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The application of physiologically-based pharmacokinetic modelling to assess the impact of antiretroviral-mediated drug-drug interactions on piperaquine antimalarial therapy during pregnancy

ABSTRACT

Antimalarial therapy during pregnancy poses important safety concerns due to potential teratogenicity and maternal physiological and biochemical changes during gestation. Piperaquine (PQ) has gained interest for use in pregnancy in response to increasing resistance towards sulfadoxine–pyrimethamine in sub-Saharan Africa. Co-infection with HIV is common in many developing countries, however, little is known about the impact of anti-retroviral (ARV) mediated drug-drug interaction (DDI) on PQ pharmacokinetics during pregnancy. This study applied mechanistic pharmacokinetic modelling to predict pharmacokinetics in non-pregnant and pregnant patients, which was validated in distinct customised population groups from Thailand, Sudan and Papua New Guinea. In each population group, no significant difference in day 7 concentrations were observed during different gestational weeks (GW) (weeks 10-40), supporting the notion that PQ is safe throughout pregnancy with consistent pharmacokinetics, although possible teratogenicity may limit this. Antiretroviral-mediated DDIs (efavirenz and ritonavir) had moderate effects on PQ during different gestational weeks with a predicted AUC_{ratio} ranging from 0.56-0.8 and 1.64-1.79 for efavirenz and ritonavir respectively over GW 10-40, with a reduction in circulating human serum albumin significantly reducing the number of subjects attaining the day 7 (post-dose) therapeutic efficacy concentrations under both efavirenz and ritonavir DDIs.

This present model successfully mechanistically predicted the pharmacokinetics of PQ in pregnancy to be unchanged with respect to non-pregnant women, in the light of factors such as malaria/HIV co-infection. However, ART-mediated DDIs could significantly alter PQ pharmacokinetics. Further model refinement will include collation of relevant physiological and biochemical alterations common to HIV/malaria patients.

KEYWORDS

Physiologically-based pharmacokinetics; malaria; anti-retroviral; drug-drug interaction; pregnancy.

1. INTRODUCTION

The problem of malaria-induced maternal morbidity and mortality in endemic areas for the disease is far reaching, particularly with respect to the unborn child. Maternal death due to malaria was reported to account for up to 25% of maternal deaths due to all causes in malaria endemic regions while close to a million children born to malaria-infected mothers had low birth weights (Consortium, 2017).

Malarial infection in pregnancy triples the maternal risk of suffering from severe diseases compared with non-pregnant women (Murray & Bennett, 2009). This is further confounded by the added complication of coinfection with human immunodeficiency virus (HIV) as a result of the immunocompromised nature of pregnancy (Menendez et al., 2008; Ofori et al., 2009; Schantz-Dunn & Nour, 2009).

The treatment of malaria during pregnancy possess major challenges to healthcare systems. This is because antimalarial treatments (AMT) which yields satisfactory safety and efficacy profiles are often found to be unsafe during the early stages of pregnancy (Nosten et al., 2006). The WHO's current recommendations for AMT chemoprophylaxis are based on intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) (Organization., 2015). This recommendation was based on a review (Kayentao et al., 2013) of seven trials which assessed the use of monthly SP administration for malaria prevention in pregnant women across six African countries. The result of the review demonstrated that there was a significant reduction in both low birth weights and placental and maternal parasitaemia following administration of no less than two doses of SP monthly during pregnancy (Kayentao et al., 2013).

However, with the spread of SP resistance, new interventions have been sought. In high transmission settings where there may be widespread resistance to SP-IPTp, dihydroarteminisin-piperaquine (DHA-PQ) has been demonstrated to result in a lower malarial burden (Kakuru et al., 2016). A recent study showed that, when compared to the use of SP in pregnant women, the

administration of DHA-PQ provided significantly higher protection against placental malaria; significantly lowered maternal parasitaemia and reduced prevalence of composite adverse birth consequences (Kakuru et al., 2016). More so, the safety of DHA-PQ in pregnancy is evident in numerous studies. In 2015, a randomised controlled superiority trial showed that in addition to the observed efficacy of DHA-PQ for the preventing malaria in pregnancy, DHA-PQ resulted in fewer detrimental maternal and infant side effects compared with SP-IPTp (Desai et al., 2015). Similarly, another study revealed that compared to quinine, DHA-PQ used for the treatment of multi-resistant malaria in 2^{nd} and 3^{rd} trimester of pregnancy resulted in less perinatal mortality, though in the 1^{st} trimester, quinine appeared to be safer (Poespoprodjo et al., 2014).

Infectious diseases such as HIV are prevalent in malaria endemic regions (Benjamin et al., 2015; Tarning et al., 2012). Pregnant women with HIV and malaria coinfection are more vulnerable to all the complications of malaria in pregnancy such as anaemia, placental parasitaemia and low birth weights (Hayes et al., 2015). This can be further confounded by the potential for many antiretroviral (ART) drugs to elicit drug-drug interactions (DDIs) on common Cytochrome P450 isozymes, e.g. 3A4 (Fichtenbaum & Gerber, 2002; Horita & Doi, 2014; Renjifo et al., 2015). Hence, these factors are significant causes for concern when treating this population. The reduced systemic concentration of DHA-PQ, due to co-administration with efavirenz in HIV infected pregnant women, has been demonstrated in a recent study which showed that in Ugandan pregnant women, $AUC_{0\text{-}8hr}$ and $AUC_{0\text{-}21d}$ of piperaquine was 50% and 40% lower respectively when DHA-PQ was co-administered with efavirenz compared to when DHA-PQ was taken alone (Kajubi et al., 2017a). A systemic review of data involving DDI between ARV drugs and AMT further accentuated the likelihood of a range of such DDIs (Seden et al., 2017).

Addressing the problem of AMT in malaria endemic areas requires consideration of physiological peculiarities in subjects that might impact upon the efficacy of the antimalarial treatment. Some example of factors that can impact upon the efficacy of AMT include, but not limited to,

geographical region differences in body weight (Hayes et al., 2015) and biochemistry (e.g serum albumin (Nanjul, 2007) and haematocrit (Newton et al., 2013; Othman, 2014)). Elucidating these factors individually in a clinical setting may be difficult due to the presence of other confounding factors and/or the ethical constraints of recruiting large number of pregnant women into clinical studies. With the aid of PBPK modelling techniques, these factors can be investigated separately to suggest the effect the impacts can make on the antimalarial therapy clinically.

