1   Population Pharmacokinetics of Praziquantel in Pregnant and Lactating

2   Filipino Women infected with *Schistosoma japonicum*

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35 ABSTRACT

36 An estimated 40 million women of reproductive age are infected with one of three species of the

37 waterborne parasite *Schistosoma* (*S.*) spp. Treatment with praziquantel (PZQ) via mass drug

38 administration (MDA) campaigns is the mainstay of schistosomiasis control for populations living

39 in endemic areas. The World Health Organization recommends that pregnant and lactating

40 women be included in schistosomiasis MDA programs and several recent studies have evaluated

41 the safety and efficacy of PZQ use during pregnancy. To date, there are no data describing PZQ

42 pharmacokinetics (PK) during pregnancy or among lactating postpartum women. As part of a

43 randomized controlled trial investigating the safety and efficacy of PZQ during human pregnancy,

44 we examined the PK of this therapeutic drug among three distinct cohorts of women infected

45 with *S. japonicum* in Leyte, The Philippines. Specifically, we studied the PK properties of PZQ

46 among early and late gestation pregnant women (N= 15 each) and lactating post-partum women

47 (N=15) with schistosomiasis. We found that women in early pregnancy had increased apparent

48 clearance and lower Area-Under-the-Curve (AUC_{0-24}) that may be related to physiological

49 changes in drug clearance and/or changes in oral bioavailability. There was no relationship

50 between body weight and apparent clearance. The mean ± standard deviation partition ratio of

51 plasma to breast milk was 0.36. ± 0.13 . The estimated median infant PZQ daily dose would be

52 0.037 mg/kg ingested from breast milk, which is significantly lower than the dosage required for

53 anti-schistosomal activity and not known to be harmful to the infant. Our PK data do not

54 support suggestion to delay breastfeeding 72 hours after taking PZQ. Results can help inform

55 future drug efficacy studies in pregnant and lactating women with schistosomiasis.

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61   INTRODUCTION

62   Over 240 million people are infected with one of three species of the waterborne

63   parasite *Schistosoma (S.)* spp., including ~40 million women of reproductive age. More than 700

64   million people are at risk of infection (1, 2). Schistosomiasis caused by the most common

65   *Schistosoma* spp. (i.e. *S. mansoni, S. japonicum, S. haematobium*) is responsible for 1.86 million

66   disability adjusted life years (DALYS) (3). Schistosomiasis remains a significant cause of morbidity

67   and mortality in endemic countries, despite the availability of praziquantel (PZQ), which is the

68   only widely available anti-schistosomal drug (4). PZQ is a first-line agent for the control of

69   schistosomiasis in populations living in endemic areas and is administered via mass drug

70   administration (MDA) programs (4). Despite WHO endorsement of inclusion of pregnant women

71   in MDA programs, this is not necessarily practised in many affected countries (5).

72   PZQ is orally bioavailable. Absorption is higher with carbohydrate and fat-rich foods.

73   PZQ undergoes significant first pass metabolism and is predominantly cleared by oxidative

74   mechanisms via CYP3A4 and CYP19A (6). There is high inter-individual PK variability, which is

75   further exacerbated in individuals with liver disease (7). When PZQ was first licensed in 1979, it

76   had not been formally studied in any pregnant or lactating women. PZQ is classified as a Class B

77   agent for use in pregnant women by the Food and Drug Administration (FDA). This classification

78   is based on demonstrated safety in laboratory animal studies, but a lack of definitive data in

79   humans. There is a paucity of information related to the PZQ PK in pregnant women (8) despite

80   the high likelihood that the physiologic changes of pregnancy may affect PZQ absorption,

81   distribution and clearance (9). Physiological changes related to pregnancy may result in altered

82   drug exposures, which may have an impact on the probability of therapeutic success (10).

83   In 2002 and 2006, the World Health Organization (WHO) recommended that all

84   schistosomiasis-infected pregnant and breastfeeding women be treated with PZQ individually or

85   during MDA programs (11). This was based on the expected accrued morbidity from

86   schistosomiasis during cycles of pregnancy and lactation without treatment.   These

87   recommendations were made despite a lack of data describing PZQ pharmacokinetics in

88   pregnancy and lactating women. Furthermore, there are no available data available regarding

89   the concentration of PZQ in human breast milk following maternal treatment to support the

90 current recommendation to stop breastfeeding for 72 hours after taking PZQ. Since that time,

91 two randomized controlled trials (RCTs) (12, 13) support the safety of PZQ in pregnancy, and one

92 (12) suggests a potential beneficial impact on the iron status of both the mother and infant (12).

93 Although many countries have included pregnant and lactating women in MDA campaigns, many

94 others are waiting for further data on the safety and PK of PZQ during pregnancy and lactation

95 (5, 14).

96   As part of a RCT examining the safety and efficacy of PZQ during human pregnancy we

97 examined the PK of PZQ in pregnant and lactating women infected with *S. japonicum* living in the

98 northeast of the Province of Leyte, the Philippines. The primary objective was to evaluate and

99 compare the PK and safety of PZQ in early and late gestation pregnant women (N=15 each) and

100 in lactating post-partum women (N=15) with schistosomiasis.

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103   RESULTS

104   *Study Design and Patient Demographics*

105   The study design is shown in **Figure 1.** A total of 47 women that were *S. japonicum*

106   positive by parasitological examination were enrolled and received a PZQ split dose of 60 mg/kg,

107   3 hours apart (i.e. two split dosages of 30 mg/kg) (12). Two patients in the early pregnancy

108   group vomited shortly after receiving PZQ and did not have PK sampling performed. This left a

109   total of 45 patients who were divided evenly among the 3 groups: (1) early pregnancy (i.e. 12-16

110   weeks gestation); n=15; (2) late pregnancy (i.e. 30-36 weeks gestation); n=15; and (3) lactating

111   non-pregnant women (i.e. 5-7 months postpartum); n=15. The weight and height for all women

112   enrolled in the study are summarized in **Table 1.**

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114   *Population PK of PZQ in Plasma*

115   A population methodology was used to fit a structural PK model to the overserved

116   plasma concentration-time data to enable robust estimates of interpatient variability. The

117   median and individual PZQ concentration-time profiles for each study group are shown in **Figure**

118   **2.** There was marked variability in the PZQ concentrations in both plasma and breast milk in all

119   study groups. The fact that the dosing of PZQ was split, 3 hours apart, resulted in more than

120   one peak concentration for each patient.

