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Pharmacokinetics, Safety, and Biologic Effects of Azithromycin in Extremely Preterm Infants at Risk for *Ureaplasma* Colonization and Bronchopulmonary Dysplasia

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Abstract

Ureaplasma spp. respiratory tract colonization is a significant risk factor for bronchopulmonary dysplasia (BPD), a chronic lung disorder in preterm infants. As an initial step preparatory to future clinical trials to evaluate the clinical efficacy of azithromycin to prevent BPD, we characterized the pharmacokinetics, safety, and biological effects of a single intravenous dose of azithromycin (10 mg/kg) in preterm neonates (n=12) 24–28 weeks gestation at risk for *Ureaplasma* infection and BPD. A two-compartment structural model with the clearance and volume of peripheral compartment (V2) allometrically scaled on body weight (WT) best described the pharmacokinetics of azithromycin in preterm neonates. The estimated parameters were: clearance [0.18 L/h × WT(Kg)$^{0.75}$], intercompartmental clearance [1.0 L/h], volume of distribution of central compartment [0.93 L] and V2 [14.2 L × WT(Kg)]. There were no serious adverse events attributed to azithromycin. A single dose of azithromycin did not suppress inflammatory cytokines

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Conflict of Interest/Disclosure

The authors declare no conflict of interest that may interfere with data interpretation. Dr. Ahmed Othman was an employee of University of Maryland at the time of the design and initiation of the study and is currently an employee of Abbott Laboratories.

or myeloperoxidase activity in tracheal aspirates. Our results demonstrated the safety of azithromycin and developed a PK model that is useful for future simulation-based clinical trials for eradicating *Ureaplasma* and preventing BPD in preterm neonates.

Keywords

azithromycin; *Ureaplasma*; prematurity; pharmacokinetics; Bronchopulmonary Dysplasia

Introduction

Accumulating data suggest that respiratory colonization with the genital mycoplasma species, *Ureaplasma parvum* and *Ureaplasma urealyticum* is a risk factor for the development of the chronic lung disorder bronchopulmonary dysplasia (BPD) in preterm neonates[1–7]. Human and experimental studies confirmed that *Ureaplasma* spp. cause an augmented and dysregulated inflammatory response in the developing lung that contributes to arrested alveolarization and angiogenesis, chronic inflammation, and interstitial fibrosis that characterize the pathologic findings of BPD[8–16]. Although, early detection and eradication of *Ureaplasma* may prevent or ameliorate the severity of BPD in infected preterm neonates, there is currently no consensus among neonatologists concerning appropriate therapy. Development of effective therapies to eradicate *Ureaplasma* from the respiratory tract and/or prevent infection-mediated lung inflammation is critically important for the prevention of BPD.

Despite *in vitro* susceptibility of *Ureaplasma* species to erythromycin (a macrolide antibiotic)[17], clinical trials involving erythromycin therapy in *Ureaplasma* colonized preterm infants have failed to demonstrate any efficacy to prevent BPD[18, 19] or to eradicate respiratory tract colonization[20]. The 14-membered macrolides that are derivatives of erythromycin and the related 15-member azalides (e.g., azithromycin) are promising candidates for modulating the inflammatory effects associated with *Ureaplasma* infection. They affect neutrophil function (e.g. chemotaxis, cell adhesion, oxidative burst and phagocytosis), cytokine release (e.g., IL-1β, IL-8, IL-6)[21] and nitric oxide production *in vitro*[22]. The macrolide antibiotics may exert these immunomodulatory effects in the setting of infection, and may occur independently of a direct bactericidal effect[23]. Azithromycin inhibits neutrophil influx and chemoattractant/cytokine release in murine lung noninfectious[24, 25], as well as pneumonia[26, 27] injury models. Pharmacokinetic studies in mice[28] and humans have shown that azithromycin is preferentially concentrated in pulmonary epithelial lining fluid and alveolar macrophages[29, 30]. Since neutrophil recruitment and activation has been implicated in BPD pathogenesis[31–33], the experimental effects observed with azithromycin *in vitro* and *in vivo* indicate that this drug may be beneficial in the treatment of *Ureaplasma* infection and the prevention of BPD in preterm infants.

Azithromycin exhibits bacteriostatic activity with higher potency than erythromycin against *Ureaplasma* isolates *in vitro*[17, 34, 35] and suppresses cytokine release in cultured tracheal aspirate cells from preterm infants[36]. We hypothesize that intravenous azithromycin therapy

will prevent BPD in *Ureaplasma*-colonized preterm infants by accelerating pathogen clearance and/or down-regulating the pulmonary inflammatory response. Although the safety and pharmacokinetics of intravenous azithromycin have been evaluated in children >6 months of age[37], there are no safety or pharmacokinetic data in preterm neonates from which appropriate age-based dosing can be derived. In order to design appropriate randomized clinical trials to evaluate clinical efficacy of azithromycin to eradicate *Ureaplasma* and/or prevent BPD, we conducted a pilot study to evaluate the safety and pharmacokinetics of a single intravenous dose of azithromycin in mechanically ventilated, preterm neonates at high risk for *Ureaplasma* respiratory tract colonization and BPD development. In addition, we evaluated pulmonary inflammatory cytokines and neutrophil myeloperoxidase activity as potential azithromycin response biomarkers and the feasibility of a central reference laboratory for *Ureaplasma* cultures and PCR genotyping for planning for future randomized clinical trials.

