Safety, tolerability and pharmacokinetic properties of co-administered azithromycin and piperaquine in pregnant Papua New Guinean women

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Running title: Pharmacokinetics of AZI plus PQP in pregnancy

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Summary

Aims: To investigate the safety, tolerability and pharmacokinetics of co-administered azithromycin (AZI) and piperaquine (PQ) in pregnant Papua New Guinean women.

Methods: Thirty women (median age 22 years; 16-32 weeks gestation) were given three daily doses of 1 g AZI plus 960 mg PQ tetraphosphate with detailed monitoring/blood sampling over 42 days. Plasma AZI and PQ were assayed using liquid chromatography-mass spectrometry and high performance liquid chromatography, respectively. Pharmacokinetic analysis was by population-based compartmental models.

Results: The treatment was well tolerated. The median [inter-quartile range] increase in rate-corrected QT interval 4 h post-dose (12 [6-26] $msec^{0.5}$) was similar to that in previous studies of AZI given in pregnancy with other partner drugs. Six women with asymptomatic malaria cleared their parasitaemias within 72 h. Two apararasitaemic women developed late uncomplicated *P. falciparum* infections on Days 42 and 83. Compared with previous pregnancy studies, the area under concentration time curve ($AUC_{0-\infty}$) for PQ (38,818 [24,354-52,299] μg h L^{-1}) was similar to published values, but there was a 52% increase in relative bioavailability with each dose. The $AUC_{0-\infty}$ for AZI (46,799 [43,526-49,462] μg h L^{-1}) was at least as high as reported for higher dose regimens, suggesting saturable absorption and/or concentration-dependent tissue uptake and clearance from the central compartment.

Conclusions: AZI-PQ appears well tolerated and safe in pregnancy. Based on the present/other data, total AZI doses higher than 3 g for treatment and prevention of malaria may be unnecessary in pregnant women, while clearance of parasitaemia could improve the relative bioavailability of PQ.

What is known about this subject

• Azithromycin has been used as part of combination antimalarial therapy in pregnancy with conventional drugs including chloroquine and sulphadoxine-pyrimethamine

• Piperaquine has also been used in pregnancy and has advantages in areas of known chloroquine and sulphadoxine-pyrimethamine parasite resistance

• Both drugs cause nausea, prolong the electrocardiographic QT interval, and whether they have a pharmacokinetic interaction has not been established

What this study adds

• Combination azithromycin-piperaquine was well tolerated and showed promising efficacy in treating malaria in Papua New Guinean women beyond the first trimester of pregnancy

• There was no evidence of greater cardiotoxicity than with either drug alone

• Based on relative drug exposure, doses of azithromycin greater than 1 g daily for three days may be unnecessary for treatment of malaria in pregnancy

Introduction

Malaria in pregnancy remains a major cause of maternal anaemia, low birthweight and increased perinatal mortality [1]. One of the major pharmacological strategies used to improve obstetric outcome is intermittent preventive treatment of malaria in pregnancy (IPTp) [2, 3]. The World Health Organisation-recommended IPTp is sulphadoxine-pyrimethamine (SP) [4], but residual maternal/foetal morbidity despite adherence to SP IPTp regimens, the spread of SP-resistant *Plasmodium falciparum*, limited efficacy against *P. vivax*, and the relatively short half-lives of the component drugs (6-11 days for sulphadoxine and 3-15 days for pyrimethamine [5-7]) have prompted the search for alternatives [8-10]. Prominent amongst these are combinations that include azithromycin (AZI) [11-15].

Azithromycin is an azalide antibiotic with unique pharmacokinetic (PK) properties characterised by prolonged tissue and phagocytic concentrations [16, 17]. It has an elimination half-life ($t_{½}$) of 12-78 h in pregnant [14, 18] and 54-102 h in non-pregnant [19-23] women. It has a slow onset of antimalarial action and modest efficacy as monotherapy [24], but a potentially valuable role as part of combination treatment [11, 24-26]. The advantages of AZI-based IPTp include its safety [2] and efficacy against other pathogens that can affect obstetric outcomes such as those causing sexually transmitted diseases [27]. In addition, its PK properties show that dose adjustment is probably unnecessary in pregnancy [14]. The choice of a partner drug depends on tolerability, safety and PK/pharmacodynamic interactions. Both SP [15] and chloroquine (CQ) [12] added to AZI have proved effective in clinical trials, but, as with adding artemisinin derivatives to SP or CQ [28], the effectiveness of these combinations is compromised by using failing drugs as partners.

Piperaquine (PQ) combined with dihydroartemisinin (DHA) is effective treatment for uncomplicated malaria [29, 30]. No concerns have been raised in animal reproductive toxicity studies of PQ [31, 32] and it appears safe and well tolerated in human pregnancy [33-38]. Like other 4-aminoquinolines, PQ prolongs the electrocardiographic QT interval at therapeutic concentrations [39] and this may have implications for safety. As a result, the manufacturers recommend that DHA-PQ is avoided in patients with a history of arrhythmias, long QT syndrome or who are taking other drugs known to prolong the QT interval [40, 41]. It has a long elimination $t_{½}$ (16-22 days in pregnancy and 21-28 days in non-pregnant women [33, 35, 36, 42, 43]) which may be advantageous if ensuring regular IPTp dosing is problematic [2]. There have, however, been no studies of AZI-PQ in pregnancy.

From available studies, the AZI dose that is both efficacious as part of antimalarial therapy with a longer-acting drug such as CQ and not associated with tolerability issues, especially nausea, is 1 g daily for three days [12]. Although higher doses have been used in pregnancy, including 2 g daily for two days in an IPTp trial in Papua New Guinea (PNG) [13], gastrointestinal symptoms are comparatively frequent with these regimens [14, 24]. Given that PQ is also a drug associated with nausea and vomiting [32], a conservative AZI dose would seem appropriate in initial interaction studies. In addition, both PQ [32] and AZI [44] can prolong the QT interval and, although their individual pro-arrhythmic effects are unclear [44, 45], whether they have additive or synergistic cardiotoxicity is unknown. This further suggests caution in selecting an AZI dose for human interaction studies.

In view of these considerations, we have assessed the safety, tolerability and pharmacokinetic properties of AZI-PQ in pregnant women from PNG. Given the possibility that some women could have malarial parasitaemia, we used a mid-range but previously effective dose of AZI (1 g daily for 3 days) and a conventional adult dose of PQ.

