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CLINICAL THERAPEUTICS

Population Pharmacokinetics of Pyrazinamide in Patients with Tuberculosis

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ABSTRACT The current treatment used for tuberculosis (TB) is lengthy and needs to be shortened and improved. Pyrazinamide (PZA) has potent sterilizing activity and has the potential to shorten the TB treatment duration, if treatment is optimized. The goals of this study were (i) to develop a population pharmacokinetic (PK) model for PZA among patients enrolled in PK substudies of Tuberculosis Trial Consortium (TBTC) trials 27 and 28 and (ii) to determine covariates that affect PZA PK. (iii) We also performed simulations and target attainment analysis using the proposed targets of a maximum plasma concentration (C_{max}) of >35 μg/ml or an area under the concentration-versus-time curve (AUC) of >363 μg · h/ml to see if higher weight-based dosing could improve PZA efficacy. Seventy-two patients participated in the substudies. The mean (standard deviation [SD]) C_{max} was 30.8 (7.4) μg/ml, and the mean (SD) AUC from time zero to 24 h (AUC_{0-24}) was 307 (83) μg · h/ml. A one-compartment open model best described PZA PK. Only body weight was a significant covariate for PZA clearance. Women had a lower volume of distribution (*V*/*F*) than men, and both clearance (CL/*F*) and *V*/*F* increased with body weight. Our simulations show that higher doses of PZA (>50 mg/kg of body weight) are needed to achieve the therapeutic target of an AUC/MIC of >11.3 in >80% of patients, while doses of >80 mg/kg are needed for target attainment in 90% of patients, given specific assumptions about MIC determinations. For the therapeutic targets of a C_{max} of >35 μg/ml and/or an AUC of >363 μg · h/ml, doses in the range of 30 to 40 mg/kg are needed to achieve the therapeutic target in >90% of the patients. Further clinical trials are needed to evaluate the safety and efficacy of higher doses of PZA.

KEYWORDS pyrazinamide, tuberculosis, pharmacokinetics, simulation

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The standard regimen for drug-susceptible tuberculosis (TB) consists of rifampin, isoniazid, pyrazinamide (PZA), and ethambutol for 2 months, followed by 4 months of isoniazid and rifampin. If used properly, this regimen is highly effective, with cure rates approaching 95% within clinical trials; however, in program settings, treatment is successful in only 70 to 85% of patients (1). Despite the widespread availability of this regimen, TB is still a worldwide pandemic; it surpassed HIV as a leading cause of death from an infectious agent in 2014. Approximately 9.6 million people develop TB annually, and 1.5 million die from it (1). Also, multidrug-resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) pose threats to public health efforts to control TB. Treatment of MDR-TB and XDR-TB requires the use of less effective and

TABLE 1 Baseline demographics of patients in TBTC studies 27 and 28

Characteristic	Value(s)
% male patients	82
Mean (range) wt (kg)	59.8 (40–101.9)
Mean (range) age (yr)	36.7 (19–76)
% of sites in Africa	51.4
Mean (range) serum creatinine concn (mg/dl)	0.75 (0.5–1.2)
Mean (range) dose (mg)	1,351 (1,000–2,000)

more toxic drugs for a prolonged period of time (18 to 24 months). The success of treatment of MDR-TB and XDR-TB compared to that of drug-susceptible TB is low.

A major limitation of the current regimen is the length of treatment. In the United States, the reported TB treatment completion rate at 6 months is 18%, and at 1 year it is 89% (Centers for Disease Control and Prevention [CDC] surveillance slides [http://www.cdc.gov/tb/statistics/surv/surv2013/default.htm] and CDC communication to C.A.P., 24 June 2014). Longer treatment durations risk nonadherence and may lead to treatment failures and acquired drug resistance. Shorter treatment regimens for TB may enhance adherence, improve TB treatment outcomes, and reduce programmatic health costs.

One approach to improve the treatment of TB is to optimize the dosing of current drugs. PZA has excellent sterilizing activity and is the only anti-TB drug showing bactericidal activity against *Mycobacterium tuberculosis* organisms in acidic environments (2). When PZA is added to isoniazid, rifampin, and ethambutol during the first 2 months of treatment, it allows for the shortening of treatment from 9 to 6 months (3, 4). Also, PZA is synergistic with the new anti-TB drugs pretomanid and bedaquiline (5, 6). Therefore, PZA is likely to be a component of novel regimens intended to shorten the treatment of TB in the future and is a prime candidate for dose optimization.

Currently, the CDC and World Health Organization (WHO) recommend a PZA dose of 20 to 25 mg/kg of body weight. In the earlier clinical studies establishing PZA's role, doses of 30 to 40 mg/kg were used, with the minimum daily dose being 1,500 mg (7–9). Recent *in vitro*, animal, and clinical studies suggest that the current dosing for PZA may be too low (7, 10–14). Chideya et al. showed that maximum plasma concentrations (C_{max}s) of PZA of less than 35 μg/ml were associated with treatment failure and death (12). Pasipanodya et al. showed that PZA area under the concentration-versus-time curve (AUC) values of less than 363 μg · h/ml were associated with treatment relapse, failure, or death (10). The same authors conducted a more detailed pharmacokinetic (PK)-pharmacodynamic (PD) study including average MIC and PK data. They showed that an AUC/MIC ratio of $>$11.3 for PZA was a significant predictor of sterilizing activity in patients with low rifampin concentrations, given certain assumptions about MIC determinations (14). A high proportion of patients with TB do not achieve these PK targets with current dosing, suggesting that higher doses of PZA will be required for optimal sterilizing activity.

