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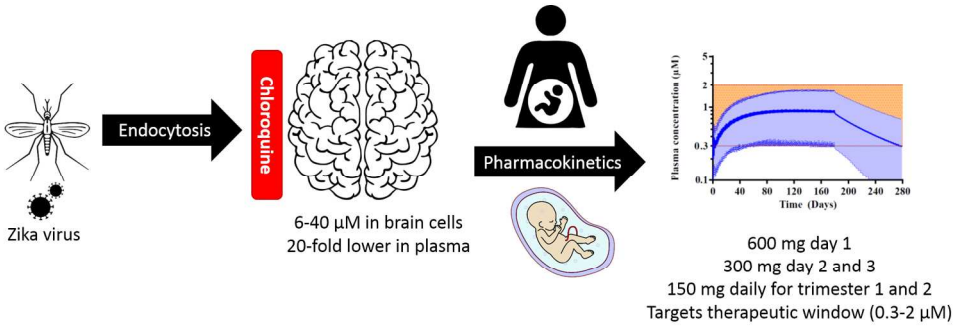

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Olusola Olafuyi[1] and Raj K. S. Badhan[1,2]

[1] Aston Health Research Group, Aston Pharmacy School, Aston University, Birmingham, B4 7ET, United Kingdom.

[2] Aston Pharmacy School, Aston University, Birmingham, B4 7ET, United Kingdom.

Corresponding Author

Raj K. S. Badhan

Aston Pharmacy School

Life and Health Sciences

Aston University

Birmingham

B4 7ET

UK

Telephone: +44 121 204 3288

E-mail: r.k.s.badhan@aston.ac.uk

ABSTRACT

The insidious nature of Zika virus (ZIKV) infections can have a devastating consequence for foetal development. Recent reports have highlighted that chloroquine (CQ) is capable of inhibiting ZIKV endocytosis in brain cells. We applied pharmacokinetic modelling to develop a predictive model for CQ exposure to identify an optimal maternal/foetal dosing regimen to prevent ZIKV endocytosis in brain cells. Model validation utilised 13 non-pregnancy and 3 pregnancy clinical studies and a therapeutic CQ plasma window of 0.3-2 μM was derived. Dosing regimens used in rheumatoid arthritis, systemic lupus erythematosus and malaria were assessed for their ability to target this window. Dosing regimen identified that weekly doses used in malaria were not sufficient to reach the lower therapeutic window, however daily doses of 150 mg achieved this therapeutic window. The impact of gestational age was further assessed and culminated in a final proposed regimen of 600 mg on day 1, 300 mg on day 2 and 3 and 150 mg thereafter until the end of trimester 2, which resulted in maintaining 65 % and 94 % of subjects with a trough plasma concentration above the lower therapeutic window on day 6 and at term, respectively.

KEYWORDS

Physiologically-based pharmacokinetics; Zika; malaria; pregnancy.

1. INTRODUCTION

First isolated in an infected Ugandan monkey in 1947 [1], the Zika virus (ZIKV), is a single stranded RNA virus originating from the *Flaviviridae* family and is transmitted to its host through bites from various *Aedes* species mosquito. Other members of the *Flaviviridae* family include West Nile virus, dengue and yellow fever [2]. An epidemic of Zika broke out in the Yap Islands in 2007 and was later known to have been reported in French Polynesia between 2013 and 2014, followed by a spread to the Americas in 2015 [1,3] and subsequently countries from Africa, Asia and the Pacific [3]. The Pan American Health Organisation (PAHO) and the World Health Organisation (WHO) reports that, aside from the States within the USA, 48 other Central and South American countries have now become affected by the transmission of the disease, and there have been a total of 3720 cases of congenital syndrome associated with the infection [4]. The impact of ZIKV on foetal development can be described by five comment features: (i) severe microcephaly, (ii) reduced brain tissue, (iii) ocular damage, (iv) congenital contractures and (v) hypertonia restricting body movements [5]. The South American country of Brazil has particularly been affected by the largest number of congenital syndrome cases associated with ZIKV, currently 2952 confirmed cases (4th January 2018) [4,6 7,8]. With cases reported in several countries across the world, the spread of ZIKV disease may now be referred to as a pandemic [1].

ZIKV has an incubation period thought to be between 3 and 12 days. A key symptom of the disease is a maculopapular rash in the face, palm, sole and trunk which is expected to be seen within the seven days of infection and infection may last for weeks. Other symptoms of the disease include the fever, joint pain, conjunctivitis and retroocular headache and usually resolves in one week [9-11]. However, acute inflammatory polyradiculoneuropathy known as the Guillian-Barre syndrome, is a complication and can consequently cause weakness and reduced reflexes in victims [9,11]. Further, ZIKV is capable of crossing the placenta and brings

about congenital anomalies such as microcephaly and other ophthalmologic abnormalities in the foetus. ZIKV strains has been found to be present in the placenta and foetal tissue as well as the amniotic fluid of mothers with new-borns [6,8,12-16]. Therefore, a major concern for ZIKV infections is the progression of the virus towards into foetal tissue and the subsequent devastating consequences on foetal brain development.

Currently there are no viable treatment options to prevent the spread of ZIKV. However, in a study by Delvecchio *et al* (2016) [17], the potential for the antimalarial agent chloroquine (CQ) to inhibit the endocytosis of ZIKV within human brain microvascular endothelial and neural stem cells was demonstrated *in-vitro*. Chloroquine has been approved by the Food and Drug Administration (FDA) for the treatment of malaria and for prophylactic treatment in pregnant women at risk of *Plasmodium* parasites [18]. In addition to its antimalarial benefits, CQ has been used as a suppressant of autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [19]. Further, CQ has been demonstrated to prevent the pH-dependant steps in viral replications for human HIV [20], human influenza A, [21] Japanese encephalitis virus [22] and dengue virus type 2 [23]. Given that CQ is widely used in non-pregnant and pregnant subjects for a range of therapeutic interventions, it represents a viable candidate for the potential repurposing in the prevention of ZIKV disease, particularly prior to and during pregnancy. Further, any potential teratogenic risk associated with CQ use in pregnancy has been investigated at doses used for malaria, SLE and RA [18]. Wolfe *et al.* (1985) [24] demonstrated no significant teratogenic consequences of CQ in pregnant women in 169 births from women who had received 300 mg weekly of chloroquine for malaria during their pregnancy. In a further study, Levy *et al* (1991) [18] demonstrated that infants born to the majority of pregnant women being treated with CQ for RA and SLE, who received up to 250 mg of CQ salt daily in the first trimester or 500 mg CQ salt daily for three days during early pregnancy, did not develop adverse conditions likely to be related to the use of CQ in their

mothers. Furthermore, Mackenzie *et al* (1983) [25] demonstrated no retinal damage associated with CQ in 900 SLE patients (non-pregnant) who were treated with less than 4 mg/kg/day of CQ salt, and suggested that the threshold for ocular toxicity was 5.1 mg/kg/day [25]. Finally, although very few studies have examined foetal CQ pharmacokinetics, two separate studies, Law *et al* (2008) [26] and Akintonwa *et al* (1998) [27], reported the CQ ratio between the foetal cord concentrations and the maternal plasma concentrations (C:M ratio) as being approximately 1, suggesting that the placenta is not a major barrier to the partitioning of CQ into the foetus and that maternal plasma concentrations would be similar to those exposed to the foetus.

Despite being used worldwide for over half of a century for anti-malaria prophylaxis [28,29], there has been no significant evidence suggesting foetal damage caused from the use of CQ in pregnant women [30-33], adding to the possibility of repurposing CQ for prevent of ZIKV endocytosis in the developing foetal brain.

Given the distinct physiological changes occurring during gestation[34-37], any attempt at providing potential doses for ZIKV during pregnancy should account for the subsequent alterations in CQ pharmacokinetics during each trimester.

In this current study, a physiologically based pharmacokinetic (PBPK) model was developed to describe the deposition of CQ in non-pregnant and pregnant subjects, with a particular emphasis on: (i) identifying an appropriate plasma therapeutic window for ZIKV, (ii) optimising dosing regimens to attain this therapeutic window and (iii) assessing an optimal dosing regimen for use in different stages of gestation.

2. METHODS

The virtual clinical trial simulator Simcyp (Simcyp® Ltd, a Certara company, Sheffield, UK, Version 16) was used to create all the population based PBPK models used in this study

through the implementation of a pre-validated 'Healthy Volunteer' (HV) population. For simulations requiring the use of pregnant subjects, we utilised the Simcyp 'Pregnancy' population group [38-40], which incorporates gestational-phase dependant physiological changes associated with pregnancy that may alter the pharmacokinetics of drugs such as a change in blood volume and organ/tissue blood flows; change in enzyme/protein expressions [38,41-45].

A 3-stage workflow model was utilised and is detailed in Figure 1. We adopted a robust validation approach utilising 16 clinical studies for CQ, a summary of which is described within the supplementary material (Section A, Table S1). Further, unless otherwise stated, population sizes used in validation steps simulations and those utilised within Steps 3 included a 10x10 trial design with 100 subjects.

2.1 Step 1: Development and validation of a CQ model in non-pregnant subjects

Model development utilised a total of 13 clinical studies reporting CQ pharmacokinetic across a range of Caucasian and non-Caucasian population groups (see Supplementary Materials: Section A). These studies incorporated both single and multiple dose studies for CQ model development and validation. CQ physicochemical parameters were obtained from published studies and are detailed in Table 1. During model development, where the model did not appropriately recover the shape of plasma concentration-time profile and/or pharmacokinetic parameters, a parameter estimation methodology was employed. Further details on validation and optimisation can be found in the Supplementary Materials: Section B.

For clinical studies conducted in non-Caucasian populations (Filiopino, Papuan, Nigerian, Pakistani, and Thai), the Simcyp HV population group was adapted to incorporate appropriate age-body weight relationships reported in non-Caucasian subjects [46]. Adaptation

to the age-weight relationships for non-Caucasian populations are reported in Supplementary Materials: Section B.

2.2 Step 2: Development and validation of a CQ model in pregnant subjects

For simulations involving pregnant women, the non-pregnant CQ model was revised to incorporate a full PBPK distribution model, which allows for consideration of gestation-phase dependant changes in maternal physiology [41,42,44,45] for pharmacokinetic modelling studies [38-40]. To recover the distribution phase profile, an appropriate volume of distribution (Vss) was empirically fixed at the mean of the range reported in literature studies utilising pregnant subjects [31,32,47], following by parameter estimation using a Weighted Least Square (WLS) method and the Nelder-Mead minimisation approach. Parameter estimates for the final optimised pregnancy model are detailed in Table 1. This optimised CQ model was subsequently validated utilising three clinical studies, details of which can be found in Supplementary Materials: Section C Table S2.

2.3 Step 3: Identification of a suitable CQ prophylactic dose regimen for ZIKV

In order to propose a plasma therapeutic window for CQ which could be used to identify an optimal dosing regimen against ZIKV, we utilised reported *in vitro* and *in vivo* concentrations for the inhibition of ZIKV into cells. Delvecchio *et al*. (2016) reported a CQ EC_{50} for the inhibition of ZIKV uptake within Vero cells, human brain microvascular endothelial cells (hBMEC) and human neural stem cells (hNSC) being within the range of 9-15 μM [17]. Further, in ZIKV-infected interferon signalling-deficient AG129 mice, Shiryaev *et al* (2017) [48], reported that CQ extended their lifespan and confirmed that concentrations up to 40 μM were able to reduce ZIKV uptake in primary human foetal neural progenitor cells (NPCs) (with 90 % inhibition at 6 μM).

In other studies, the correlation between brain and plasma concentrations have been identified, with a suggested 10-to-30-fold greater brain concentration compared to plasma

concentrations for CQ [49,50] and a 4-to-30-fold difference for the CQ analogue hydroxychloroquine [51], highlighting the ability of CQ of adequately partition into brain tissue.

Furthermore, in the study reported by Shiryaev *et al* (2017) [48], doses of 30 mg/kg were used in their ZIKV-infected interferon signalling-deficient AG129 mice to demonstrate uptake inhibition, this dose was similar to those used in arthritic patients where 5 mg/kg CQ salt was administered daily for one week which resulted in a CQ plasma concentrations of 10 µM [25]. A total dose of about of 30 mg/kg would be achieved in humans, which would comparable to doses used in the study by Shiryaev *et al* (2017) [48], suggesting that such plasma concentrations are attainable using similar dosing regimens employed for existing therapeutic indication for CQ.

