed to quantify the implications of our findings for the ability to prevent *P. falciparum* transmission by PQ treatment and to inform better dosing strategies.

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544 **Tables**

545 **Table 1.** Baseline Characteristics

	Study arm	
	0.25 mg/kg PQ	*0.40 mg/kg PQ*
Number of participants	20	20
Gender (%, female)	55	60
	Median (IQR[¥])	Median (IQR)
Age (years)	8 (6 - 10)	10 (6.5 - 12)
Body weight (kg)	19.8 (16.2 - 26.3)	24.9 (15.9 - 31.6)
Height (cm)	118 (104 - 133.5)	129 (104 - 144.5)
Temperature (°C)	36.6 (36.4 – 37.0)	36.7 (36.1 – 36.9)
Asexual parasites (per µL)	991 (635.5 - 2066.5)	1188 (284.5 - 3340)
Haemoglobin (g/dL)[¤]	11.4 (10.6 - 12.5)	11.9 (11.1 - 12.7)
Alanine transaminase (U/L)	22.5 (17.5 - 34.5)	22 (17 - 29)
Aspartate transaminate (U/L)	38 (32 - 46)	36 (27 - 41)
Total bilirubin (µmol/L)	7.4 (5.4 - 9.9)	10.8 (8 - 13.8)
Creatinine (µmol/L)	37.8 (35.5 - 40.8)	38.4 (32.7 - 45.2)

[¥] *Interquartile range*

[¤] *Haemoglobin levels measure by Hemocue*

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548 **Table 2.** Pharmacokinetic model parameters. All flow and volume parameters were
549 allometrically scaled to a body weight of 70 kg with an allometric exponent of 0.75 for flow
550 parameters and an exponent of 1 for volume parameters. CV = coefficient of variation.

Parameter	Estimate	Relative standard error of estimate (% CV)	Shrinkage (%)
Mean absorption time (*MAT*)	0.706 h	12%	-
PQ volume of distribution (V_{PQ}) (70kg)	127 L	20%	-
Inter-individual variability V_{PQ}(%)	82.9%	38%	12.2%
C-PQ volume of distribution (V_{CPQ}) (70 kg)	21.7 L	39%	-
$CL_{int,MAO}$ (70 kg)	7.35 L/h	41%	-
Inter-individual variability $CL_{int,MAO}$(%)	65.3%	33%	13.1%
$CL_{int,CYP2D6,pop}$ (70 kg)	6.70 L/h	69%	-
CL_{CPQ} (70 kg)	1.50 L/h	35%	-
F_{50}	4.27 y	44%	-
Residual error PQ Proportional Additive	32.7% 2 ng/mL (FIX)	42%	19.5%
Residual error CPQ Proportional Additive	45.2% 2 ng/ml (FIX)	20%	21.6%

551 FIX: values were fixed during modelling

552

553 **Table 3.** Model-derived pharmacokinetic parameters for PQ and C-PQ in African children receiving a
554 single PQ dose of 0.25 (n=18) or 0.40 mg/kg (n=20). Values are reported as medians (minimum –
555 maximum).

Parameters	0.25 mg/kg PQ		0.40 mg/kg PQ	
	PQ	C-PQ	PQ	C-PQ
T_{max} *(h)*	1.6 (1.2 – 1.9)	7.1 (4.3 – 10.1)	1.6 (1.0 – 2.1)	6.6 (2.7 – 16.0)
C_{max} *(ng/ml)*	50.2 (20.2 – 138.7)	108.3 (25.6 – 240.2)	88.9 (24.4 – 391.2)	196.8 (29.3 – 404.0)
$AUC_{0-\infty}$ *(h x ng/mL)*	450.4 (259.9 – 875.9)	2912.3 (962.1 – 5076.3)	730.7 (334.2 – 3315.4)	5091.7 (1075.0 – 10506)

556

557

Figure legends

Figure 1. PQ and C-PQ plasma levels (y-axes) after PQ administration (x-axes). In A and C, PQ levels are presented for participants who received the 0.25 and 0.40 mg/kg PQ dose, respectively. In B and D, C-PQ levels are presented for the 0.25 and 0.40 mg/kg PQ study arms, respectively. Assay results of all samples collected after PQ administration, including those with PQ or C-PQ levels below the limit of detection (i.e. with assigned level of 0 ng/mL), are presented.

Figure 2. Schematic representation of the model. The mass transport of this model can be described with the following rate constants:

$k_{12} = 3/MAT$

$k_{23} = 3/MAT$

$k_{34} = 3/MAT$

$k_{40} = CL_{H,\ CYP2D6}/V_L$

$k_{45} = CL_{H,\ MAO}/V_L$

$k_{50} = CL_{CPQ}/V_{CPQ}$

$k_{46} = (Q_H\ (1-E_H))/V_L$

$k_{64} = Q_H/V_{PQ}$.