The goals of this study were (i) to develop a population PK model for PZA among patients enrolled in PK substudies of Tuberculosis Trial Consortium (TBTC) studies 27 and 28 and (ii) to determine covariates that affect PZA PK. (iii) We also performed simulations and target attainment analysis using the proposed targets of a C_{max} of $>$35 μg/ml or an AUC of $>$363 μg · h/ml to see if higher weight-based dosing could improve PZA efficacy.

RESULTS

We evaluated the PK of PZA in 72 patients with TB, using 499 observations. The study included 60 males and 12 females with a mean weight of 59.8 kg and a mean age of 36.7 years (Table 1). Participants received a mean dose of 22.7 mg/kg. Twenty-seven patients received a dose of 1,000 mg, 2 patients received a dose of 1,250 mg, 35 patients received a dose of 1,500 mg, and 8 patients received a dose of 2,000 mg.

TABLE 2 PK parameters estimated from noncompartmental analysis

Parameter	Mean (range) value
C_{max} (μg/ml)	30.8 (16–54)
AUC_{0-24} (μg · h/ml)	307 (136–579)
T_{max} (h)	1.6 (0.8–6)
$t_{1/2}$ (h)	7.5 (4.5–11)
CL/*F* (liters/h)	4.6 (2.3–7.6)

Noncompartmental analysis. Results for the noncompartmental analysis are shown in Table 2. PZA demonstrated rapid absorption after oral administration, with all but three patients reaching C_{max} at 2 h or less. The mean (standard deviation [SD]) time to C_{max} (T_{max}) was 1.6 (1.2) h, and the mean (SD) C_{max} was 30.8 (7.4) μg/ml. Females had slightly higher C_{max}s than males (33.2 versus 30.3 μg/ml). The mean (SD) AUC from time zero to 24 h (AUC_{0-24}) was 307 (83) μg · h/ml. Dose (in milligrams per kilogram of body weight) showed a modest association with C_{max} (coefficient of determination [R^2] = 0.12) and with AUC ($R^2 = 0.16$). Figure 1 shows the frequency distribution for C_{max} and AUC_{0-24}. The mean (SD) half-life ($t_{1/2}$) was 7.5 (1.2) h.

Most patients in our study did not achieve the proposed C_{max} and/or AUC target. Only 18 (25%) of 72 patients achieved a C_{max} of 35 μg/ml, and only 15 (21%) patients achieved an AUC of 363 μg · h/ml. There was a strong correlation between C_{max} and AUC ($R^2 = 0.62$). On the basis of the results of our study, a C_{max} of 35 μg/ml corresponds to an AUC of 344 μg · h/ml, very close to our 363 μg · h/ml target, indicating that achievement of one target (AUC or C_{max}) was likely to result in achievement of the other target as well.

Population pharmacokinetics. PZA was best described by a one-compartment open model with first-order elimination and first-order absorption. A combined error model best described the residual variability. Because PZA was rapidly absorbed and there were limited data prior to 2 h postdosing, the final population estimate for the absorption rate constant (k_a) was high. Therefore, the distribution for k_a was capped at 4.5 h^{-1}. Significant covariates included sex and body weight for the volume of

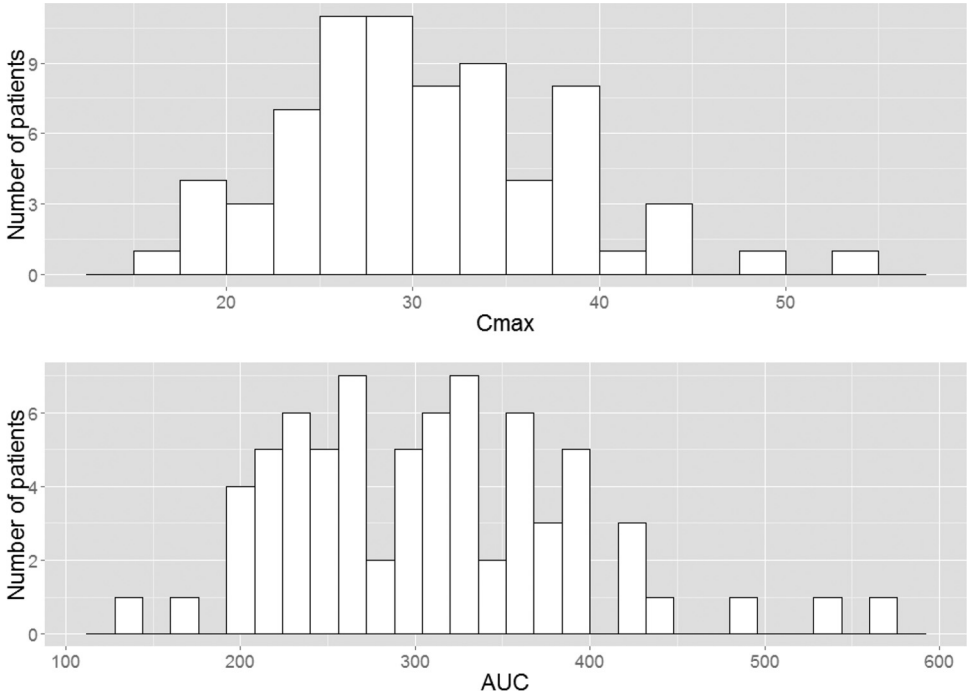

FIG 1 Frequency distribution for both C_{max} (in micrograms per milliliter) and AUC (in micrograms · hours per milliliter).