Methods

Study design and infant enrollment

This study was a single-dose (10 mg/kg), pharmacokinetic study of i.v. azithromycin in mechanically ventilated preterm neonates born between 24–28 wk of gestation conducted from February to December, 2008. This dose was selected since it was previously reported to be safe in older children[37]. The Institutional Review Boards of the participating sites (University of Maryland, School of Medicine and the University of Virginia, School of Medicine) approved the study protocol. Neonatal intensive care unit admissions with gestational age 24wk 0d to 28wk 6d were screened for study eligibility. Inclusion criteria were: 1) appropriate weight for gestational age as determined by best obstetrical estimates[38]; 2) intubation and mechanical ventilation for any duration during the first 48 hours after birth; 3) presence of an indwelling intravenous line for drug administration; and 4) presence of an indwelling clinically indicated arterial line for blood sampling. Exclusion criteria were: 1) non-viability or planned withdrawal of life support; 2) major lethal congenital anomalies; 3) delivery for maternal indications (low risk of *Ureaplasma* colonization); 4) hypotension defined as mean arterial blood pressure < the gestational age in wks[39] that persists for >6 h during the first 24 h of life; 5) electrocardiogram QT interval corrected for heart rate (Qtc) ≥450 ms; 6) significant renal impairment (urine output <0.5 mL/kg/hr); 7) significant hepatic impairment (liver function tests >2x upper limit normal range); 8) exposure to another macrolide antibiotic; 9) clinically suspected systemic *Ureaplasma* spp. infection or other confirmed systemic bacterial/viral infection; 10) maternal receipt of a macrolide antibiotic within 7 days prior to delivery and 11) participation in other clinical trials involving investigational or marketed products. Infants surviving to 36 wks post-menstrual age (PMA) were assessed for physiologic BPD. A timed oxygen reduction test was conducted for those infants on low fractional inspired oxygen[40]. Infants who could be weaned off of supplemental oxygen and remain on room air for 30 minutes were classified as non-BPD infants while those who were receiving positive pressure support at 36 wks PMA or failed the timed oxygen reduction test were classified as BPD infants.

Pharmacokinetic study design

After baseline laboratory tests and pre-dose cultures had been obtained, infants received an azithromycin dose of 10 mg/kg at a concentration of 2 mg/mL as a single intravenous infusion over 1 h using a functioning intravenous line. Blood draws were performed using an existing indwelling arterial catheter inserted for clinical indications. Sparse sampling technique was employed. Six total samples (0.5 mL each) were collected between 0–1, 1–4, 6–8, 24–48, 48–96, and 96–144 h post-dose with the exact time of each sample recorded. The sampling windows were selected to allow precise characterization of the distribution and the elimination phases of azithromycin and are based on the results of a previously published report in older infants and children[37]. The blood samples were collected into tubes containing anticoagulant EDTA, centrifuged, and the plasma aspirated and frozen at –80°C until analyzed for azithromycin concentrations using a validated LC/MS/MS detection method[41].

Pharmacokinetic data analysis

Plasma concentration data were modeled using the non-linear mixed-effects modeling software NONMEM (version VI) (ICON Development Solutions, Ellicott City, MD). The first-order conditional estimation method with interaction was used throughout the modeling procedure. A two compartment structural model (ADVAN3 TRANS4 NONMEM subroutine) was used for the analysis. The model selection was based on evaluation of the objective function (OF) value, pharmacokinetic parameter estimates and their relative standard errors, physiologic plausibility of the parameter estimates, and inspection of goodness-of-fit plots. The Likelihood Ratio Test was used for comparing rival hierarchical models where a decrease in OF (−2 log likelihood) of 6.6 points was necessary to consider the improvement in model performance statistically significant at p =0.01 and 1 degree of freedom[42]. An exponential error model was used to describe the inter-individual variability in CL and V2 as follows:

$$P_i = TVP \exp(\eta_i)$$

Where η_i is the proportional difference between the hypothetical true parameter estimate of the i^{th} subject (P_i) and the typical population parameter value (TVP) and is assumed to be normally distributed with a mean of 0 and a variance of ω^2. The residual error (which includes model misspecification, intra-subject variability as well as errors in dosing, sampling times and sample analysis) was described using a proportional error model as follows:

$$Y_{obs} = Y_{pred} * (1+\varepsilon)$$

Where Y_{obs} is the observed plasma concentration, Y_{pred} is the model predicted plasma concentration and ε is a normally distributed parameter with a mean of 0 and variance of σ^2. The CL and V2 were allometrically scaled on body weight in the final model as follows

$$TVCL_i = TVCL \cdot (WT_i)^{0.75}$$
$$TVVp_i = TVVp \cdot (WT_i)$$

Were $TVCL_i$ and $TVVp_i$ are the typical values for clearance and volume of peripheral compartment for a subject i with a certain body weight (WT_i). Such scaling resulted in statistically significant (p <0.01) drop in the OF (9 and 10 points drop in OF for scaling CL and V2, respectively, on body weight). The final pharmacokinetic model was then used to simulate different regimens of i.v. azithromycin dosing (combinations of different doses and treatment durations) using Pharsight® Trial Simulator software (version 2.2.1, Pharsight Corporation, Mountain View, CA). For each regimen, 1000 neonates were simulated and the median and 90 % prediction intervals (calculated from the 5th and 95th percentiles) for the simulated azithromycin concentrations were determined.

Respiratory specimen collection

Tracheal aspirates (TA) for *Ureaplasma* culture and cytokines were obtained at the time of clinically indicated endotracheal tube suctioning. Two tracheal aspirate samples were obtained 2–6 h apart pre-dose and, in infants who remain intubated additional aspirates were obtained at 2, 4–5 and 21 d post-dose. A single nasopharyngeal (NP) culture was obtained pre-dose and additional NP samples were obtained using the same schedule if the infant was extubated. For tracheal aspirate sampling, the first instillation of saline was collected for cytokine analysis and second instillation for culture. Lung lavage was performed by a standardized technique as previously described[10]. Culture specimens were immediately inoculated in 10B urea broth[43] and kept on ice until processed.