In this study, through virtual clinical trials simulations, we investigate the impact changes in PQ plasma concentrations in the absence and presence of ARV-mediated DDIs in three malaria-specific geographical regions (Thailand, Papua New Guinea and Sudan) pregnant population groups, whereby changes in biochemical and haematology were incorporated into the design of the population groups.

2. METHODS

All population based PBPK modelling was conducted using the virtual clinical trials simulator Simcyp (Simcyp Ltd, a Certara company, Sheffield, UK, Version 16).

2.1 Model development

A four-stage stepwise approach was employed for model development (Figure 1) which is fully described in the supplementary materials and briefly summarised below.

2.1.1 Base model development (Step 1)

The base model was developed from two reported studies of PQ dosed in fasted Caucasian healthy volunteers (Ahmed et al., 2008; Sim, Davis, & Ilett, 2005). Given the high lipophilicity and expected wide-spread tissue distribution of PQ, a full PBPK model was employed for all model simulations.

2.1.2 Non-pregnant malaria population groups (Step 2)

To assess the predictive performance of the model in non-Caucasian non-complicated malaria population groups, we identified four studies where PQ was dosed to non-pregnant females in Thailand (Rijken et al., 2011; Tarning et al., 2012) [Tarning *et al* 2008 (Tarning et al., 2008) was excluded from thus study due to the difficulty in obtaining individual data points for the study duration], Papua New Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012). The 'Healthy Volunteer' (HV) population group within Simcyp was adapted. In order to address the differences in patient demographics (primarily body weight) and biochemistry (haematocrit/plasma proteins) between healthy-subjects and malaria-subjects (see supplementary materials Table S1).

2.1.3 Pregnant malaria population groups (Step 3)

The 'Pregnancy' population group within Simcyp was adapted (see supplementary materials) to create 'Malaria-Pregnancy' population groups based upon the three regional populations originating from the four clinical studies highlighted in section 2.1.2. These studies also detailed the pharmacokinetics of PQ in pregnant women and this was used as a basis to further validate the 'Malaria-Pregnancy' population groups. Final model parameters for PQ are detailed in supplementary materials Table S2.

2.1.4. 'What-If' scenarios (Step 4)

To assess 'What-If' scenarios (Figure 1), case studies were included, in order to demonstrate the impact of possible drug-drug interactions mediated by efavirenz (EFV) or ritonavir (RTV); the former was selected due to the potential for CYP3A4 induction and the latter for its CYP3A4 inhibitory effects.

2.1.4.1 Efavirenz/Ritonavir-mediated drug-drug interactions

Validation of DDIs mediated by ART was considered through the only published DDI study available (Kajubi et al., 2017b) with EFV and PQ in a Ugandan population group. A Ugandan population group was developed (see supplementary materials for details) and the trial design was replicated for the three arms of the study within the reported trial, namely non-pregnant women with no-DDI, pregnant women with no-DDI and pregnant women with a DDI scenarios, in order to compare the ability of the PQ PBKP model to capture the extent of the reported DDIs.

Subsequently, DDIs were simulated in a 10x10 virtual clinical trial with each population group described previously (see section 2.1.2). A standard daily dose approach was employed for EFV (600 mg once daily) and RTV (100 mg twice daily, in line with ritonavir/lopinavir combination dosing of 100mg/400mg twice daily) with EFV/RTV dosed for 14 days and PQ dosed on days 3,4 and 5 (10 mg/kg PQ base). The malaria-pregnancy population groups were redefined during the

simulation duration on a daily basis to account for physiological/biochemical changes, and studies conducted across gestational weeks (GW) 10 to 40. EFV and RTV are pre-validated compounds developed by Simcyp and included into the Simcyp Simulator® compound library. The RTV compound file has been widely used as a CYP3A4 inhibitor in mechanistic modelling (Colbers, Greupink, Litjens, Burger, & Russel, 2016; Hyland, Dickins, Collins, Jones, & Jones, 2008; Kaspera et al., 2014; Marsousi et al., 2016; Wang, 2010), and compound-specific parameters and validation data for the use of EFZ as a CYP3A4 and 2B6 inducer within the Simcyp Simulator®, have recently been published (Ke, Barter, Rowland-Yeo, & Almond, 2016).

2.1.4.2. Human serum albumin

Human serum albumin (HSA) concentrations was set at 20 g/L and 50 g/L within population groups, to mimic the reduction in serum albumin reported at different stages of malaria infection, with 20 g/L representing severe malaria (Sagaki et al., 2013) . The Simcyp 'Pregnancy' population includes a description for alterations in HSA during pregnancy and the baseline initial HSA concentration was fixed at the aforementioned concentrations.

2.1.4.3. Gestational week

The impact of gestation week on PQ pharmacokinetic during EFV/RTV-mediated DDI was further assessed at weeks 10, 20 and 30 for all population groups.

2.2 Predictive performance

Although no uniform criterion has been accepted for defining an 'optimal' predictive performance range, a prediction to within 2-fold of the observed data is generally accepted in this context [40] and was employed as our criterion for C_{max} and AUC comparisons to those clinically reported. For EFV/RTV DDI simulation, as the clinical efficacy of PQ is determined by its day-7 concentration (post-first dose) of 30 ng/mL (Price et al., 2007), the impact of a DDI of PQ pharmacokinetics was assessed by direct analysis of the day-7 concentration.

2.3 Data analysis

Unless otherwise stated, all simulations of plasma concentration-time profiles were presented as arithmetic mean and 5-95th percentiles. Reported concentration-time profiles from clinical studies were digitally retrieved using the WebPlotDigitizer v3.10 [41] and superimposed onto simulated profiles for visual predictive checks.