TABLE 3 PK parameter estimates for final population PK model[a]

PK parameter	Estimate	
	Base model	Final model
k_a (h^{-1} [% RSE])	3.94 (7)	3.63 (12)
IIV for k_a (% CV)	233 (18)	220 (22)
V/*F* (liters [% RSE])	44.9 (3)	
Females		46.5 (4)
Males		54 (2)
IIV for *V*/*F* (% CV)	21 (10)	10.9 (15)
CL/*F* (liters/h [% RSE])	4.44 (3)	5.06 (3)
IIV for CL/*F* (% CV)	25.3 (9)	23 (9)
Residual variability		
a (% RSE)	0.921 (8)	0.94 (8)
b (% [% RSE])	10.5 (8)	10 (7)

[a]Both *V*/*F* and CL/*F* are scaled for a 70-kg individual in the final model. Log (CL/*F*) = log(5.06) + 0.75 · [log(weight) − log(70 kg)]. Log (*V*/*F*) = log(46.5) + 0.148 · sex (male) + [log(weight) − log(70 kg)]; female was considered the reference. RSE, relative standard error; IIV interindividual variability; CV, coefficient of variation.

distribution (*V*/*F*) and body weight for clearance (CL/*F*). Allometric scaling was used to describe the effect of body weight on both *V*/*F* and CL/*F*, using fixed exponents of 1 and 0.75, respectively. The typical value of CL/*F* in a 70-kg individual was 5.06 liters/h, while the typical values of *V*/*F* were 54.2 liters for a 70-kg male and 46.5 liters for a 70-kg female (Table 3). Goodness-of-fit plots are shown in Fig. 2 and 3. Figure 2 shows the observed and predicted concentrations, while Fig. 3 shows the visual predictive check (VPC) plot.

External validation. We validated the final model with an external data set. The average age in the external data set was 48 years, and 73% of the patients were male. These values are comparable to those for our population, where the average age was 37 years and 82% of the patients were males. The average dose was 1,750 mg. There was a good correlation between the population predicted and observed concentrations ($R^2 = 0.73$). However, our model underpredicted C_{max} (bias = 12%, precision = 35%). There was excellent agreement between the observed and individual predicted concentrations ($R^2 = 0.97$) (Fig. 4). Our model also showed low levels of bias and precision (bias = −4.7%, precision = 12.1%).

Simulation and target attainment. The final model estimates for CL/*F* with body weight as a covariate were used for the simulations. The model-predicted change in AUC between the lower and upper bounds of each group was <21%, indicating comparable exposures within groups (Table 4). The model-predicted change in AUC from a 40-kg to a 90-kg adult was approximately 100% if all patients were given the same dose on a milligram basis (i.e., if dosing was not weight adjusted). Therefore, it seemed reasonable to dose patients by these three weight groups instead of using a fixed dose for all patients.

For simulations based on an AUC/MIC of >11.3, very high doses were needed to achieve the therapeutic target (Table 5). If we convert these doses to doses in milligrams per kilogram of body weight, the dose needed to achieve a rate of target attainment of 80% across all groups was approximately 50 mg/kg, while doses of >80 mg/kg were needed to achieve target attainment in 90% of patients.

Simulations based on an AUC of >363 μg · h/ml and on a C_{max} of >35 μg/ml produced similar and more achievable results (Tables 6 and 7). Depending on the weight group, doses in the range of 35 to 45 mg/kg were needed to achieve the therapeutic targets in >90% of the patients. Patients at the lower bound of body weight (weight groups 1 and 2) needed higher doses (about 40 to 45 mg/kg), while patients at the upper bound of body weight needed lower doses (about 35 mg/kg) to achieve the therapeutic targets (Fig. 5).