Therefore, in order to define a therapeutic window for CQ, we assumed that the target brain concentration of a maximum of 40 µM was required and to theoretically achieve this concentration in the human brain, a plasma concentration of less than an average of at least 20-folds of the brain concentration may be required, that is, approximately 2 µM. This was defined as the upper plasma therapeutic window. Given that concentration in excess of 6 µM have been reported to prevent ZIKV uptake in brain derived cells, we set the lower plasma therapeutic window at 20-fold less, that is 0.3 µM. Therefore a plasma concentration therapeutic range of 0.3 µM to 2 µM was assumed in this study.

To identify an appropriate dosing regimen to target this therapeutic window, plasma concentration-time profiles for CQ were simulated in 100 pregnant subjects (during the entire gestational phase of 280 days) using the validated CQ model at doses used for the prophylaxis of malaria and RA, i.e. 150 mg-300 mg weekly or 150 mg daily, respectively.

During this optimisation phase, the dose regimen which was able to maintain trough plasma concentrations above the lower limit of the plasma therapeutic window was identified as the optimal predicted dosing regimen.

2.4 Predictive performance

There are currently no universally agreed measure of predictive performance range when comparing observed data to predicted data in PBPK pharmacokinetic studies, however, a 2-fold prediction of observed data is largely accepted [52-54].

2.5 Visual Predictive Checks

Model predictions were compared to existing clinical studies using visual predictive checking (VPC), and approach described at the 2012 FDA Pediatric Advisory Committee (US Food and Drug Administration, 2012) [55]. In brief, the predictability of the simulations was confirmed by comparing the predicted 5th and 95th percentiles of predicted concentration–time profiles with the observed data for any validation data sets. When the predicted data points overlapped with those from the observed data sets, which should (normally) contain a measure of spread of observed plasma concentration data (e.g., a standard deviation for each mean concentration point), the prediction was assumed to be valid.

2.6 Data analysis

Retrospective clinical data used for VPC were extracted using WebPlotDigitizer v.3.10 (http://arohatgi.info/WebPlotDigitizer/). Where applicable, statistical analysis was conducted using paired t-tests with a $P < 0.05$ indicating statistical significance.

3. RESULTS

3.1 Step 1: Development and validation of a CQ model in non-pregnant subjects

Following the optimisation of the model, the final model predicted C_{max}, AUC and t_{max} were within 2-fold of the reported parameters across all thirteen published single and multiple dose clinical studies in Caucasian and non-Caucasian subjects (Table 2). Further, for these studies the model was able to appropriately recover the plasma concentration-time profiles (Figure 2).

The Mzayek *et al* (2007) [56] study demonstrated a wide variability in the absorption phase of the reported plasma concentration-time profiles, and the optimised model developed was able to recover this, with resultant predicted pharmacokinetic parameters within 2-fold of those reported (Table 2). Further, for the Walker *et al* (1987) [57] study, the terminal elimination phase was slightly over predicted, however, the resultant AUC predicted by the model was within 2-fold of that reported (Table 2).

3.2 Step 2: Development and validation of an optimised model of CQ in pregnant subjects

The model was next optimised for use in pregnant population groups, utilising matching gestational weeks (where possible) to published studies, and subsequently was able to satisfactorily predict the plasma concentration-time profiles of CQ in pregnant women (Figure 4) with all pharmacokinetic parameters predicted to within 2-fold of those reported in clinical studies (Table 2).

3.3 Step 3: Identification of a CQ prophylactic dosing regimen for ZIKV during pregnancy

In order to identify a dosing regimen appropriate for maintaining maternal plasma (and foetal exposure) levels, such that a sufficient concentration would be achieved to prevent foetal ZIKA brain endocytosis, standard CQ dosing regimens commonly used for malaria, RA and SLE (150 mg and 300 mg weekly, and 150 mg or 300 mg daily, respectively), were simulated during the first trimester.

For malaria prophylaxis doses, 150 mg and 300 mg weekly, the simulated plasma concentration-time profiles did not achieve the lower target therapeutic limit until the end of trimester 1 (Supplementary Materials: Section C Figure S3) and were not considered for further optimisation.

For doses used in RA and SLE, with a 150 mg daily dose the mean trough plasma-concentration did not reach the lower limit of 0.3 μM until 12 days post first dose (Figure 5A) where 96 % of subjects achieved a trough concentration in excess of the lower therapeutic window (Table 3). At steady-state, a C_{max} of 0.9 ± 0.4 μM was achieved. For the 300 mg daily dose, the trough plasma-concentration doubled (Table 3) (Figure 5B), with a shortening of the time taken to reach the target plasma concentration to 5 days (Table 3). However, the mean steady-state C_{max} was 1.8 ± 0.8 μM with 59 % of subject demonstrating a peak plasma-concentration in excess of 2 μM (Table 3)

Dose optimisation was considered to identify an appropriate dosing regimen for trimester 1 to (i) achieve rapid attainment of the lower plasma therapeutic window and (ii) to maintain this concentration for the longest duration possible. The dosing regimen identified was a loading dose of 600 mg on day 1 followed by 300 mg for 2 days and subsequently 150 mg daily during trimester 1 (Figure 5C). Under this regimen, the time taken for trough plasma concentration to be maintained within the therapeutic window shorted by six days (Figure 5C) (Table 3) compared to a 150 mg daily dose (Figure 5A) (Table 3). Further, only 1 % of

subjects possessed a plasma concentration above the upper therapeutic limit of 2 μM (Table 3).

Subsequently, the impact of initiating the optimal dosing regimen at the start of each trimester on the pharmacokinetics of CQ was assessed. CQ pharmacokinetic were simulated for each trimester period. In comparison to results from trimester 1, dosing this optimal regimen during either trimester 2 or 3 resulted in a progressive and statistically significant decrease ($P < 0.001$) in AUC, from 20.9 ± 9.6 μM.h to 11.8 ± 4.8 μM.h (calculated on the final day of the trimester) (Figure 6) with an increase in the time to reach target trough plasma concentration from 6 days for trimester 1 to 35 days for trimester 3 (Table 4) (Figure 6). Further, at trimester 3, only 79 % of subjects possessed a trough plasma concentration above the lower therapeutic limit (Table 4).

In order to finally identify an appropriate CQ dosing regimen for the entire duration of pregnancy, CQ treatment was extended from the end of the first trimester to the end of the second trimester. During trimester 1 (days 1 to 84), the predicted mean plasma concentration was maintained above the lower therapeutic window (Figure 7A) with a steady-state C_{max} of 0.92 ± 0.41 ng/mL (Table 5). Assuming CQ was halted at the end of trimester 1, mean plasma concentration reached the lower therapeutic window on day 150 with 95 % of subjects possessing a trough plasma concentration above the lower therapeutic window (Table 5). When dosing was continued throughout trimester 2 (Figure 7B), steady-state plasma concentrations were maintained with a C_{max} of 0.92 ± 0.41 ng/mL (Table 5) until the end of trimester 2 (day 168), at which point CQ was halted. Mean plasma concentrations reached the lower therapeutic window on day 279 with 94 % of subjects possessing a trough

plasma concentration above the lower therapeutic window and 5 % of subjects possessing a peak plasma concentration below the lower therapeutic window the (Table 5).

4. DISCUSSION

The Zika virus (ZIKV) is an infectious disease that began spreading at an alarming and unprecedented way in the early part of the current decade, and its spread has been classified as a pandemic [1]. Perhaps alarmingly (and importantly) a prominent feature of ZIKV which gained much publicity was the foetal and neurological consequences on infants born to infected mothers, and which primarily exhibited as microcephaly and Guillian-Barr syndrome. Although no current treatment options are available for the prevention of the spread of ZIKV, the opportunity for repurposing existing treatments towards ZIKV exists for the antimalarial drug chloroquine (CQ). This study addressed the potential to repurpose CQ for use in ZIKV, with a focus on developing potential dosing regimens for use in pregnancy.

During model development and validation (Step 1), model performance depends largely upon the certainty of model input parameters describing CQ absorption, distribution and metabolism and elimination (ADME) [58]. When using literature reported pharmacokinetics parameters for absorption (ka), distribution (Vss) and elimination (clearance), the model performance was poor. This may be, in part, due to the wide variability reported for these parameters, for instance, ka has been reported in different studies as 1.8 h^{-1} (0.27-3.4 h^{-1}) [59]; 1.19 h^{-1} ± 1.44 h^{-1} [60] and 0.51 h^{-1} ± 0.11 h^{-1} [61] and Vss has been reported as 204-800 L/kg [51]; 128 L/kg (112-137 L/kg) [47] and 100-1000 L/kg [62]. Further, the reported Vss of CQ depends on whether they were estimated based on the plasma concentration or blood concentrations, particularly given that CQ has a high blood-to-plasma partitioning ratio of >5:1, therefore the

blood Vss is likely to differ from that of the plasma Vss by up to 10-folds [63,64]. Therefore, we utilised a parameter estimation approach with the application of Weighted Least Square (WLS) and the Nelder-Mead minimisation, final optimised parameter value were obtained (Table 1), and this model was used for subsequently validation purposes.

The application of this optimised model with retrospective clinical studies conducted in Caucasian subjects [56,65,66] resulted in model predictions of pharmacokinetic parameters to within 2-fold of that reports (Table 2), with VPC confirming appropriate predictions of the plasma concentration-time profiles for each study (Figure 2).

In non-Caucasian subjects, physiological parameters, such as body-weight, vary significantly from typical Caucasian subjects and these differences may alter the pharmacokinetics of the drugs [46]. We have previously demonstrated the impact of this in Thai [67,68], Sudanese and Papua New Guinea [68], Ugandan [69] and Malaysians [70] population groups, and these alterations were made to the Simcyp HV population groups (Supplementary Materials: Section B). Following these revision, in all single and multidose simulations involving Caucasians and non-Caucasian subjects, plasma concentration-time profiles and resultant pharmacokinetic parameters were well predicted (Figure 3) and within 2-fold of the reported parameters (Table 2) [32,47,71-75].

Having successfully validated a non-pregnant model in Caucasian and non-Caucasian subjects, the model was extended to pregnancy subjects. The non-pregnant model was adapted by the inclusion of a full PBPK distribution model, which enables the consideration of gestational-age related alterations in maternal physiology. This is important considering that physiological alterations such as blood volume, tissue perfusion, plasma protein binding [41,42,44,45] and CYP450 metabolic capacity [43] can occur. One strength of PBPK modelling is in its ability to incorporate these changes into predictive modelling approaches [38]. When using

this pregnancy model, key pharmacokinetic parameters were predicted to within 2-fold of the reported clinical parameters (Table 2), with plasma concentration-time profiles well recovered for all studies (Figure 4) [31,32,47]. The altered blood volumes, blood flows and albumin binding capacity expected in pregnancy led to a reduction in the overall exposure of CQ in pregnant subjects compared to non-pregnant subjects (Table 2), and a similar decrease in exposure (AUC) and associated C_{max} has been reported in studies where was used in pregnant subjects [31,32,47].

Current dose regimens for CQ use in antimalarial prophylaxis, RA and SLE treatment were next examined to determine the ability of these regimens to drive steady state concentrations within the proposed therapeutic window for ZIKV disease. With a malaria prophylactic weekly CQ dose of 150 mg and 300 mg administered to pregnant subjects during the first trimester, the proposed therapeutic range for ZIKV was not achieved (Supplementary Materials: Section C Figure S3). However, when CQ doses commonly used for SLE, that is, 150 mg daily (Figure 5A) or 300 mg daily (Figure 5B) was administered, the 150 mg daily dose achieved a satisfactory mean steady-state C_{max} (Table 3) within the proposed therapeutic window for ZIKV (Figure 5A), and attained the lower therapeutic window on day 12 (Table 3). However, despite the higher daily dose of 300 mg achieving the target lower therapeutic limit after 5 days, this regimen was not selected as a result of peak plasma concentrations exceeding the upper therapeutic window (Figure 5B) (Table 3).

Based upon the 150 mg daily dose regimen, an optimal dosing regimen was derived to reduce the time taken for trough concentration of CQ to fall within the therapeutic range proposed, and consist of: (i) an initial loading dose of 600 mg on day 1; (ii) 300 mg daily on day 2 an 3; (iii) 150 mg daily thereafter (Figure 5C). This dosing regimen attained the target lower limit within 6 days with the majority of subjects, 96 %, possessing trough plasma concentrations within above the lower therapeutic window (Table 3). In order to improve the clinical

plausibility of the optimised dose, the optimised dose was adapted from doses used for malaria treatment (600mg at 0 hour, 6 hours, 24 hours and 36 hours) [71-74] and SLE/RA prophylaxis (long-term 150 mg daily dose) [18] because there is sufficient evidence to show at these doses, CQ administration is safe for both mother and foetus [18 24].

