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ORIGINAL ARTICLE

Steady-state population pharmacokinetics of terizidone and its metabolite cycloserine in patients with drug-resistant tuberculosis

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Aims: Despite terizidone being part of the second-line recommended drugs for treatment of drug-resistant tuberculosis (DR-TB), information on its pharmacokinetics is scarce. The aim of this study was to describe the steady-state population pharmacokinetics (PPK) of terizidone and its primary metabolite cycloserine in patients with DR-TB and determine the effect of patient characteristics.

Methods: This clinical study involved 39 adult DR-TB patients admitted to Brewelskloof Hospital in Cape Town, South Africa for intensive treatment phase. Blood samples were collected at predose and 0.5, 1, 2, 3, 3.5, 4, 8, 16 and 24 hours after drug administration. The estimation of PPK parameters was performed using nonlinear mixed-effects modelling software Monolix 2018R1. Free-fat mass was used to perform allometric scaling on disposition parameters.

Results: A 1-compartment model best described the pharmacokinetics of terizidone and cycloserine. A modified transit compartment model described the absorption of terizidone. The parameters of terizidone model were mean transit time (1.7 h), absorption rate constant (2.97 h^{-1}), apparent volume of distribution (*Vp/F*: 13.4 L) and apparent total clearance (0.51 L h^{-1}). In the joint model, apparent fraction of terizidone converted to cycloserine was 0.29 while apparent clearance of terizidone via other routes and apparent cycloserine clearance was 0.1 L h^{-1} and 2.94 L h^{-1}, respectively. Serum albumin had significant effect on *Vp/F*.

Conclusions: The developed PPK model described well the concentration–time profile for terizidone and cycloserine in DR-TB patients. High albumin concentration was associated with low *Vp/F*.

KEYWORDS

cycloserine, drug-resistant tuberculosis, population pharmacokinetics, terizidone

1 | INTRODUCTION

Drug-resistant tuberculosis, which includes rifampicin mono-resistant and multidrug-resistant tuberculosis, is a persistent global threat and is linked to inadequate tuberculosis treatment.[1,2] Usually, the suboptimal drug treatment influences spontaneous mutations in the *Mycobacterium tuberculosis* chromosomal genes, which lead to the emergence of resistant strains.[3] To prevent further drug resistance, a multidrug treatment regimen consisting of 5–7 drugs is used in the treatment of drug-resistant tuberculosis.[4]

The authors confirm that the PI for this paper is Prof. Pierre Mugabo and that he had direct clinical responsibility for patients.

Terizidone, a condensation product of two cycloserine molecules, is one of the medicines used to treat drug-resistant tuberculosis.[5,6] Information on how terizidone is metabolised into cycloserine and the enzymes involved is not available in literature. Nevertheless, it seems to undergo hydrolysis into cycloserine presystemically.[7] Terizidone and cycloserine exert their respective antibacterial effect by disrupting the synthesis of peptidoglycan needed for bacterial cell wall formation through inhibition of D-alanine ligase and L-alanine racemase.[8] Terizidone is a potential drug for treatment of extra-pulmonary tuberculosis[9] and has been reported to have fewer central nervous system side effects than cycloserine and well tolerated in patients on dialysis.[10,11] A recent study indicated that both terizidone and cycloserine were clinically effective in the intensive treatment phase of drug-resistant tuberculosis.[12]

Despite terizidone being recommended and currently used in the treatment of drug-resistant tuberculosis,[4] information on its pharmacokinetics in literature is hardly found or poorly described. There appears to be only one study published in which terizidone and cycloserine pharmacokinetics were compared after a single-dose administration of each drug in tuberculosis patients.[13] After oral administration of 250–750 mg, terizidone reaches maximum concentration within 3 hours with absorption rate constant (*ka*) in the range 1.17–1.36 h^{-1}. The distribution volume is high, ranging between 113 and 246 L, while clearance is in the range 2.49–6.4 L h^{-1}. Thirty-nine percent of the administered dose is excreted in urine after 30 hours.[13] Terizidone plasma and urine concentrations in this study were not measured but estimated based on cycloserine using colorimetric method.[13] By contrast, the population pharmacokinetics of cycloserine in multidrug-resistant tuberculosis patients have been described[14] as well as noncompartmental pharmacokinetics in which cycloserine was measured as terizidone metabolite.[15] Cycloserine reaches maximum concentration within 2–3 hours with *ka* of 0.135 h^{-1} after oral daily dose of 500–1000 mg. It is widely distributed in most body fluids and tissues with distribution volume of 10.5 L. Its clearance is 1.38 L h^{-1} and primarily eliminated via renal route with 50–70% excreted unchanged within 12–24 hours.[14,16] The primary and secondary pharmacokinetic parameters of terizidone at steady state in drug-resistant tuberculosis patients are still unknown.