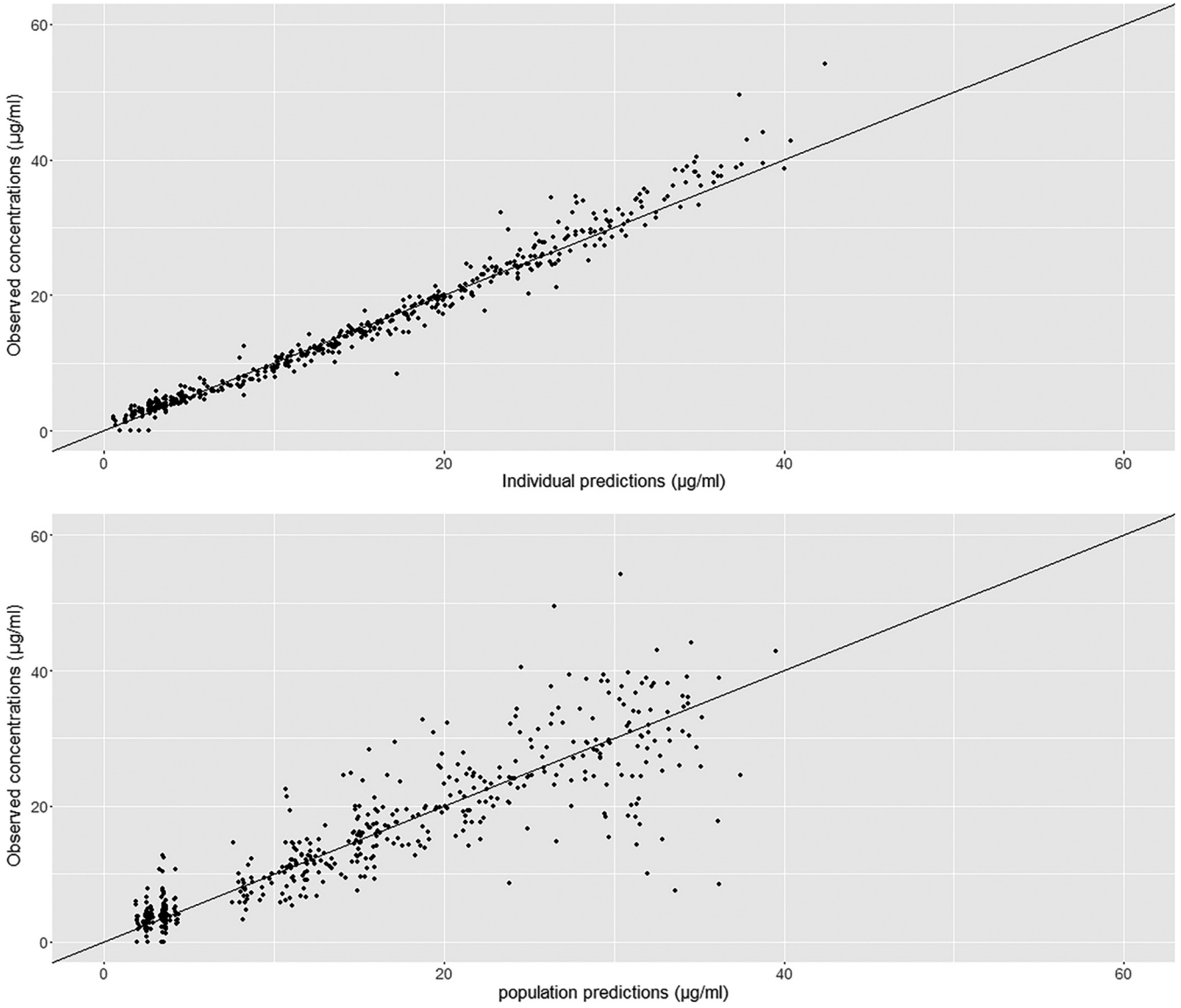

FIG 2 Goodness-of-fit plots for the final population pharmacokinetic model. (Top) Individual predictions of PZA concentrations versus observed concentrations; (bottom) population predictions of PZA concentrations versus observed concentrations.

DISCUSSION

We described the population PK of PZA in patients with TB. The one-compartment structural model that we used is consistent with the models used in previous studies (15–17). PZA showed rapid absorption, as expected. PZA probably has the most predictable absorption among all anti-TB drugs. Both body weight and sex independently influenced PZA PK. Because these two variables significantly affect the PZA *V*/*F*, the inclusion of both of them in the model decreased the variability by approximately 50%. Body weight significantly influenced PZA CL/*F* and explained 9% of its variability. Our models predict that AUC would decrease by approximately 2-fold in a 90-kg patient compared to that in a 40-kg individual if the patients were given the same fixed dose (rather than weight-based dosing). Therefore, administration of a fixed dose to all patients is not appropriate. We suggest dividing patients into three different weight groups (Table 5), where the exposure is comparable across each weight group.

Both *V*/*F* and CL/*F* estimates were modestly higher in our analysis than previous studies (15, 17). Of note, most of the patients in our study did not achieve the proposed C_{max} or AUC targets of 35 μg/ml and 363 μg · h/ml, respectively. This indicates that the

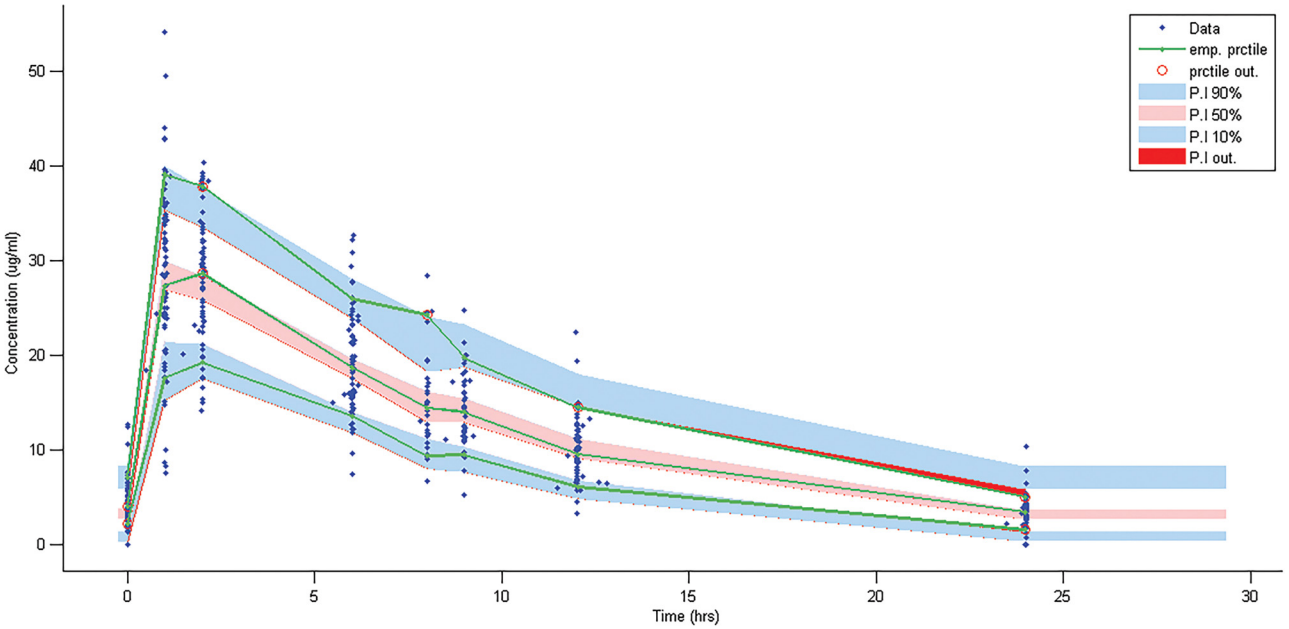

FIG 3 Visual predictive check (VPC) for PZA concentration versus time on the basis of 1,000 Monte Carlo simulations. Solid green line, the 10th, 50th, and 90th percentiles of the observed data; shaded regions, 90% confidence interval around the 10th, 50th, and 90th percentiles of simulated data; blue diamonds, observed concentrations. emp. prctile, empiric percentile; prctile out., percentile outside of the bounds; and P.I, prediction interval.

current dosing might be too low to achieve PK targets. In the study by Zhu et al., the average C_{max} and AUC values were 38 μg/ml and 373 μg · h/ml, respectively, whereas they were 30.8 μg/ml and 307 μg · h/ml, respectively, in our study, despite the use of similar doses on a milligram-per-kilogram basis (15).