Having identified an optimal dosing regimen, we next assessed its application at different trimesters, and when comparing trimester 1 to trimester 3, identified a concurrent decrease in C_{max} (0.92 ± 0.41 μM to 0.53 ± 0.21 μM) and AUC (21.8 ± 9.6 μM.h to 11.8 ± 4.8 μM.h) (Table 4) (Figure 6A), which resulted in an increased in the time take to achieve trough plasma concentrations at the lower limited of the therapeutic window (Figure 6B) (Table 4). The physiological changes during pregnancy, primarily alterations in the body weight, plasma proteins and plasma volume [76-80], will drive this decrease in exposure as gestation progresses.

Finally, when assessing the optimised dosing regimen for its use throughout pregnancy, we first considered dosing through trimester 1 only (Figure 7A), which resulted in mean plasma concentrations decreasing below the lower window at 150 days gestation (Figure 7A) where 95 % of subjects possessing tough plasma concentrations above the lower window (Table 5).

On extension of this optimised dosing regimen throughout trimester 2 (Figure 7B), mean plasma concentrations were maintained within the therapeutic window until day 279, which exceeded the start of the 'at term' phase, commencing from week 38 onwards (Figure 7B), and where 94 % of subjects possessed trough plasma concentrations above the lower therapeutic window.

In summary, we have identified a possible therapeutic regent that would be capable of proving sufficient plasma exposure for the duration of the gestations period to potential limit ZIKV uptake into the developing foetus. However, this study is not without limitations.

An obvious limitation to our work is the current inability to accurately predict the pharmacokinetics of CQ in the foetus. Only two clinical studies have reported CQ as being able to reach the foetus through sampling cord blood [26,27], but foetal drug levels were not recorded. However, the reported foetal: maternal concentration ratio was reported to be near unity, suggesting overall foetal exposure would be similar to that within the mother. Therefore we assumed that the driving force for overall foetal exposure would be the material plasma concentration, which was used as a measure of the 'target' concentration within the foetus also. It further goes without saying that this assumption would therefore need to also consider the gestational-related changes in foetal physiology. However, the placenta plays a vital ADME role in controlling delivery of xenobiotics to the foetus, and given the similarity in exposure of CQ between both the mother and foetus [26,27], the Simcyp Pregnancy model incorporated these key gestation related changes in anthropometric features of the mother and the foetus (implemented as a pooled feto-placental compartment within Simcyp) [38-40].

Recently Abduljalil *et al* (2018) [81] have collated foetal biometry and tissue composition data which may drive future studies to better describe and drive, from a mechanistic pharmacokinetic viewpoint, the development of a more appropriate and detailed foetal PBPK model which could predict overall foetal CQ brain exposure, however without CQ foetal tissue sampling data, any validation of such prediction would be difficult.

Our modelling approach utilised a 'worst-case' scenario in deriving a possible plasma therapeutic window for CQ. Our upper and lower plasma concentrations, 2 μM and 0.3 μM respectively, was based on assuming that a 10-to-30-fold greater brain concentration existing when compared to plasma concentrations for CQ [49,50]. We utilised the average of this range, a 20-fold lower plasma concentration compared to reported range of inhibitory concentration of 6-40 μM [17,48]. Whilst not being able to directly verify human foetal brain concentrations, our range of predicted peak plasma concentrations (0.1-1.88 μM) for the final optimised

dosing regimen in pregnant subjects spanned this range and would potentially provide an overall peak brain exposure of 18.8 μM to 56.4 μM. This is assuming that CQ is capable of partitioning across the blood-brain barrier (BBB), with reports suggesting the BBB does not provide a permeability barrier to CQ [50]. However, it is known that the foetal BBB develops from gestational week 8 with tight junction formation by week 18 [82]. Therefore further characterisation of the role of the foetal BBB is warrented to estimate the likely CQ foetal brain exposure.

In relation to the dosing regimen proposed, chloroquine has been in use for at least 50 years, having been introduced as an alternative to quinine [28,29]. Its use in pregnancy has therefore been examined by various groups for safety and efficacy with little reported concerns. At doses proposed in this simulation, Klinger *et al* (2001)[83] reported no ophthalmic abnormalities in children born followed the maternal use of CQ during a mean duration of 7.2 months of gestation for doses of up to 332 mg daily. In a further study by Rukaria-Kaumbutho *et al* (1996)[84], at doses of 25 mg/kg over 3 days, no safety concerns were identified in births at term. Further, a review by Nosten *et al* (2006) [85] identified 755 cases of first trimester exposure to chloroquine with no significant abortion risk or foetal risk. Finally, a study by Wolfe and Cordero (1985) [24] examined a cohort of 168 births to women treated with 300 mg CQ once weekly during the duration of pregnancy and identified no significant increase in the proportion of birth defects when compared to a control group who were not treated with CQ. Therefore, the proposed dosing regimen would provide a level of exposure similar to those reported in existing studies of CQ in pregnant women.

5. CONCLUSION

With the CQ model developed in this study, a CQ dose of 600 mg on day one, followed by 2 days treatment of 300 mg daily and thereafter 150 mg daily from day 3 until the end of

trimester 2 would provide a plasma concentration within the range of 0.3-2 μM, potentially providing protection against ZIKV throughout pregnancy. Though the results from this study are subject to clinical confirmation, it is serves as a guide for future clinical studies.

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Figure 1: Workflow of PBPK model

The workflow based approach implemented in the development and validation of a CQ model for use in non-pregnant and pregnant subjects.

Figure 2: Simulated blood or plasma concentration-time profiles of single dose CQ in non-pregnant subjects

Simulated blood or plasma concentrations for CQ following single dose studies in healthy Caucasian (Frisk-Holmberg, Gustaffson and Mzayek only) subjects and non-Caucasian subjects. Solid lines represent mean predicted concentration-time profile with dotted lines representing 5^{th} and 95^{th} percentile range. Open red circles represent observed clinical data from each study. For the Mzayek *et al* study, red circles indicate data extracted from complete plasma concentrations profile 'lines' for individual subjects rather than discrete time-points. Where presented, error bars indicate standard deviation.

Figure 3: Simulated blood or plasma concentration-time profiles of multiple dose CQ in non-pregnant subjects

Simulated mean blood or plasma concentrations for CQ following multi- dose studies in healthy Caucasian subjects (Wetsteyn only) and non-Caucasian subjects. Solid lines represent mean predicted concentration-time profile with dotted lines representing 5^{th} and 95^{th} percentile range. Open red circles represent observed clinical data from each study. Error bars indicate standard deviation in the Lee; Na-Bangchang; Tanariya; Bustos and Wetsteyn

studies. Individual plasma or blood concentration data point are represented by open red circles in Karunajeewa and Hoglund studies. Left-hand side panels indicate simulations for the total study duration and right-hand side panels illustrate the first three dosing days.

Figure 4: Simulated plasma concentration-time profiles of multiple dose CQ in pregnant subjects

Simulated mean plasma concentrations for CQ following multidose studies in pregnant subjects. Solid lines represent mean predicted plasma concentration-time profile with dotted lines representing 5th and 95th percentile range. Open red circles represent observed clinical data from each study. Error bars indicate standard deviation. Left-hand side panels indicate simulations for the total study duration and right-hand side panels illustrate the dosing period only.

Figure 5: Simulated plasma concentration-time profiles for CQ dosed during the first trimester.

Simulated CQ plasma concentration-time profiles during trimester 1 for: (A) a 150 mg daily dose; (B) a 300 mg daily dose; (C) a proposed optimised daily dose. Dark green lines indicate mean plasma concentration-time profiles; light green shaded area bordered by the dash lines indicate the area within the 5th and 95th percentile of predicted mean plasma concentration-time profiles; light brown shaded area represents the proposed therapeutic range of CQ for ZIKV (0.3-2 μM); dashed dash vertical lines indicates the time at which trough concentration are maintained above the lower therapeutic window.

Figure 6: Simulated plasma concentration-time profiles for CQ dosed during each trimester

Simulated CQ plasma concentration-time profile utilising the optimised dosing regimen, during each trimester. (A) Simulated profiles for the entire duration of each trimester; (B) Simulated profiles for the first 40 days of each trimester. Dark green, red and blue lines indicate mean plasma concentration-time profiles during the 1st, 2nd and 3rd trimesters respectively; lighter shaded areas indicate the area within the 95th and 5th percentile of the predicted mean plasma concentration-time profiles during trimester 1 (upper, light green) and trimester 3 (lower, light blue); light brown shaded area represents the proposed plasma therapeutic window of CQ for ZIKV (0.3-2 μM); dashed dash vertical lines indicates the time at which trough concentration are maintained above the lower therapeutic window.

Figure 7: Simulated plasma concentration-time profiles for CQ dosed during trimester 1 and 2

Simulated CQ plasma concentration-time profile utilising the optimised dosing regimen during (A) trimester 1 and (B) throughout trimester 1 and 2. Left panels indicate the entire duration of gestation (day 0 to 280) and right panels indicate periods from the end of the trimester to the point at which mean trough plasma concentrations fall below the lower therapeutic window. Dark green and blue lines indicate mean plasma concentration-time profiles during the 1st and 2nd trimesters respectively with lighter shaded areas indicating the area within the 95th and 5th percentile of the predicted mean plasma concentration-time profiles; light brown shaded area represent the therapeutic range of CQ proposed to be effective against ZIKV. The time at which the mean trough plasma concentrations fall below the lower therapeutic window is indicated by the arrows. Red dashed lines indicates the 'at term' phase.

Table 1: Model parameter values for base and optimised model of CQ in non-pregnant and pregnant subjects

Parameter	Optimised model (non-pregnant)	Optimised model (pregnant)
Compound type	Diprotic base [43]	Diprotic base [43]
Molecular weight (g/mol)	319.9 [44]	319.9 [44]
log P	4.72 [45]	4.72 [45]
fu	0.55 [46]	0.55 [46]
pKa 1	10.1 [45]	10.1 [45]
pKa 2	8.38 [45]	8.38 [45]
Vss (L/kg)	125 (CV: 40 %) [b]	130 [b]
Vsac (L/kg)	52.9 [b]	-
Q (L/h)	5 [b]	-
Kp scalar	-	3.35 [c]
fa	0.8 [d]	0.8 [d]
k_a (h^{-1})	1.2 [d]	0.5 [d]
Solubility (mg/mL)	0.0175 [44]	0.0175 [44]
$Vmax_{2D6}$ (pmol/min/pmol)	2.10 [47]	2.10 [47]
$Vmax_{3A4}$ (pmol/min/pmol)	2.94 [47]	2.94 [47]
$Vmax_{2C8}$ (pmol/min/pmol)	8.33 [47]	8.33 [47]
Km_{2D6} (µM)	19.5 [47]	19.5 [47]
Km_{3A4} (µM)	294 [47]	294 [47]
Km_{2C8} (µM)	111 [47]	111 [47]
fumic	0.13 [e]	0.13 [e]
ISEF CYP 2D6	0.5 [f]	0.8 [f]
ISEF CYP 3A4	0.42 [f]	0.7 [f]
ISEF CYP 2C8	1.1 [f]	1.6 [f]
Cl_{renal} (L/h)	4.6 [g]	5.5 [g]
Absorption model	first order	first order
Distribution model	minimal PBPK	full PBPK

[a] Simcyp® mechanistic prediction; [b] parameter estimated using a minimal PBPK model with a single adjusting compartment (SAC); [c] an appropriate Kp scalar was empirically optimised for a full PBPK model in

pregnancy; [d] parameter estimated using a first order absorption kinetic model; [e] parameter estimated; [f] parameter estimated for use in optimisation of clearance kinetics; [g] parameter estimated. logP: the logarithm of the n-octanol:buffer partition coefficient; fu: unbound fraction; B/P: blood-to-plasma ratio; Vss: steady state volume of distribution; Vsac: volume of single adjusting compartment; Q: blood flow to the single adjusting compartment; ka: absorption rate constant; Kp scalar: scalar applied to all predicted tissue partition values fa: fraction dose absorbed; k_a: absorption rate constant; Vmax: maximum rate of metabolite formation; Km: Michaelis-Menten constant; fu_{mic}: fraction of unbound drug in the invitro microsomal incubation; ISEF: Intersystem extrapolation factor for scaling CYP *in-vitro* kinetic data; CL_{renal}: renal clearance.