Microbiological Methods

***Ureaplasma* culture and antimicrobial susceptibility testing—**Screening cultures for *Ureaplasma* spp. on specimens of endotracheal or nasal secretions obtained as described above were performed on site at the University of Maryland, Baltimore (UMB). For each specimen, 4 ten-fold serial dilutions were performed in 10B broth and incubated under atmospheric conditions at 37°C. Cultures were observed for up to 72 h for broth color change from yellow to pink without turbidity, indicating pH change due to urease activity. Aliquots of all original TA and NP cultures from both sites and all positive cultures obtained at UMB were immediately frozen at −80°C and batch-shipped to the Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham (UAB) for definitive culture and organism identification. Colonies of *Ureaplasma* spp. were identified presumptively by their characteristic granular brown appearance on A8 agar in the presence of the $CaCl_2$ indicator[43]. *Ureaplasma* isolates from all culture-positive neonates were tested by microbroth dilution as described previously[44] to determine their minimal inhibitory concentrations (MICs) for azithromycin. Treatment failure was defined as a confirmed positive culture at any time point post-dose.

***Ureaplasma* Genotyping by Real-time PCR Assay—**In addition to culture, all clinical specimens were tested directly for the presence of *U. urealyticum* and *U. parvum* by

a real-time PCR assay. Individual *Ureaplasma* isolates obtained by culture were also tested by PCR to determine their species designations. Genomic DNA from clinical specimens and the *Ureaplasma* isolates was extracted by the proteinase K method as described previously[45]. A multiplex real-time PCR assay was employed to detect and differentiate the two *Ureaplasma* species simultaneously using the Roche LightCycler 2.0 (Roche Diagnostics, Indianapolis, IN)[46]. The *U. parvum* primer/probe set anneals to the 477 bp UP063 gene (NP_077893), which encodes a conserved hypothetical protein that is identical in all 4 *U. parvum* serovars. The *U. urealyticum* primer/probe set anneals to a 15,072 bp open reading frame (ORF) that is almost perfectly (>99.97%) conserved in all 10 *U. urealyticum* serovars (serovar 10 Genbank ID ACI59931.1).

Cytokine and Myeloperoxidase analysis

Lung lavage fluid for cytokines was placed on ice, immediately centrifuged at 4000×g to pellet cells, the supernatant aliquoted and frozen at –80°C for later cytokine analysis by the UMB Cytokine Core Laboratory. IL-1β, IL-8, and IL-6 were measured by Luminex™ multianalyte immunoassay using reagents from Upstate Biotechnology. The cell pellet was resuspended in 100 μl lysis buffer (50 mM potassium phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide (HTAB)), then frozen at −80°C for later myeloperoxidase assay (MPO). MPO activity was assayed in cell lysates as previously described[47, 48].

Safety evaluation

Safety and tolerance evaluation in each infant included vital signs, frequency of apnea and bradycardia, cardiac rhythm, pulse oximetry, clinical laboratory testing and adverse events. Infants were assessed for morbidities associated with prematurity, concomitant medications, and adverse events on study days 3, 7 (± 3 d), 14 (± 3 d) and every 2 wks thereafter until discharge or transfer. Clinically relevant AE that were specifically evaluated included: local site reaction including phlebitis, gastrointestinal abnormalities (abdominal distension, gastric aspirates, emesis, heme-positive stools), intestinal obstruction including radiographic-confirmed pyloric stenosis, culture-proven sepsis with other bacterial or nonbacterial pathogens, necrotizing enterocolitis (NEC), and cardiac arrhythmias. A hearing screening test as part of clinical care was performed before discharge. Serial clinical laboratory tests (complete blood count with manual differential, electrolytes, liver function, and renal function) were performed pre- and post-dose.

Results

Clinical outcomes

Out of sixty-nine potential infants 24wk 0d to 28wk 6d gestation screened, 41 infants were eligible for the study. Parental consent was obtained for 14 infants, 19 refused consent, and 8 were not approached within the enrollment time window. Plasma samples were available from twelve subjects only since one subject was withdrawn prior to dosing secondary to clinical deterioration, and one subject was not included in the PK analysis secondary to receipt of an additional dose of azithromycin in violation of the protocol. Since pre-dose cultures were successfully obtained and processed for all 14 enrolled subjects and all

subjects were followed for safety data and were assessed for BPD outcome, demographic data for all subjects are included in Table 1. The postnatal age at time of study drug dosing was 47 ± 28 h (mean±SD). As summarized in Table 2, three *Ureaplasma*-positive infants died (including the infant withdrawn prior to receiving study drug), while all *Ureaplasma*-negative infants survived. Sixty percent of surviving *Ureaplasma*-positive infants were classified as physiologic BPD compared to 17% of *Ureaplasma*-negative infants (Table 2). Overall, 75% of *Ureaplasma*-positive infants either died or developed BPD (Table 2).