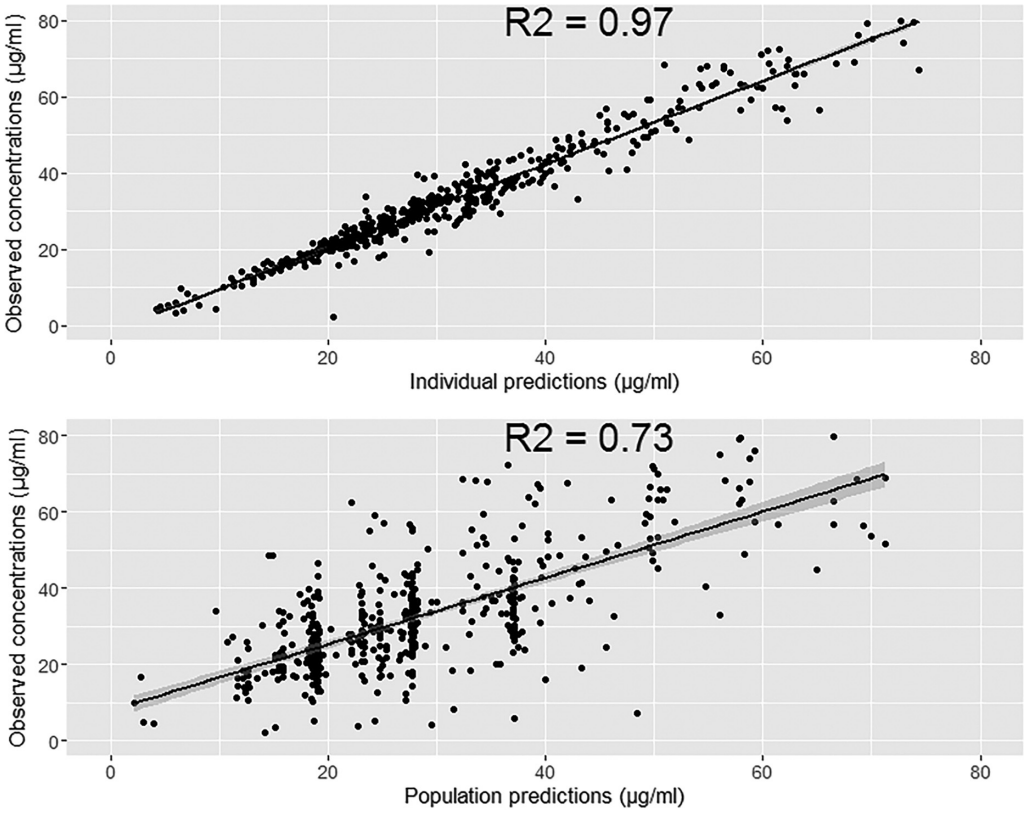

FIG 4 External validation of the final model. The individual predictions (top) and population predictions (bottom) of the PZA concentrations versus the concentrations observed in the external data set are shown.

TABLE 4 Model-predicted AUC stratified by different weights[a]

Wt (kg)	CL/*F* (liters/h)	AUC (μg · h/ml)	% change compared to:	
			Value in previous row	Value for a 40-kg individual
40	3.3	601		
55	4.2	473	79	79
75	5.3	375	79	62
90	6.1	327	87	54

[a]All data are based on a dose of 2,000 mg.

Previous clinical studies have shown that a C_{max} of $<$35 μg/ml or an AUC of $<$363 mg · h/ml is associated with treatment failure (10, 12, 18). However, it is important to note that antimicrobial activity is not linked to the drug concentration alone but is linked to a combination of the drug concentration and the susceptibility of the infecting strain to the antibiotic of interest (the MIC). A clinical study by Chigutsa et al. evaluated the PK/PD of first-line anti-TB drugs (14). This study was unique in that MIC values were available for the patients' infecting isolates, allowing for a more detailed PK/PD analysis. An AUC/MIC of $>$11.3 for PZA was a key predictor of sterilizing activity in patients with a rifampin AUC of $<$35.4 μg · h/ml. An *in vitro* study by Gumbo et al. also showed that PZA sterilizing activity is linked to the AUC/MIC (11). To further evaluate the dosing of PZA, we used Monte Carlo simulations and target attainment analysis. We evaluated target attainment at three different targets: an AUC/MIC ratio of $>$11.3, a C_{max} of $>$35 μg/ml, and an AUC of $>$363 mg · h/ml. To account for the effect of weight on PZA CL/*F* and *V*/*F*, simulations were performed for three different weight groups. The purpose was to minimize the variability in exposure for PZA and simplify its dosing. The predicted change in AUC between the upper and lower end of each weight band was $<$21%. To achieve the therapeutic targets of a C_{max} of $>$35 μg/ml and an AUC of $>$363 μg · h/ml in $>$90% of the patients, doses of 35 to 45 mg/kg were needed. To achieve the therapeutic target of an AUC/MIC of $>$11.3, we show that doses higher than those currently used (1,000 to 2,500 mg or 15 to 25 mg/kg) are needed. Across all three weight groups, doses of 50 mg/kg were needed to achieve target attainment in 80% of the patients, while doses of 60 to 70 mg/kg were needed to achieve target attainment in 90% of the patients. Our results are similar to the findings of Gumbo et al., who suggested a dose of $>$60 mg/kg (11). However, although the relationship between PZA dose and hepatotoxicity remains debatable, patient safety may be a concern at doses of $>$50 mg/kg (19).