Table 2. Summary of predicted and observed pharmacokinetic parameters of for CQ

		C_{max}		t_{max} (h)		AUC	
		Predicted	**Observed**	**Predicted**	**Observed**	**Predicted**	**Observed**
Caucasian Single Dose	**Gustafsson** [a, e]	56.8 ± 23.8	76 ± 14	4.9 ± 2.6	3.6 ± 2.0	9315 ± 3951	6111 ± 1315
	Mzayek [b, f*]	1.45 (0.3 – 6.1)	1.8 (1.3-5.2)	4.2 (1.5 – 7.1)	3.0 (1.0-8.0)	112 (31.5 - 225)	90 (48.9-212
	Frisk (150 mg dose) [b, i]	0.11	NR	5.2 ± 2.4	NR	3.14 ± 1.3	2.54 ± 0.55
	Frisk (300 mg dose) [b, i]	0.94	NR	5.2 ± 2.4	NR	6.28 ± 2.5	6.19 ± 1.39
	Frisk (600 mg dose) [b, i]	1.9 ± 7.4	NR	5.2 ± 2.4	NR	12.6 ± 5.1	11.6 ± 2.4
Non-Caucasian Single Dose	**Chukwuani** [a, e]	177 ± 170	391 ± 91	4.7 ± 2.4	5.6 ± 0.8	7408 ± 4622	10820 ± 2714
	Najmi [c, g,**]	172 ± 166	201 ± 15	4.6 ± 2.3	6.10 ± 0.66	12775 ± 5835	10827 ± 1340
	Walker [a, e]	159 ± 149	374 ± 56	4.8 ± 2.4	5 ± 3	25865 ± 10608	18609 ± 4254
Non-Caucasian Multi Dose	**Karunajeewa** [c, g,**]	297 (79.1-769)	376 [#]	-	-	57014 (11218-112760)	47892 (43486-53746)
	Na-Bangchang [a, j.***]	883.6 (266-2306)	838 (656-1587)	-	-	167 (35.4-315)	122 (103-182)
	Bustos [a, k,**]	166.4 (63.47-335.7)	285 (186-422)	-	-	2189 (525-4760)	2299 (1149 -39908)
	Lee [a, e,**]	836 (244-3006)	700 (403 - 1625)[##]	-	-	189024 (47160 - 334210)	134087 (62940 - 229695)
	Hoglund [b, l,**]	2.7 (2.04)	NR	3.8	NR	24.2 (10.3)	NR

		Tanariya [a, k,**]	994 (666)	NR	4.3	NR	7897 (3245)	NR
Caucasian	Multi Dose	**Wetsteyn** [d, l,**]	85.6 (56.1)	NR	3.6	NR	1429 (590)	NR
	Pregnant	**Fakeye** [a, e]	123.9 ± 56.0	204.36 ± 134.7	4.6 ± 1.68	2	2113.9 ± 38.3	NR
		Karunjeewa [d, h,**]	145.7 (53.4-240.5)	296**	-	-	38585 (14236-65641)	35750 (31343-39729)
		Lee [a, e,**]	482.7 (166.5-921.8)	960.5 (297-1835)	3.84 (2.4- 4.8)	3 (1.5- 8)	156 847 (54768-349488)	122216 (74145-269600)

Units for C_{max} are as follows: [a] ng/mL; [b] µM; [c] mg/L; [d] µg/L; Units for AUC are as follows: [e] ng/mL.h; [f] µM.h; [g] mg/L.h; [h] µg/L.h; [i] µM.day; [j] µg/mL.h ; [k] ng/mL.Day; [l] µg/L.Day. Unless otherwise stated, data represent means ± SD or median (range). * Data represents median (range); ** $AUC_{0-\infty}$ (AUC calculated from the start of the study and extrapolated to infinity); *** AUC_{0-28d}: AUC calculated 28 days period only; **** AUC_{0-48d}: AUC calculated 48 days period only. [#] No SD or median was reported; [##] C_{max} reported for the first dose only

Table 3: Steady-state pharmacokinetic parameters of CQ during pregnancy

Dose (mg)	**C_{max}** (μM)	**tmax** (h)	**AUC** (μM.h)	Time to lower window [a] (days)	Percentage of subjects with C_{min} > 0.3 μM at SS [b]	Percentage of subjects with C_{max} < 2 μM at SS [c]
150	0.9 ± 0.4	2.4 ± 0.5	21.8 ± 9.4	12	96	99
300	1.8 ± 0.8	2.4 ± 0.5	43.9 ± 18.7	5	99	59
Optimised	0.9 ± 0.4	2.4 ± 0.5	48.8 ± 30.9	6	96	99

Data represents mean ± standard deviation. [a] Time taken for mean trough plasma concentrations to be maintained above 0.3 μM; [b] Percentage of subjects with trough plasma concentrations above 0.3 μM at steady-state; [c] Percentage of subjects with peak plasma concentrations below 2 μM at steady-state. AUC was calculated for the final dosing day.

Table 4: Steady-state pharmacokinetic parameters of the optimised CQ regimen during pregnancy

Trimester	C_{max} (μM)	tmax (h)	AUC (μM.h)	Time to lower window [a] (days)	Percentage of subjects with $C_{min} > 0.3$ μM at SS [b]	Percentage of subjects with $C_{max} < 2$ μM at SS [b]
1	0.92 ± 0.41	2.2 ± 0.5	21.8 ± 9.6	6	96	99
2	0.75 ± 0.32	2.2 ± 0.5	17 ± 7.2	14	93	100
3	0.53 ± 0.21	2.6 ± 0.7	11.8 ± 4.8	35	79	100

Data represents mean ± standard deviation. [a] Time taken for mean trough plasma concentrations to be maintained above 0.3 μM; [b] Percentage of subjects with trough plasma concentrations above 0.3 μM at steady-state; [c] Percentage of subjects with peak plasma concentrations below 2 μM at steady-state. AUC was calculated for the final dosing day.

Table 5: Steady-state pharmacokinetic parameters of the optimised CQ regimen during pregnancy

	Trimester	
	1	**1 and 2** *
C_{max} (μM)	0.92 ± 0.41	0.92 ± 0.41
tmax (h)	2.2 ± 0.5	2.2 ± 0.5
AUC (μM.h)	20.9 ± 9.6	21.0 ± 9.5
Time to increase to lower window [a] (Days)	6	6
Subjects with C_{min} above window (%) [b]	*67*	*65*
Time to decrease to lower window [c] (Days)	150	279
Subjects with C_{min} above window (%) [b]	*95*	*94*
Subjects with C_{max} below window (%) [d]	*1*	*5*

Data represents mean ± standard deviation from the final dose. * C_{max}, tmax and AUC collected on the final dosing day. [a] Time taken for mean concentrations to be reach 0.3 μM; [b] Percentage of subjects with trough plasma concentrations above 0.3 μM; AUC was calculated for the final dosing day; [c] Time taken for mean plasma concentrations to decrease to 0.3 μM; [d] Percentage of subjects with peak plasma concentrations below 0.3 μM.

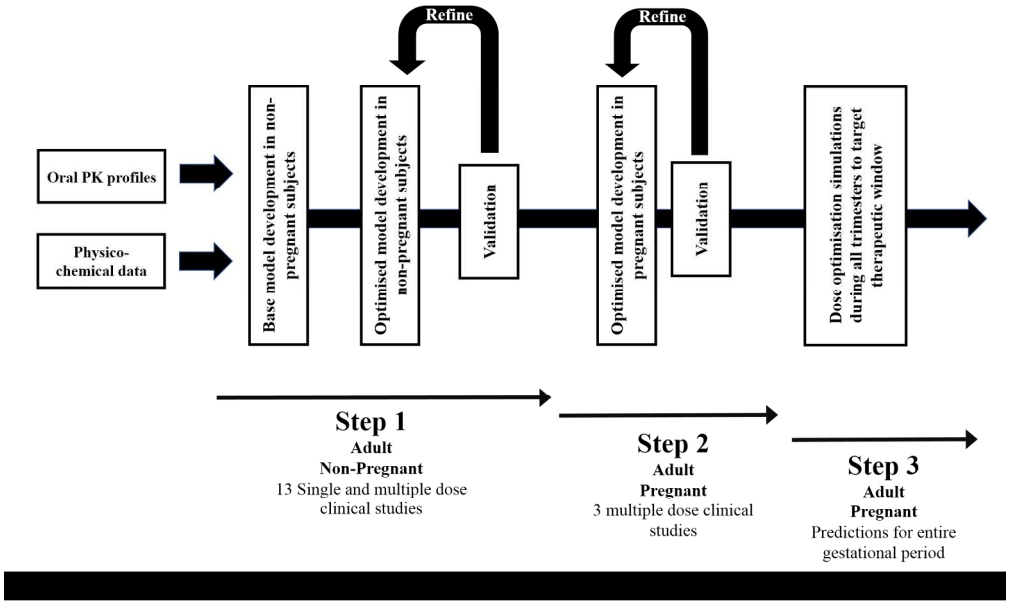

Figure 1

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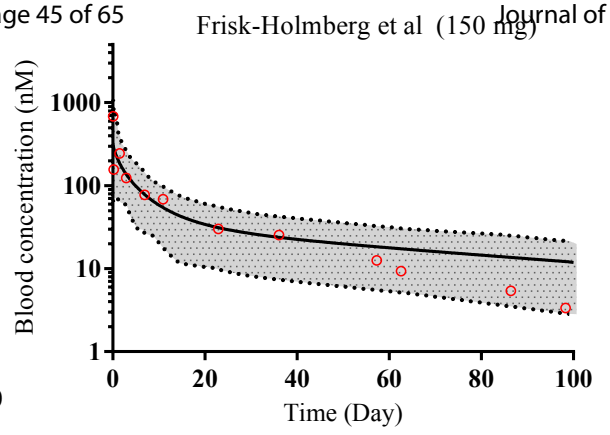
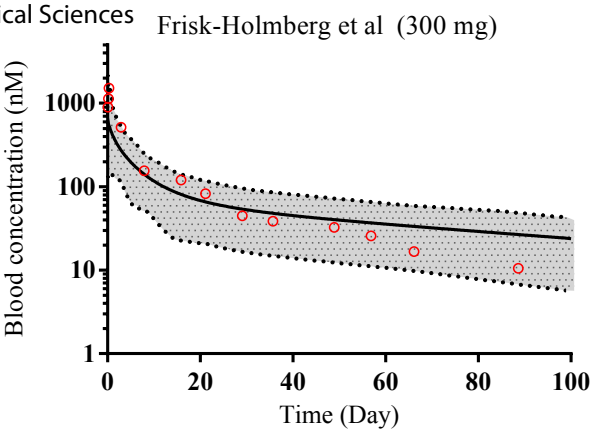
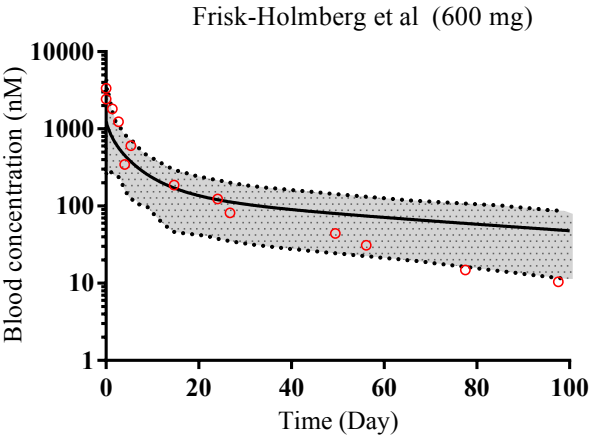
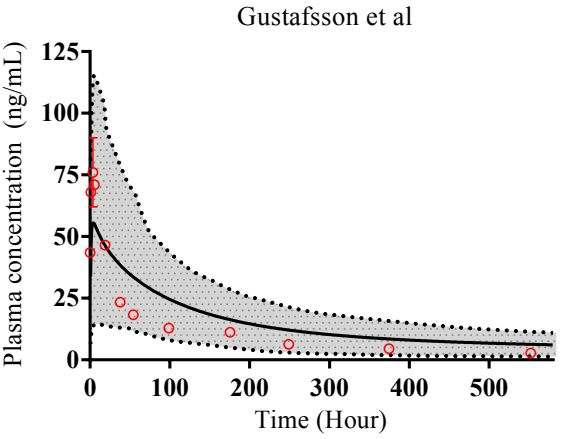
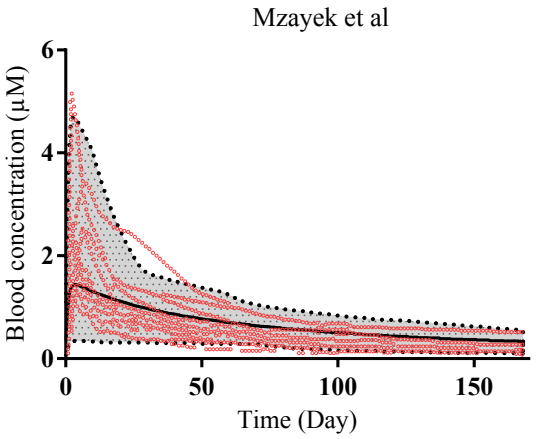
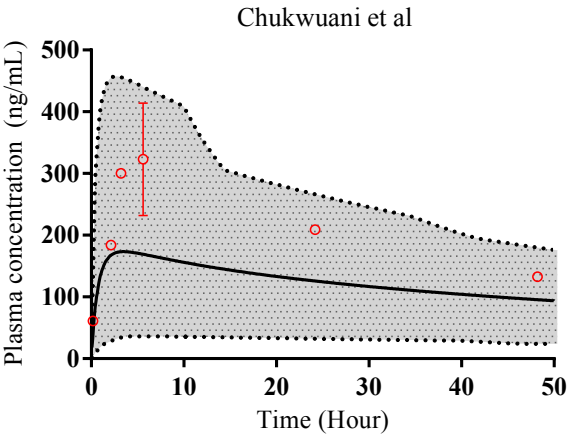