Methods

Study site, approval and participants

The present study was conducted at the Alexishafen Health Centre, Madang Province, where there is endemic transmission of *P. falciparum* and *P. vivax* [46]. Pregnant women at 14-32 weeks' gestation and attending their first antenatal visit were eligible if i) they had not taken study drugs in the previous 28 days, ii) they had no history of allergy to study drugs, iii) there was no significant co-morbidity or features of severe malaria, and iv) they could attend all follow-up assessments. Witnessed informed consent was obtained from each participant, and verbal consent from their husband/father as culturally appropriate in PNG. Ethics approval was obtained from the PNG Institute of Medical Research Institutional Review Board, the Medical Research Advisory Committee of the PNG Health Department, and the Human Research Ethics Committee of the University of Western Australia.

Baseline assessment and treatment allocation

A detailed medical history including a standardised symptom questionnaire was completed, a physical examination (including estimation of gestational age by fundal height) was performed, and a 12-lead electrocardiogram (ECG) was taken. Thick and thin blood smears were prepared. An intravenous cannula was inserted and a 2 mL baseline sample collected for haemoglobin and blood glucose. The remaining blood was centrifuged within 15 minutes of collection. The separated plasma was placed promptly in a foil-covered tube, stored immediately at -20^{o}C and then transferred to -80^{o}C for long-term storage prior to drug assay.

All participants received three daily doses (0, 24 and 48 h) of i) 1 g AZI as film-coated 500 mg tablets (Sandoz, Pyrmont, NSW, Australia) given with ii) three PQ tetraphosphate 320 mg tablets (Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Italy) at a dose of 58 mg kg^{-1} (or 33 mg kg^{-1} PQ base for a 50 kg woman), consistent with the manufacturer's and WHO recommendations at the time the study was done [3]. Because of the possibility of hypoglycaemia especially in the parasitaemic women, participants were fasted for ≥2 h before and after dosing rather than the ≥3 h recommended by the manufacturer. All treatments were administered with water and directly observed. Women vomiting within 30 minutes of dosing were to be retreated. After a 48-72 h inpatient stay, each participant was given an insecticide-treated bed-net and instructed on its use for the rest of the pregnancy as an alternative to SP IPTp.

Monitoring, sampling and follow-up

Detailed assessment, including lying/standing blood pressure and pulse rate, respiratory rate, axillary temperature, haemoglobin, and thick/thin blood smears, was carried out at follow-up visits on Days 1, 2, 3, 7, 14, 28 and 42. Additional monitoring comprised i) completion of the standardised side-effect questionnaire at 6 h and on Days 1, 2, 3 and 7, ii) blood glucose on Days 1, 2 and 3, iii) ECG at 4, 12 and 24 h, iv) assessment of foetal viability (maternally-detected movements and foetal heart beat on auscultation) daily on Days 1-4 and then at each subsequent sampling time-point, and v) ultrasound scans if required to confirm gestational age and to determine foetal lie and presentation and. Clinical review on Days 14, 28 and 42 included assessment of symptoms even though the standardised questionnaire was not administered at these follow-up visits.

The timing of the ECGs relative to dosing and the use of Bazett's method to determine the rate-corrected QT interval in lead II ($QT_c=QT/\sqrt{RR}$) were selected to allow comparison with previous studies in pregnant PNG women [36]. Each QT interval was determined by manual measurement from the ECG tracing. All blood smears were examined, and parasitaemia quantified, on site by study staff and subsequently by two skilled independent microscopists in a central laboratory. Discrepancies were adjudicated by a senior microscopist. Parasite densities were calculated from the number of parasites/200 white cells (or /500 white cells if there were <25 parasites/200 white cells) and an assumed total white cell count of 8,000 μL^{-1}, with the final density calculated as the geometric mean of the two or three values from expert microscopy [47].

Since AZI-PQ is not yet a WHO-recommended treatment for malaria in pregnancy, all monitoring data were reviewed for each participant at each study visit by the on-site study clinician (JMB). Those women in whom clinical concerns emerged were to be transferred to Modilon Hospital in Madang Town (the provincial referral facility) for further assessment and treatment.

A sparse blood sampling protocol was used based on previous studies of AZI-CQ [14], AZI-SP [14], PQ-SP [36] and PQ-DHA [36]. Participants were randomly assigned to seven of 18 time-points (1, 2, 3, 6, 12, 24, 32, 40, 48 and 72 h, and Days 4, 5, 7, 10, 14, 21, 28 and 42) at which 3 mL blood samples were drawn either through an intravenous cannula (to 72 h) or by venepuncture. The 24 and 48 h samples were taken immediately before the second and third doses, respectively.

Participants were returned to usual care after the Day 42 assessment but they were requested to attend Alexishafen Health Centre when they went into labour or to notify study staff if they delivered in an alternative local healthcare facility or at home. If delivery occurred at the Alexishafen Health Centre, maternal thick/thin blood films were prepared and a maternal haemoglobin concentration was measured. A 3 mL sample of maternal and cord blood was taken for AZI and PQ assay, three thick/thin cord blood smears and a placental smear were prepared, and a section of placenta was collected into 10% neutral buffered formalin for evaluation if cord/placental smears were positive for malaria.

Drug assays

Plasma PQ was measured by a validated high-performance liquid chromatography assay [48]. Briefly, extracted plasma was injected on to a Gemini 5µm C_6-phenyl column coupled with a SecurityGuard column (Phenomenex®, Lane Cove, Australia). Analytes were detected at 346 nm and quantified using Chemstation Software (version 9; Agilent Technology, Waldbronn, Germany). For concentrations ranging from 2.5 to 500 µg L^{-1}, intra-day relative standard deviations (RSDs) were 2.5%-8.1% and inter-day RSDs were 3.6%-9.6%. The limits of quantification (LOQ) and detection (LOD) were 2 µg L^{-1} and 1 µg L^{-1}, respectively, with a signal-to-noise ratio of 3.0.