3. RESULTS

3.1. Healthy volunteer: base model development (Step 1)

The initial model development for health-volunteer (Caucasian) subjects focussed on addressing model validation to recover appropriate absorption kinetics coupled with an appropriate prediction of steady state volume of distribution (V_{ss}) and CYP3A4 and CYP2C8-mediated metabolic clearance (see supplementary materials). The resultant model was found to be appropriate to capture C_{max} and t_{max} and resulted in a broadly consistent simulated C_{max} (21.5 ng/mL ± 9.2 ng/mL), t_{max} (5.3 hours) and AUC (AUC_{0-24}: 384.2 ng h/mL ± 145.9 ng h/mL; AUC_{0-last}: 3207.3 ng h/mL ± 1121 ng h/mL) when compared to Ahmed *et al* (Ahmed et al., 2008) (C_{max}: 41.6 ng/mL ± 29.5 µg/L; t_{max}: 4.0 hours; AUC_{0-24}: 393 ng h/mL ± 149 ng h/mL; AUC_{0-last}: 2312 ng h/mL ± 790 ng h/mL) (Figure 2A) and Sim *et al* (Sim et al., 2005) (C_{max} [range]: 21.0 µg/L [14-31.4 µg/L]; t_{max}: 6.8 hours [2.1-11.5 hours]; AUC_{0-last}: 2818 µg h/L [1566-5070 µg h/L]) for a 500 mg PQP dose (Figure 2C). For a higher 1500 mg PQP dose, consistent simulated C_{max} (76.1 ng/mL ± 69 ng/mL), t_{max} (5.1 hours) and AUC (AUC_{0-24}: 1243 ng h/mL ± 193.8 ng h/mL; AUC_{0-last}: 9065 ng h/mL ± 1299 ng h/mL) were simulated when compared to Ahmed *et al* (Ahmed et al., 2008) (C_{max}: 147 ng/mL ± 110 µg/L; t_{max}: 2.5 hours; AUC_{0-24}: 1418 ng h/mL ± 775 ng h/mL; AUC_{0-t}: 6399 ng h/mL ± 2067 ng h/mL) (Figure 2B).

These predictions supported the successful model development in healthy-volunteer population groups for fasted single dose studies only.

3.2 Non-Caucasian, non-pregnant malaria population groups (Step 2)

In order to assess the predictive performance in multi-dose studies, three-population groups were developed for Thailand, Papua New Guinea and Sudan females based on published clinical studies

within these groups, under conditions of standard multi-dose regimens (10 mg/kg PQ base once daily for 3 days) (Figure 3).

For all population groups, the majority of estimated parameters (Table 1) fell within 2-3 fold of the reported metrics (Table 2). Notability however, for the Thailand population group, the predicted increase in median C_{max} following each dose was only moderately correlated with that reported by Rijken *et al* (Rijken et al., 2011) (Figure 3). However, the clinical end-point marker of successful antimalarial therapy (day 7 concentration) (Price et al., 2007) were all simulated (Table 1) to with 2-fold of the reported clinical measures (Table 2), in addition to day 14 and day 28 concentrations.

Furthermore, the model predictions were also able to capture the differences in day 7 concentration across population groups, despite similar dosing strategies, e.g. Thai 24.74 ng/mL (4.42-64.93 ng/mL) *vs.* Sudanese 34.0 ng/mL (6.8-86.7 ng/mL) population groups. A one-way ANOVA indicated statistical differences in the median day 7 concentrations, when comparing all 4 predicted population studies, with the Sudanese population group demonstrating a statistically higher median C_{max} ($p = 0.0415$) compared to the other population groups.

This highlighted the successful creation of sub-population group's validation in each population group.

3.3 Non-Caucasian, pregnant malaria population groups (Step 3)

The PBPK model was further adapted to evaluate PQ pharmacokinetics in non-Caucasian pregnant population groups (Figure 4). For all population groups, the majority of estimated parameters (Table 3) fell within 2-fold of the reported metrics (Table 4), with predictions of the median day 7, 14 and 28 concentrations all simulated to with 2-fold of the reported clinical measures (Table 4). These predicted point markers were not significantly different than those for non-pregnant subjects ($p > 0.05$) (Tables 1 and 2)

A one-way ANOVA indicated statistical differences in the median day 7 concentrations, when comparing all 4 predicted population studies, with the Sudanese population group demonstrating a statistically higher median C_{max} ($p = 0.0392$) compared to the other population groups (Figure 4). However, when comparing non-pregnant to pregnant population groups, no significant difference in the median day 7 concentration was identified for each population. Further, predicted half-life in pregnancy population groups were significantly different ($p < 0.01$ for all population groups [t-test]) from those in non-pregnancy population groups (Table 3).

This highlighted the successful creation of sub-population group's validation in each population group.

3.4 'What-If' scenarios (Step 4)

To evaluate and validate the impact of ART on PQ systemic exposure, the only known recent study investigating the impact of ART (efavirenz) on PQ systemic exposure in Ugandan pregnant women (Kajubi et al., 2017b) was replicated, following creation of a Uganda pregnancy-malaria population group (see supplementary materials) where EFV was orally dosed at 600 mg once daily (see supplementary materials Figure S1). The predicated day 7, 14 and 21 PQ concentrations were all within 2-fold of that reported by Kajubi *et al (Kajubi et al., 2017b),* with a similar approximate 50 % decrease in the predicted mean day 7 concentrations (No EFV: 20.5 ng/mL; EFV: 9.2 ng/mL) (see supplementary materials Table S3). Furthermore, our predicted $AUC_{0\text{-}d21}$ was within 2-fold of that reported by Kajubi *et al* (this study: 0.51; Kajubi: 0.62).

3.4.1 The impact of change in HSA on the extent of ART-DDIs

In all population groups (absence and presence of a DDI) an increase in HSA from 20 g/L to 50 g/L significantly increased the median day 7 (total) plasma concentration of PQ (Figure 5). This

was associated with a significant increase in the number of subjects with a day 7 concentration > 30 ng/mL in the absence of an ART (Thailand: 8 to 45, p = 0.007; PNG: 11 to 53, p = 0.00009; Sudan: 9 to 49, p = 0.0006), and in the presence of EFV (Thailand: 7 to 48, p = 0.006; PNG: 4 to 24, p = 0.0003; Sudan: 1 to 16, p = 0.0005) or RTV (Thailand: 41 to 80, p = 0.0009; PNG: 49 to 85, p = 0.0008; Sudan: 47 to 80, p = 0.00003) (Figure 5).

Additionally, the presence of EFV or RTV significantly reduced or increased, respectively, the day 7 PQ concentration across all population groups, however the overall impact of the DDI across population groups for both EFV and RTV were broadly similarly (Figure 5). This resulted in a similar number of subjects attaining a day 7 concentration ≥ 30 ng/mL except for the Sudanese population with a EFV-mediated DDI, where a statistically significant difference in the median day 7 concentration across the three population groups was identified (One-Way ANOVA, p = 0.0023).

3.4.3 The impact of gestation on the extent of an ART-mediated DDI

In the absence of an ART-mediated DDIs, the median predicted day 7 concentration was broadly consistent across all gestational weeks investigated (Thailand: 20.2-21.2 ng/mL; PNG: 22.5-23.5 ng/mL); Sudan: 25.1-26.2 ng/mL) and demonstrated no significant difference across gestational weeks within the same population group (Figure 6).

