Pharmacokinetic Properties of Azithromycin in Pregnancy

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Azithromycin (AZI) is an azalide antibiotic with antimalarial activity that is considered safe in pregnancy. To assess its pharmacokinetic properties when administered as intermittent preventive treatment in pregnancy (IPTp), two 2-g doses were given 24 h apart to 31 pregnant and 29 age-matched nonpregnant Papua New Guinean women. All subjects also received single-dose sulfadoxine-pyrimethamine (SP) (1,500 mg or 75 mg) or chloroquine (450-mg base daily for 3 days). Blood samples were taken at 0, 1, 2, 3, 6, 12, 24, 32, 40, 48, and 72 h and on days 4, 5, 7, 10, and 14 for AZI assay by ultra-high-performance liquid chromatography-tandem mass spectrometry. The treatments were well tolerated. Using population pharmacokinetic modeling, a three-compartment model with zero-order followed by first-order absorption and no lag time provided the best fit. The areas under the plasma concentration-time curve (AUC0–t) (28.7 and 31.8 mg·h liter−1 for pregnant and nonpregnant subjects, respectively) were consistent with the results of previous studies, but the estimated terminal elimination half-lives (78 and 77 h, respectively) were generally longer. The only significant relationship for a potential of covariates, including malarial parasitemia, was with pregnancy, which accounted for an 86% increase in the volume of distribution of the central compartment relative to bioavailability without a significant change in the AUC0–t. These data suggest that AZI can be combined with compounds with longer half-lives, such as SP, in combination IPTp without the need for dose adjustment.

Azithromycin (AZI) is a semisynthetic azalide antibiotic that is structurally related to erythromycin but has a broader spectrum of antibacterial activity and a more favorable pharmacokinetic profile (13, 22). It is widely used in the treatment of respiratory and sexually transmitted infections, including those in HIV-infected patients (32, 34). AZI also inhibits protein synthesis in the plasmodial apicoplast (39, 40) and thus has activity against both Plasmodium falciparum and Plasmodium vivax (3, 12, 16, 27–30, 41). It acts mainly against the progeny of parasites that inherit a nonfunctioning apicoplast after exposure, with the result that its antimalarial effect has a slow onset and is relatively weak. Therefore, AZI is best used in combination with other antimalarial compounds as both treatment (20, 27, 29) and chemoprophylaxis (5, 19), with likely additive or synergistic effects (28, 30, 31).

Malaria in pregnancy can result in adverse outcomes for both mother and fetus (14). Intermittent preventive treatment in pregnancy (IPTp) aims to reduce the burden of malaria by administering treatment doses of antimalarial drugs at predetermined intervals as part of routine antenatal care in areas of endemicity (44). Because AZI is considered safe in pregnancy and could have activity against other clinically significant pathogens (8, 38), it has been suggested as a candidate for IPTp. Although the pharmacokinetics of AZI have been investigated (2, 6, 7, 11, 13, 23–26, 35–37, 45), only one study included pregnant women (36), and most focused on its antibacterial properties. In addition, AZI is likely to be partnered with conventional antimalarial drugs if given as IPTp, and there is evidence that such combinations are safe and well tolerated in studies with chloroquine (CQ) in healthy volunteers (11) and with sulfadoxine-pyrimethamine (SP) in pregnant women (20). Although there does not appear to be a clinically significant pharmacokinetic interaction with CQ (11), AZI interactions with other conventional IPTp treatments are unknown. Therefore, we investigated the pharmacokinetic properties of AZI in combination with CQ or SP in pregnant and nonpregnant women from an area of Papua New Guinea (PNG) with intense transmission of both P. falciparum and P. vivax malaria.

MATERIALS AND METHODS

Study site, sample, and approvals. The present study was conducted at Alexander Health Centre, Madang Province, on the north coast of PNG. The pregnant women were recruited at their first antenatal clinic visit, and the age-matched nonpregnant volunteers were from the same communities as the pregnant participants. Women were eligible if (i) they had not taken any of the study drugs in the previous 28 days, (ii) they had no history of significant allergy to any study drug, (iii) there was no significant comorbidity or clinical evidence of severe malaria, and (iv) follow-up was possible for the duration of the study. The study was approved by the Medical Research Advisory Committee of PNG and the Human Ethics Research Committee at the University of Western Australia. Written informed consent was obtained from all participants.