Frisk-Holmberg et al (150 mg)
Blood concentration (nM)
1000
100
10
1
0
20
40
60
80
100
Time (Day)

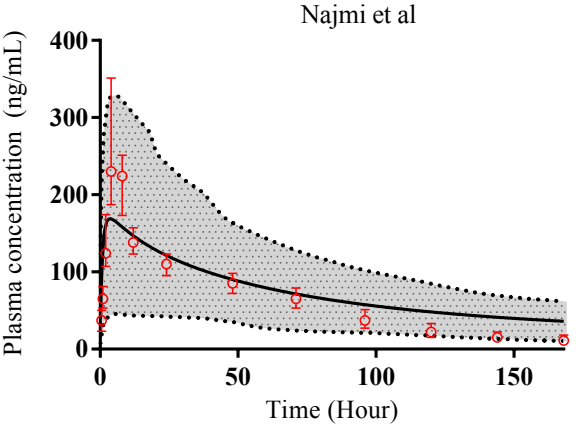

Frisk-Holmberg et al (300 mg)
Blood concentration (nM)
1000
100
10
1
0
20
40
60
80
100
Time (Day)

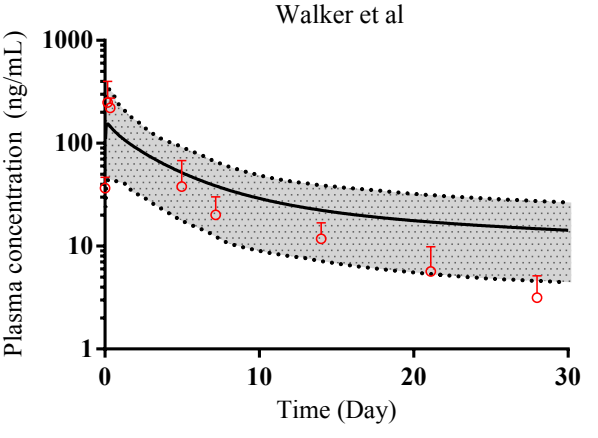

Frisk-Holmberg et al (600 mg)
Blood concentration (nM)
10000
1000
100
10
1
0
20
40
60
80
100
Time (Day)

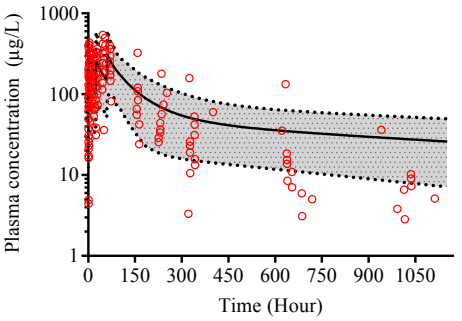

Gustafsson et al
Plasma concentration (ng/mL)
125
100
75
50
25
0
0
100
200
300
400
500
Time (Hour)

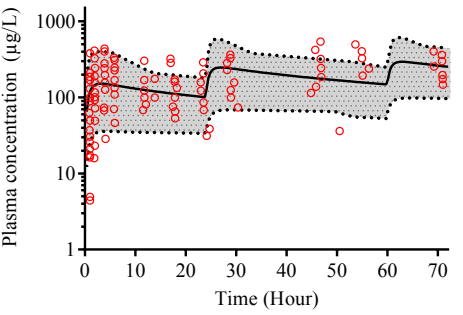

Mzayek et al
Blood concentration (μM)
6
4
2
0
0
50
100
150
Time (Day)


Chukwuani et al
Plasma concentration (ng/mL)
500
400
300
200
100
0
0
10
20
30
40
50
Time (Hour)

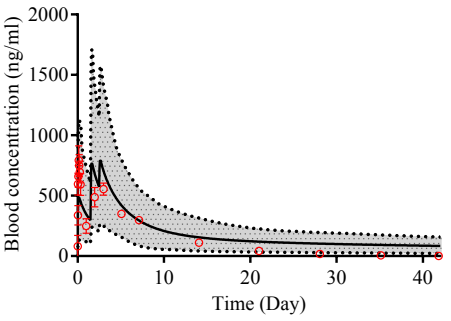

Najmi et al
Plasma concentration (ng/mL)
400
300
200
100
0
0
50
100
150
Time (Hour)

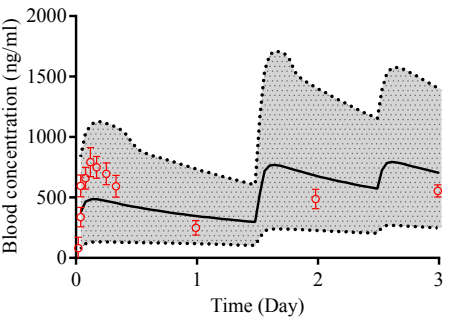

Walker et al
Plasma concentration (ng/mL)
1000
100
10
1
0
10
20
30
Time (Day)