In the presence of EFV, a significant decrease in PQ concentrations (p < 0.0001) was simulated across all gestational weeks within each population group (Thailand: 9.8-11.3 ng/mL; PNG: 13.2-21.5 ng/mL; Sudan: 15.4-18.3 ng/mL), except for gestational week 40 with the PNG population group (Figure 6). In the presence of RTV, a significant increase in PQ concentrations (p < 0.0001) were simulated across all gestational weeks within each population group (Thailand: 34.2-37.9 ng/mL; PNG: 40.7-42.2 ng/mL; Sudan: 41.3-46.1 ng/mL). Furthermore, a trend in increasing median concentration with increasing gestational week was observed for all population groups, although this was not statistically significant (Figure 6).

4. DISCUSSION

The treatment of malaria in special populations, such as pregnant women and young children, is complicated by the 'moving-target' nature of such population groups and their associated reduced immune function with which to resist the individual impact and spread of malaria. Attempting to address the problem of AMT in malaria endemic areas requires careful consideration of the disease pathophysiology in those population groups which may in turn impact upon the efficacy endpoint of the antimalarial treatment. With the aid of PBPK modelling techniques, these factors can be investigated separately to suggest the effect specific impacts can make on antimalarial therapy clinically. Hence, the applied 4-stage workflow model (Figure 1) was aimed at developing and validating a PBPK model to assess the pharmacokinetics of PQ during pregnancy, as well as under conditions of altered serum albumin (mimicking severe malaria), and during potential DDIs; these were mediated by common ARTs available for use in pregnant women (efavirenz and ritonavir).

Despite the advantages of PBPK/mechanistic modelling, the application of modelling approaches to the prediction of plasma concentration profiles has largely been based around systems-parameters derived from Caucasian healthy subjects. The base model development in step 1 followed this similar approach, but only to identify and optimise parameters for single dose PQ studies. A common feature of many antimalarials are the large variability in absorption kinetic processes, represented by a highly variable C_{max}, and it was important to capture this, where possible (Borrmann et al., 2010; Sim et al., 2005; Tarning et al., 2014; White, 2013). To this end, in the absence of appropriate *in-vitro* Caco-2 derived passive permeability (P_{app}) measures for PQ, we applied a first-order absorption model with final estimates of 0.50 for fa and 0.45 h^{-1} for k_a which were able to recover the C_{max} and t_{max} compared to the single-dose studies (Figure 2). However, to capture the range of reported values (e.g. C_{max}: 14-31.5 µg/L and t_{max}: 2.1-11.7 h) a 50 % CV was applied. It should be noted that an inclusion of a transit absorption model, such as the Simcyp ADAM module, may improve predictions (Tarning et al., 2012) but the lack of

appropriate *in-vitro* permeability measures makes this less attractive over a first order model. The development of the base model was successful for single dose studies.

Malaria is endemic in developing countries and this is reflecting by the availability of reported clinical studies we identified where PQ was administrated to pregnant and non-pregnant women from studies conducted with subjects from Thailand (Rijken et al., 2011; Tarning et al., 2012), Sudan (Hoglund et al., 2012) and Papua New Guinea (Benjamin et al., 2015). Application of the 'Healthy-Volunteer' population group to simulate PQ pharmacokinetic would not be appropriate given the difference in adult age across these geographic regions (Walpole et al., 2012) and therefore custom age-weight relationships (Hayes et al., 2015) were generated for each population group to develop non-pregnant and pregnant populations from these regions, which incorporated alterations in blood parameters (haematocrit, human serum albumin and alpha-1-acidic glycoprotein) (see supplementary materials Table S1). Change in haematological biochemistry are also common in malaria and it plays a major role in pathogenesis (Bakhubaira, 2013; Maina et al., 2010; van Wolfswinkel et al., 2013). From a pharmacokinetic perspective, such changes are likely impact upon the blood:plasma ratio, but more importantly the unbound fraction, a key driver for the prediction of clearance, Vss and the DDIs.

The development of appropriate systems-based population groups, specific to the study design is important and highlighted by the stark differences in body weights compared to 'Healthy Volunteer' population. Using the customised age-weigh relationships for malaria population (see supplementary material), body weight for Thai (49.65 ± 7.13 kg), PNG (58.32 ± 12.2 kg) and Sudan (53.2 kg ± 14.46 kg) were generally consistent and significant difference ($p < 0.01$) from a standard 'Healthy Volunteer' population group giving an average weight of 66.7 ± 13.1 kg. As dosing for many AMT is based on body weight, this may have a direct effect on the dose administered and the resultant determination of endpoint concentrations (Terlouw, Courval, et al., 2003; Terlouw,

Nahlen, et al., 2003), particularly considered dosing in many developing countries is based on age as a surrogate for body weight, in situations where weight facilities are unavailable.

Further, the inclusion of potential alterations in biochemistry- based changes during pregnancy or because of a disease state are important if they are thought to directly impact upon the resultant pharmacokinetics of AMT. Haematological alterations are common in malaria and a marker of the severity of malaria is often determined from changes in serum albumin. Equally, albumin binding is a direct driver for the free fraction of drugs, and any alterations in this may directly impact upon drug distribution and metabolic pathways. Indeed, the stark difference in HSA in Sudanese (45.5 g/L) and Thai (33.7 g/L) illustrates this difference across population groups (see supplementary materials Table S1).

For the three population groups developed, model predictions of key pharmacokinetics metrics were within 2-fold of those reported and illustrate the successful prediction of PQ in non-pregnancy (Table 1) (Figure 3) and pregnant women (Table 3; Figure 4). Notably, no significant differences in key pharmacokinetic parameters, including day 7 concentrations, were observed between non-pregnant or pregnant population groups, suggesting the systemic exposure of PQ is relatively unchanged between the two groups and concurs with other reports of unchanged PQ pharmacokinetics in non-pregnant and pregnant populations (Adam et al., 2012; Rijken et al., 2011; Tarning et al., 2012). However, model predictions were less successful at predicting the interindividual variability in the range of C_{max} for population groups for each dosing period (Figure 3 and 4). This may be partially due to poor control of food intake during the trial study, e.g. Tarning *et al* (2012) (Tarning et al., 2012), but may also be a feature of the spare collection points around the expected C_{max} for each dosing day compared to the much richer collection over the longer elimination phases (Tarning et al., 2012). Further, the larger predicted C_{max} for each dosing period could be a result of the splanchnic blood flow (as a result of increased cardiac out) seen in pregnancy (Dawes & Chowienczyk, 2001), and which is altered using 'Pregnancy' population

groups in Simcyp, and hence represent a slight increase in the bioavailability of PQ (Tarning et al., 2012).