121   The PK of PZQ in plasma and breast milk was co-modelled using a population methodology with

122   the program Pmetrics (15) A standard 3-compartment PK model consisting of an absorptive

123   compartment (i.e. gut), central compartment (i.e. bloodstream) and peripheral compartment

124   (i.e. rest of the body) was initially fitted to the data before the potential impact of covariates on

125   the PK was assessed. The mean, median and dispersions for the population PK parameters from

126   the base model are summarized in **Table 2.** The fit of the model to the data was acceptable.

127   There was an acceptable degree of bias and imprecision as determined by a normalized

128   prediction distribution error (NPDE) analysis for both plasma and breast milk concentrations

129   (data not shown; a standard VPC was not performed because women received different

130   absolute amounts of drug). The observed-predicted values are shown in **Figure 3** and the

131   individual plots in Supplemental figure 1. The residuals are shown in Figure 4. The mean of the

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132   weighted residuals was not statistically different from zero and were normally distributed. The

133   Bayesian posterior estimates for each patient were calculated and these were used to assess

134   the impact of covariates on the PK as well as estimating drug exposure of PZQ in each individual

135   patient.

136   There was no relationship between weight and Bayesian estimates for the apparent

137   clearance (i.e. clearance/F), and weight and the apparent volume of the central compartment

138   (i.e. V/F (**Figure 4**). The correlation coefficient for these relationships was r = 0.0303 (95% CI -

139   0.2656, 0.3210), p-value = 0.8435 and r = 0.1617 (95% CI -0.1384, 0.4346), p = 0.2885). Hence,

140   covariates were not incorporated into the structural model.

141   There were no differences in the absolute dosage received by women within the three

142   study groups (p=0.21, Kruskal Wallis test; **Figure 6A**). Furthermore, there was no relationship

143   between the Bayesian estimates for the apparent volume of the central compartment and the

144   study groups (**Figure 6B**). However, there was a significant relationship between the apparent

145   clearance and the stage of pregnancy. Women in the early pregnancy group had higher

146   apparent clearances than the other groups (**Figure 6C**). Women in the early pregnancy group

147   had faster apparent clearance of PZQ compared with postpartum women (p=0.02). There were

148   also differences between early and late stage pregnancy that approached, but did not achieve

149   statistical significance (p=0.056).

150   Women in the early pregnancy group had significantly lower AUC_{0-24} compared with late

151   pregnancy (p= 0.0144) and postpartum women (p-value 0.0378). Since there were no

152   differences in absolute dosage received by women in these groups, the lower AUC_{0-24} in early

153   pregnancy can only be explained by the faster clearance that was observed in this group or by

154   lower oral bioavailability. There were significant differences in the observed Cmax values

155   between the groups. The overall differences were statistically significant using ANOVA

156   (p=0.019). There was a difference between early and late pregnancy group (p=0.017 after

157   Bonferroni correction), but not between early pregnancy and post-partum women (p=0.236) or

158   late pregnancy and post-partum women (p=0.814). Further evidence of the potential

159   importance of oral bioavailability affecting drug exposure (i.e. AUC_{0-24}) was obtained from

160   relationship between SCL/F and V/F. Both were highly correlated (*r*=0.636, 45 observations,

161   p<0.001) suggesting F may have an impact on both parameters. Given the uncertainty regarding

162   the impact of altered oxidative metabolism versus oral biovailablity as an explanation for the

163   lower AUC_{0-24} we did not further complicate the structural model that was fitted to the data.

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Population PK of PZQ in Breast Milk

166   The concentration-time course of PZQ in breast milk was variable (**Figure 2**). The

167   elimination of drug in breast milk was similar to that of plasma. The AUCplasma:AUCbreast milk

168   mean +/- SD calculated from the Bayesian posterior estimates was 0.36. ± 0.13 with a range in

169   the 15 lactating women of 0.19-0.55. The average concentration in breast milk was 0.185 mg/L

170   (i.e. $AUC_{0-24}/24$). Therefore, the estimated average ingestion of PZQ by a new-born infant that

171   consumes 150 ml/kg of breast milk per day was approximately 0.028 mg/kg per day (i.e. 0.185

172   mg/L * 0.15 L/kg).

173   The elimination half-life of PZQ from breast milk was 1.90 hours. For a lactating woman

174   of average weight observed in this study receiving 60 mg/kg in two divided dosages of 30 mg/kg,

175   the estimated PZQ concentration in breast milk 24 and 48 hours post dose was 0.0004 mg/L and

176   3×10^{-7} mg/L, respectively. Hence, 24 hours post dose there is only 0.01% of the maximal

177   concentration of PZQ in breast milk and at 48 hours the concentrations of drug were negligible.

Monte Carlo Simulations

179   Monte Carlo simulations were performed using the median weight of study participants

180   (47.9 kg). Pmetrics was used to generate a total of 1,000 lactating women. The concentration-

181   time profile for each patient was determined. The 5th, 25th, 50th, 75th and 95th centiles and their

182   95% confidence bound in plasma and breast milk is shown in **Figure 7**. The AUC_{0-24} in plasma and

183   breast milk was calculated from the median Bayesian posterior estimates using the trapezoidal

184   rule in the first 24 hours following the initiation of therapy. A plot of the simulated AUCplasma-

185   versus-AUCbreast milk is shown in **Figure 7,** which is overlaid with the observed AUCs from the

186   15 lactating women in the study. The partitioning of PZQ into breast milk was comparable

187   between the observed data and the simulations and was approximately 30%.