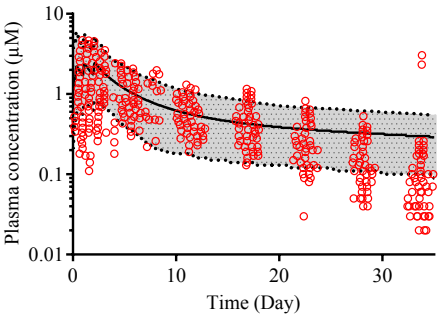


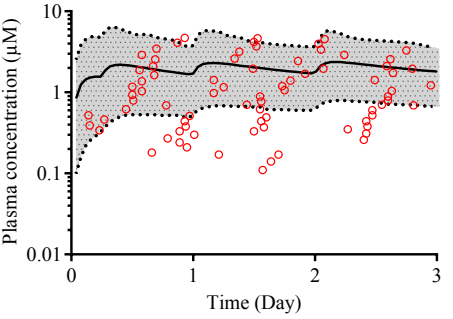
Lee et al



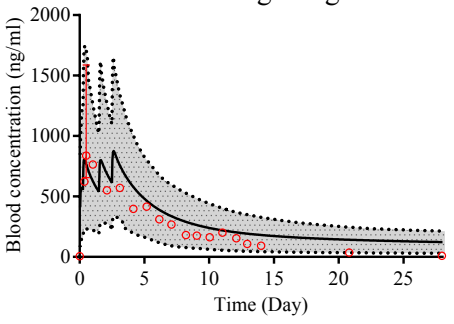


Hoglund et al

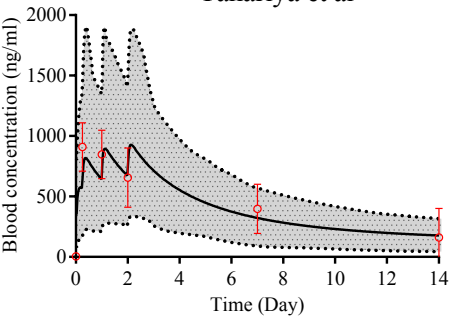




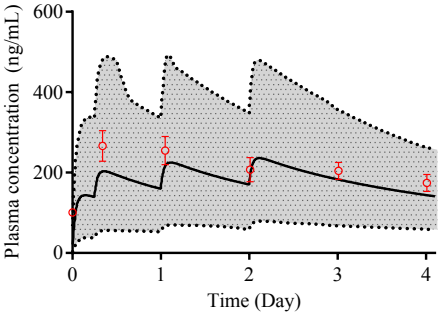
Na-Bangchang et al



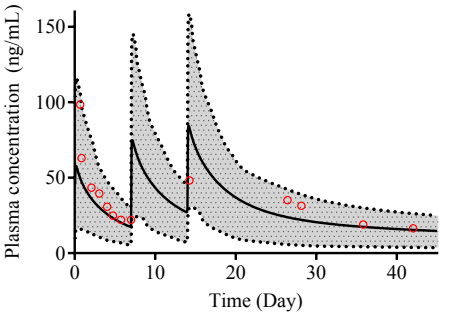
Tanariya et al



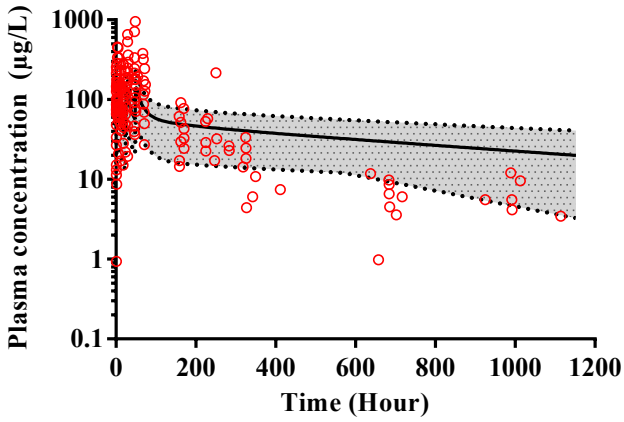
Bustos et al

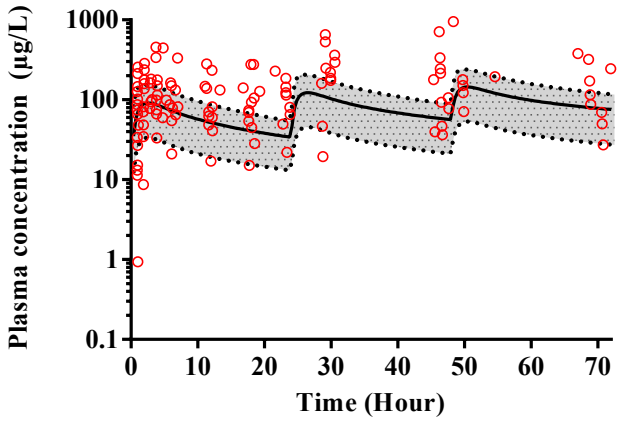


Wetsteyn et al

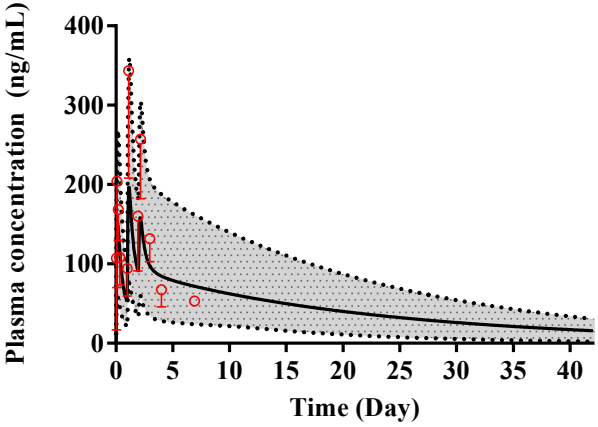


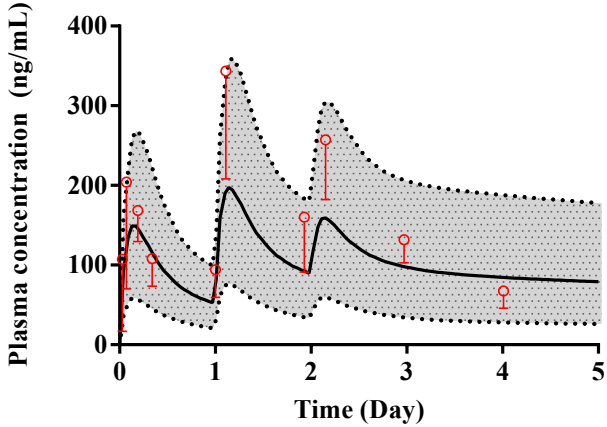
Kartnajeewa et al



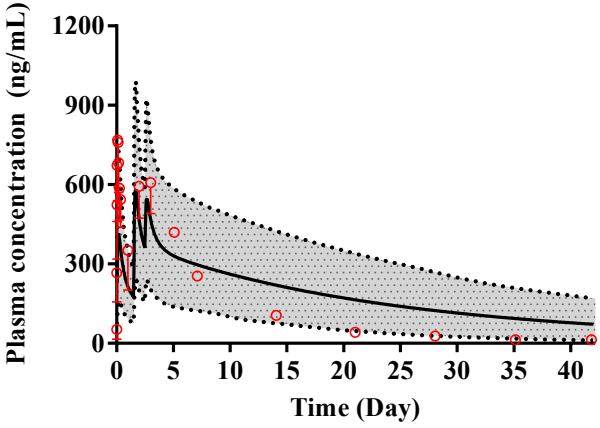


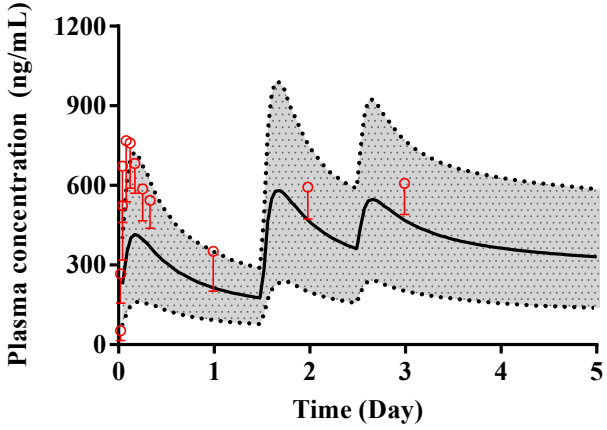
Fakeye et al



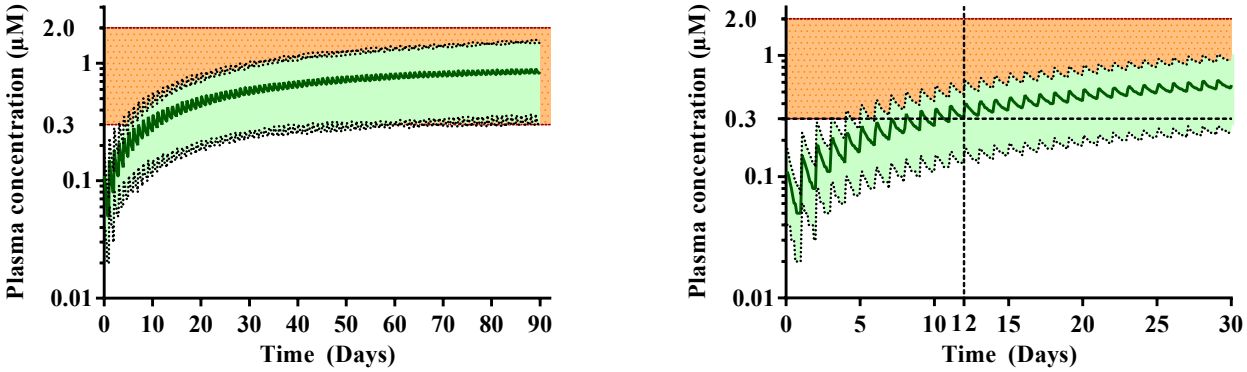


Lee et al

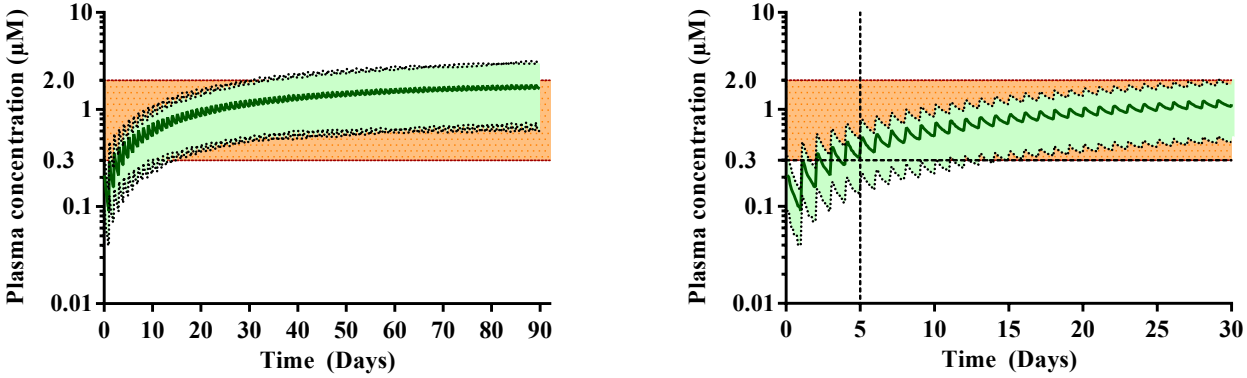




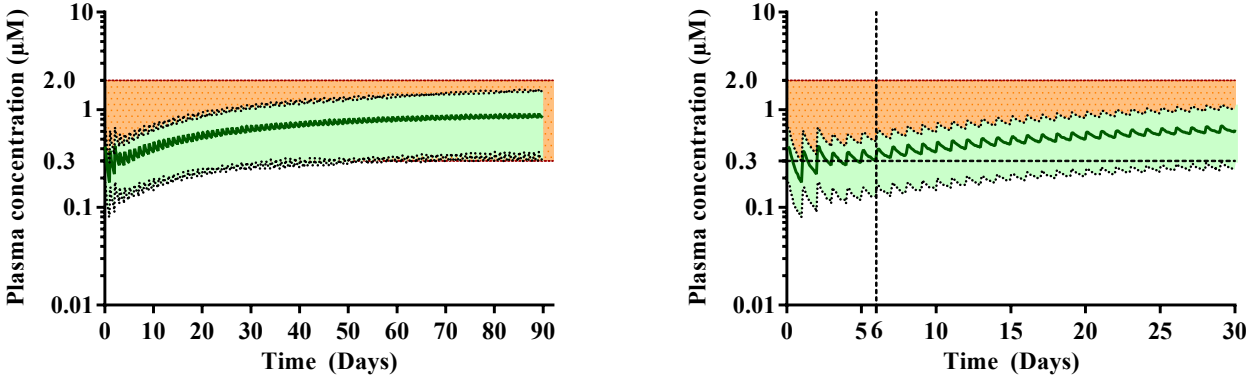
(A)


150 mg daily
Plasma concentration (µM)
2.0
1
0.3
0.1
0.01
0 10 20 30 40 50 60 70 80 90
Time (Days)
Plasma concentration (µM)
2.0
1
0.3
0.1
0.01
0 5 10 12 15 20 25 30
Time (Days)

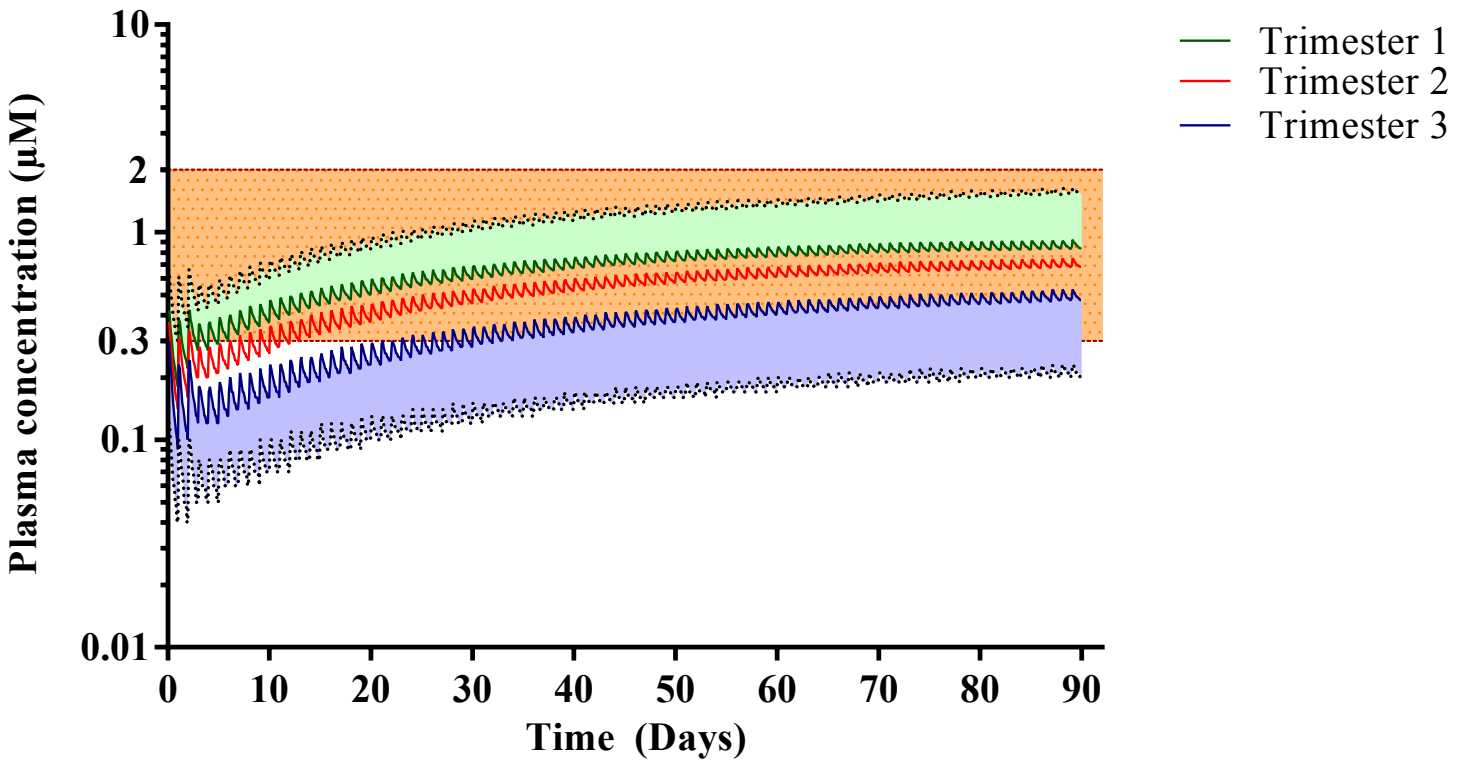
(B)


300 mg daily
Plasma concentration (µM)
10
2.0
1
0.3
0.1
0.01
0 10 20 30 40 50 60 70 80 90
Time (Days)
Plasma concentration (µM)
10
2.0
1
0.3
0.1
0.01
0 5 10 15 20 25 30
Time (Days)

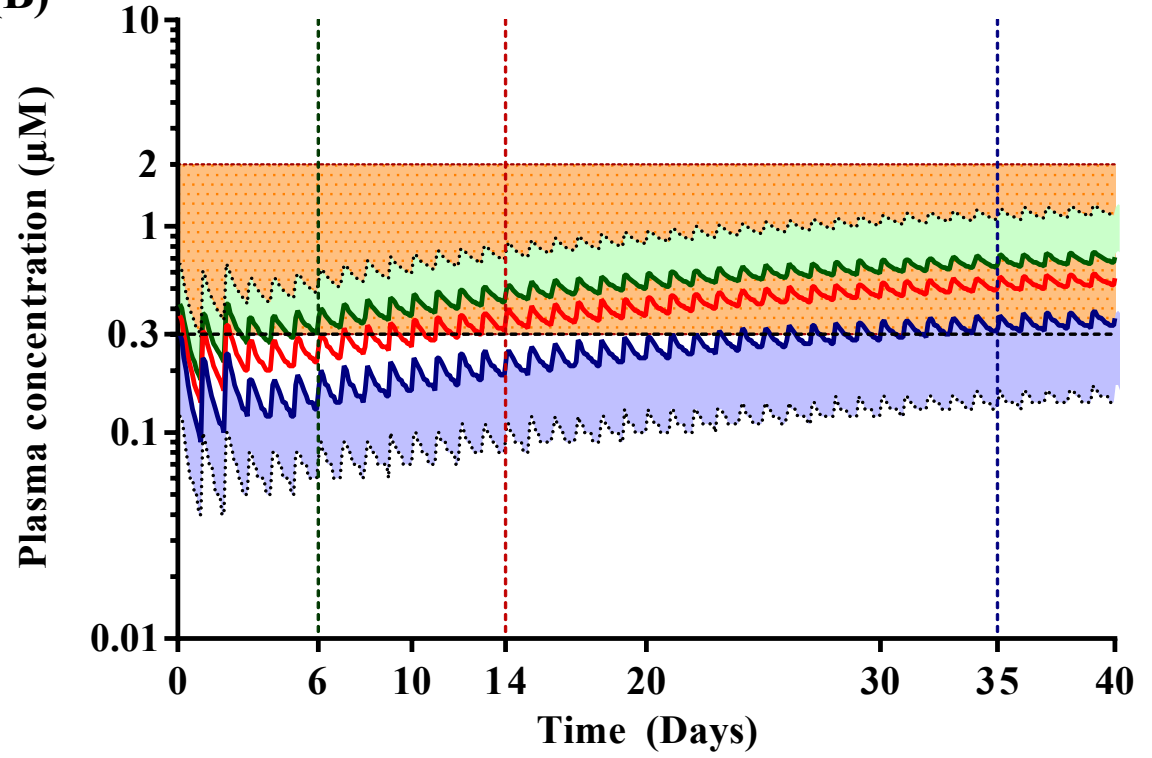
(C)