During pregnancy, the activity of CYP3A4 is also known to increase (Little, 1999), directly impacting upon the metabolic clearance of any CYP3A4 substrates. In our simulations, increasing gestational week had a noticeable impact of the terminal elimination of the PQ (see supplementary materials Figure S2), as quantified by a decrease in the terminal elimination half-life of PQ during pregnancy populations (Table 3) compared to non-pregnancy populations (Table 1).

Having established a working PBPK for PQ in pregnant females, the importance of the risk of DDIs was next assessed. The only existing study assessing the risk of DDI with PQ and antiretroviral (efavirenz) was recently published by Kajubi *et al* (Kajubi et al., 2017b), and demonstrated a 40% reduction in $AUC_{0\text{-}21d}$ along with a 50 % reduction in day-7 concentrations, highlighting the potential risk EFV-mediated DDIs pose, and our model was able to recapitulate these changes (see supplementary materials Figure S1). As EFV is known to induce CYP3A4, the reduction in AUC and day-7 concentration is likely to be a result of this effect (Hariparsad et al., 2004). Further, this effect would be augmented by the induction of CYP3A4 itself during pregnancy, as noted for other drugs (Costantine, 2014; Dawes & Chowienczyk, 2001), and therefore would likely reduce day-7 concentrations below the clinical efficacy end-point of 30 ng/mL (Price et al., 2007). Indeed, our model simulation demonstrated the impact of this in non-pregnant, pregnant and pregnant +EFV populations, demonstrating the additive effect of EFV-mediated DDI and pregnancy-related induction of CYP3A4 expression (see supplementary materials Figure S1).

Although pregnancy has been associated with a reduction in haematological parameters, e.g. human serum albumin (decrease by 1% at week 8 and 12% at week 32 (Murphy, Scott, McPartlin, & Fernandez-Ballart, 2002)), the impact of such changes on the pharmacokinetics of highly bound drugs is not well characterised in malarial infected subjects. Further, as demonstrated by the development of specific populations, the overall haematological levels are often reduced in such

364 populations. It has also been speculated that *P. falciparum* plays a major role in proteolysis of
365 albumin (El Tahir, Malhotra, & Chauhan, 2003; Kolakovich, Gluzman, Duffin, & Goldberg, 1997).
366 Further, previous reports have demonstrated alteration in fu_{plasma} for quinine (Mansor et al., 1991)
367 and halofantrine (Cenni, Meyer, Brandt, & Betschart, 1995) during the progression of malaria. In
368 attempting to assess the impact of potential changes in human serum, albumin on the overall extent
369 of ART-mediated DDIs (via assessing change in PQ day 7 concentration), we set the HSA
370 concentration to 20 g/L (severe malaria) and 50 g/L (healthy subjects). In all cases (absence and
371 presence of an ART) and in all population groups, the change from 20 g/L to 50 g/L had a direct
372 on day 7 concentrations, leading to a statistically significant increase (Figure 5). For all population
373 groups developed, the reduction in HSA to 20 g/L, generally resulted in a statistically significant
374 increase in PQ fu_{plasma} ($p < 0.001$) which subsequently propagated to an increase in the hepatic
375 clearance ($p < 0.001$), when compared to compared to healthy volunteer population groups. This
376 trend was also demonstrated under conditions of efavirenz/ritonavir exposure when compared to
377 non-DDI studies (see table 5 for representative illustration in the Thailand non-pregnant
378 population). The impact of this combined reduction in baseline HSA concentration in malaria
379 population coupled with the pregnancy-related reduction in HSA is important considering as it can
380 directly impact upon the elimination of the drug.

381

382 As expected, the impact of EFV and RTV on day 7 concentration were in-line with their function
383 as CYP3A4 inducer (EFV) and inhibitors (RTV) resulting in a direct effect on day-7
384 concentration following the interaction (Figure 5). A reduction in AMT concentrations as a result
385 of induction will lead to parasite recrudescence, as has been demonstrated for lumefantrine
386 (Huang et al., 2012; WorldWide Antimalarial Resistance Network Lumefantrine, 2015),
387 dihydroartemisinin (Lamorde et al., 2013) and piperaquine (Kajubi et al., 2017b). Further,
388 piperaquine is known to prolong QTc in a concentration dependant manner (Darpo et al., 2015),

and increased concentration following inhibition of metabolic clearance may potentially lead to QTc prolongation, as demonstrated with an adapted 2-day treatment with DHA-PQ (Manning et al., 2014).

Physiological changes during gestation can result in significant changes in plasma volume, CYP expression and cardiac output (Costantine, 2014; Dawes & Chowienczyk, 2001), it would therefore be expected that significant changes in the pharmacokinetics would be expected during pregnancy. We explored the impact of gestation on the predicted day-7 concentrations in the three pregnancy population groups.

In all three populations, the baseline median day 7 concentration was consistent across all population groups and with increasing GW, approximately 20-26 ng/mL, with no significant differences when GW increased (Figure 6). Given the long half-life of PQ, the impact of gestation on day 7 concentrations may not be significantly noticeable. However, CYP3A4 activity is thought to increase during pregnancy, reaching a peak at approximately week 20-24 (Hebert et al., 2008; Hirt et al., 2006; Villani et al., 2006). When considering the Thai population as an example, at baseline, GW20 corresponded with the lowest median day-7 concentration and highest hepatic CLint (week 17-27) (see supplementary materials Figure S3). However the impact of this may be negligible given the long half-life and large volume of distribution of PQ (Adam et al., 2012; Benjamin et al., 2015; Rijken et al., 2011; Tarning et al., 2013).

When ARVs were concomitantly dosed with PQ, statistically significant decreases (efavirenz) or increases (ritvonair) in PQ median day-7 concentrations were predicted, in-line with the role of EFV and RTV as inducer/inhibitors of CYP3A4 expression. Surprisingly, there were no significant difference across GW for either ART, suggesting the magnitude of the DDI would be similar, irrespective of the gestation period of the mother. Further, when considering the Thai population as an example, although each ART resulted in a significant change ($p < 0.0001$) in the hepatic CLint in the absence of ART (~290-320 L/h) and presence of ART (EFV: 1230-1271 L/h; RTV:

6.3-10.7 L/h), no significant differences across gestational weeks were simulated (see supplementary materials Figure S3). Similarly, although each ART resulted in a significant change ($p < 0.0001$) in the oral CL in the absence of ART (~2000-2135 L/d) and presence of ART (EFV: 4125-5142 L/d; RTV: 899-1239 L/d), no significant differences across gestational weeks were simulated (see supplementary materials Figure S3). The resultant AUC_{ratio} (last dose-to-end) in the presence of EFV was consistent across GW 7 to 27 (0.56-0.58) but increased to 0.72 at GW 37 (One way ANOVA, Tukey post hoc analysis $p < 0.01$) (see supplementary materials Figure S4). Similarly, inhibition results in an AUC_{ratio} across GW 7 to 27 (1.71-1.79) which decreased to 1.64 at GW 37 (not significant) (see supplementary materials Figure S4).