Adverse events

189   There were no severe adverse events documented in any of the women. Only two

190   women had mild side-effects with vomiting documented within two hours of PZQ dosing and

191   were excluded from PK analysis. The adverse events are summarized in **Table 3**.

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194   DISCUSSION

195   This is the first study to describe the PK of PZQ in pregnant and lactating women infected

196   with *Schistosoma japonicum.* Women in early pregnancy had significantly lower AUC_{0-24}

197   compared with women in late pregnancy and lactating postpartum women. The most likely

198   explanation for the differences in clearance relate to pregnancy-induced increases in hepatic

199   enzyme activity related to hormonal changes associated with pregnancy (9, 16). The absorption

200   of PZQ is limited by grapefruit juice suggesting the importance of oxidative mechanisms in the

201   gut wall. PZQ is known to undergo high first-pass metabolism. (6, 17) Estradiol and

202   progesterone are both known to induce CYP3A4 in pregnancy (18) and are responsible for

203   increased clearance of drugs such as midazolam (19, 20). However, these changes are typically

204   more pronounced later in pregnancy, which is not consistent with the raw data or the estimates

205   of clearance in this study. This observation raises that possibility that some of the changes may

206   be related to differences in oral bioavailability in the study groups. There were differences in

207   Cmax between the groups (significantly lower in early pregnancy) and a high degree of

208   correlation between SCL/F and V/F. It is possible another pregnancy related hormone or

209   transporter expressed in early pregnancy has an impact on clearance and drug exposure.

210   We did not investigate the potential impact of hepatic metabolism on the PK variability of

211   PZQ. Liver function may be potentially altered from schistosomiasis due to *S. japonicum* (21).

212   The clearance of PZQ may also be affected by pharmacogenetic polymorphisms in CYP enzymes

213   (e.g. CYP1A2, CYP3A4, CYP2B1, CYP3A5 and CYP2C19) and/or interactions with drugs or

214   substances taken concomitantly that induce or inhibit specific isoenzymes of the CYP system

215   (e.g. rifampicin (22)). Several studies have reported a decrease in CYP1A2 (23) and estrogen

216   inhibition of CYP2C19 (24) during pregnancy, requiring a dose adjustment of certain drugs (16).

217   The AUC_{0-24} is a measure of drug exposure (10) that has been used to link dosage with

218   clinical outcomes in a recent PK/PD model in children with schistosomiasis in Uganda (25). In a

219   recent study [24] the mean PZQ AUC_{0-24} values ranged from 8.2-14.6 mg*h/L. These values are

220   higher than the PZQ AUC_{0-24} mean estimated from 60 Ugandan children 3 to 9 year of age with

221   intestinal schistosomiasis (2.71 mg*h/L) (25). The relevance of this observation depends on

222   whether the pharmacodynamics of PZQ against schistosomiasis in children and pregnant women

223   are comparable. While women in early pregnancy have lower AUC_{0-24} than women in late

224   pregnancy or postpartum, these values are significantly higher than children receiving

225   comparable dose for whom the efficacy of PZQ has been established. Hence in principle, there

226   does not appear to be any requirement to adjust the dosage according to the stage of

227   pregnancy. However, further studies are required to document the clinical response in pregnant

228   women with schistosomiasis.

229   There are limited studies on the partitioning of drugs into breast milk (26-29). A single

230   previous study has examined PZQ concentrations in the breast milk of healthy lactating women

231   (30). Our study provides further insights into the pharmacokinetics of PZQ in lactating women

232   and the potential implications for mass drug administration programs. Firstly, the amount of

233   drug an infant ingests depends on the concentration of drug in breast milk. This changes rapidly

234   over the initial 24 hours post-dose. The amount of drug that is ingested by an infant depends on

235   the time of feeding relative to the administration of PZQ as well as the volume of milk that is

236   consumed. Using estimates for an average concentration and volume of milk, the weight-based

237   intake of 0.037 mg/kg is significantly less than that required for therapeutic efficacy (circa 40-60

238   mg/kg). Second, there is relatively little variability in the AUC in breast milk. We observed

239   approximately a 2-fold variation in the 15 lactating women in this study and the Monte Carlo

240   simulations suggest up to 10-fold variability may be expected if a larger number of women had

241   been studied. Hence, the small amount from ingestion of breast milk is unlikely to be clinically

242   relevant. The benefits of treating lactating women to prevent them from further developing

243   schistosomiasis-related morbidity would seem to outweigh any potential risks. The PK data do

244   not support the manufacturer's suggestion to delay breastfeeding 72 hours after taking PZQ

245   (31).

246   Women have been systematically excluded both from studies and MDA efforts (14). We

247   contend that pregnant and lactating women should not be excluded from any treatment efforts

248   because of the demonstrated safety and efficacy of PZQ during gestation (12, 13). This study

249   further demonstrates there are unlikely to be clinically relevant pharmacokinetic differences in

250   pregnant and lactating women. Untreated schistosomiasis may lead to more severe disease and

251   chronic disability. For example, female genital schistosomiasis may lead to infertility and

252   disruption of a healthy reproductive life (32). Women with intestinal schistosomiasis may have

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253   worsening anemia and liver fibrosis. Early treatment with PZQ is known to mitigate these late

254   complications of schistosomiasis (33). A concern about the theoretical risks related to PZQ has

255   led to pregnant women being excluded from mass drug administration programs (5), however

256   recent trials in pregnant women and this pharmacokinetic study suggest that the withholding of

257   PZQ during pregnancy and lactation is not justified. (12, 13). Our PK results can help inform

258   future drug efficacy studies in pregnant and lactating women with schistosomiasis.

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261   METHODS

Study Protocols and Permissions

263   The study was separately approved by the ethics review board of the Research Institute

264   of Tropical Medicine in Manila, Philippines (#2010-39) and the Institutional Review Boards from

265   the Rhode Island Hospital in Providence, RI, USA (#415810), Boston University Medical Center in

266   Boston, MA, USA (#H30043) and the University of California at San Diego in San Diego, CA, USA

267   (#120559X). Informed consent was obtained from all study participants prior to enrolment.