Clinical procedures. A detailed assessment was performed prior to drug administration, including a side effects questionnaire, point-of-care hemoglobin and blood glucose (HemoCue, Angelholm, Sweden), thick and thin blood films, and (for pregnant participants) estimation of gestational age by fundal height. A 3-ml blood sample was taken for subsequent antimalarial drug assay. All women...
received 2 g AZI (Zithromax; Pfizer, New York, NY) both at enrolment and 24 h later. Subjects were also randomized to receive single-dose SP (1,500 mg or 75 mg; Fansidar; Roche, Basel, Switzerland) at enrolment (AZI-SP arm) or CQ (Chloroquin; Astra, Sydney, Australia) (450 mg base daily for 3 days; AZI-CQ arm) in accordance with regimens recommended for PNG (15). The administration of all doses was directly observed. The dosing schedule for AZI was chosen as the simplest regimen that would be likely to ensure effective drug concentrations during the first 4 days of treatment (40).

Following the first dose of AZI (day 0), additional blood samples were taken at 1, 2, 3, 6, 12, 24, 32, 40, 48, and 72 h and then on days 4, 5, 7, 10, and 14 for drug assay. The exact timing of each blood sample was recorded. All samples were centrifuged promptly, with red cells and separated plasma stored frozen at −80°C. The side effects questionnaire was readministered at 6 h and then at 1, 2, 3, and 7 days. Hemoglobin, erect and supine heart rates and blood pressure, respiratory rate, temperature, and blood slides were taken on days 1, 2, 3, 7, 14, 28, and 42, and head glucose was measured on days 1, 2, and 3. After the completion of follow-up, pregnant patients were returned to the usual antenatal care.

Laboratory methods. Giemsa-stained thick blood smears were examined independently by at least two skilled microscopists who were blinded to pregnancy and treatment status. Each microscopist viewed >100 fields at ×1,000 magnification before a slide was considered negative. Any slide discrepant for positivity negativity or species identification was referred to a third microscopist.

AZI levels were measured using a validated ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-LCMS-MS) method using a deuterated internal standard. The samples were retained for subsequent SP assay. AZI USP was obtained from APAC Pharmaceutical LLC (Ellicott City, MD) and deuterated AZI from Toronto Research Chemicals (North York, Canada). In brief, following the addition of an internal standard, AZI was extracted from 5 μl of plasma by protein precipitation. After centrifugation, supernatant (5 μl) was injected onto a 2795/Quattro Premier XE UPLC-ESI-MS/MS (Waters Corp, MA) using a Waters BEH C18 1.7-μm, 2.1- by 100-mm column. Gradient elution was performed using mobile phases A (45/55 [vol/vol], containing 1 glitler ammonium bicarbonate in 50/50 [vol/vol] methanol-water and acetonitrile) and B (50/50 [vol/vol] methanol-acetonitrile) at 0.4 ml/min. Adduct transitions were monitored using positive electrospray ionization with multiple reaction monitoring for AZI and d3-AZI and were set at 284 to 252 and 572.6 to 594.4, respectively. The method was linear to 1,012 ng/l (r2 = 0.9997) with a limit of quantification of 2.5 μg/liter AZI. All inter- and intraday coefficients of variation were <10%, and the between-subject variability (BSV) was <5% when matrix effects were investigated at three concentrations.