Further, median day 7 concentration in the absence and presence of an ART-mediated DDI were consistently higher in Sudanese population compared to Thai and PNG populations, and this can be attributed to the lower body-weight corrected doses administered to Thai subjects (see supplementary materials) coupled with the higher HSA concentrations in Sudanese populations (see supplementary materials table S1).

Thus, although the impact of ARV on PQ pharmacokinetics in pregnancy may lead to treatment failure or an increase in the adverse effects, the overall effect and magnitude of the DDI during pregnancy is largely minimal, with little change in day-7 concentrations.

It should be noted that the population groups developed altered only the age-weight relationships and haematological parameters, and reflecting changes predominantly malaria-infected subjects. Genetic polymorphisms in CYP2B6 are common (Haas et al., 2009; Lang et al., 2001; To et al., 2009) and may impact upon the clearance of EFV and hence its ability to elicit a DDI, and CYP2B6 population-based polymorphic changes were not incorporated into our customised population groups. Further, changes in the abundance of CYP-isozymes across population groups have also not been incorporated and this may enable better predictions of the terminal elimination phases for PQ across population groups (Bains, 2013). It should also be noted that only one previous study

reported PQ metabolic pathways (Lee et al., 2012) and our assumption of the fraction metabolised by CYP3A4 and CYP2B6 of 0.99 and 0.01, alongside parameter estimated CLint, may be optimised at a later date with *in-vitro* metabolic clearance data to enhance the application of the model, when such data becomes available.

Further, the complexity of diseases states which can present differently depending upon disease progress, as is common with malaria and HIV (Wanke et al., 2000), would dictate that the developed population groups should address these different stages of disease progression. Finally, the studies used for validation utilised two DHA-PQ combination formulation regimens, Eurartesim® (Sigma-Tau, Rome, Italy) or Artekin® (Holleykin Pharmaceutical Co., Guangzhou, China). However only Eurartesim® has gained Good Manufacturing Practice compliance, having being developed without the Medicines for Malaria Venture (MMV) (Ubben & Poll, 2013). Therefore, batch-to-batch variability in the manufacture of the Artekin® fixed-dose combination tablet, may lead to variability in disintegration/dissolution process resulting in altered absorption kinetics and this should be considered in the context of further validation of the absorption kinetics of the model development. Finally, estimates of PQ *in-vitro* Caco-2 permeability are currently lacking and therefore this precludes the application of the Simcyp ADAM model, to appropriately model the biopharmaceutics processes in greater mechanistic detail. The modelling of the absorption phase of PQ pharmacokinetics may therefore be improved when such information becomes available.

5. CONCLUSION

The present PBPK model provides the ability to mechanistically predict the pharmacokinetic of PQ in non-pregnancy and pregnant women, whilst also considering possible population differences in malaria-HIV co-infected subjects. The present model demonstrated that PQ pharmacokinetics in pregnancy is consistent and was relatively unchanged, compared to non-pregnant women and that the impact of ART-mediated DDIs can significantly alter the PQ pharmacokinetics, the magnitude

of which was generally consistent across GW. Further adaptions of the model presented is warranted and would require further detailed collation of relevant physiological and biochemical alterations common to HIV/malaria patients and which would further enhance the clinical application of the proposed model.

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697 **LIST OF FIGURES**

698

699 **Figure 1: A four-stage workflow approach for model development, validation and**
700 **predictions**

701

702 **Figure 2: The simulated plasma fasted single-dose concentration-time profile of piperaquine**
703 **in healthy-volunteers**

704 (A) Simulation of PQ plasma concentration-time profile following a single oral dose of 500 mg
705 PQP (left panel: open circles are observed mean points) and 1500 mg (right panel: open squares
706 are observed mean points) to healthy volunteers (n=6). Observed data was obtained from Ahmed
707 *et al* (2008) (Ahmed et al., 2008). (B) Simulation of PQ plasma concentration-time profile
708 following a single oral dose of 500 mg PQP to healthy volunteers (n=8). Observed data is
709 represented by open circles and represents the 3 individual subject concentration-time points only
710 that were reported by Sim *et al* (2005)(Sim et al., 2005) out of a total study size of 8 subject. Insert
711 graphs illustrate plasma concentration profiles in the first 24-hours post-dosing. Errors bars
712 indicate either (A) lower SD at C_{max} for observed data or (B) reported range of C_{max} (vertical red
713 line) or t_{max} (horizontal red line) values. Solid lines represent population mean prediction with
714 dashed lines representing the 5^{th} and 95^{th} percentiles of prediction.

715

716 **Figure 3: The simulated plasma fasted multi-dose concentration-time profile of piperaquine**
717 **in non-pregnant malaria-female subjects**

718 Multidose simulations of PQ (10 mg/kg base once daily for 3 days) were conducted on malaria-
719 non-pregnant female population groups (Thailand (Rijken et al., 2011; Tarning et al., 2012), Papua
720 New Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012), adapted from the 'Healthy
721 Volunteer' population group with Simcyp with adaptations to the age-weight relationships and
722 blood biochemistry and matching (where possible) the clinical trial design (subject numbers and
723 age range) within Simcyp. Crosses indicate observed data obtained from reported individual subject
724 plasma concentration-time profile lines, open circles indicated observed data sampling points
725 obtained from individual plasma concentration points. Insert graphs illustrate plasma concentration
726 profiles in the first 24-hours post-dosing. Solid lines represent population median predictions with
727 dashed lines representing the 5^{th} and 95^{th} percentiles of prediction.