Plasma AZI was measured using a triple quadrupole mass spectrometer (LCMS-8030 plus; Shimadzu, Kyoto, Japan). Plasma samples were extracted as described [14], and 20 µL aliquots were spiked with AZI-d_3 1,000 µg L^{-1} (internal standard), added to 50 µL ammonium hydroxide (40 mM) and 500 µL methanol, mixed for 30 s, and centrifuged at 10,000 x g for 5 min. Supernatant (100 µL) was mixed with 100 µL water and 5 µL injected onto an Acquity UPLC BEH C_{18} column connected to a VanGuard Acquity BEH C_{18} guard column (Waters Corp., Wexford, Ireland) at 50°C. The mobile phase (acetonitrile:methanol:40 mM ammonium hydroxide (50:25:25) and acetonitrile:methanol (50:50) in equal volumes) was run in isocratic mode at 0.2 mL min^{-1}. Retention times for AZI and AZI-d_3 were both 1.38 min. Quantitation was performed in multiple reaction monitoring mode using an ESI^+ ion source. The precursor-product ion pairs were AZI *m/z* 749.4→591.6 and AZI-d_3 *m/z* 752.5→594.5. Optimised mass spectra were acquired with a 4.5kV interface voltage, a 1.0 kV detector voltage, and heat block and desolvation line temperatures of 400°C and 250°C, respectively. Nitrogen was used as the nebulizer and drying gas at flow rates of 3 L min^{-1} and 10 L min^{-1}, respectively. Argon was used as the collision gas at 230 kPa. Dwell time for both AZI and AZI-d_3 was 100 msec, and their collision energies were -32 V and -33 V, respectively. For concentrations 10 to 2000 µg L^{-1}, intra-day RSDs were 3.2%-7.5% and inter-day RSDs were 4.3%-9.1%. The LOQ and LOD were 2.5 µg L^{-1} and 1.3 µg L^{-1}, respectively, with signal-to-noise ratios of ≥10:1 and ≥3:1, respectively.

Pharmacokinetic modelling

The package NONMEM (v 7.2.0, ICON Development Solutions, Ellicott City, MD, USA) with an Intel Visual FORTRAN 10.0 compiler employing nonlinear mixed effects modelling was used to analyse $\log_e$ plasma concentration-time PQ and AZI data. The first-order conditional estimation with interaction estimation method was used and a *P*-value <0.05 was set as the significance level for a comparison of the nested models (ΔOFV=-6.635, χ^2 df=1). *A priori* allometric scaling was employed, with volume terms multiplied by (body weight/70)$^{1.0}$ and clearance terms by (body weight/70)$^{0.75}$ [49]. For residual variability (RV), two structures were tested using the log-transformed data. These were equivalent to exponential and combined RV structures on the normal scale [50]. Using final model parameters, *post hoc* Bayesian prediction in NONMEM was utilised to derive secondary PK parameters including area under the plasma concentration-time curve ($AUC_{0-\infty}$) and $t_{½}$s . Simulated plasma concentrations after the final dose were used to obtain estimates of the maximum plasma concentration (C_{max}) Base models included the parameters absorption rate constant (k_a), central volume of distribution relative to bioavailability (F) (V_C/F), clearance (CL/F), and peripheral volumes of distribution and the associated inter-compartmental clearances (V_P/F and Q/F, respectively).

In the case of PQ, models with two- and three- compartments (ADVAN 4 and 12) were tested, as were various absorption models including those with zero-, first- or mixed-order absorption with and without lag time. There was significant variability in absorption, as reported in other studies [36, 51], which necessitated the assessment of a transit compartment model (the dose passing through a series of compartments before delayed entry into the absorption compartment [36, 51]). In this model, there is a single rate constant (k_{tr}) that describe the entry and exit for all transit compartments. The additional continuous variables in this model include the number of transit compartments (NN) and the mean transit time ($MTT=(1+NN)/k_{tr}$) [52]. Similar models were tested for AZI including zero-, first- and mixed-order absorption with and without a lag time linked with two- and three- compartment models. A transit compartment was not included as a mixed order model has been shown to best describe the absorption kinetics of AZI in this patient group [14].

When model structures had been determined, variability parameters were added. These included inter-individual variability (IIV), and inter-occasion variability (IOV) for F and absorption parameters. Correlations between IIV terms were also estimated where possible. Relationships between all model parameters and malaria status, dose number and gestational age were assessed through inspection of scatterplots and boxplots of eta (IIV code used in NONMEM) versus covariate. These associations were then quantified within NONMEM using a stepwise forward inclusion and backward elimination method with $P<0.05$ required for inclusion of, and $P<0.01$ for retention of, a covariate relationship.

Model evaluation

The median and 2.5th and 97.5th percentiles (95% empirical confidence intervals) were obtained from a bootstrap using Perl speaks NONMEM using 500 samples to evaluate final model parameter estimates. The predictive performance of the model was evaluated using prediction corrected visual predictive checks of 1,000 datasets simulated from the final models from which the simulated 95% CIs for the 10th, 50th and 90th percentiles were plotted together with the corresponding observed data. Numerical predictive checks were also used in the evaluation of the predictive performance of the model.

Results

Subject characteristics

Thirty women (median age 22 years, median gestational age 26 weeks) were recruited, five of whom (16.7%) were aged <20 years (see Table 1). Twelve (40.0%) had been treated with SP between 14 and 28 days before recruitment but none of the others had taken antimalarial drugs in the previous month. All women were asymptomatic but blood film examination showed that six (20.0%) had an asexual parasitaemia (*P. falciparum* n=4, *P. vivax,* n=1, mixed *P. falciparum/vivax*, n=1) and one had *P. falciparum* gametocytes. The median and interquartile range [IQR] asexual parasite densities at recruitment were 77 [41-113]/µL and 48 [39-56] /µL for *P. falciparum* and *P. vivax*, respectively. Consistent with PNG policy at the time of recruitment, routine HIV screening was not performed but no participant had clinical evidence of HIV and the prevalence in pregnant PNG women is 1.5% [53].

Tolerability, safety and efficacy

The treatment was well tolerated and all doses were taken without incident. Side-effects during the first 7 days are summarised in Table 2. All were mild (did not interfere with daily activities) and self-limiting (resolution within 2 days), with nausea, anorexia and diarrhoea the most frequent. Two women developed skin rashes (on Days 1 and 2, respectively), one in woman who had contact with a plant to which she had known sensitivity and the other of uncertain aetiology; both rashes resolved within 24 h without requiring treatment. All women were asymptomatic by Day 7. No participant developed a blood glucose <2.5 mmol L^{-1} during follow-up. One woman had a baseline haemoglobin of 73 g L^{-1} which fell to 48 g L^{-1} on Day 14 without clinical evidence of blood loss. She responded to oral iron/folate (haemoglobin 74 g L^{-1} on Day 28).