Population pharmacokinetic analysis. Concentration-time data sets were analyzed by nonlinear mixed-effect modeling using NONMEM (version 6.2; Icon Development Solutions, Ellicott City, MD) with an Intel Visual FORTRAN 10.0 compiler. Linearized model building was conducted within NONMEM (ADVENT and -12 used with TRAN4 in the PREDDPP library), first-order conditional estimation (FOCE) with f-η interaction, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) without a lag interaction, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models. Unless otherwise specified, a difference in the OFV of 3.84 in the OFV (P < 0.05) and −12 used with TRANS4 in the PREDPP library), first-order conditional estimation, and the objective function value (OFV) (a NONMEM-calculated global goodness-of-fit indicator equal to −2 log-likelihood value of data) were used to construct and compare plausible models.
not significant and there were no associated symptoms. After completion of the study, one of the study participants had a stillbirth. A medical review of her case notes by three independent physicians concluded that it was unlikely to be the result of the study medication.

**Pharmacokinetic modeling.** A three-compartment model had a lower OFV than a two-compartment model (8,700.058 vs 8,185.104; \( P < 0.05 \)) and a more favorable distribution of WRES over time. Zero-order followed by first-order absorption without a lag time provided the favorable distribution of WRES over time. Zero-order fraction from the central compartment; \( k_a \) (the first-order absorption rate constant); \( \text{CL/F} \) (clearance from the central compartment); \( V_{C1/F}, V_{P1/F}, \) and \( V_{P2/F} \) (volumes of distribution of the central, first peripheral, and second peripheral compartments, respectively); and \( Q_{1/F} \) and \( Q_{2/F} \) (intercompartment clearances for \( V_{P1/F} \) and \( V_{P2/F} \), respectively). The model structure is shown in Fig. 1. BSV could be estimated for DUR, \( \text{CL/F}, V_{C1/F}, \) and \( V_{P2/F} \), while a proportional-error model was best for RUV. After testing the various covariates, only pregnancy on \( V_{P1/F} \) produced a significant decrease in the OFV (\( \chi^2 \text{df} = 1; P < 0.05 \)) accompanied by a decrease in the BSV of \( V_{P1/F} \) from 111.0% to 99.6%.

The results of the parameter estimates and their relative standard errors (RSE) are summarized in Table 3 and secondary parameter estimates in Table 4. All drug concentrations after day 2 were strongly correlated with the AUC_{0-\infty} (\( r > 0.7; P < 0.001 \)), with 96-h levels showing the strongest association (\( r = 0.78 \)). The bootstrap results (Table 3) demonstrate a robust estimation of both fixed and random parameters with bias < 4% and < 5%, respectively. Goodness-of-fit plots of observed versus population and individual predicted concentrations and WRES versus time are shown in Fig. 2 and 3. The VPC results, stratified for pregnancy status, are presented in Fig. 4 and show reasonable predictive performance of the model while demonstrating some difficulty in capturing post-absorption plasma concentrations peaks.

### DISCUSSION

The present study is the first pharmacokinetic evaluation of AZI in pregnant and nonpregnant women living in a malaria-endemic area. We found that a three-compartment model with a combined absorption process best described the disposition of AZI in our subjects. Both two-compartment (23, 26, 37) and three-compartment (6, 35) models have been found to best describe AZI plasma concentration-time profiles in other contexts. Our ability to differentiate the triexponential elimination of AZI may have been facilitated by the relatively long sampling duration. This may also explain why our estimated terminal elimination half-lives (78 and 77 h for pregnant and nonpregnant participants, respectively) were longer than those in most previous studies (range, 27 to 79 h) (6, 7, 13, 23, 35, 37). The overall drug exposure (AUC_{0-\infty} 28.7 and 31.8 mg·h liter\(^{-1}\) for pregnant and nonpregnant subjects, respectively)
was within the range expected from dose-scaled results from previous studies in other contexts (26.5 to 46.4 mg h \(^{-1}\)) (2, 7, 11, 13, 23, 24, 26, 35, 37), suggesting that the bioavailability of AZI is not dose dependent.

Both zero-order (23, 37) and first-order (6, 26) absorption have been reported previously for AZI, but neither was appropriate for our data. A combined absorption process in which the drug enters the absorption compartment in a zero-order manner and then is absorbed according to first-order kinetics provided the best model in the present study. This is analogous to the twin processes of (i) gastric emptying of the drug into the small intestine (the zero-order process) and (ii) absorption in the small intestine proportional to the amount present (the first-order process). Despite this more complex model, AZI absorption was still not well characterized in our final model. This has been reported previously (37) but is unlikely to be significant in the treatment of uncomplicated malaria, where exposure of the parasite to therapeutic drug concentrations over several life cycles is more important than that immediately after drug administration.