Optimised
Plasma concentration (µM)
10
2.0
1
0.3
0.1
0.01
0 10 20 30 40 50 60 70 80 90
Time (Days)
Plasma concentration (µM)
10
2.0
1
0.3
0.1
0.01
0 5 6 10 15 20 25 30
Time (Days)

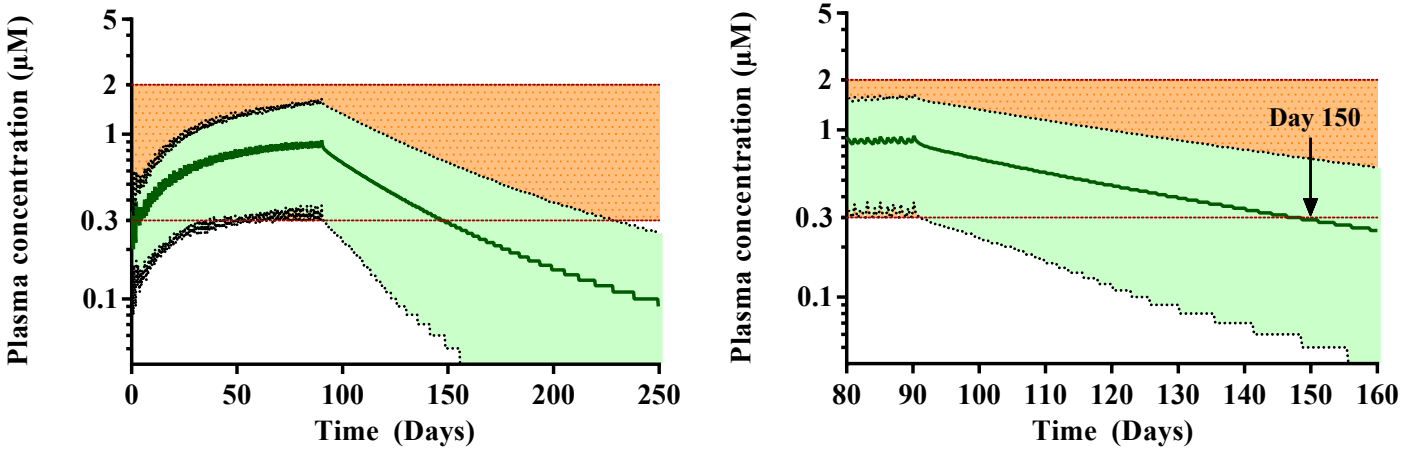
(A)


Trimester 1
Trimester 2
Trimester 3
Plasma concentration (µM)
10
2
1
0.3
0.1
0.01
0
10
20
30
40
50
60
70
80
90
Time (Days)

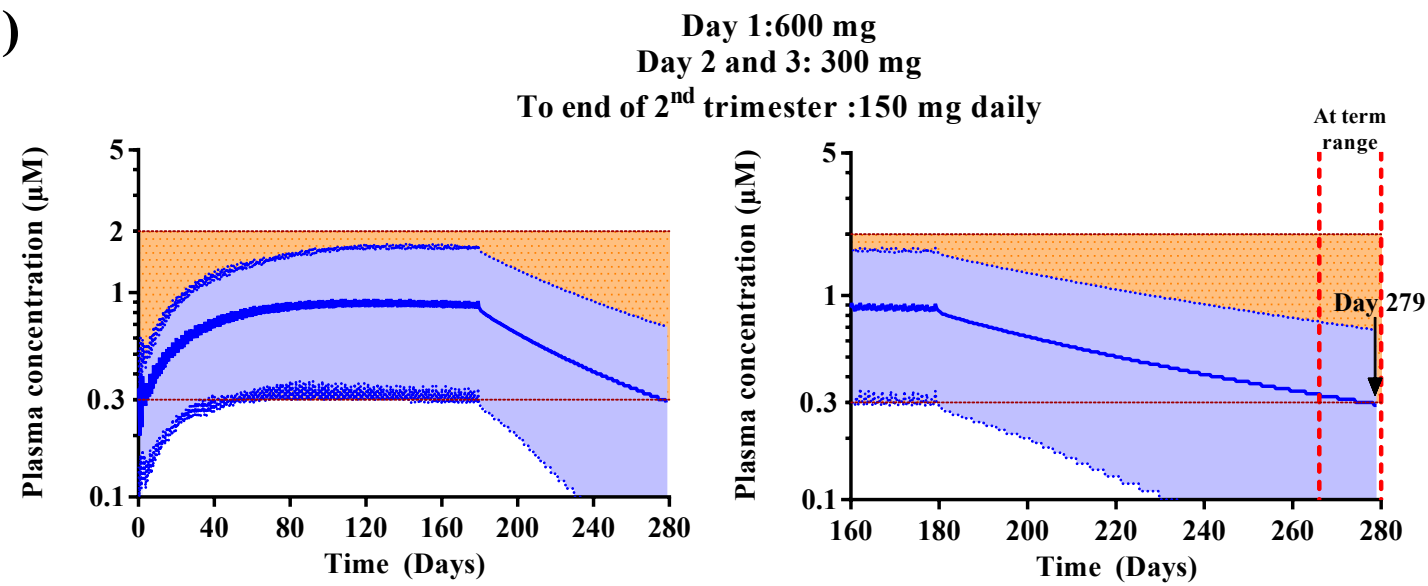
(B)


Plasma concentration (µM)
10
2
1
0.3
0.1
0.01
0
6
10
14
20
30
35
40
Time (Days)

(A)


Day 1:600 mg
Day 2 and 3: 300 mg
To end of 1st trimester :150 mg daily
Plasma concentration (µM)
5
2
1
0.3
0.1
0
50
100
150
200
250
Time (Days)
Day 150
80
90
100
110
120
130
140
150
160

(B)


Day 1:600 mg
Day 2 and 3: 300 mg
To end of 2nd trimester :150 mg daily
Plasma concentration (µM)
5
2
1
0.3
0.1
0
40
80
120
160
200
240
280
Time (Days)
At term range
Day 279
160
180
200
220
240
260

Dose optimisation of chloroquine by pharmacokinetic modelling during pregnancy for the treatment of Zika virus infection

Olusola Olafuyi[1] and Raj K. S. Badhan[1,2]

Supplementary Information

Section A

Table S1. Summary of single and multiple dose studies used in the validation of CQ pharmacokinetics in non-pregnant subjects

Study	Number of subjects	Ethnic group	Age (Years)	Weight (kg)	Gender (M/F)	Dosing regimen	Concentration matrix
Mzayek *et al* (2007)[1]	24	Mixed (Caucasian and Black American)	28.7 ± 5.3	75.8 ± 18.6	M, F	600 mg (single oral)	Blood
Gustaffson *et al* (1983)[2]	11	Caucasian	20 - 36	65 - 91	M	300 mg (single oral)	Plasma
Najmi *et al* (2008)[3]	10	Pakistani	33.5	66	M	600 mg (single oral)	Plasma
Höglund *et al* (2016)[4]	75	Thai	17 - 52	NR	M, F	10 and 5 mg/kg at 0 and 6–12 h on day 0, and 5 mg/kg each on day 1 and day 2	Plasma
Karunajeewa *et al* (2010)[5]	30	Papuan	25.5 ± 8.9	51.8 ± 5.5	F	450 mg once daily for 3 days	Plasma
Tanariya e*t al* (1995)[6]	57	Thai	26.4 ± 8.7	56.4 ± 7.1	M, F	600 mg initially, followed by 300 mg at hours 6, 24 and 48 hours)	Blood
Na-Bangchang *et al* (1994)[7]	7	Thai	18 - 35	45 - 68	M	600 mg initially, followed by 300 mg at hours 6, 24 and 48 hours)	Blood
Chukwuani *et al* (2004)[8]	5	Nigerian	23 - 37	56 - 66	F	600 mg (single oral)	Plasma
Lee *et al* (2008)[9]	13	Thai	29 (15 - 40)	46 ± 4.9	F	10, 10, and 5 mg/kg given at 0, 24, and	Blood

						48 hours	
Bustos *et al* (2002)[10]	11	Filipino	35 (13 - 63)	60 (40-63)	M, F	10 and 5 mg/kg at 0 and 6 hours on day 0, and 5 mg/kg each on day 1 and day 2	Plasma
Wetsteyn *et al* (1995)[11]	5	Caucasian	41	64 ± 10	M, F	300 mg weekly for 3 weeks	Plasma
Frisk-Holmberg *et al* (1984)[12]	5	Caucasian	37 - 42	72 ± 8	M, F	150 mg (single oral); 300 mg (single oral); 600 mg (single oral) given to each subject on 3 separate occasions	Blood
Walker *et al* (1987)[11]	8	Nigerian	19 - 55	53 - 66	M, F	600 mg (single oral)	Plasma

Data represented as: range, mean (range) or mean ± SD.

Section B

Step 1: Development and validation of a CQ model in non-pregnant subjects

In this step, the CQ model was optimised to adequately recover the general shape of the plasma concentration-time profiles and accurately predict pharmacokinetic parameters of CQ in non-pregnant populations. Where the initial model did not appropriately recover pharmacokinetic phases and parameters, a parameter estimation methodology was employed.

To recover the shape of the absorption phase, a First Order absorption model was utilised to identify an appropriate maximum plasma/blood concentrations (C_{max}) and time to reach the C_{max} (*tmax*). Clinically reported absorption rate constants (*ka*) and fraction absorbed (*fa*) values were selected with *ka* reported in literature as ranging from 0.27 to 3.4 h^{-1} and *fa* reported as 0.9. These were empirically fixed (with *ka* fixed as the mean of the reported range), and subsequently optimised by parameter estimation methodology implementing a Weighted Least Square (WLS) approach and the Nelder-Mead minimisation method to arrive at parameters which appropriately recovered the absorption phase (*fa*: 0.8; *ka*: 1.2 h^{-1}), and were within the range reported from clinical studies [13-15].

The volume of distribution at steady-state (*Vss*) was estimated by a similar methodology as that applied for the absorption phase, with *Vss* reported in clinical studies as ranging from 100 L/kg to 1000 L/kg [5,16,17], and empirically fixed as the mean of this range prior to parameter estimation. As the reported *Vss* was large, a minimal PBPK model was utilised with the incorporation of a 'single adjusting compartment' (SAC) to capture the correct distribution phases of the plasma concentration-time profile (Figure S1). Final parameter estimates of 125 L/kg for the

central compartment (V_{SS}) and 52.9 L/kg for the SAC (V_{sac}) were able to appropriately recover the distribution phase of the profile. It should be noted that this was achieved following the incorporation of a change in the mean dispersion parameter applied to the central compartment (i.e. the coefficient of variation), which was adjusted from the Simcyp default of 30 % to a revised 40 %.

Finally, the rate of metabolite formation, V_{max} and Michaelis-Menten constant (*Km*) for CYP2D6, 3A4 and 2C8 elimination pathways were obtained from a literature reported study [18] using recombinant P450 systems. However, to achieve satisfactory recovery of the elimination phase, the Inter-System Extrapolation Factor (*ISEF*) for scaling recombinant cytochrome (CYP) P450 enzymes from *in vitro* kinetic data were parameter estimated for all three metabolism pathways. In addition, CQ elimination has contributions from both hepatic and renal pathways, with the latter contribution approximately 30-50 % of the total clearance of CQ [2,19]. Therefore, a renal clearance was parameter estimated based on an empirically fixed mean estimate.

Parameter sensitivity analysis was subsequently conducted on ISEF for CYP2D6 and CYP3A4 (the two isozymes requiring significant changes in ISEF). When conducted over a range of 0.2-2, there was minimal sensitivity of CL, C_{max} and AUC to changes in ISEF (Figure S2) confirming appropriate estimates of ISEF for CYP2D6 and CYP3A4.

These revisions were confirmed in against 13 published clinical studies conducted in Caucasian and non-Caucasian subjects. Final parameter estimates are detailed within Table of the manuscript.