Pharmacokinetics of azithromycin in preterm neonates

Data from 12 infants were included in the pharmacokinetic (PK) analysis. Each infant contributed 6 plasma samples collected in pre-specified intervals over 144 h post-dose. A population approach using NONMEM was used to analyze the relatively sparse PK data. Such approach is best suited for analyzing PK data derived from designs with sparse sampling[42]. The estimated population pharmacokinetic parameters of azithromycin in preterm infants are presented in Table 3. The structural model parameters were estimated with good precision. The clearance (CL) and the volume of the peripheral compartment (V2) were allometrically scaled on body weight (using an exponent of 0.75 for CL and 1 for V2) in the final model[49]. Using body weight as a covariate for CL and V2 accounted for ∼ 38 and 43%, respectively, of the initially estimated inter-subject variability in these parameters. The homogeneous nature of the dataset (narrow range of age) and the small number of neonates in the different categories of other covariates (e.g., sex and race) precluded robust examination of the influence of these covariates on the PK of azithromycin. Based on the final model parameters, the typical value for the elimination half-life ($t_{1/2}$) is estimated to be 58 hours for a neonate weighing 1 kg. Goodness-of-fit plots for the final population PK model depicted in Figure 1 indicate that the model described the data well with no systematic bias. It is noteworthy that there was large variability in the plasma concentrations at the first sampling time. Sampling errors may have contributed to such variability when the plasma levels were declining rapidly with time. Figure 2 represents the observed and the model predicted plasma concentrations for each neonate (n=12) based on the empirical Bayesian estimates of the individual PK parameters. Non compartmental analysis of the data (details not shown) indicated that the harmonic mean $t_{1/2}$ is 61 hours while the mean AUC_{24} and AUC_{inf} were 17 and 56 μg.h/mL, respectively. Assuming 30% plasma-protein binding of azithromycin[50], the AUC_{24} for unbound fraction of azithromycin ($AUC_{24(u)}$) is ∼12 μg.h/mL. Neither single 10 mg/kg/day × 1 day (Figure 2, observed) nor multiple 10 mg/kg/day × 3 days (Figure 3A, simulated) azithromycin dosage regimens were adequate for maintaining azithromycin plasma concentrations above the MIC_{50} (1 μg/mL) derived from *Ureaplasma* isolates obtained from 25 neonates who received care at the University of Alabama at Birmingham. However, a dosage regimen of 20 mg/kg/day × 3 days i.v. azithromycin seems sufficient to maintain azithromycin plasma levels above the MIC_{50} (Figure 3B, simulated).

Ureaplasma culture and polymerase chain reaction (PCR) results

Tracheal aspirate and nasopharyngeal samples were obtained pre-dose (day 0) and on study days 2, 4–5, and 21 of age for both *Ureaplasma* culture and PCR analysis. Prior to azithromycin dosing, eight of the fourteen enrolled infants (57%) were both *Ureaplasma*

culture- and PCR-positive. Screening cultures performed at the University of Maryland, Baltimore (UMB) and confirmative cultures performed at the University of Alabama at Birmingham (UAB) agreed in all 8 positive infants. *U. parvum* alone was the most common species isolated from culture-positive infants (n=5, 63%). *U. urealyticum* alone was isolated from 1 culture-positive infant (13%) and both species were isolated from 2 infants (25%). Of the seven subjects who were culture-positive pre-dose and completed the study, 3 had at least one positive culture post-dose and were considered treatment failures (43%).

Azithromycin effects on indices of inflammation

Analyses of TAs pre- and post-dose for concentrations of pro-inflammatory cytokines (IL-1β), chemokines (IL-8), counter-regulatory cytokines (IL-6) and neutrophil myeloperoxidase (MPO) activity revealed a trend towards higher IL-6, IL-8, and IL-1β concentrations in TAs pre-dose in the azithromycin-treated *Ureaplasma*–positive infants compared to concentrations pre-dose in *Ureaplasma*-negative infants. However, a single 10 mg/kg dose of azithromycin did not significantly ($p > 0.05$) suppress the TA cytokines or MPO of the treated neonates who remained intubated at later time points (Table 4).

Safety and serious adverse events

All infants were monitored until discharge for adverse events and serious adverse events (SAE). The SAEs observed were common morbidities of extreme prematurity and none was assessed as being related to azithromycin exposure. The SAEs were: a) one bilateral Grade 3 intraventricular hemorrhage (IVH) complicated by posthemorrhagic hydrocephalus treated with ventriculo-peritoneal shunt placement, b) one pneumomediastinum treated with increased ventilator support with high frequency oscillation, c) one worsening Respiratory Distress Syndrome treated with high frequency oscillator ventilation and nitric oxide administration, d) one event of direct hyperbilirubinemia one day post-dose that returned to baseline within 24 h, and e) two events of rapid progression of intestinal necrotizing enterocolitis (NEC). Both NEC events were followed by life support withdrawal and death. One infant failed the hearing screening test.

Discussion

To the best of our knowledge, this is the first study to evaluate azithromycin pharmacokinetics in preterm neonates. Azithromycin pharmacokinetics in this preterm sample followed bi-exponential disposition with a rapid initial decline in the plasma levels followed by a slower elimination phase (Figure 2). A population model for azithromycin pharmacokinetics in preterm neonates was developed using NONMEM. Incorporating body weight as a covariate resulted in statistically significant improvement in the model fit ($p < 0.01$) and reduced the estimated random inter-subject variability in both CL and V2 (Table 3). The results from the present study indicate that preterm neonates have reduced azithromycin clearance and increased volume of distribution compared to older children[37, 51], with considerable inter-subject variability (Table 3). The estimated clearance of azithromycin in the present study was 0.18 L/h for a typical preterm neonate weighing 1 kg. In a previous pharmacokinetic study involving 32 older children (0.5–16 yr) who received a single 10 mg/kg intravenous dose, azithromycin clearance was estimated to be

0.98 L/h/kg[37]. The reduced clearance of azithromycin in preterm neonates may be attributed to the immature or deficient biliary excretion pathways in preterm neonates relative to older children, which is the major route of azithromycin elimination[52]. Consistently, the systemic exposure of azithromycin was ∼ 4-fold greater in preterm neonates than previously reported in older children (AUC_{inf} = 56 μg.h/mL in preterm neonates versus 15 μg.h/mL for 0.5 to 2 year old children[37] at the same 10 mg/kg i.v. dose level). A wide range of azithromycin half-life values in older children (0.5–16 yr) was reported (approximately 26 to 83 h)[37, 51]. In our study, based on the final model, the estimated half-life of azithromycin in preterm neonates is approximately 58 hours for a typical 1 kg neonate.