Figure 3. Prediction-corrected visual predictive check of observed data. Left and right panels depict the prediction-corrected visual predictive checks for PQ and C-PQ, respectively, based on 1000 simulations. Prediction-corrected simulated (shaded areas) and observed (circles and lines) PQ and C-PQ concentrations are presented over time (h; y-axes). The thick red line connects the observed median values per bin. The dotted red lines connect the 5^{th} and 95^{th}

582 percentiles of the observations. The light blue areas are the 95 % confidence interval of the
583 5th and 95th percentiles, and the light red area indicates the confidence interval of the median.
584
585 **Figure 4.** Area under the plasma concentration – time curve for both PQ (left panel) and C-
586 PQ (right panel) by weight (x-axes).
587
588 **Figure 5.** Effect of age and CYP2D6 Activity Score (AS) on PQ and C-PQ plasma
589 concentrations over time after 0.25 mg/kg single dose of PQ. Panels A and B respectively
590 show the effect of age on PQ and C-PQ concentrations over time, in two hypothetical
591 children of different ages (2-year-old 12kg and 14-year-old 40 kg body weight), with
592 CYP2D6 AS=1.0. Panels C and D respectively show the effect of genetically determined
593 CYP2D6 AS, in four hypothetical 14-year-old children, on PQ and C-PQ concentration over
594 time. The values use to construct curves are model derived.
595
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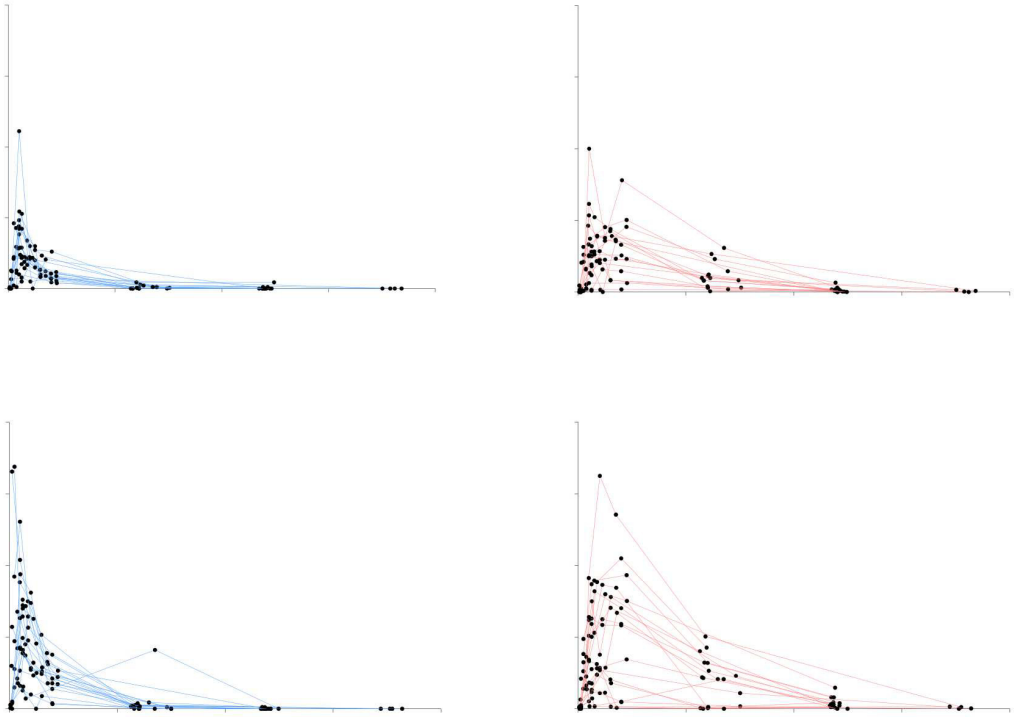