The objective of this study was to describe the population pharmacokinetics of terizidone and cycloserine at steady state in patients with drug-resistant tuberculosis and assess the influence of patient characteristics on pharmacokinetic parameters. We also estimated the associated secondary pharmacokinetic parameters.

What is already known about this subject

- Single-dose pharmacokinetics of terizidone.

What this study adds

- The study outlines the first description of population pharmacokinetics of terizidone and cycloserine at steady state in drug-resistant tuberculosis patients with and without human immunodeficiency virus infection.
- The study characterises for the first time the secondary pharmacokinetic parameters of terizidone at steady state.
- The first description of terizidone fraction undergoing biotransformation into cycloserine and the influence of serum albumin on apparent volume of distribution of terizidone.

2 | METHODS

2.1 | Study design

This was a non-randomised observational clinical study involving adult patients admitted for intensive treatment phase of drug-resistant tuberculosis at Brewelskloof Hospital, Western Cape province, South Africa. All patients were taking second-line anti-tuberculosis drugs such as pyrazinamide, ethionamide, kanamycin, moxifloxacin or ofloxacin, and ethambutol in addition to terizidone. In patients with tuberculosis monoresistant to rifampicin, isoniazid was added to their treatment. The doses were administered according to the local treatment guideline for management of drug-resistant tuberculosis.[17]

The patients included in this study were on anti-tuberculosis treatment for at least 2 weeks and had consented to participate in the study. Patients were excluded from the study if they requested so, were pregnant, breast-feeding, severely dehydrated or intolerant to terizidone. The demographic information was captured from patients on the day of the study while medical and treatment history was obtained from patients' folders. The ethics committees of the University of the Western Cape (Ref: 07/6/12) and University of Cape Town (Ref: 777/2014) approved this study. The patients' information was treated with confidentiality and the study was conducted in conformity with the principles outlined in the declaration of Helsinki.[18]

2.2 | Pharmacokinetic blood sampling

The patients were in fasting state from 22:00 hours prior to the morning of the blood-sampling day. Using an intravenous catheter placed in a vein of the forearm, 5 mL of blood from each patient was collected in heparinised tubes at baseline (predose) and at 0.5, 1, 2, 3, 3.5, 4, 8, 16 and 24 hours after drug administration. After centrifugation, the plasma was stored at −80°C until the date of analysis. Other blood samples were also collected for renal and liver function tests, virological and immunological tests. Patients then took their usual dose of anti-tuberculosis medications, including terizidone, and the time was noted. Human immunodeficiency virus (HIV) coinfected patients also received antiretroviral drugs as prescribed. Patients were then allowed to eat and drink as usual.

2.3 | Plasma quantification of terizidone and cycloserine

Plasma concentration of terizidone was analysed using high-performance liquid chromatography–UV method. It was extracted from plasma using protein precipitation method. The average inter- and intraday precision was 3.3 and 7%, respectively, while the mean accuracy was 107%. Calibration curves were linear with coefficient of determination ranging between 0.9988 and 0.9999. The lower limit of quantification and limit of detection was 3.125 and 0.78 μg mL^{-1}, respectively.[19] Cycloserine concentrations were analysed using ultra-performance liquid chromatography–tandem mass spectrometry method and validated according to the international guidelines.[20] Extraction was achieved through plasma protein precipitation with propranolol as internal standard. The mean inter- and intraday precision was 10.2 and 7.3%, respectively. The inter- and intraday accuracy was 103.8 and 108.7%, respectively. The curves were linear over the concentration range of 0.01–50 μg mL^{-1} with mean coefficient of determination of 0.9994. The quantification and detection limits were 0.01 and 0.004 μg mL^{-1}, respectively, while the carry-over was 0.0021 μg mL^{-1}. The matrix effect was insignificant. Cycloserine was stable after 3 freeze–thaw cycles and extraction efficiency ranged between 68.7 and 71.2%.

2.4 | Pharmacokinetic modelling

At the time of pharmacokinetic blood sampling, all patients had already achieved steady-state concentration for terizidone. The previous dose of terizidone was administered 24 hours before the sampling day. Therefore, the predose sampling time of 0 hours was set to 23.9 hours for modelling purposes. Conversion of concentration from μg mL^{-1} to μmol L^{-1} (molar units) was performed by using molar mass of 302.3 g mol^{-1} and 102.1 g mol^{-1} for terizidone and cycloserine, respectively. The doses for terizidone were also converted from mg to μmol.