Study site and participants

269   The study design is summarised in **Figure 1.** Eligible patients that were living in villages in

270   northeastern Leyte, the Philippines, where *S. japonicum* is endemic, were identified and

271   screened by local midwives. Patients with at least one positive stool samples for *S. japonicum*

272   were then assessed by a study obstetrician at the Remedios Trinidad Romualdez Hospital

273   (Tacloban, Leyte, Philippines). The methodology for detection of parasites in stool is described

274   elsewhere (12). Women were eligible if they met the following inclusion criteria: (1) infected

275   with *S. japonicum*; (2) aged 18 years or older; (3) otherwise healthy as established by physician

276   history, physical examination and laboratory studies; (4) had a normal obstetrical ultrasound, if

277   pregnant; and (5) provided informed consent. Post-partum women were recruited from study

278   villages; many had been considered for enrolment in the main RCT, but were beyond the

279   gestational age criteria. Eligibility criteria were the same as for pregnant women with the

280   exception of the criterion for pregnancy. Early pregnancy was defined as women in their 12-16

281   week of gestation, and late pregnancy as women in their 30 -36 weeks of gestation.

PZQ PK sampling

283   Within 4 weeks of enrolment, patients received PZQ (Schering-Plough, Kenilworth, NJ,

284   USA) in two dosages of 30 mg/kg administered approximately 3 hours apart for a total dose of 60

285   mg/kg. Women were given local foods that consisted of a carbohydrate-rich snack prior to PZQ

286   dosing, as this enhances absorption of the drug (7). After receiving PZQ, patients remained in

287   hospital for PK sampling and monitoring for adverse events. Patients were discharged

288   approximately 24 hours after the first dose. An indwelling venous catheter was placed to draw

289   blood samples for assay for praziquantel concentrations, which were collected at the following

290   times: for pregnant and post-partum women prior to PZQ dosing, 1, 2, 3 (prior to administration

291   of the 2nd dose of PZQ), 4, 5, 6, 7, 8, 9, 12, 15 and 24 hours after the first dose of PZQ.

292   For lactating women, women hand-expressed breast milk and samples were collected

293   within 15 minutes of collection of the blood samples scheduled for 3, 6, 9, 12, 15 and 24 hours

294   after the first dose of PZQ. Blood samples for toxicity monitoring (complete blood count, BUN

295   and creatinine, liver function tests) in blood samples were collected just before the first dose, 24

296   hours after the dose and at approximately 32 weeks gestation (early gestation subjects only) or

297   10-14 days after the PZQ dose (late gestation and lactating post-partum subjects). Newborns

298   were monitored for clinical signs of toxicity until 28 days after delivery for early and late

299   pregnancy subjects with the final study visit at 28 days of life with the study pediatrician at RTR

300   Hospital in Tacloban. Post-partum women were seen at RTR Hospital 2 weeks after

301   administration of study drug.

302   Venous blood was drawn and samples were spun for 15 minutes at ~5,000 x g, 20

303   degrees C in Eppendorf centrifuge. Plasma was removed and stored in two separate aliquots and

304   at -80 degrees C. Breast milk was stored at -80 degrees C. Both plasma and breast milk samples

305   were shipped on dry ice to the University of California at San Diego (UCSD) Pediatric Clinical

306   Pharmacology Laboratory where they were assayed for PZQ using high performance liquid

307   chromatography–electrospray mass spectrometry according to the methods of Bonato et al (34).

308   The lower limits of quantitation of the assay were 31.3 ng/mL for plasma and 4.3 ng/mL for

309   breast milk.

310   *Quantification and resolution of PZQ and 4-OH PZQ in plasma and breastmilk*

311   Praziquantel (PZQ) concentrations were quantified in plasma and breast milk by liquid

312   chromatography   mass   spectrometry   (LC/MS),   using   an   Agilent   liquid

313   chromatograph/autosampler interfaced with a Sciex API 4000 mass spectrometer.   Prior to

314   analysis, proteins were removed from plasma/milk samples by precipitation with acetonitrile.

315   Analytical grade PZQ was obtained from Sigma Aldrich. Separation of PZQ from other matrix

316   constituents was obtained with an isocratic HPLC mobile phase consisting of 80% methanol and

317   20% formic acid (0.1%) in water, in conjunction with a 2.1mm x 15cm MacMod Ace-5 C18

318   reverse phase column. Mass transitions 313.2>203.1 1 served as quantification ions for PZQ

319   detection, while mass transitions 313.2>174.1 served as qualification ion verification of PZQ.

320   Quantitation was by means of external calibration using Analyst 1.6.1 software, with a

321   qualification ion ratio threshold of ≤10% (deviation from expected). The dynamic range of the

322   assay was 0.1-4000 ng/mL and 2-2200 ng/mL, for plasma and breast milk, respectively. The

323   precision of the assay was <11% and <15% at all calibration concentrations, for plasma and

324   breast milk, respectively. Assay accuracy was ≤ ± 8% and ≤ ± 13% for plasma and breast milk,

325   respectively. Recovery from plasma was >91%, and >87% for breast milk, at all calibration

326   concentrations.

327

328   *Population Pharmacokinetics*

329   A population methodology was used to fit a structural model to the data. PZQ was allowed

330   to redistribute back to the maternal plasma without terminal elimination via expression of breast

331   milk. The model was structured in this way to avoid an unidentifiable solution, but also because

332   the excretion of drug in breast milk was assumed to be minimal and the equilibrium was rapid.