For effective *Ureaplasma* eradication, the plasma concentration of free unbound azithromycin must be maintained above the minimum inhibitory concentration that is required to inhibit 50% (MIC_{50}) of *Ureaplasma*. Although the MIC_{50} of azithromycin against *Ureaplasma* is determined *in vitro*, it provides target unbound drug concentrations to be reached/exceeded for desired clinical outcome. The MIC_{50} and MIC_{90} of azithromycin against neonatal *Ureaplasma* isolates at the University of Alabama at Birmingham are 1 and 4 μg/mL, respectively, and the MIC range from the isolates obtained from the 8 tested neonates in this study is 0.5 – 2 μg/mL. In the current study, a rapid immediate decline of azithromycin plasma concentrations below the MIC_{50} was observed (Figure 2) which may explain the 43% treatment failure rate of a single i.v. dose of azithromycin to eradicate *Ureaplasma* in culture-positive study subjects. The long elimination half-life of azithromycin (∼58 hr) enables short course azithromycin administration to be clinically effective[53]. Indeed, the recommended dosing duration of azithromycin in children and adults is 3–5 days[51, 54–56]. During this time interval, steady-state azithromycin plasma levels are not achieved since treatment for 5 half-lives (∼12 days) is required for steady state azithromycin plasma levels to be achieved. Therefore, we conducted our simulation analysis for not more than a 3-day period which indicated that even multiple dose administration of 10 mg/kg/day × 3 days azithromycin would be inadequate to maintain azithromycin plasma concentrations above the MIC_{50}. Hence, we predict that multiple dose administration of 10 mg/kg will be insufficient for eradicating *Ureaplasma*. On the other hand, a dosage regimen of 20 mg/kg/day × 3 days would be sufficient to maintain azithromycin plasma concentration above the MIC_{50} as demonstrated by our simulation analysis (Figure 3B).

The pharmacodynamic parameter that is a good predictor for achieving azithromycin efficacy is the ratio of $AUC_{24(u)}/MIC_{90}$ [50]. The optimal $AUC_{24(u)}/MIC_{90}$ ratios for azithromycin to eradicate *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, and Moraxella catarrhalis* were reported to be 0.5, 0.9, 14.8, 14.8, and 14.8 h, respectively[50] however, the optimal $AUC_{24(u)}/MIC_{90}$ for azithromycin to effectively eradicate *Ureaplasma* is still unknown. Based on our calculated $AUC_{24(u)}$ (12 μg.h/mL) for azithromycin in the preterm sample and the MIC_{90} of 4 μg/mL, we predict that this ratio needs to be significantly higher than 3 h. This can be achieved either by accelerated azithromycin dosing or “front-loading” azithromycin (administering all intended therapeutic doses as one single bolus dose) to increase the 24 h systemic exposure of unbound azithromycin ($AUC_{24(u)}$) as demonstrated in a previous preclinical study[57].

Azithromycin has unique pharmacokinetic characteristics. Once administered, very little of the administered dose resides in the plasma and the vast majority of azithromycin accumulates intracellularly leading to a prolonged elimination $t_{1/2}$ and extended mean residence time (MRT). These characteristics favor administering higher dosage regimens of azithromycin, a strategy that is commonly used for the management of acute otitis media in older children (10 mg/kg/day × 3 days or 30 mg/kg as single dose). In the current study with sparse information about the pharmacokinetics, safety and toxicity of azithromycin in preterm neonates, we would not have found it acceptable to evaluate the effects of a higher dosage regimen without first assuring the safety in this population. As such, an azithromycin dose previously evaluated in older children was used[37]. Infants in this study were closely monitored for signs of intolerance to azithromycin. No SAEs attributed to the drug were observed following single dose administration. Single (10 mg/kg/day) and multiple (10 mg/kg/day × 3 days) dosage regimens of azithromycin were deemed safe for older children and no azithromycin-related SAEs were reported[37, 55].

In the current study, 57% of the mechanically ventilated preterm infants were both *Ureaplasma* culture and PCR positive pre-dose. Seventy-five percent of *Ureaplasma*-positive infants died or developed BPD while only seventeen percent of *Ureaplasma*-negative infants developed BPD and none died, highlighting the adverse outcomes in *Ureaplasma*-positive infants (Table 2). The dosage regimen used in this study (10 mg/kg/day) is likely insufficient against *Ureaplasma*, as three of seven *Ureaplasma*-positive infants remained culture positive post-dosing. Likewise, this dosage regimen was ineffective in suppressing the levels of inflammatory cytokines or the MPO activity in the lungs of these infants. In a single center RCT pilot study of 43 subjects, azithromycin (10 mg/kg/day × 7 days followed by 5 mg/kg/day up to 6 wks) did not reduce the incidence of BPD in infants <1000 g birthweight[58]. In a follow-up larger trial at the same institution, *Ureaplasma* clearance and BPD rates were similar in the placebo and azithromycin-treated groups[59]. These results suggest that 10 mg/kg azithromycin as a single or multiple dose is likely inadequate for *Ureaplasma* eradication and BPD prevention. Furthermore, they demonstrate the importance of performing appropriate PK/PD studies preparatory to phase II/III trials to assess microbiological and clinical efficacy of azithromycin.