The limitations of our study include inadequate early samples to estimate k_a precisely, forcing us to cap the k_a distribution at 4.5. It should be noted that MIC determinations for PZA are particularly challenging and highly dependent upon the technique used (20). Therefore, a note of caution is needed when evaluating PZA MIC values. Further, the targets that we evaluated in our study are based on a limited

TABLE 5 Probability of target attainment analysis by weight group on the basis of an AUC/MIC of $>$11.3

Dose (mg)	Probability of target attainment (%) in the following wt group (kg):		
	40–55	56–75	76–90
1000	29	15	10
1,500	54	41	28
2,000	66	56	48
2,500	77	66	58
3,000	83	74	66
3,500	87	80	74
4,000	90	84	79
4,500	94	87	83
5,000	96	90	86

TABLE 6 Probability of target attainment analysis by weight group on the basis of an AUC of >363 μg · h/ml

Dose (mg)	Probability of target attainment (%) in the following wt group (kg):		
	40–55	56–75	76–90
1,000	10	1	0
1,500	66	27	8
2,000	94	72	43
2,500	100	94	78
3,000	100	99	94
3,500	100	100	99
4,000	100	100	100

number of clinical studies, and they have not been prospectively validated. More PK/PD studies are needed to better establish therapeutic and safety targets for PZA.

In conclusion, we described the population PK of PZA using a one-compartment model. Both sex and body weight influenced the PK of PZA. Our simulations show that the current dosing used for PZA might be too low for the majority of patients. Our results are consistent with those of previous studies that established the efficacy of PZA. In those studies, doses in the 30- to 40-mg/kg range were used, and the minimum dose used was 1,500 mg (7–9). Clinical studies are needed to evaluate the efficacy and safety of higher doses of PZA.

MATERIALS AND METHODS

Study design. Data from PK substudies of TBTC studies 27 and 28 were used. Both studies were phase 2 prospective, placebo-controlled, randomized clinical trials (21, 22). The studies included adult patients from Uganda, South Africa, and the United States with drug-susceptible, smear-positive pulmonary tuberculosis. Participants in TBTC study 27 were randomized to receive ethambutol or moxifloxacin once daily, in addition to rifampin, isoniazid, and pyrazinamide. Patients in TBTC study 28 were randomized to receive isoniazid or moxifloxacin once daily, in addition to rifampin, pyrazinamide, and ethambutol. In both studies, PZA was given in weight-banded doses of 1,000 mg for patients weighing 40 to 55 kg, 1,500 mg for patients weighing 56 to 75 kg, and 2,000 mg for patients weighing 76 to 90 kg. The result was a dose ranging from 18 to 27 mg/kg daily. Seventy-two patients participated in the PK substudies. Institutional review board approvals were obtained at all participating research sites.

Sample collection and analytical assay. Blood was collected for plasma predosing and then at 1, 2, 6, 8, 12, and 24 h postdosing. Blood sampling was performed after the fourth or fifth dose of PZA. Each dose was administered as directly observed therapy. PZA concentrations were measured using a validated gas chromatography assay with mass selective detection, as previously described (15). The standard curve ranged from 0.5 to 100 μg/ml. The intraday precision ranged from 0.20% to 11.11%, while the interday precision ranged from 0.32% to 5.00%.

Pharmacokinetic analysis. (i) Noncompartmental analysis. A noncompartmental PK analysis was performed using Phoenix software (Phoenix Build, version 6.2.1.51, containing WinNonlin, version 6.3; Pharsight). C_{max} was the highest observed concentration, and T_{max} was the time at which C_{max} occurred. AUC_{0-24} was estimated using the trapezoidal rule. The elimination rate constant (k_{el}) was estimated from the terminal elimination slope, and the half-life ($t_{1/2}$) was equal to $0.693/k_{el}$. We also estimated the fraction of patients that achieved the proposed C_{max} or AUC target of 35 μg/ml and 363 μg · h/ml, respectively (10, 12,

TABLE 7 Probability of target attainment analysis by weight group on the basis of a C_{max} of >35 μg/ml

Dose (mg)	Probability of target attainment (%) in the following wt group (kg):		
	40–55	56–75	76–90
1,000	10	0	0
1,500	83	29	3
2,000	97	86	47
2,500	99	97	89
3,000	100	99	97
3,500	100	99	97
4,000	100	100	99
4,500	100	100	100
5,000	100	100	100

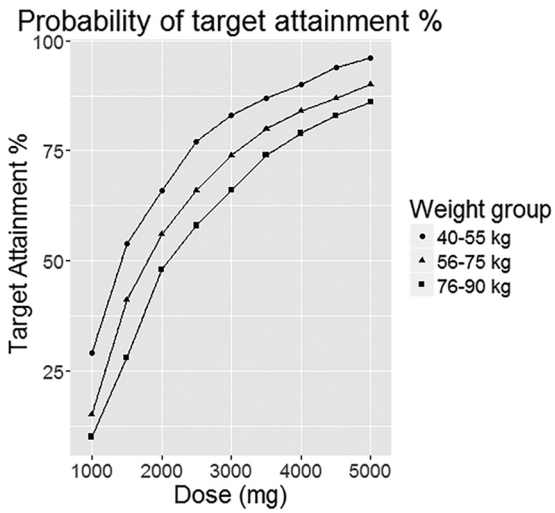

FIG 5 Target attainment analysis for PZA (AUC/MIC > 11.3) for all four dosing groups.

18). Descriptive statistics were estimated using R statistical software, version 3.1.1 (23). Continuous variables are presented as the mean (range). Categorical variables are presented as frequencies.