333   The structural model took the form:

334

335   $$XP(1) = Bolus\text{-}Ka{\times}X(1) \hspace{4cm} \text{Eq. 1}$$

336   $$XP(2) = Ka{\times}X(1)\text{-}\frac{(SCL)}{Vc}{\times}X(2) + Kpc{\times}X(3) + Kbc^{*}X(4)\text{-}Kcb^{*}X(2) \hspace{1cm} \text{Eq. 2}$$

337   $$XP(3) = Kcp{\times}X(2)\text{-}Kcp{\times}X(3) \hspace{4cm} \text{Eq. 3}$$

338   $$XP(4) = \text{-}Kbc^{*}X(4) + Kcb^{*}X(2) \hspace{4cm} \text{Eq. 4}$$

339

340   Where: XP(1), XP(2), XP(3) and XP(4) is the rate of change of PZQ mass in the gut, central

341   compartment, peripheral compartment and breast milk, respectively. Similarly, X(1), X(2), X(3)

342   and X(4) represent the mass (mg) of PZQ in the respective compartments. Bolus refers to the

343   oral administration of PZQ; SCL is the first-order clearance of PZQ from the central compartment,

344   Vc is the volume of the central compartment; Kpc, Kpc, Kcb and Kbc are the first-order

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345   intercompartmental rate constants. A lag function (not shown in the differential equations) was

346   applied between the oral administration of PZQ and the appearance of drug in the central

347   compartment.

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349   The output equations were given by:

350   Y(1)=X(1)/Vc (for the plasma concentrations)

351   Y(2)=X(4)/Vb (for the concentrations in breast milk)

352   Where Vb is the volume of breast milk compartment.

353

354   The fit of the model to the data was informed by a linear regression of observed-

355   predicted values before and after the Bayesian step, the log likelihood ratio and a normalised

356   prediction distribution error. The latter was used in place of a more traditional visual predictive

357   plot because women received different dosages of PZQ. Both the mean and median parameter

358   values were interrogated to see which measure of central tendency better described the data.

359   Weighted residuals were calculated and plotted against predicted concentrations, time and

360   assessed for normality using D'Agostino, Shapiro-Wilk and Kolmogorov-Smirnof tests   Drug

361   exposure was quantified in terms of the AUC_{0-24} as previously described by us(25).This was

362   estimated using the trapezoidal rule using Pmetrics and estimated from the Bayesian posterior

363   estimates from each study patient or from simulated patients.

364

365   *Statistical Modelling*

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367   The Bayesian estimates for clearance and AUC_{0-24} were modelled for study groups using

368   univariate analysis of variance (ANOVA). Since both Bayesian estimates for clearance and AUC_{0-24}

369   were not distributed normally, they were fitted on natural log scale. The estimated means of

370   clearance and AUC_{0-24} between individual study groups were compared in a post-hoc analysis

371   using Tukey's Test, and the reported p-values were corrected for multiple comparisons.

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471   **Table 1:** Characteristics of the patients at enrolment

	Early pregnancy N=17	Late pregnancy N=15	Post-partum N=15	All (N=47)
Weight (Kg) Mean (SD)	47.6 (8.12)	51.5 (7.08)	46.6 (7.40)	48.5 (7.69)
Height (cm) Mean (SD)	152.0 (5.45)	149.8 (5.63)	152.3 (4.56)	151.4 (5.24)
Age (Years)				
Mean (SD)	23.8 (5.98)	26.5 (6.61)	26.5 (6.65)	25.5 (6.39)
Median	21.0	25.0	24.0	24.0
Min, Max	18, 37	18, 37	20, 44	18, 44
Ethnicity N (%)				
Non-Hispanic nor Non-Latino	17 (100.0)	15 (100.0)	15 (100.0)	47 (100.0)
Hispanic or Latino	0 (0)	0 (0)	0 (0)	0 (0)
Race N (%)				
American Indian/Alaskan Native	0 (0)	0 (0)	0 (0)	0 (0)
Asian	17 (100.0)	15 (100.0)	15 (100.0)	47 (100.0)
Hawaiian/Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)
Black/African American	0 (0)	0 (0)	0 (0)	0 (0)
White	0 (0)	0 (0)	0 (0)	0 (0)
Multi-Racial	0 (0)	0 (0)	0 (0)	0 (0)
Number of prior live births N (%)				
0	6 (35.3)	4 (26.7)	1 (6.7)	11 (23.4)
1 - 5	10 (58.8)	9 (60.0)	11 (73.3)	30 (63.8)
6 - 10	1 (5.9)	2 (13.3)	3 (20.0)	6 (12.8)
> 10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Current smoking status N (%)				
No	17 (100.0)	15 (100.0)	13 (86.7)	45 (95.7)
Yes	0 (0.0)	0 (0.0)	2 (13.3)	2 (4.3)
Current alcohol consumption N (%)				
No	5 (29.4)	2 (13.3)	2 (13.3)	9 (19.1)
Yes	12 (70.6)	13 (86.7)	13 (86.7)	38 (80.9)
Intensity of *S. japonicum* infection N (%)				
Low (<100 eggs per gram of stool)	16 (94)	15 (100)	15 (100)	46 (97.8)

Moderate (100-399 eggs per gram of stool)	1 (6)	0 (0)	0 (0)	1 (2.2)
Heavy (≥ 400 eggs per gram of stool)	0 (0)	0 (0)	0 (0)	0 (0)

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501   **Table 2.** Parameter Values from the population PK model

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Parameter (Units)[a]	Mean	Median	SD	CV%
Ka (h^{-1})	2.012	0.395	4.301	213.750
SCL/F (L/h)	324.075	277.447	175.373	54.115
Vc/F (L)	183.006	142.618	93.211	50.933
Kcp (h^{-1})	19.313	18.941	10.167	52.644
Kpc (h^{-1})	15.816	13.996	9.447	59.733
Kcb (h^{-1})	18.750	19.301	9.387	50.067
Kbc (h^{-1})	17.816	17.077	7.845	44.031
Vb/F (L)	612.130	563.802	395.661	64.637
Lag (h)	0.772	0.868	0.233	30.202

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505   [a]Note: Parameters are as follows: Ka is the first-order absorption constant; SCL/F is the apparent
506   clearance; Vc/F and Vb/F are the apparent volumes of the central and breast compartments,
507   respectively; Kcp, Kpc, Kbc and Kcb are the first-order intercompartmental rate constants; Lag is
508   the delay between drug administration and the appearance of drug in the central compartment.