Plasma AZI concentrations appeared to differ between pregnant and nonpregnant women only in the first 48 h after the first dose. This was confirmed by the population pharmacokinetic modeling, in which pregnancy, the only significant covariate relationship, accounted for an 86% increase in \(V_{C/F}\). Despite significant differences in the secondary parameters \(V_{C/F}, V_{P2/F}, V_{SS/F}, \) and \(t_{1/2u}\) (first-distribution half-life) between pregnant and nonpregnant subjects, no difference was

![Diagram](attachment:image.png)

**FIG. 1.** Structural model used in the final pharmacokinetic analysis of plasma azithromycin concentrations in the central compartment versus time. GUT, gastrointestinal tract.

### TABLE 3. Model building, final parameter estimates, and bootstrap results from the AZI population pharmacokinetic modeling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Bootstrap (n = 1,000) (median [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFV</td>
<td>7,999.870</td>
<td>7,993.646</td>
</tr>
<tr>
<td>Pharmacokinetic (estimate [% RSE])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DUR) (h)</td>
<td>1.66 [10.4]</td>
<td>1.55 [3.3]</td>
</tr>
<tr>
<td>(k_a) (h(^{-1}))</td>
<td>0.513 [2.2]</td>
<td>0.525 [14.8]</td>
</tr>
<tr>
<td>Pregnancy on (V_{C/F}) (liters)</td>
<td>330 [69.4]</td>
<td>318 [48–604]</td>
</tr>
<tr>
<td>(CL/F) (liters h(^{-1}))</td>
<td>158 [3.9]</td>
<td>158 [6.7]</td>
</tr>
<tr>
<td>(Q_1/F) (liters h(^{-1}))</td>
<td>327 [5.7]</td>
<td>325 [12.7]</td>
</tr>
<tr>
<td>(V_{P2/F}) (liters)</td>
<td>5,070 [5.6]</td>
<td>5,040 [7.3]</td>
</tr>
<tr>
<td>(Q_2/F) (liters h(^{-1}))</td>
<td>67.2 [11.5]</td>
<td>66.4 [12.4]</td>
</tr>
<tr>
<td>Random (CV [% RSE])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSV (V_{C/F})</td>
<td>111.4 [20.9]</td>
<td>99.6 [35.5]</td>
</tr>
<tr>
<td>BSV (CL/F)</td>
<td>28.3 [24.1]</td>
<td>28.3 [33.1]</td>
</tr>
<tr>
<td>BSV (V_{P1/F})</td>
<td>35.8 [27.0]</td>
<td>35.6 [27.2]</td>
</tr>
<tr>
<td>BSV (DUR)</td>
<td>73.0 [21.4]</td>
<td>76.9 [22]</td>
</tr>
<tr>
<td>RUV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportional error (CV [% RSE])</td>
<td>31.3 [9.5]</td>
<td>31.2 [15.1]</td>
</tr>
</tbody>
</table>

\(a\) CV, coefficient of variation.
seen in $t_{1/2b}$ (terminal elimination half-life) or AUC$_{0\rightarrow\infty}$. This suggests that the drug elimination and overall exposures were similar in the two groups. A much shorter AZI half-life (12 h) than in the present study was reported previously in pregnant women (36), but the study employed a shorter sampling duration (168 versus 336 h) and included pregnant women at or near term, and the analysis was constrained by relatively sparse sampling.

Because of the need for AZI to be combined with other therapies (12, 41), we included conventional antimalarial drugs currently recommended as part of IPTp in PNG and other countries (10, 20). There were no significant differences in the disposition of AZI between the AZI-CQ and AZI-SP groups, consistent with a study of the interaction of CQ and AZI in healthy volunteers (11). We conclude that AZI dose modification is unnecessary in these combinations. In addition, the lack of an effect of malaria status as a covariate on AZI disposition suggests that, unlike drugs such as quinine (21), the dose may not have to be adjusted when parasitemia is present.