(ii) Population PK analysis. Population PK modeling was performed using the nonlinear mixed effects modeling software Monolix (version 4.3; Lixoft) to estimate primary PK parameters, characterize the effects of covariates on key PK parameters, and support the dose simulations. Monolix estimates PK parameters via a maximum likelihood approach using the stochastic approximation expectation maximization (SAEM) algorithm (24). Various structural models, including one- and two-compartment open models with linear or nonlinear elimination, were tested. For the absorption process, first- and zero-order, gastrointestinal transit compartment and simultaneous first- and zero-absorption models with or without lag time were tested. PK parameters were assumed to be log normally distributed, as follows: $p_i = p \cdot \exp(\eta_i)$, where p_i is the PK parameter for the ith individual, p is the typical value of the PK parameter in the population, and η_i is the random effect for the ith individual with a mean of zero and a variance of ω^2. The following statistical models were tested to describe the unexplained residual variability: constant error, proportional error, combined error, and exponential error models.

The covariates included in the data set were sex, body weight, age, serum creatinine concentration, and study site location (classified as Africa versus non-Africa). The initial step was to plot the *post hoc* individual PK parameters versus covariates for data visualization and to identify potential covariates for inclusion in the model. Later, we performed a stepwise regression analysis to test the significance of covariates using the −2 log-likelihood ratio test (a P value of 0.1 was used for initial inclusion in the model, and a P value of 0.05 was used for retention in the final model). The reduction of the between-subject variability for PK parameters and the improvement in diagnostic plots were taken into consideration when adding covariates. Throughout the analysis process, model selection was guided by goodness-of-fit plots, log-likelihood ratio testing, the Bayesian information criterion (BIC), physiologic plausibility, and the standard errors of the parameter estimates.

External validation. The final model was externally validated using an independent data set. Observations on PZA concentrations from an additional 498 samples collected from 297 patients with TB (average, 1.7 observations per subject) collected at the University of Florida Infectious Disease Pharmacokinetic Laboratory comprised the external data set. These were patients who underwent routine therapeutic drug monitoring (TDM) for PZA. Most were sampled at 2 and 6 h postdosing. The same assay was used to measure the PZA concentration in both the model-building data and the external data. Age and sex were the only covariates included in the external data set. Therefore, we imputed the values for body weight for these patients to be 66 kg for males and 53 kg for females; these are the average weights by sex that we observed in the TBTC data sets for North American patients.

The final population parameter estimates were fixed for all parameters, and population and individual predictions were made for each patient. The individual predictions were estimated using the maximum *a posteriori* (MAP) Bayesian estimator (25). To assess the predictive ability of the model, we estimated the correlation between predicted and observed concentrations. We calculated percent bias (mean prediction error) and percent precision (root mean squared error) as follows (26):

$$\text{percent bias} = \frac{\Sigma\,(y_{\text{predicted}} - y_{\text{observed}})}{N} \cdot \left(\frac{100}{y_{\text{mean}}}\right) \quad (1)$$

$$\text{percent precision} = \sqrt{\frac{\Sigma\,(y_{\text{predicted}} - y_{\text{observed}})^2}{N}} \cdot \left(\frac{100}{y_{\text{mean}}}\right) \quad (2)$$

where $y_{\text{predicted}}$, y_{observed}, and y_{mean} are concentrations, and N is the number of concentrations.

Simulation and target attainment. We performed target attainment analyses to evaluate the dosing of PZA. To assess the influence of body weight on dosing and the probability of target attainment, simulations were performed for three different weight groups as follows: group 1 consisted of weights of 40 to 55 kg, group 2 consisted of weights of 56 to 75 kg, and group 3 consisted of weights of 76 to 90 kg. Since there is no clear target for PZA, we evaluated target attainment at three different targets: an AUC/MIC of >11.3, an AUC of >363 μg · h/ml, and a C_{max} of >35 μg/ml (10, 12, 14).

For the AUC/MIC simulation, we simulated 10,000 random values for CL/*F* and MIC values per weight group. The simulations were performed so that the body weights for all 10,000 virtual patients were within the limits of the weight group. A log-normal distribution was used for CL/*F*, using the mean and variance values obtained in the final model. We used a discrete distribution for MIC on the basis of the findings of the study by Chigutsa et al. (14). In that study, the MIC distribution ranged from 12.5 to 100 μg/ml, and the probability associated with each MIC value was 11.5% for 12.5 μg/ml, 44.2% for 25 μg/ml, 28.8% for 50 μg/ml, and 15.3% for 100 μg/ml. We calculated target attainment to be the fraction of patients that achieved an AUC/MIC of >11.3 μg/ml. AUC/MIC was calculated as follows: (*D*/CL)/MIC, where *D* is dose (in milligrams).

Simulations for an AUC of >363 μg · h/ml were performed as described above but did not include an MIC distribution. We calculated target attainment as the fraction of patients that achieved an AUC of >363 μg · h/ml. AUC was calculated as follows: *D*/CL.

For target attainment on the basis of C_{max}, we simulated the concentration-versus-time profiles for 10,000 patients in each weight group using Monolix software. Then, C_{max} was estimated for each virtual patient. We calculated target attainment as the fraction of patients that achieved a C_{max} of >35 μg/ml. All simulations were performed using R software (23). All the targets that we evaluated came from clinical studies that measured total and free PZA concentrations; we used the total concentration for the simulations, because PZA is minimally bound to plasma proteins and the total drug concentration is a reasonable approximation (27).