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511   **Table 3.** Adverse Events by severity and cohort

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Reactogenicity	Cohort 1 (N=17)				Cohort 2 (N=15)				Cohort 3 (N=15)			
	Severity				Severity				Severity			
	None n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	None n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	None n (%)	Mild n (%)	Moderate n (%)	Severe n (%)
Fever	16 (94.1)	0 (0)	1 (5.9)	0 (0)	13 (86.7)	2 (13.3)	0 (0)	0 (0)	13 (86.7)	1 (6.7)	1 (6.7)	0 (0)
Headache	9 (52.9)	6 (35.3)	2 (11.8)	0 (0)	9 (60.0)	5 (33.3)	1 (6.7)	0 (0)	6 (40.0)	8 (53.3)	0 (0)	1 (6.7)
Malaise	11 (64.7)	5 (29.4)	1 (5.9)	0 (0)	13 (86.7)	2 (13.3)	0 (0)	0 (0)	9 (60.0)	5 (33.3)	0 (0)	1 (6.7)
Abdominal Pain	13 (76.5)	2 (11.8)	1 (5.9)	1 (5.9)	11 (73.3)	4 (26.7)	0 (0)	0 (0)	10 (66.7)	4 (26.7)	1 (6.7)	0 (0)
Nausea	5 (29.4)	11 (64.7)	1 (5.9)	0 (0)	9 (60.0)	6 (40.0)	0 (0)	0 (0)	8 (53.3)	6 (40.0)	1 (6.7)	0 (0)
Vomiting	11 (64.7)	5 (29.4)	1 (5.9)	0 (0)	12 (80.0)	3 (20.0)	0 (0)	0 (0)	14 (93.3)	1 (6.7)	0 (0)	0 (0)
Shortness of Breath	16 (94.1)	1 (5.9)	0 (0)	0 (0)	14 (93.3)	1 (6.7)	0 (0)	0 (0)	15 (100.0)	0 (0)	0 (0)	0 (0)
Dizziness	10 (58.8)	7 (41.2)	0 (0)	0 (0)	13 (86.7)	2 (13.3)	0 (0)	0 (0)	12 (80.0)	3 (20.0)	0 (0)	0 (0)
Rashes	15 (88.2)	2 (11.8)	0 (0)	0 (0)	13 (86.7)	2 (13.3)	0 (0)	0 (0)	13 (86.7)	1 (6.7)	1 (6.7)	0 (0)
Urticaria	16 (94.1)	1 (5.9)	0 (0)	0 (0)	15 (100.0)	0 (0)	0 (0)	0 (0)	14 (93.3)	1 (6.7)	0 (0)	0 (0)
Bloody Stools	17 (100.0)	0 (0)	0 (0)	0 (0)	14 (93.3)	1 (6.7)	0 (0)	0 (0)	14 (93.3)	1 (6.7)	0 (0)	0 (0)
Any Symptoms	4 (23.5)	9 (52.9)	3 (17.6)	1 (5.9)	5 (33.3)	9 (60.0)	1 (6.7)	0 (0)	3 (20.0)	8 (53.3)	3 (20.0)	1 (6.7)

513   N=Number of subjects in population; n=Number of subjects with at least one occurrence of an
514   adverse event in the specified category.

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518 Figures

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537 **Figure 1:** Study flow design

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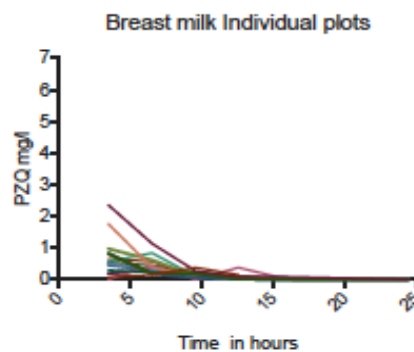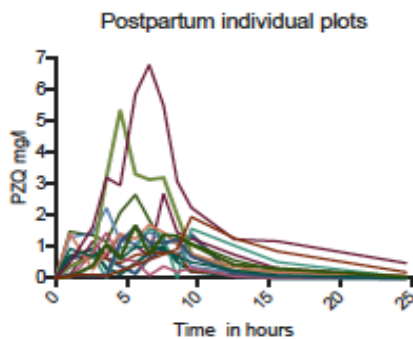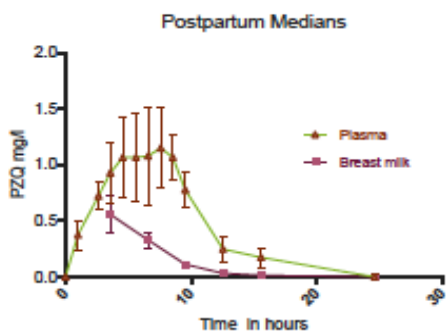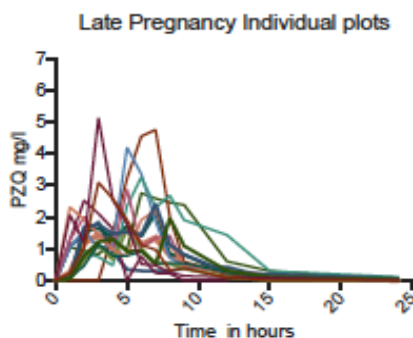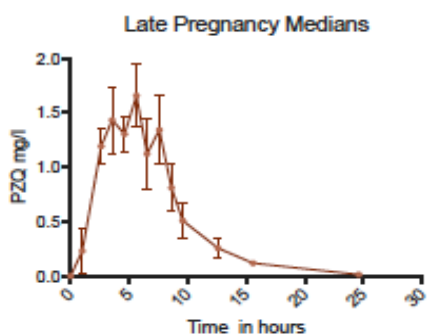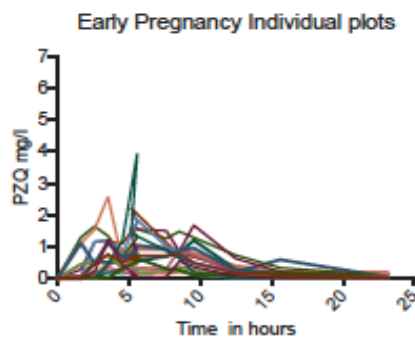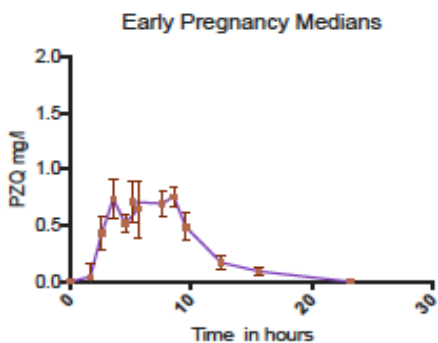
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Figure 2: Median and individual PZQ concentration time profiles.