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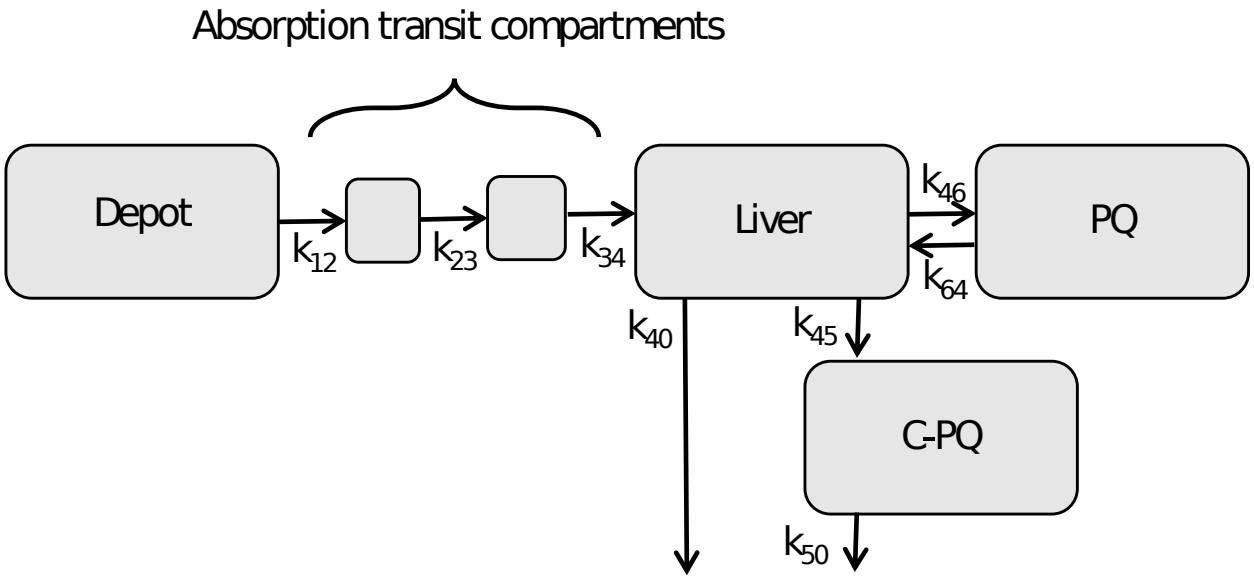

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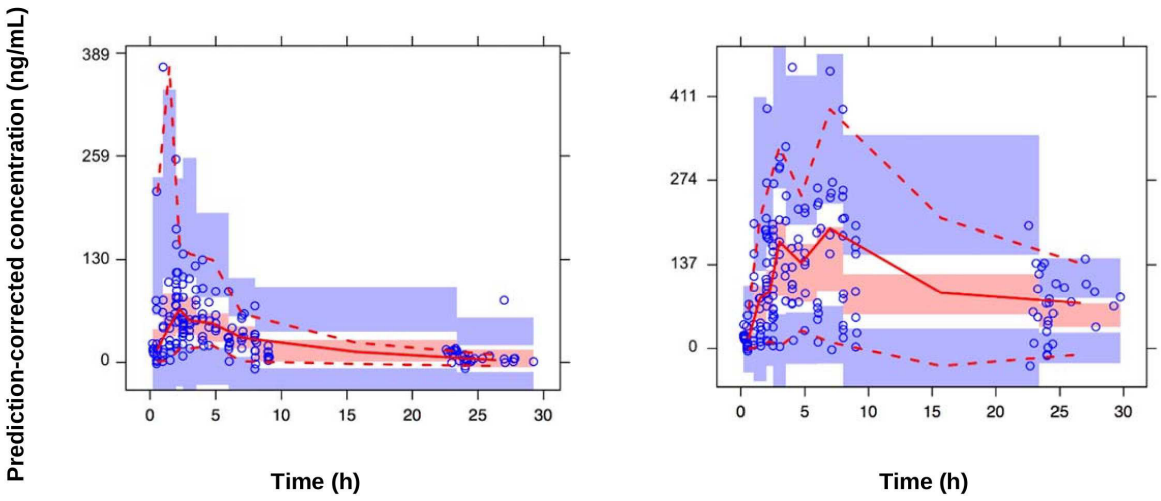

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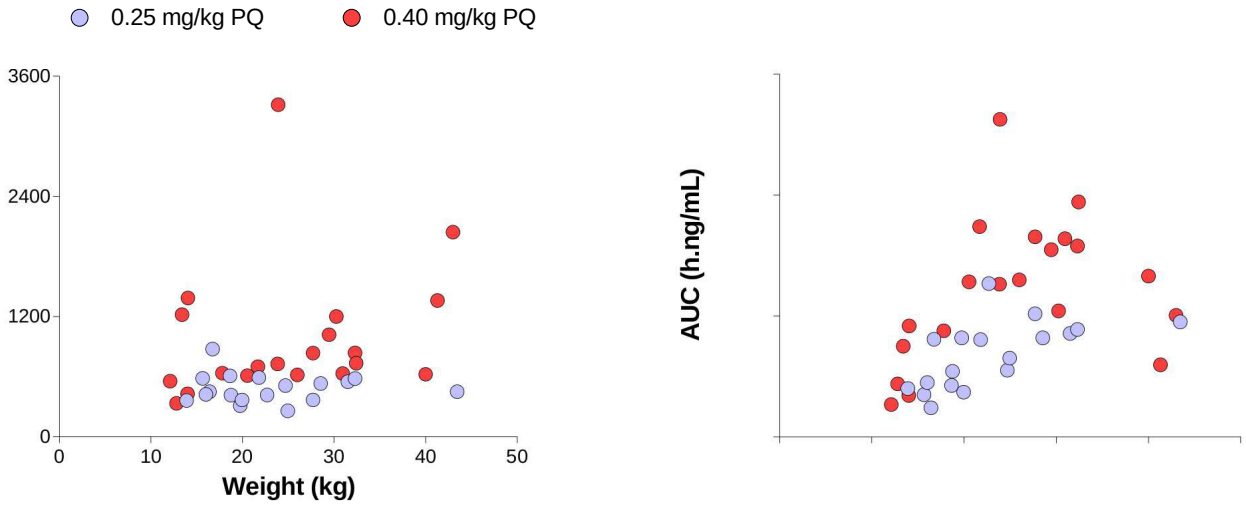