All modelled was conducted within the Simcyp Simulator (Version 17). This is available under a free licence for academic research (non-profit) from Certara (www.certata.com). However, for those unable to obtain a licence from Certara, the open source package PK-Sim (Open Systems Pharmacology) (https://github.com/Open-Systems-Pharmacology) allows simulations in both non-pregnant and pregnant subjects and provides a detailed summary of 'systems' related parameters for model building. The 'Compound' related parameters described within Table 1 of the manuscript will allow re-creation and re-validation of CQ within PK-SIM.

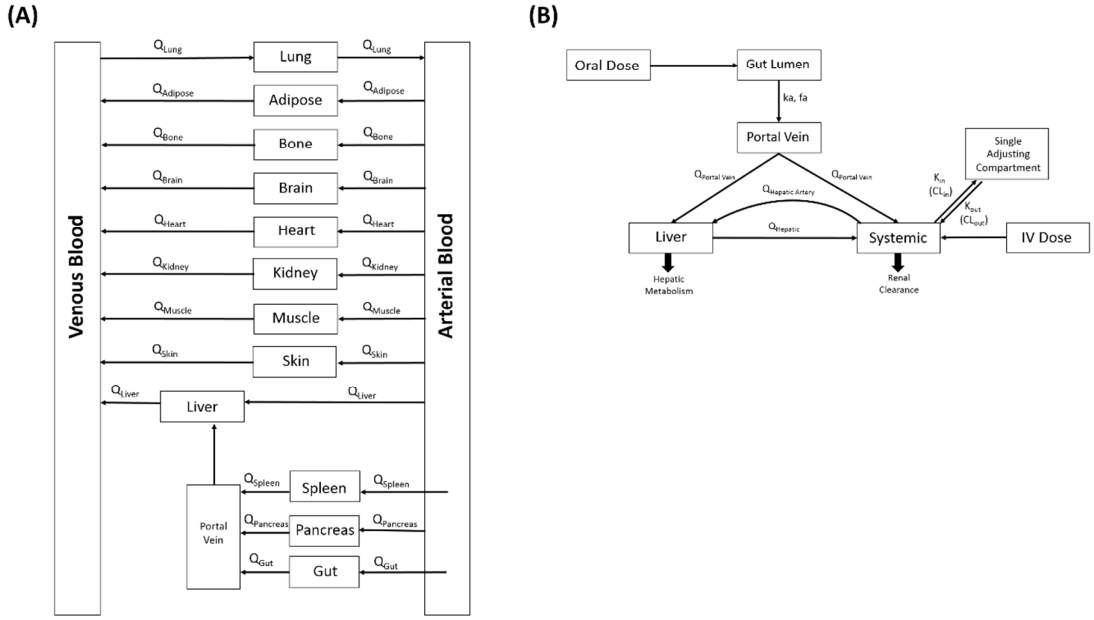


Figure S1: PBPK models utilised within Simcyp.

Structure of the full (A) and minimal (B) PBPK models used within the Simcyp Simulator. *Q* refers to tissue perfusion; K_{in}/K_{out} (CL_{int}/CL_{out}): refer to transfer processes between the systemic compartment and the single adjusting compartment.

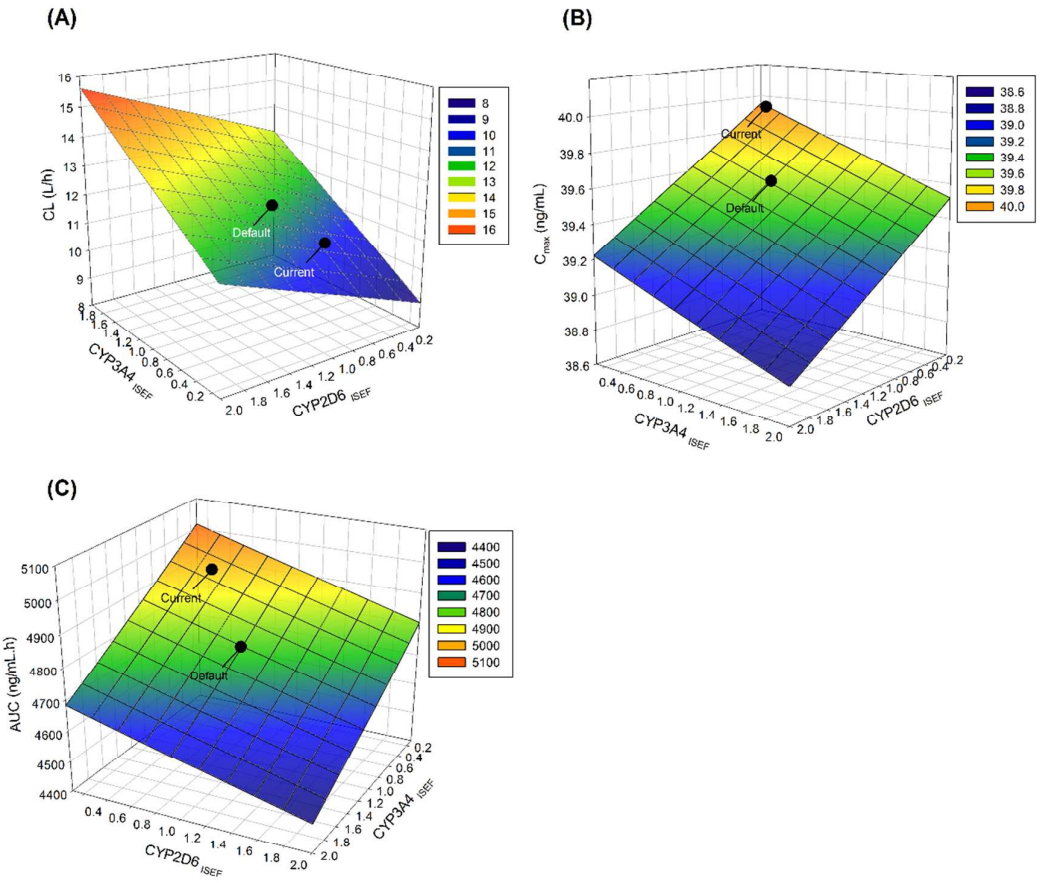


Figure S2: Sensitivity analysis of CYP3A4 and CYP2D6 ISEF

Sensitivity analysis of (A) clearance, (B) first dose Cmax and (C) AUC following alterations in CYP3A4 and CYP2D6 ISEF (over a range of 0.2-2) for non-pregnant adults. Current optimised model estimates (CYP3A4 ISEF = 0.42 and CYP2D6 = 0.5) and default estimates (ISEF = 1) are illustrated.

Adaptation of the age-weight relationships for non-Caucasian groups

Customised age-weight relationships for non-Caucasian subjects were incorporated through adaptation of the Simcyp Healthy Volunteer population group and utilising reported age-weight relationships [20] for specific countries of origin relating to each clinical study used, which included Filipinos, Nigerians, Pakistani, Papuans and Thais. The reported median age-weight reference charts for the specific population groups were used to establish mathematical (polynomial regression) relationships to predict body weight from age, using TableCurve2D (Systat Software, San Jose, CA, USA)

The final mathematical relationships are detailed below:

Filipinos and Papuans

Due to geographical locations, an age-weight relationship for Filipinos and Papuans were assumed to be similar and the age weight relationship was shown below:

Adult males:

Body weight = $(6.0000871 + (1.8363904*age) + (-0.28876641*age^2) + (0.011482471*age^3)) / (1 + (-0.06584622*age) + (-0.0016572488*age^2) + (0.00016955778*age^3))$

Adult females:

Body weight = $(6.03 + 0.197*age^2 + 0.0012*age^4) / (1+0.00127*age^2 + 0.0000255*age^4)$

Nigerians:

Adult males:

Body weight = (3.1190351 + (2.7547707*age$^{0.5}$)+(-1.9861521*age)+(0.29731577*age$^{1.5}$))/(1+(-0.63494158*age$^{0.5}$)+(0.15239313*age)+(-0.017751472*age$^{1.5}$)+(0.0010549434*age^{2}))

Adult females:

Body weight = (3.9015149+(0.280026178*age$^{0.5}$) + (-0.92347063*age) + (0.16145376*age$^{1.5}$)) / (1 + (-0.75349793*age$^{0.5}$) + (0.2157188*age) + (-0.028738874*age$^{1.5}$) + (0.0016167479*age^{2}))

Pakistani and Thais:

Due to geographical locations, an age-weight relationship for Pakistani and Thais were assumed to be similar and the age weight relationship was shown below:

Adult males:

Body weight = 33.46 + (-0.3569*age^{2}) + (0.001522*age^{4}) / (1 + (-0.00755*age^{2}) + (2.78x10 - 5*age^{4}) + (-1.07x10-9*age^{6}))

Adult Females:

Body weight = -920.66 + (-188.63*age) + (22.48*age$^{1.5}$) + (-0.999*age^{2}) + (700.23*age$^{0.5}$).

Section C

Table S2. Summary of single and multiple dose studies used in the validation of CQ pharmacokinetics in pregnant subjects

Study	Number of subjects	Ethnic group	Age (Years)	Weight (kg)	Gestation (Weeks)	Dosing regimen	Concentration matrix
Karunajeewa *et al* 2010[5]	30	Papuan	26.0 ± 5.9	54.0 ± 6.4	NR	450 mg once daily for 3 days	Plasma
Lee *et al* 2008[9]	12	Thai	25 (15 - 37)	49.5 ± 5.6	20 -32	10, 10, and 5 mg/kg given at 0, 24, and 48 hours	Blood
Fakeye *et al*, 2002[21]	4	Nigerian	30 ± 2.3	60.3 ± 8.9		10, 10, and 5 mg/kg given at 0, 24, and 48 hours	Plasma

Data represented as: range, mean (range) or mean ± SD.

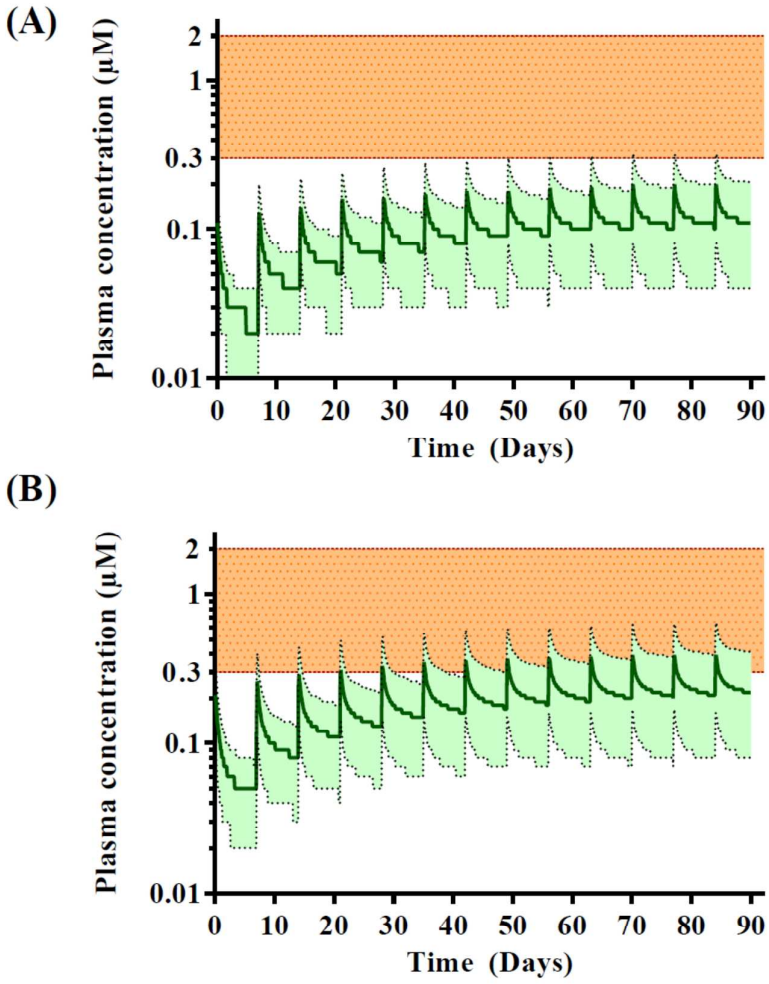


Figure S3: Simulated plasma concentration-time profiles for CQ dosed once weekly during the first trimester.

Simulated CQ plasma concentration-time profiles during trimester 1 for: (A) a 150 mg weekly dose; (B) a 300 mg weekly dose. Dark green lines indicate mean plasma concentration-time profiles; light green shaded area bordered by the dash lines indicate the area within the 5th and 95th percentile of predicted mean plasma concentration-time profiles; light brown shaded area represents the proposed therapeutic range of CQ for ZIKV (0.3-2 µM).

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