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REFERENCES

1. World Health Organization. 2015. Global tuberculosis report. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/publications/global_report/en/.
2. Zhang Y, Mitchison D. 2003. The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis 7:6–21.
3. Anonymous. 1982. A controlled trial of six months chemotherapy in pulmonary tuberculosis. Second report: results during the 24 months after the end of chemotherapy. British Thoracic Association. Am Rev Respir Dis 126:460–462.
4. Combs DL, O'Brien RJ, Geiter LJ. 1990. USPHS Tuberculosis Short-Course Chemotherapy Trial 21: effectiveness, toxicity, and acceptability. The report of final results. Ann Intern Med 112:397–406.
5. Ibrahim M, Andries K, Lounis N, Chauffour A, Truffot-Pernot C, Jarlier V, Veziris N. 2007. Synergistic activity of R207910 combined with pyrazinamide against murine tuberculosis. Antimicrob Agents Chemother 51:1011–1015. https://doi.org/10.1128/AAC.00898-06.
6. Tasneen R, Tyagi S, Williams K, Grosset J, Nuermberger E. 2008. Enhanced bactericidal activity of rifampin and/or pyrazinamide when combined with PA-824 in a murine model of tuberculosis. Antimicrob Agents Chemother 52:3664–3668. https://doi.org/10.1128/AAC.00686-08.
7. Donald PR, Maritz JS, Diacon AH. 2012. Pyrazinamide pharmacokinetics and efficacy in adults and children. Tuberculosis (Edinb) 92:1–8. https://doi.org/10.1016/j.tube.2011.05.006.
8. Anonymous. 1974. Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Third report. East African-British Medical Research Councils. Lancet ii:237–240.
9. Anonymous. 1972. Controlled clinical trial of short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis. Lancet i:1079–1085.
10. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis 208:1464–1473. https://doi.org/10.1093/infdis/jit352.
11. Gumbo T, Dona CS, Meek C, Leff R. 2009. Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel in vitro model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs. Antimicrob Agents Chemother 53:3197–3204. https://doi.org/10.1128/AAC.01681-08.
12. Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, Wells CD, Reingold AL, Kenyon TA, Moeti TL, Tappero JW. 2009. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among a predominantly HIV-infected cohort of adults with tuberculosis from Botswana. Clin Infect Dis 48:1685–1694. https://doi.org/10.1086/599040.
13. Ahmad Z, Fraig MM, Bisson GP, Nuermberger EL, Grosset JH, Karakousis PC. 2011. Dose-dependent activity of pyrazinamide in animal models of intracellular and extracellular tuberculosis infections. Antimicrob Agents Chemother 55:1527–1532. https://doi.org/10.1128/AAC.01524-10.
14. Chigutsa E, Pasipanodya JG, Visser ME, van Helden PD, Smith PJ, Sirgel FA, Gumbo T, McIlleron H. 2015. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. Antimicrob Agents Chemother 59:38–45. https://doi.org/10.1128/AAC.03931-14.
15. Zhu M, Starke JR, Burman WJ, Steiner P, Stambaugh JJ, Ashkin D, Bulpitt AE, Berning SE, Peloquin CA. 2002. Population pharmacokinetic modeling of pyrazinamide in children and adults with tuberculosis. Pharmacotherapy 22:686–695. https://doi.org/10.1592/phco.22.9.686.34067.
16. Peloquin CA, Jaresko GS, Yong CL, Keung AC, Bulpitt AE, Jelliffe RW. 1997. Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide. Antimicrob Agents Chemother 41:2670–2679.
17. Wilkins JJ, Langdon G, McIlleron H, Pillai GC, Smith PJ, Simonsson US. 2006. Variability in the population pharmacokinetics of pyrazinamide in

South African tuberculosis patients. Eur J Clin Pharmacol 62:727–735. https://doi.org/10.1007/s00228-006-0141-z.
18. Burhan E, Ruesen C, Ruslami R, Ginanjar A, Mangunnegoro H, Ascobat P, Donders R, van Crevel R, Aarnoutse R. 2013. Isoniazid, rifampin, and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. Antimicrob Agents Chemother 57:3614–3619. https://doi.org/10.1128/AAC.02468-12.
19. Girling DJ. 1978. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. Tubercle 59:13–32.
20. Cui Z, Wang J, Lu J, Huang X, Zheng R, Hu Z. 2013. Evaluation of methods for testing the susceptibility of clinical Mycobacterium tuberculosis isolates to pyrazinamide. J Clin Microbiol 51:1374–1380. https://doi.org/10.1128/JCM.03197-12.
21. Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, Bozeman L, Heilig CM, Bernardo J, Choudhri S, Grosset JH, Guy E, Guyadeen P, Leus MC, Maltas G, Menzies D, Nuermberger EL, Villarino M, Vernon A, Chaisson RE, Tuberculosis Trials Consortium. 2009. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. Am J Respir Crit Care Med 180:273–280. https://doi.org/10.1164/rccm.200901-0078OC.
22. Burman WJ, Goldberg S, Johnson JL, Muzanye G, Engle M, Mosher AW, Choudhri S, Daley CL, Munsiff SS, Zhao Z, Vernon A, Chaisson RE. 2006. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. Am J Respir Crit Care Med 174:331–338. https://doi.org/10.1164/rccm.200603-360OC.
23. R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
24. Lavielle M, Mentre F. 2007. Estimation of population pharmacokinetic parameters of saquinavir in HIV patients with the MONOLIX software. J Pharmacokinet Pharmacodyn 34:229–249. https://doi.org/10.1007/s10928-006-9043-z.
25. Sheiner LB, Beal SL. 1982. Bayesian individualization of pharmacokinetics: simple implementation and comparison with non-Bayesian methods. J Pharm Sci 71:1344–1348. https://doi.org/10.1002/jps.2600711209.
26. Sheiner LB, Beal SL. 1981. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 9:503–512. https://doi.org/10.1007/BF01060893.
27. Holdiness MR. 1984. Clinical pharmacokinetics of the antituberculosis drugs. Clin Pharmacokinet 9:511–544. https://doi.org/10.2165/00003088-198409060-00003.