Changes in QT_c after the first dose are summarised in Table 3, together with equivalent data from a previous study of DHA-PQ and CQ-SP in pregnant PNG women [36]. There was an overall median increase in QT_c from baseline of 12 $msec^{0.5}$ at 4 h in the present participants, with a progressive reduction at 12 h and 24 h, but no significant difference between parasitaemic and non-parasitaemic women at any time-point (P>0.13 by ANOVA). These data were similar to those in the previous study of DHA-PQ and CQ-SP. Twenty one of the present 30 women (70.0%) had a 4 h QT_c >440 $msec^{0.5}$, with a longest recorded QT_c of 487 $msec^{0.5}$. No woman experienced palpitations or breathlessness during follow-up. One complained of mild dizziness 6 h after the first dose (simultaneous QT_c 453 $msec^{0.5}$) which had resolved by 12 h. Postural hypotension (>20 mmHg fall in systolic blood pressure after standing) occurred in 7 participants (23.3%) during follow-up but there were no associated symptoms and no clear temporal relationship with the time of drug administration.

All asexual parasitaemias cleared by Day 3, although one woman developed a gametocytaemia that persisted to Day 7. Two women, both slide-negative at baseline, developed falciparum malaria during follow-up, one at Day 42 (with fever) and the other at delivery on Day 83 (asymptomatic but with positive peripheral and placental blood smears).

In both cases, the women responded to a three-day course of artemether-lumefantrine prescribed under PNG treatment guidelines [54].

Obstetric outcomes

Of the 25 participants (83.3%) followed to parturition, seven successfully delivered at home without medical assistance, two delivered healthy babies at Modilon Hospital, and the remaining 16 delivered at the Alexishafen Health Centre (mean±SD birth weight 2.9±0.5 kg, 48% males). All deliveries were vaginal and two were footling presentations. One involved a posterior presentation accompanied by heavy meconium staining. The baby required respiratory support during transfer to the Modilon Hospital, but was discharged well the following day.

Pharmacokinetic modeling

None of the 189 PQ and 177 AZI plasma concentrations were below the LOQs. For PQ disposition, a three-compartment model was superior to a two-compartment model with a reduction in the OFV (P<0.001) and resolution of bias in CWRES plots. The structural model parameters were NN, MTT, V_C/F_{PQ}, V_{P1}/F_{PQ}, V_{P2}/F_{PQ}, CL/F_{PQ}, Q_1/F_{PQ} and Q_2/F_{PQ}. Of these, the IIV of MTT, V_C/F_{PQ} and CL/F_{PQ} were estimable. As k_a was poorly estimated (high residual standard error, high correlation with MTT) for models using transit compartments, it was set at k_{tr} (1+NN)/MTT). Since correlations between IIV terms for V_C/F_{PQ} and CL/F_{PQ} were 0.99, it was fixed at unity to facilitate successful minimisation of model estimation. IOV in F_{PQ} (relative bioavailability) was estimated to examine potential differences in between-dose relative PQ bioavailability, as noted previously for PQ and other lipophilic antimalarials

[51, 55, 56]. There was a significant increase in F_{PQ} for subsequent doses (ΔOFV=-8.414, *P*<0.01). No other significant covariate relationships were identified.

A three-compartment model for AZI was also chosen given a significant reduction in OFV (*P* <0.001) and no identified bias on CWRES plots. Mixed zero- and first-order absorption without a lag time adequately described the absorption phase and more complex models were not required. The structural model parameters were, therefore, k_a, DUR (duration of zero-order absorption), V_C/F_{AZI}, V_{P1}/F_{AZI}, V_{P2}/F_{AZI}, CL/F_{AZI}, Q_1/F_{AZI} and Q_2/F_{AZI}. IIV was estimable for DUR, V_C/F_{AZI} and CL/F_{AZI} along with the correlation between IIV terms for V_C/F_{AZI} and CL/F_{AZI}. The addition of an IOV term for relative bioavailability did not result in a significant fall in the OFV (*P* >0.05). No significant covariate relationships were identified.

The final model parameter estimates and the bootstrap results for PQ and AZI are summarised in Tables 4 and 5, respectively. Bias was <12% for all structural and random model parameters for both models. Figures 1 and 2 show goodness-of-fit plots and Figures 3 and 4 show pcVPCs for PQ and AZI, respectively. Secondary PK parameters for PQ and AZI are shown in Table 6.

Discussion

The present study is the first to have investigated the potential of AZI-PQ as IPTp or for pregnant women with malarial parasitaemia. The regimen selected, which involved a lower total AZI dose than in some previous studies [13, 14] in view of adverse effect concerns, was well tolerated with no evidence of greater cardiotoxicity than with PQ in combination with other antimalarial drugs in pregnant PNG women [36]. In the relatively few women who were parasitaemic, parasite clearance was prompt. Malaria during follow-up was infrequent and, reflecting the relatively long elimination $t_{½}$ of PQ [32], occurred ≥6 weeks after treatment. Consistent with this observation, drug exposure assessed from individual $AUC_{0-\infty}$s was at least that in other PK studies of AZI [14] and PQ [36] in pregnancy.

Nausea affected 17% of the present participants, a percentage within the range (7-24%) seen in previous studies of pregnant PNG women treated AZI or PQ combined with other drugs including CQ [14], SP [14, 36] or DHA [36]. There was no vomiting and other side-effects were mild and infrequent (≤6.7%). The median QT_c prolongation 4 h after the first dose (12 $msec^{0.5}$) was similar to that in a previous study from our group (14 $msec^{0.5}$) in which the same mg kg^{-1} PQ dose was given to pregnant PNG women in combination with SP or DHA [36]. In the latter [36] and present studies, QT_c prolongation was resolving by 12 h post-dose, no symptoms suggestive of arrhythmia were recorded during follow-up, and symptomatic postural hypotension was not observed. Although we did not have QT_c data beyond 24 h, we interpret these findings as evidence that the addition of AZI to PQ in the present doses is safe despite the fact that both drugs affect ventricular repolarisation [32, 44]. The lack of adverse outcomes in the majority of women who were followed to parturition is also reassuring.