728

729 **Figure 4: The simulated plasma fasted multi-dose concentration-time profile of piperaquine**
730 **in pregnant malaria subjects**

731 Multidose simulations of PQ (10 mg/kg base once daily for 3 days) were conducted on malaria-
732 pregnant female population groups (Thailand (Rijken et al., 2011; Tarning et al., 2012), Papua New
733 Guinea (Benjamin et al., 2015) and Sudan (Hoglund et al., 2012), from, adapted from the
734 'Pregnancy' population group within Simcyp with adaptations to the age-weight relationships and
735 blood biochemistry and matching (where possible) the clinical trial design (subject numbers and
736 age range). Crosses indicate observed data obtained from reported individual subject plasma
737 concentration-time profile lines, open circles indicated observed data sampling points obtained
738 from individual plasma concentration points. Insert graphs illustrate plasma concentration profiles
739 in the first 24-hours post-dosing. Solid lines (black: non-pregnant [for comparison]; red: pregnant)

represent population median prediction with dashed lines representing the 5th and 95th percentiles of prediction.

Figure 5. The impact of changes in human serum albumin concentrations on the piperaquine median day 7 concentration in the absence and presence of an EFZ or RTV-mediated DDI

Multidose simulations of PQP (10 mg/kg base once daily for 3 days) were conducted on malaria-pregnant female population groups (Thailand, Papua New Guinea and Sudan). The human serum albumin concentration was fixed at 20 g/L or 50 g/L. EFV (600 mg once daily) (red bars) or RTV (100 mg twice daily) (green bars) were orally dosed throughout the simulation time period (30 days) with piperaquine dosed on days 10, 11 and 12. Box and whisker plots represent minimal, 25th percentile, median, 75th percentile and maximum values. Dashed lines indicate the 30 ng/mL clinical efficacy cut-off. Numbers above the box and whisker are median values and the number (n) of subjects with a predicted concentration of over 30 ng/mL is indicated. Vertical drop-lines indicated statistical comparisons between 20g/L or 50 g/L simulation. Asterisks above the maximum bar indicate statistical significance when compared to black (no DDI) simulations. ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Figure 6. The impact of changes in gestational week on median day 7 PQ concentration in the absence and presence of a DDI mediated by EFZ (induction) or RTV (inhibition)

Multidose simulations of PQP (10 mg/kg base once daily for 3 days) were conducted on malaria-pregnant female population groups (Thailand, Papua New Guinea and Sudan over gestational weeks (GW) 10, 20, 30 and 40. EFV (600 mg once daily) or RTV (100 mg twice daily) were orally dosed throughout the simulation time period (30 days) with PQ dosed on days 10, 11 and 12. Box and whisker plots represent minimal, 25th percentile, median, 75th percentile and maximum values. Dashed lines indicate the 30 ng/mL clinical efficacy cut-off. Numbers above the box and whisker

765 are median values and the number (n) of subjects with a predicted concentration of over 30 ng/mL
766 is indicated. Horizontal drop-lines indicate statistical comparisons between each GW in the absence
767 and presence of the ART. ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

TABLES

Table 1: Simulated PQ pharmacokinetics in non-Caucasian non-pregnant females

	Thailand		PNG	Sudan
	Rijken **Median (Range)**	*Tarning* **Median (Range)**	*Benjamin* **Median (Range)**	*Hoglund* **Median (Range)**
C_{max} 1st (ng/mL)	66.14 (17.2-182.1)	64.59 (18.7-189.5)	87.70 (20.76-216.39)	92.15 (25.11-261.7)
C_{max} 2nd (ng/mL)	85.25 (21.23-241.46)	88.17 (23.74-251.49)	116.15 (25.89-292.40)	116.8 (30.71-337.6)
C_{max} 3rd (ng/mL)	98.5 (23.9-280.62)	99.2 (24.2-284.45)	134.6 (29.25-342.48)	134.6 (34.51-388.5)
T_{max} (h)				
T_{max} 1st (h)	4.80 (2.88-6.48)	4.81 (2.82-6.12)	4.67 (2.91-6.51)	3.84 (2.16-5.04)
T_{max} 2st (h)	4.56 (2.64-6.01)	4.61 (2.54-6.32)	4.46 (2.72-6.06)	3.6 (2.16-4.8)
T_{max} 3st (h)	4.56 (2.64-7.62)	4.62 (2.71-7.20)	4.39 (2.7-6.12)	3.6 (2.16-4.8)
AUC_{0-24} (ng/mL.h)	998.64 (240-2830.8)	993.56 (248-2824.9)	1372.8 (296.83-3466.7)	1261.4 (318.24-3678.7)
AUC_{24-48} (ng/mL.h)	1409.3 (324.5-4074.9)	1429.3 (331.5-4107.34)	1968.7 (404.8-5064.4)	1790.6 (436.08-5254.3)
AUC_{48-72} (ng/mL.h)	1712.4 (384-4945.9)	1762.1 (381-4995.2)	2378.4 (479.4-6175.7)	2194 (520.6-6383.8)
$AUC_{0-\infty}$ (µg/L.h)			21715 (4463-53645.8)	35201.3 (303.9-74180.6)
Day 7 Conc. (ng/mL)	24.74 (4.42-64.93)	25.11 (4.21-65.14)	29.17 (5.31-80.85)	34.0 (6.8-86.7)
Day 14 Conc. (ng/mL)	17.01 (3.17-41.63)	16.78 (3.22-42.99)	18.75 (3.67-49.9)	24.2 (5.3-54.9)
Day 28 Conc. (ng/mL)	12.03 (2.34-26.28)	11.63 (2.23-27.27)	11.70 (2.05-26.80)	17.5 (4.02-38.7)
Half-life (d)	27.3 (21.3-40.9)	28.5 (20.8-39.8)	18.3 (16.1-23.6)	32.1 (21.6-43.3)

772 **Table 2**: Literature reported PQ pharmacokinetics in non-Caucasian non-pregnant females

	Thailand		PNG	Sudan
	Rijken **Median (Range)**	*Tarning* **Median (IQR)**	*Benjamin* **Median (Range)**	*Hoglund* **Median (Range)**
C_{max} 1st (ng/mL)		291 (194–362)		185 (109–363)
C_{max} 2nd (ng/mL)	138 (39.3–328)			
C_{max} 3rd (ng/mL)	201 (58.2–455)			
T_{max} (h)	309 (138–575)			
T_{max} 1st (h)		3.14 (2.84–3.84)		3.07 (1.65-4.64)
T_{max} 2st (h)				
T_{max} 3st (h)				
AUC_{0-24} (ng/mL.h)				
AUC_{24-48} (ng/mL.h)	1480 (506–3,270)			
AUC_{48-72} (ng/mL.h)	2400 (734–4,400)			
$AUC_{0-\infty}$ (µg/L.h)	3660 (1,160–5,010)			
Day 7 Conc. (ng/mL)			23721 (21481–27951)	42700 (27100–68700)
Day 14 Conc. (ng/mL)	31.8 (13.3–80.2)	28.8 (23.6–34.6)		60.7 (40.1-103)
Day 28 Conc. (ng/mL)	19.5 (7.76–49.3)			
C_{max} 1st (ng/mL)	10.7 (3.70–31.4)	10.3 (9.18–14.4)		16.1 (9.68-26.8)
Half-life (d)[a]	4.78-39.9	22-26.1	20.3	20.9-33.3