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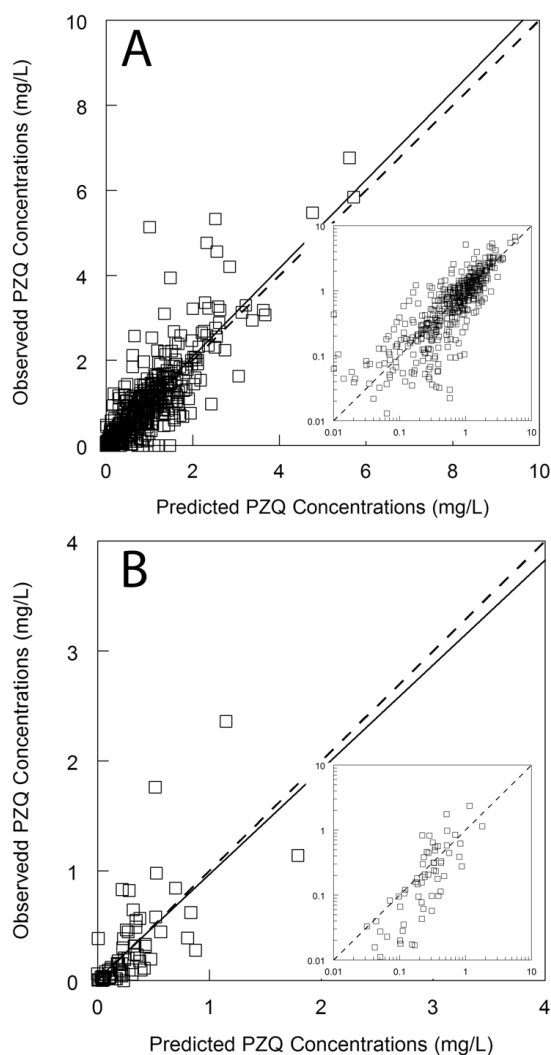
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Figure 3. The observed-predicted plots for the PZQ concentrations in plasma (Panel A) and breast
milk (Panel B) after the Bayesian step. The median parameter values for each patient have been
used. The observed-predicted data is plotted on a log-log plot for both outputs and is shown in
the inserts.　　The regression line for plasma in Panel A is given by Observed =
0.016+1.04*Predicted; r^2=0.604.　The regression line for breast milk in Panel B is given by
Observed = 0.015+0.953*Predicted; r^2=0.468.