The AZI PK parameters in the present study differed from those in a previous study in pregnant PNG women [14]. The present total AZI dose was lower (3 g over 3 days vs 4 g over 2 days) but the $AUC_{0-\infty}$ was greater (46,799 vs 28,713 μg h L^{-1} [14]). Although there were some minor differences in characteristics (the present women were younger and later in pregnancy than those in the previous study [14]), the most likely explanation for the $AUC_{0-\infty}$ difference is that there are dose-dependent effects on AZI absorption (saturation), in combination with more extensive tissue distribution, at higher doses. Indeed, Q_1/F and Q_2/F in the three-compartment model were >50% higher with the 2 g daily [14] vs the present 1 g daily dose, consistent with a substantial increase in CL/F. Protein binding diminishes as plasma AZI concentrations increase [21], perhaps facilitating greater tissue uptake and clearance from the central compartment, with a consequent reduction in $AUC_{0-\infty}$. Although dose-dependent effects have not been observed previously [16, 21], the lower individual doses (0.5 g vs 1.5 g and 0.25 g vs 0.5 g compared with 1 g vs 2 g in our present and previous [14] studies) may have masked an effect. In any case, the present $AUC_{0-\infty}$ data suggest that 1 g AZI daily for three days should be as efficacious as higher dose regimens [13, 14].

Although CL/F and $AUC_{0-\infty}$ for PQ were consistent with those in our previous study of PQ-SP and PQ-DHA in pregnant PNG women [36], there was a 52% increase in the relative bioavailability of PQ with each subsequent dose in the present study. A study of Thai women with malaria treated with PQ-DHA also showed a time-dependent increase in bioavailability [33] which we did not find in our previous study [36]. Of the potential explanations we postulated previously as an explanation for this latter difference (different sample sizes and durations of sampling, ingestion of fat at the time of PQ dosing, the effect of malaria, and/or racial differences in PQ disposition [36]), the present data suggest that the effect of malaria may be most influential.

The subjects (pregnant PNG women) and protocols (including no food for ≥2 h either side of dosing) were the same for the present and our previous study [36], but the percentage of women with malaria in the present study was greater (20.0% vs 9.4%) and in the Thai study it was 100% [33]. Data relating to the effect of malaria on the disposition of other slowly eliminated 4-aminoquinoline drugs appear inconsistent [57, 58] and malaria at study entry was not a significant co-variate in the present PQ PK model, but it is plausible that parasite clearance during PQ dosing progressively improves bioavailability, an effect not observed in our previous involving a sample of pregnant women in whom <10% were parasitaemic [36]. The V_c/F and $t_{½}$ estimates also tended to be lower, and the C_{max} higher, than in our original study [36] as well as in other published studies [33, 42, 43]. For example, the median elimination $t_{½}$ was 13.1 days in our patients compared with 16-22 days [33, 36, 42, 43]. This could reflect between-study differences in variables such as maternal age, gestational age and parasitaemia, or the present sparse sampling protocol with few taken late in an inadequately characterised elimination phase [59]. An effect of AZI on PQ disposition is also possible, but previous studies of AZI and CQ did not show an interaction [60]. Indeed, there are few recorded AZI interactions for a drug which is largely excreted unchanged rather than being metabolised [61].

The present study had limitations. It was designed as a tolerability, safety and PK study, but a larger AZI-PQ IPTp efficacy study is in progress (ClinicalTrial.gov NCT02575755). We employed a pragmatic blood sampling regimen and, as discussed, cannot exclude an effect of this on some PQ PK parameters. Although we had complete follow-up data to Day 28, three women could not be contacted subsequently and this also applied to two women after their

Day 42 assessment. However, obstetric and other outcomes were available for the remaining 25 women and were reassuring given the relatively high rate of perinatal morbidity and mortality observed in PNG [62]. An important effect of the combination of AZI and PQ accumulation on QT_c prolongation beyond 24 h, especially at the PQ C_{max} after the third dose, may have been missed. However, there was no evidence from the present and previous [36] data to 24 h that AZI amplified the QT_c effects of PQ (see Table 3). In addition, in the case of the long $t_{½}$ 4-aminoquinoline naphthoquine, studies in PNG children with malaria suggest that repeated daily doses do not lead consistently to significant further QT_c prolongation after the first dose [63, 64], while data from animals suggest that that combination of AZI-CQ is not pro-arrhythmic [65]. Nevertheless, given persistent concerns regarding the adverse cardiovascular effects of AZI [66], we recommend that future trials of AZI and 4-aminoquinoline combinations continue to assess this potential safety issue.

Given the need for novel IPTp regimens, AZI-based therapies have been recommended for development [2, 11, 12]. They have advantages over other candidate therapies, including the longer $t_{½}$ of AZI compared with the artemisinin derivatives administered with partner drugs such as PQ, and proven safety as well as efficacy against bacterial and other infections that can add to the burden of malaria in pregnancy. The present data suggest that AZI 1 g daily for 3 days in combination with three days of conventional adult PQ doses, most likely administered monthly at scheduled antenatal visits if used as IPTp [13, 33, 36], is a suitable regimen for further assessment in clinical trials.

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Conflicts of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: IM, SJR and TMED received a Malaria in Pregnancy Consortium Grant funded through Bill and Melinda Gates Foundation (#46099) for the submitted work. BRM (Early Career Fellowship #1036951), IM (Senior Research Fellowship #1043345) and TMED (Practitioner Fellowship #1058260) had support from National Health and Medical Research Council of Australia for the submitted work. JBM, SOA, SS, GY, SG, MP-S, KTB and PMS had no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years. There were no other relationships or activities that could appear to have influenced the submitted work.

Author contributions

BRM co-ordinated the study, analysed data, and drafted the manuscript. JMB collected data and reviewed/edited the manuscript. SOA performed piperaquine assays, contributed to data analysis, and reviewed/edited the manuscript. SS performed pharmacokinetic analyses and reviewed/edited the manuscript. GY collected data and reviewed/edited the manuscript. SG collected data and reviewed/edited the manuscript. MP-S performed drug assays and reviewed/edited the manuscript. KTB supervised drug assays and reviewed/edited the manuscript. PMS facilitated the approval and conduct of the study and reviewed/edited the manuscript. IM assisted with study design and reviewed/edited the manuscript. SJR assisted with study design and reviewed/edited the manuscript. TMED designed the study, assisted with data analysis and produced the final version of the manuscript.