773

774 [a] Half-life is reported as a range or median

775 **Table 3**: Simulated piperquine pharmacokinetics in non-Caucasian pregnant females

	Thailand		PNG	Sudan
	Rijken **Median (Range)**	*Tarning* **Median (Range)**	*Benjamin* **Median (Range)**	*Hoglund* **Median (Range)**
C_{max} 1st (ng/mL)	70.44 (33.56-153.08)	72.43 (31.92-167.89)	89.52 (38.20-175.42)	92.91 (41.04-202.38)
C_{max} 2nd (ng/mL)	90.47 (46.02-204.60)	86.23 (50.61-214.36)	118.35 (25.82-237.95)	116.6 (55.6-263.53)
C_{max} 3rd (ng/mL)	103.28 (53.95-237.19)	109.73 (53.54-264.38)	136.70 (61.99-276.93)	132.17 (65.25-303.4)
T_{max} (h)				
T_{max} 1st (h)	4.80 (2.9-7.68)	4.62 (3.1-7.98)	5.04 (2.9-8.16)	4.32 (2.64-6.96)
T_{max} 2st (h)	4.56 (2.88-6.96)	4.86 (2.91-7.02)	4.8 (2.9-7.2)	4.08 (2.64-6.48)
T_{max} 3st (h)	4.56 (2.88-6.96)	4.79 (2.83-7.11)	4.56 (2.9-6.96)	4.08 (2.64-6.24)
AUC_{0-24} (ng/mL.h)	1036.3 (564.96-2429)	1135.2 (536.9-2532)	1399.4 (681.1-2850)	1249 (112.72-2974.32)
AUC_{24-48} (ng/mL.h)	1450.3 (770.4-3482.4)	1424.1 (779.3-3599.8)	2002.1 (938.9-4127.52)	1745.3 (931.92-4229.76)
AUC_{48-72} (ng/mL.h)	1736.1 (912.5-4195.9)	1811.2 (964.9-4201.7)	2408.64 (1110.24-4977.1)	2424.96 (1114.1-5108.2)
$AUC_{0-\infty}$ (µg/L.h)			21633.6 (8383.9-42237.8)	30067.4 (15267-84201.1)
Day 7 Conc. (ng/mL)	25.97 (10.87-52.59)	24.17 (11.03-53.13)	29.62 (12.2-57.7)	34.04 (15.13-70.60)
Day 14 Conc. (ng/mL)	19.11 (7.88-39.46)	19.92 (8.24-40.11)	20.19 (7.89-38.8)	26.14 (11.31-56.55)
Day 28 Conc. (ng/mL)	14.40 (5.63-31.20)	15.12 (5.91-39.97)	13.60 (4.82-27.2)	20.11 (7.94-45.62)
Half-life (d)	19.4 (18.7-35.4)	19.9 (18.64-35.7)	26.3 (16.7-39.25)	24.7 (14.9-27.2)

776

777

778

779 **Table 4**: Literature reported piperquine pharmacokinetics in non-Caucasian pregnant females

	Thailand		PNG	Sudan
	Rijken **Median (Range)**	*Tarning* **Median (IQR)**	*Benjamin* **Median (Range)**	*Hoglund* **Median (Range)**
C_{max} 1st (ng/mL)		216 (139–276)		102 (40.6-235)
C_{max} 2nd (ng/mL)	71.6 (10.1–239)			
C_{max} 3rd (ng/mL)	136 (13.6–393)			
T_{max} (h)	245 (53.4–798)			
T_{max} 1st (h)		3.04 (2.36–4.13)		1.48 (0.887-4.18)
T_{max} 2st (h)				
T_{max} 3st (h)				
AUC_{0-24} (ng/mL.h)				
AUC_{24-48} (ng/mL.h)	869 (157–2,940)			
AUC_{48-72} (ng/mL.h)	1710 (167–4,740)			
$AUC_{0-\infty}$ (µg/L.h)	2750 (500–8,280)			
Day 7 Conc. (ng/mL)			35644 (29546–39541)	38000 (12400–100000)
Day 14 Conc. (ng/mL)	25.9 (6.80–56.6)	22.7 (17.6–32.8)		55.4 (16.6-146)
Day 28 Conc. (ng/mL)	16.7 (2.24–59.2)			
C_{max} 1st (ng/mL)	9.17 (5.14–47.6)	10.3 (8.06–14.9)		15.4 (4.85-38.6)
Half-life (d)[a]	8.88-24.9	16.2-19.4	15.9	19.1-25.8

780

781 [a] Half-life is reported as a range or median

782

783 **Table 5**: Impact of changes in blood biochemistry on hepatic clearance in the absence and presence of a efavirenz or ritonavir mediated drug-drug

784 interaction for a representative population group (Thailand non-pregnant).

	No DDI			*Efavirenz*			*Ritonavir*		
	Healthy	**20 g/L** [a]	**50 g/L** [a]	**Healthy**	**20 g/L**	**50 g/L**	**Healthy**	**20 g/L**	**50 g/L**
CL_H (L/h)	8.41 ± 4.48	16.24 ± 6.91	7.06 ± 3.75	33.86 ± 10.95	46.91 ± 10.26	29.94 ± 9.43	2.62 ± 1.31	5.1 ± 2.35	1.94 ± 0.87
fu_{plasma}	0.0139 ± 0.0014	0.0368 ± 0.0037	0.0135 ± 0.0013	0.0138 ± 0.0016	0.0375 ± 0.0032	0.0136 ± 0.0014	0.0137 ± 0.0016	0.0381 ± 0.0031	0.0138 ± 0.0014
HSA (g/L) [b]	45.28 ± 4.53	16.72 ± 1.73	46.81 ± 4.68	45.68 ± 4.66	16.62 ± 1.74	47.04 ± 4.63	45.16 ± 4.56	16.92 ± 1.72	47.21 ± 4.61

785

786 [a] Pre-defined fixed mean human serum albumin (HSA) concentration.

787 [b] Simcyp simulated population median HSA concentration

788 Data represented as median ± standard deviation

789 Healthy: Healthy Volunteer population group; CL_H: hepatic clearance; fu_{plasma}: unbound fraction in plasma; HSA: human serum albumin.