In conclusion, the high rate of mortality and respiratory morbidity associated with *Ureaplasma* colonization of preterm infants underscores the need to develop effective treatment to eradicate the organisms and prevent lung injury in at-risk infants. The ultimate goal of this investigation was to determine PK parameters necessary to design and conduct future clinical trials of azithromycin in preterm neonates. The data presented were consistent with the safety of azithromycin and established a PK model for refining future simulation-based clinical trials to eradicate *Ureaplasma* and prevent BPD.

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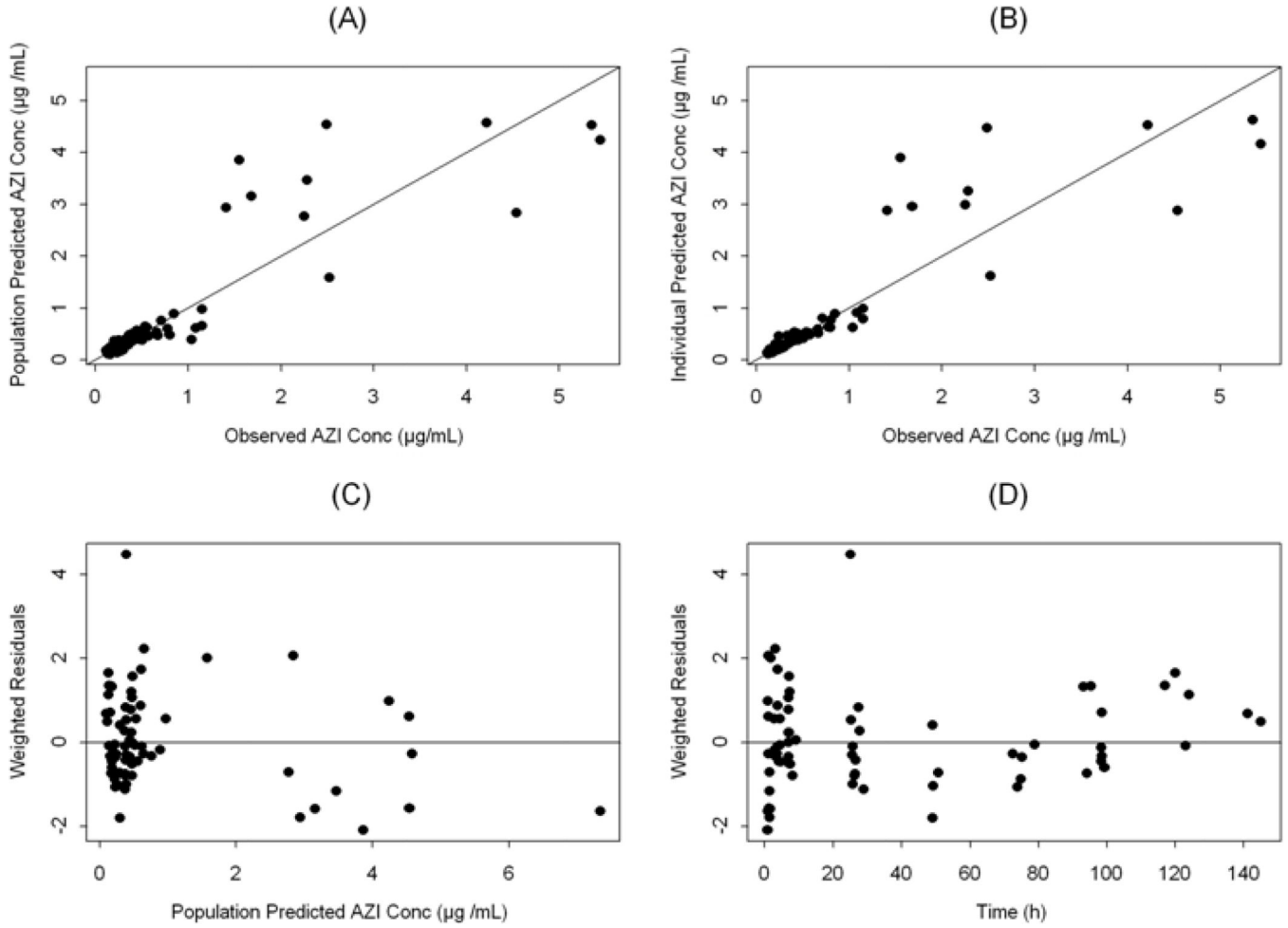

Figure 1.

Diagnostic scatter plots for the population PK model. (A) population-predicted versus observed azithromycin plasma concentrations; (B) individual-predicted versus observed azithromycin (AZI) plasma concentrations; (C) weighted residuals versus population predicted plasma concentrations; and (D) weighted residuals versus time (The solid lines represent the lines of identity in A and B and zero weighted residuals in C and D).