The most common side effects of AZI, especially with higher doses, are nausea and vomiting. These symptoms are thought to be related to the effect of AZI on the motilin receptor in the upper gastrointestinal tract (33). However, with the exception of pruritus, which tended to be associated with AZI-CQ therapy, consistent with known CQ effects (1), there were no differences in the incidences of side effects between the two treatment groups, and most reported adverse effects were mild.

### TABLE 4. Secondary pharmacokinetic parameters derived from post hoc Bayesian estimates for pregnant and nonpregnant study participants (median [IQR])

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DUR (h)</td>
<td>1.65 [0.94–2.34]</td>
<td>NS$^a$</td>
</tr>
<tr>
<td>$k_e$ (h$^{-1}$)</td>
<td>0.525 [0.525–0.525]</td>
<td>NS</td>
</tr>
<tr>
<td>$V_{e}/F$ (liters)</td>
<td>647 [422–995]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$V_{0}/F$ (liters)</td>
<td>3,620 [2,747–3,951]</td>
<td>NS</td>
</tr>
<tr>
<td>$V_{SS}/F$ (liters)</td>
<td>8,355 [7,460–8,973]</td>
<td>0.002</td>
</tr>
<tr>
<td>$t_{1/2a}$ (h)</td>
<td>0.88 [0.57–1.36]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$t_{1/2b}$ (h)</td>
<td>20.7 [18.3–22.8]</td>
<td>NS</td>
</tr>
<tr>
<td>$t_{1/2b}$ (h)</td>
<td>78.2 [74–82.5]</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{0\rightarrow\infty}$ (µg h liter$^{-1}$)</td>
<td>28,713 [25,913–32,942]</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$ NS, not significant.

$^b$ $t_{1/2a}$, $t_{1/2b}$, and $t_{1/2b}$ are the first-distribution, second-distribution, and terminal elimination half-lives respectively.

![FIG. 2. Observed versus model predicted concentrations (A) and individual predicted concentrations (B) for AZI. The solid gray lines are the lines of identity, while the dashed black lines are the linear regression lines of best fit.](http://aac.asm.org/)

![FIG. 3. Weighted residuals versus time after dose (log scale) plot for AZI.](http://aac.asm.org/)
The AZI dose regimen in both combination therapy groups in the present study (2.0 g daily for 2 days) was associated with a side effect profile similar to that reported previously after a single 2.0-g dose (35). Use of the sustained-release formulation of AZI should reduce side effects, including nausea and vomiting (9). However, this formulation has a bioavailability of 82.8% relative to conventional AZI, suggesting that a higher dose will be required to achieve the same drug exposure. As well as increasing the cost of AZI treatment, this could mean that side effects are more frequent with higher-dose sustained-release AZI administration.

Although the present study had limited subject numbers, it is encouraging that both regimens achieved a 100% uncorrected APCR. The plasma concentrations of AZI required to achieve 96-h drug levels increased potency, suggesting that either AZI renders second-generation parasites unable to establish a parasitophorous vacuole upon host cell invasion or the effect on apicoplast protein synthesis inhibits successful development of the progeny of drug-treated parasites (40).

Given the need for relatively prolonged parasite exposure to therapeutic plasma concentrations, it is unlikely that the benefit of “front loading” of AZI used in treating bacterial infections (23, 24) will be relevant in malaria. However, experience with AZI as an antimalarial agent is growing. A Cochrane review of its efficacy is currently under way (43), and promising results are being seen when it is used with SP in IPTp, such as might be given at least twice during pregnancy (20). The present study provides a pharmacokinetic foundation for the further investigation of AZI as an antimalarial agent in pregnancy, particularly in combination IPTp. Further data from the present study should also determine whether AZI influences the disposition of CQ and SP. Although there was a significant increase in AZI V_{c/F} in pregnant women, there was no significant change in the AUC_{0–t} and it is therefore likely that no dose adjustments will be required for pregnant women when AZI is given in combination with CQ or SP.

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