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Figure captions

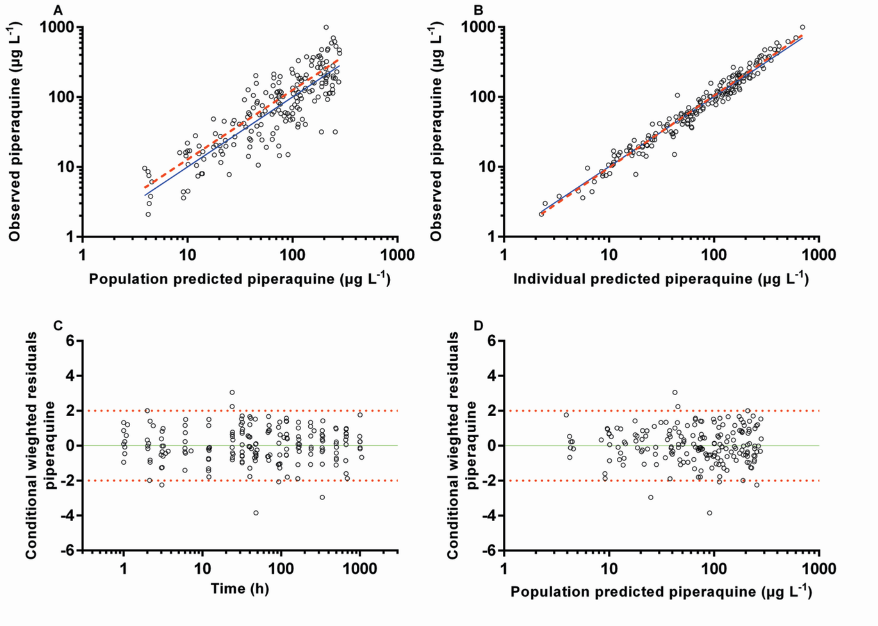
Figure 1. Diagnostic plots of the final plasma piperaquine population pharmacokinetic model. (A) observed vs. population predicted plasma concentration; (B) observed vs. individual predicted plasma concentrations; (C) conditional weighted residuals (CWRES) vs. time; (D) conditional weighted residuals (CWRES) vs. population predicted concentrations. Solid lines represent lines of identity in A and B, and zero conditional residuals in C and D. Dashed lines represent lines of best fit in A and B while dotted lines represent ± 2 SD in C and D.

Figure 2. Diagnostic plots of the final azithromycin population pharmacokinetic model. (A) observed vs. population predicted plasma concentration; (B) observed vs. individual predicted plasma concentrations; (C) conditional weighted residuals (CWRES) vs. time; (D) conditional weighted residuals (CWRES) vs. population predicted concentrations. Solid lines represent lines of identity in A and B and zero conditional residuals in C and D. Dashed lines represent lines of best fit in A and B while dotted lines represent ± 2 SD in C and D.

Figure 3. Plasma concentration vs time data (upper panel) and normalised prediction corrected visual predictive check for plasma piperaquine (lower panel). The solid line in the upper panel represents the average concentration vs time curve for the present patients overlying individual data points represented as open circles. The solid and dotted lines in the lower panel represent the normalised observed 50th percentile and its 80% prediction interval

(i.e. 10th and 90th percentiles), respectively, within their normalised simulated 95% CI represented as grey shaded areas.

Figure 4. Plasma concentration vs time data (upper panel) and normalised prediction corrected visual predictive check for plasma azithromycin (lower panel). The solid line in the upper panel represents the average concentration vs time curve for the present patients overlying individual data points represented as open circles. The solid and dotted lines in the lower panel represent the normalised observed 50th percentile and its 80% prediction interval (i.e. 10th and 90th percentiles), respectively, within their normalised simulated 95% CI represented as grey shaded areas.



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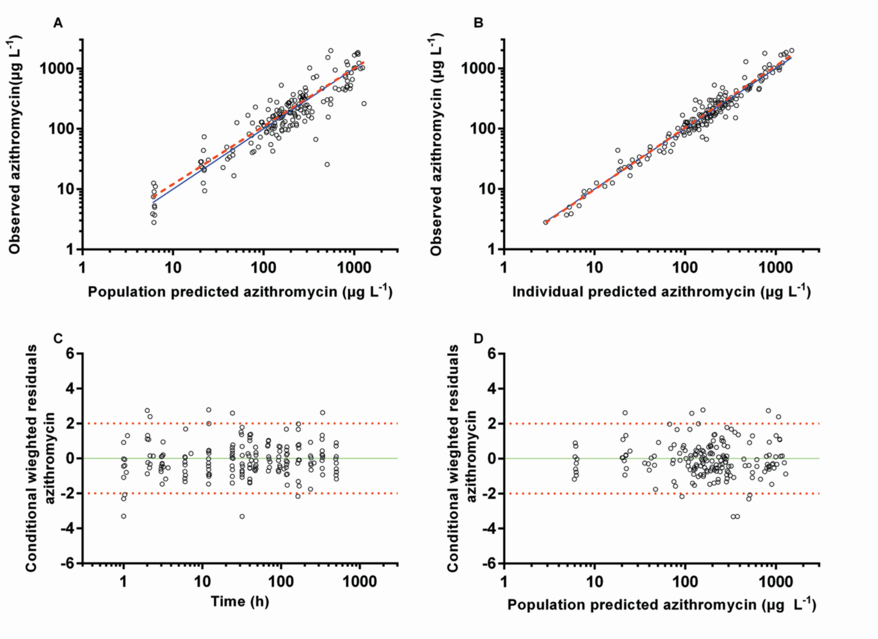

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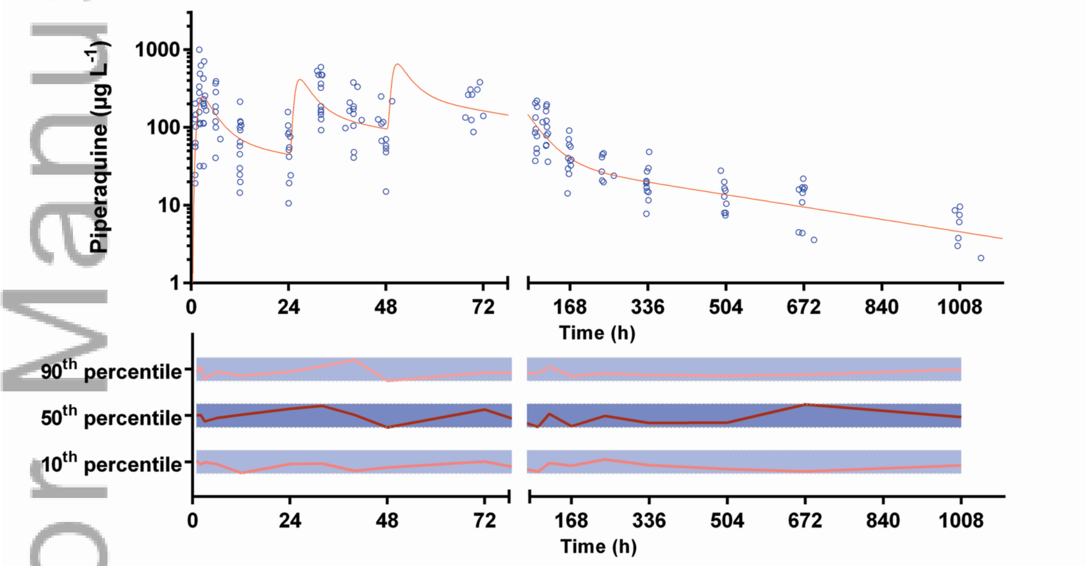