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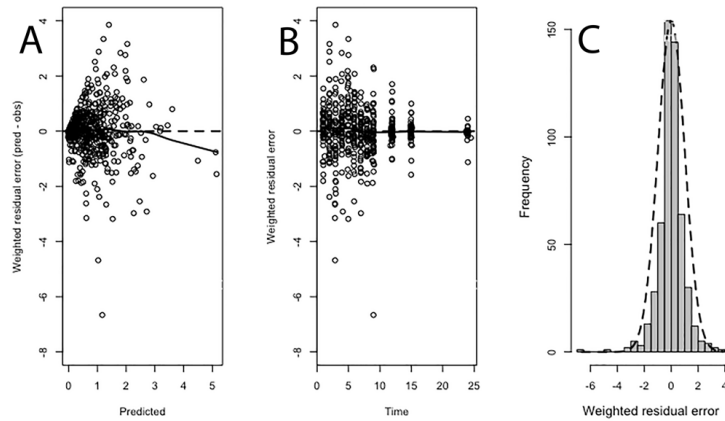
Figure 4. Residual plots for plasma concentrations. The average residuals did not vary from zero; p=0.88 for weighted residual error versus Predicted concentrations (far left panel) and for weighted residual error versus Time (middle panel). The solid line Panel A and Panel B is the loess regression. The residuals were normally distributed as assessed using D'Agostino, Shapiro-Wilk and Kolmogorov-Smirnof tests (far right panel). (p>0.05)
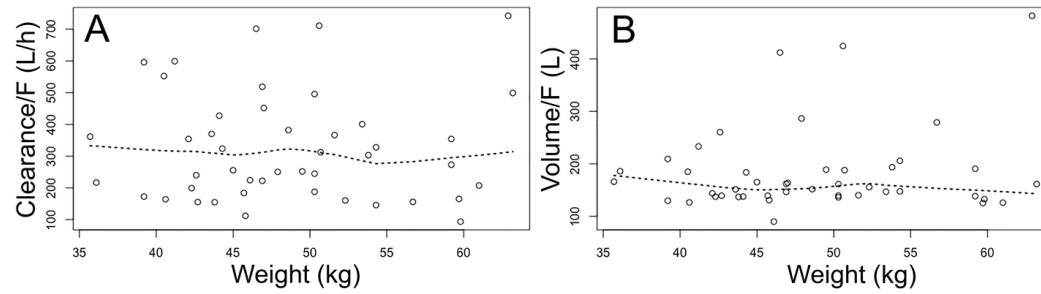
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Figure 5. The relationship between Weight and Clearance/F (Panel A) and Weight and Volume/F (Panel B). The volume is the volume of the central compartment. Neither relationship is statistically significant with r=0.03 (p=0.84) and 0.16 (p=0.29) for clearance/F and volume/F, respectively. The broken line is the loess line.
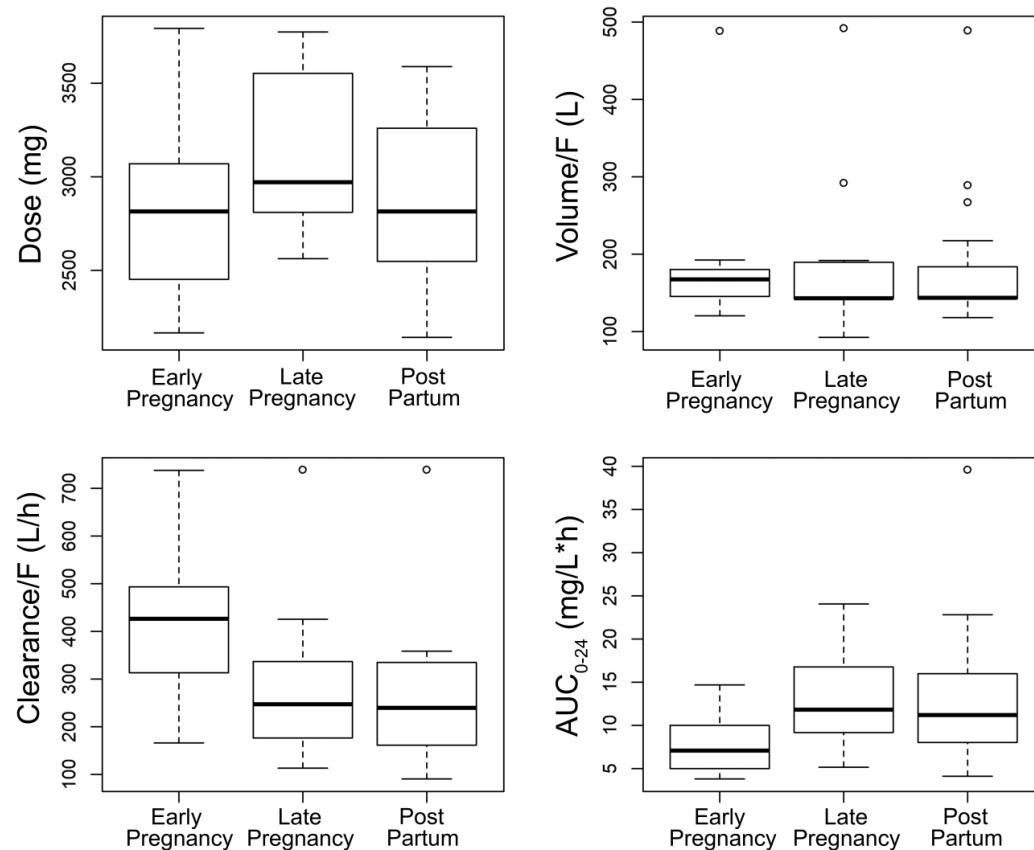
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Figure 6. Box plots showing the relationship between various stages of pregnancy and dose (Panel A), Volume of the central compartment/F (Panel B), Clearance/F (Panel C) and the area under the concentration-time curve (AUC$_{0-24}$) in Panel D. There was no relationship between the stage and pregnancy and the absolute dose (mg) and volume/F (p=0.2072 and 0.626, respectively). Women in the early pregnancy group have a higher clearance/F than other women (p=0.016 for all groups) and a lower AUC$_{0-24}$ (p=0.01 for all groups).
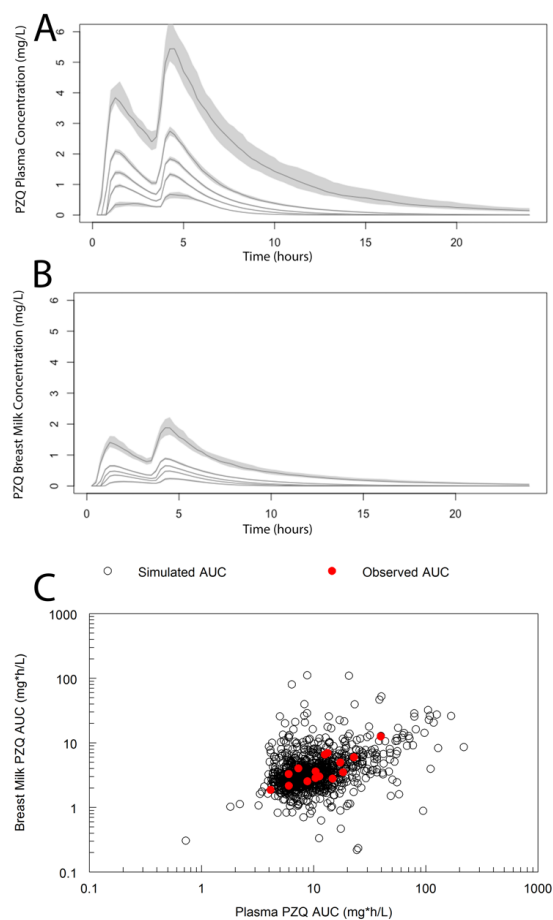
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Figure 7. Monte Carlo simulations showing the drug exposure in plasma (Panel A), breast milk (Panel B) from 1,000 lactating women. Each line represents the 5th, 25th, 50th, 75th and 95th centiles and the grey representing the confidence interval around each centile. In Panel C the AUC_{0-24} in plasma versus breast milk in each simulated woman is shown with black open circles. The AUC_{0-24} from each of the 15 patients in the study is shown with a solid red circle.

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