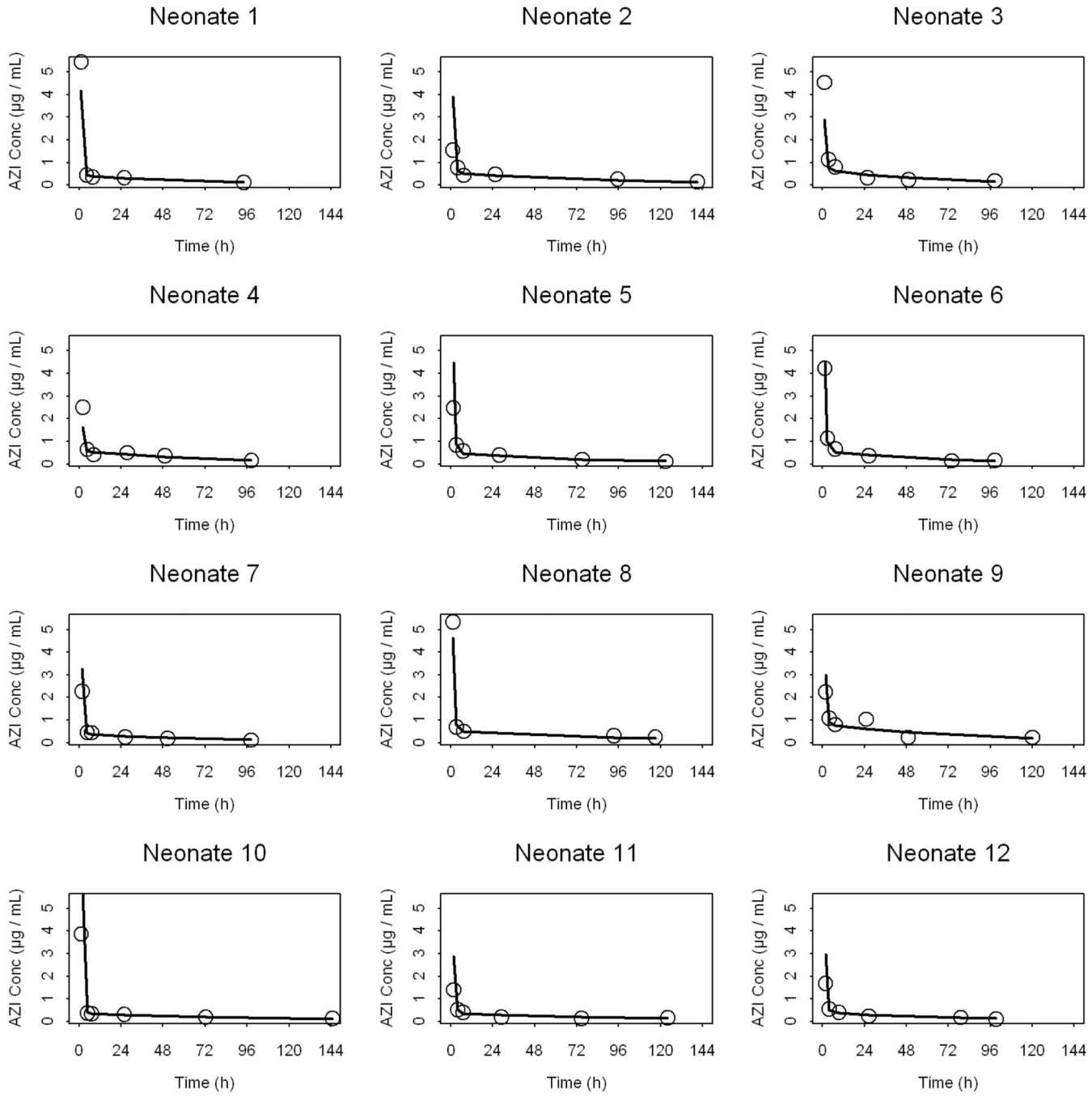

Figure 2.

Observed (closed circles) and the post-hoc predicted (solid lines) azithromycin plasma concentrations versus time profiles for each neonate.