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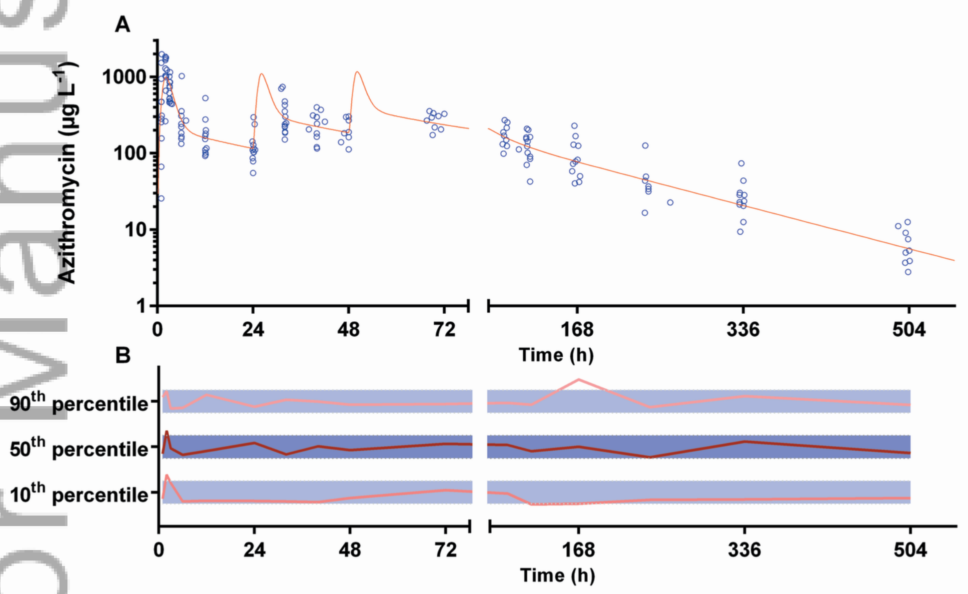

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Table 1. Baseline characteristics of the 30 pregnant participants. Data are percentages, median [IQR] and (range).

Age (years)	22 [20 – 26]	(18 to 32)
Weight (kg)	54 [51 – 60]	(42 to 74)
Height (cm)	157 [153 – 158]	(147 to 175)
Axillary temperature (^{o}C)	36.8 [36.4 – 37.0]	(36.0 to 37.1)
Plasmodium falciparum parasitaemia (%)	16.7	
Gestational age (weeks)	26 [22 – 28]	(16 to 32)
Gravidity	2 [1 – 3]	(1 to 4)
Parity	1 [0 – 2]	(0 to 3)
Respiratory rate (min^{-1})	20 [17 – 22]	(16 to 28)
Pulse rate (min^{-1})	81 [76 – 87]	(68 to 108)
Systolic blood pressure (mmHg)	93 [90 – 104]	(80 to 120)
Diastolic blood pressure (mmHg)	60 [50 – 60]	(50 to 70)
Systolic fall on standing (mmHg)	-4 [-10 to 0]	(-20 to 20)
Diastolic fall standing (mmHg)	-2 [-5 to 0]	(-10 to 10)
Haemoglobin (g L^{-1})	97 [85 – 112]	(62 to 127)
Blood glucose (mmol L^{-1})	5.4 [4.4 – 5.8]	(3.1 to 8.3)
QT_c (msec$^{0.5}$)	436 [424 – 445]	(400 to 463)

Table 2. Side-effects reported by the 30 participants during the first week after treatment was started. Data are numbers of participants and (percentages).

Fever	1 (3)
Chills	0 (0)
Headache	1 (3)
Nausea	5 (17)
Vomiting	0 (0)
Diarrhoea	2 (7)
Abdominal pain	1 (3)
Anorexia	2 (7)
Insomnia	0 (0)
Dizziness	2 (7)
Rash	2 (7)

Table 3. Prolongation of the rate-corrected electrocardiographic QT interval (QT_c) in $msec^{0.5}$ in the present patients treated with azithromycin-piperaquine (AZI-PQ), including those who were positive or negative for malaria parasites at baseline, and in pregnant PNG women recruited to a previous study [36] and treated with dihydroartemisinin-piperaquine (DHA-PQ) or sulphadoxine-pyrimethamine-piperaquine (SP-PQ). Data are median and [interquartile range].

	AZI-PQ			DHA-PQ	SP-PQ
	All	Malaria	Non-malaria	All	All
Number	30	6	24	16	16
4 h	12 [6 to 26]	11 [9 to 16]	16 [5 to 27]	14 [5 to 26]	14 [9 to 30]
12 h	8 [2 to 17]	3 [-5 to 5]	12 [3 to 19]	6 [-1 to 12]	4 [1 to 16]
24 h	8 [-1 to 18]	4 [-1 to 20]	10 [-1 to 17]	9 [-1 to 20]	12 [3 to 19]

Table 4. Final population pharmacokinetic estimates and bootstrap results for piperaquine in pregnant Papua New Guinean women.