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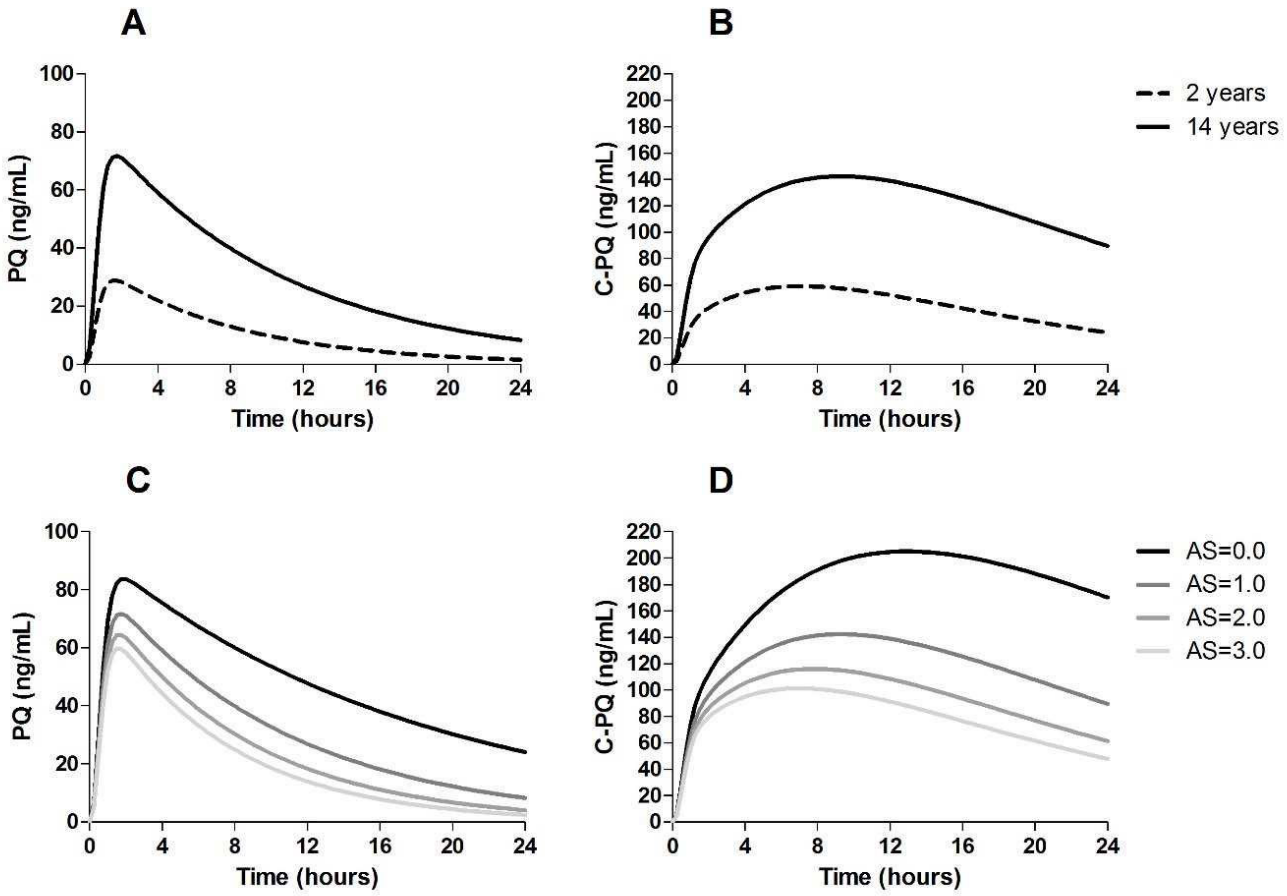

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