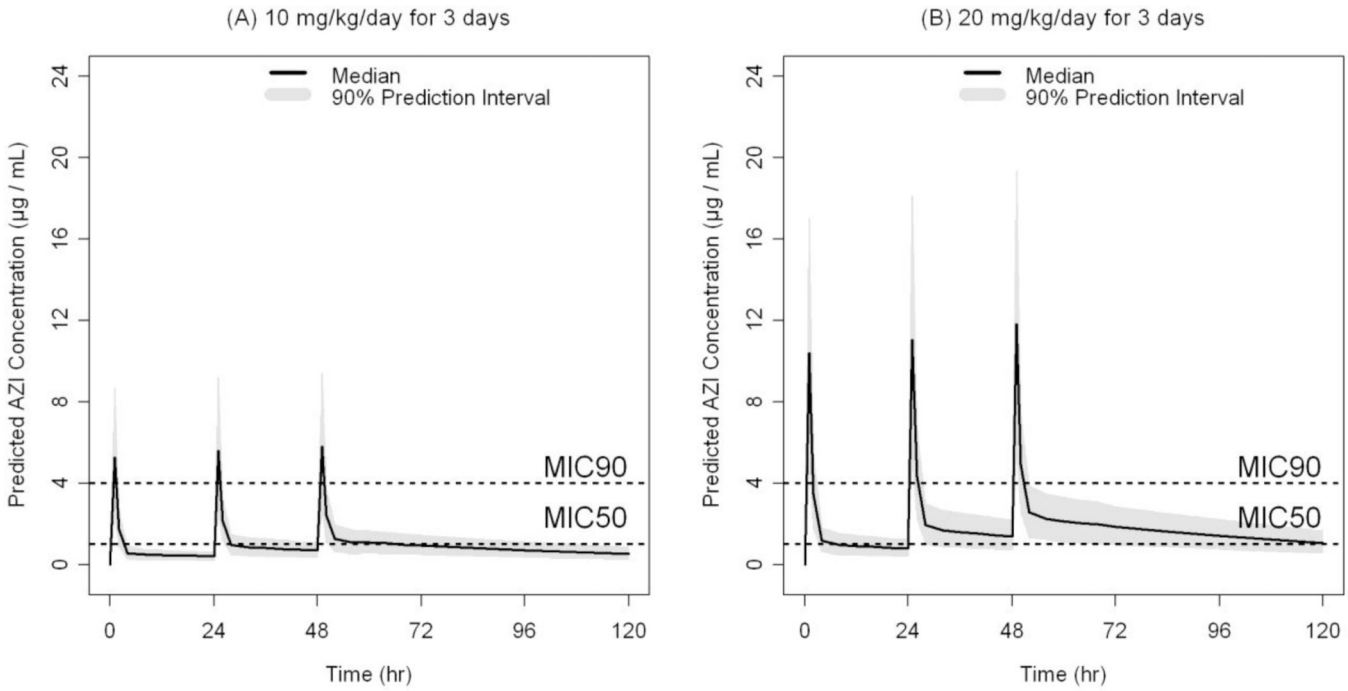

Figure 3.

Azithromycin i.v. simulated plasma concentration versus time profiles in preterm neonates after the following dosage regimens (A) 10 mg/kg/day × 3 days, n=1000, and (B) 20 mg/kg/day × 3 days, n=1000. Median plasma concentrations and 90% prediction interval (calculated from the 5^{th} and 95^{th} percentiles of the simulated concentrations) are depicted in the figure. The dotted lines represent the MIC_{50} (1 µg/mL) and MIC_{90} (4 µg/mL) of azithromycin against *Ureaplasma*.

Table 1

Summary of infants' demographics

Infant Characteristics	**mean ± SD or N (%) N=14**
Birth weight (g)	855 ± 276
Gestational age (wk)	26 ± 1
Postnatal age at dosing time (h)	47 ± 28
(wk)	26.3 ± 0.95
Length (cm)	33.5 ± 3.4
Body Surface Area (m^2)	0.09 ± 0.02
Duration of IMV at study entry (h)	29 ± 13
Male gender	7 (50)
Race:	
Black	9 (64)
White	2 (14)
Hispanic	1 (7)
Asian	1 (7)
Mixed race	1 (7)
***Ureaplasma* positive**	8 (57)

IMV: intermittent mandatory ventilation

Table 2

Clinical outcomes

Study number	*Ureaplasma* culture status	Status @ 36 wk PMA	Timed O_2 reduction test performed	Results of Timed O_2 reduction test	Physiologic BPD @ 36 wk PMA
1	Pos	Died 56 d			
2	Pos	Alive, SIMV	No		Yes
3	Pos	Alive, NC	Yes	Passed	No
4	Pos	Died 21 d			
5*	Pos	Alive, nCPAP	No		Yes
6	Pos	Alive, NC	Yes	Passed	No
7	Pos	Alive, SIMV	No		Yes
8**	Pos	Died 3 d			
All positives		3 deaths	2/5	2	3/5 (60%)
9	Neg	Alive, NC	Yes	Passed	No
10	Neg	Alive, NC	Yes	Passed	No
11	Neg	Alive, NC	Yes	Passed	No
12	Neg	Transferred, NC	No		Yes
13	Neg	Alive, room air	No		No
14	Neg	Alive, NC	Yes	Passed	No
All negatives		0 deaths	4/6	4	1/6 (17%)
Total		3 deaths	6	6	4/14 (29%)

* received 2 additional doses azithromycin (protocol violation);

** withdrawn prior to dosing due to clinical deterioration

PMA: post-menstrual age

NC: nasal cannula; nCPAP: nasal continuous positive pressure; SIMV: synchronized intermittent mandatory ventilation

Table 3

Population pharmacokinetic parameter estimates for azithromycin in preterm neonates

Parameter	Estimate (% RSE)	% ISV (%RSE)
CL (L/h)	0.18 x $WT^{0.75}$ (8.2)	23.5 (44.6)
V1 (L)	0.93 (12.1)	NE
Q (L/h)	1.0 (13.2)	NE
V2 (L)	14.2 x WT (9.2)	26.2 (50.6)
Residual Error (%)	28.7 (15.0)	---

RSE: relative standard error

ISV: intersubject variability, calculated as the square root of the estimated variance of intersubject variability (i.e., ω) x100

WT: body weight in kilograms

NE: not estimated

Table 4

Tracheal aspirate cytokine concentrations pre- and post-azithromycin therapy*

Cytokine	Pre-dose (N=12)	D2 post-dose (N=9)
IL-6 (pg/mL)	204 (92–444)	148 (67–255)
IL-6 *Ureaplasma* positive	237 (128–400)	147 (67–255)
IL-6 *Ureaplasma* negative	107 (76–1070)	148 (64–721)
IL-8 (pg/mL)	4560 (1980–6595)	2910 (2250–5850)
IL-8 *Ureaplasma* positive	5850 (2140–-5850)	3110 (2220–5850)
IL-8 *Ureaplasma* negative	2700 (1820–14200)	2750 (2250–8170)
IL-1β (pg/mL)	20.14 (2–89)	40.4 (25–91)
IL-1β *Ureaplasma* positive	24 (2–95)	63 (25–599)
IL-1β *Ureaplasma* negative	4 (2–82)	30 (5–91)
MPO (units/mg protein)	0.6 (0.3–1.5)	0.5 (0.3–0.8)
MPO *Ureaplasma* positive	0.6 (0.3–2.0)	1.2 (0.2–3.0)
MPO *Ureaplasma* negative	0.5 (0.3–1.2)	0.5 (0.1–0.8)

* Data are expressed as median with 25^{th} to 75^{th} percentiles in parentheses