Parameter	Mean	RSE%	Bootstrap median [95% CI]
Objective Function Value	-73.222		-89.430 [-141.288--34.582]
Structural model parameters			
MTT (h)	1.42	14	1.37 [1.11-2.01]
NN	3.29	45	3.71 [0.87-9.88]
CL/F_{PQ} (L h^{-1} 70 kg^{-1})	89.2	15	88.7 [68.5-115.4]
V_C/F_{PQ} (L h^{-1} 70 kg^{-1})	1,920	23	1,918 [1123-3067]
Q_1/F_{PQ} (L h^{-1} 70 kg^{-1})	69.2	22	67.2 [44.2-98.8]
V_{P1}/F_{PQ} (L h^{-1} 70 kg^{-1})	17,800	17	17,838 [12465-25335]
Q_2/F_{PQ} (L h^{-1} 70 kg^{-1})	275	24	269 [134-429]
V_{P2}/F_{PQ} (L h^{-1} 70 kg^{-1})	3,720	15	3,707 [2622-5264]
% increase in relative bioavailability with subsequent doses	51.7	30	52.0 [25.4-83.5]
Variable model parameters [shrinkage %]			
IOV in F_{PQ} (%)	62 [8,15,30]	9	60 [47-70]
IIV in MTT (%)	32 [36]	24	29 [11-50]
IIV in CL/F_{PQ} (%)	14 [49]	62	15 [2-27]
IIV in V_C/F_{PQ} (%)	21 [49]	40	20 [4-38]
$r(CL/F_{PQ}, V_{P1}/F_{PQ})$	1	FIX	FIX
RV for PQ (%)	31 [24]	12	29 [23-37]

MTT (Mean transit time), NN (number of transit compartments), CL/F_{PQ} (clearance relative to bioavailability), V_C/F_{PQ} (central volume of distribution relative to bioavailability), Q_1/F_{PQ} (inter-compartmental clearance for V_{P1}/F_{PQ}), V_{P1}/F_{PQ} (first peripheral volume of distribution relative to bioavailability), Q_2/F_{PQ} (inter-compartmental clearance for V_{P2}/F_{PQ}), V_{P2}/F_{PQ} (second peripheral volume of distribution relative to bioavailability), IOV (inter-occasion variability), IIV (inter-individual variability) and RV (residual variability). IOV and IIV are presented as 100%×√(variability estimate)

Table 5. Final population pharmacokinetic estimates and bootstrap results for azithromycin in pregnant Papua New Guinean women.

Parameter	Mean	RSE%	Bootstrap median [95% CI]
Objective Function Value	-122.715		-133.692 [-201.308 - -73.288]
Structural model parameters			
k_a (h^{-1})	0.89	12	0.92 [0.64 - 1.44]
DUR (h)	1.67	4	1.65 [0.98 - 2.06]
CL/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	76.0	5	75.7 [67.6 - 84.5]
V_C/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	365	25	393 [198 -859]
Q_1/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	228	14	223 [151 - 297]
V_{P1}/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	2,670	20	2,660 [1,457 - 3,883]
Q_2/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	59	23	58.0 [32.9 - 93.4]
V_{P2}/F_{AZI} ($L\ h^{-1}\ 70\ kg^{-1}$)	3,980	13	3,940 [2,809 - 4,943]
Variable model parameters [shrinkage%]			
IIV in DUR (%)	60 [45]	23	57 [9 - 116]
IIV in CL/F_{AZI} (%)	25 [8]	10	24 [13 - 33]
IIV in V_C/F_{AZI} (%)	127 [12]	18	119 [51 - 179]
$r(CL/F_{AZI},V_{P1}/F_{AZI})$	-0.80	10	-0.86 [-1.00 - -0.11]
RV for AZI (%)	33 [12]	10	29 [27 - 32]

k_a (rate for first order absorption), DUR (duration of zero order absorption), CL/F_{AZI} (clearance relative to bioavailability), V_C/F_{AZI} (central volume of distribution relative to bioavailability), Q_1/F_{AZI} (inter-compartmental clearance for V_{P1}/F_{AZI}), V_{P1}/F_{AZI} (first peripheral volume of distribution relative to bioavailability), Q_2/F_{AZI} (inter-compartmental clearance for V_{P2}/F_{AZI}), V_{P2}/F_{AZI} (second peripheral volume of distribution relative to bioavailability), IIV (inter-individual variability) and RV (residual variability). IIV is presented as 100%×√(variability estimate)

Table 6. *Post-hoc* Bayesian estimates of pharmacokinetic parameters and derived secondary parameters for piperaquine and azithromycin in pregnant Papua New Guinean women. Data are median [IQR].

Parameter	Piperaquine	Azithromycin
MTT (h)	3.29 [3.29 - 3.29]	-
NN	1.425 [1.331 - 1.61]	-
k_a	-	0.93 [0.93 - 0.93]
DUR	-	1.60 [1.29 - 1.93]
CL/F (L h^{-1})	75.9 [65.9 - 83.3]	64.1 [60.7 - 68.9]
V_C/F (L)	1,457 [1,122 – 2,013]	354 [152 - 628]
Q_1/F (L h^{-1})	56.9 [54.5 - 61.4]	199 [191 - 215]
V_{P1}/F (L)	13,758 [12,994 – 15,223]	1,837 [1,735 – 2,033]
Q_2/F (L h^{-1})	227 [217 - 244]	56.9 [54.5 - 61.4]
V_{P2}/F (L)	2,868 [2,709 – 3,174]	3,242 [3,062 – 3,587]
$t_{½ \alpha}$ (h)	2.33 [1.88 - 2.77]	0.738 [0.316 - 1.21]
$t_{½ \beta}$ (h)	28 [27.5 - 28.8]	15.5 [14.9 - 16.1]
$t_{½ \gamma}$ (h)	315 [303 - 331]	89.2 [86.1 – 93.0]
C_{max} (μg L^{-1})	731 [445 – 900]	1,080 [839 – 1,439]
$AUC_{0-\infty}$ (μg h L^{-1})	38,818 [24,354 – 52,299]	46,799 [43,526 – 49,462]
$AUC_{0-\infty}$ (μg h L^{-1}) per mg kg^{-1} dose	1,264 [846 – 1,873]	846 [828 – 876]

MTT (Mean transit time), NN (number of transit compartments), CL/F (clearance), V_C/F (central volume of distribution), Q_1/F (inter-compartmental clearance for V_{P1}/F), V_{P1}/F (first peripheral volume of distribution), Q_2/F (inter-compartmental clearance for V_{P2}/F), V_{P2}/F (second peripheral volume of distribution), $t_{½ \alpha}$, $t_{½ \beta}$ and $t_{½ \gamma}$ (first and second distribution, and elimination half-lives, respectively), C_{max} (maximum plasma concentration) and $AUC_{0-\infty}$ (area under the plasma concentration-time curve).