The population pharmacokinetic parameters were estimated using nonlinear mixed-effects modelling in Monolix 2018R1 software.[21] The software utilises stochastic approximation expectation maximization algorithm[22] to carry out parameter estimations. The likelihood and Fisher information matrix were computed using importance sampling and stochastic approximation, respectively. Selection of the base model was guided by the visual inspection of diagnostic plots, change in the objective function value (OFV: −2 * *loglikelihood*), plausibility and precision of the parameter estimates (percentage relative standard error–%RSE).

We performed modelling in 2 parts. In the first part, only terizidone concentration–time profile was modelled. Based on the visual inspection of terizidone concentration–time profile using Datxplore interface of Monolix 2018R1, 1- and 2-compartment models were fit to the data. Absorption process was modelled using lag-time or transit compartment model[23] and assessed if it improved the model fit. In the case of the transit compartment being over-parameterised, modification was performed by setting *ka* equal to transit rate constant (*Ktr*; http://mlxtran.lixoft.com/examples/transit-compartments-weibull-absorption/).

In the second part, terizidone and cycloserine concentration–time profiles were modelled jointly. Terizidone model was modified in order to link it to cycloserine compartment. We assumed that terizidone did not undergo first-pass metabolism but was eliminated by biotransformation into cycloserine and other routes. Additionally, oral bioavailability (F) was assumed to be one. The fraction (*Fm*) of terizidone that is converted into cycloserine is unknown and unidentifiable. The clearance of terizidone by other routes is also unidentifiable. Similarly, the apparent volume of distribution of cycloserine (*Vm/F*) is unidentifiable, as the dose was not administered directly. To circumvent this problem, we decided to fix *Vm/F* to the literature value of 10.5 L.[14] This decision allows *Fm* to be identified and to distinguish between terizidone clearance via biotransformation and other routes.[24,25] It is worth noting that *Fm* is not the true fraction but apparent fraction.[26] Another way of dealing with parameter identifiability problem is to set the volume of the parent drug equal to metabolite volume, fixing metabolite volume equal to 1 or fixing *Fm* to any value between zero and 1. However, we chose not to undertake these options.

Proportional and combined (additive and proportional) error models were explored to model residual unexplained variability. The between-subject variability (BSV) model described the random variation in population pharmacokinetic parameters. We assumed that these parameters were log-normally distributed.

2.5 | Covariate model

After the base model was selected using the criteria in previous section, we investigated the effects of covariates on pharmacokinetic model parameters. The total body weight (TBW), free-fat mass[27] (FFM) or body mass index was used as body size descriptor. In order to adjust for the expected effect of body size, allometric scaling was performed on clearance and volume parameters. The exponents were either fixed to 0.75 for clearance and 1 for volume[28] or estimated. Other covariates explored were age, sex, HIV status, alanine aminotransferase, aspartate transaminase, total bilirubin, TBW creatinine clearance and FFM creatinine clearance. Creatinine clearance was calculated using Cockcroft–Gault formula.[29] A covariate was selected if it was pharmacologically plausible; a correlation existed between a covariate and the random effects of the predicted individual parameters. Retention of the covariate in the model was based on statistical significance ($P \leq .05$ using Wald test), a decrease in OFV and BSV. Covariates, normalised by the typical population median value, were added in the model one at a time in log-linear fashion. The relationship between a pharmacokinetic value (θ_i) and continuous covariate (cov_i) and of an *i*-th individual was expressed as shown in equation 1:

$$\theta_i = \theta_{pop} * \left(\frac{cov_i}{COV_{median}}\right)^{\beta_{\theta i}} * e^{\eta_i} \quad (1)$$

where θ_{pop} represented the typical population pharmacokinetic value and COV_{median} the population median value of the continuous covariates. The random effect associated with the *i*-th individual was denoted by η_i where $\eta_i \approx N(0, \omega^2)$. The factor describing the effect of a continuous covariate on θ_i was denoted by $\beta_{\theta i}$. The relationship, in the case of the categorical covariates was expressed as shown in equation 2:

$$\theta_i = \theta_{pop} * e^{\beta_{\theta i,cat}[cat=n]} * e^{\eta_i} \quad (2)$$

where $\beta_{\theta i,cat}[cat = n]$ denoted the difference in parameter (θ_i) between an individual belonging to group n and the reference group. Correlations among random effects of estimated individual parameters were then investigated and significant ones were estimated as population parameters.

2.6 | Model evaluation

The model was evaluated visually by inspection of the diagnostic plots such as individual- and population predicted *vs* observed concentrations, individual-weighted residuals *vs* time and predicted concentrations. The visual predictive checks for both terizidone and cycloserine were inspected for possible model misspecification. The distribution of parameter estimates randomly sampled from the conditional distribution[30] were evaluated in order to ensure that the assumption of normality was met. We also performed a bootstrap procedure of 250 runs using Monolix software aided by Rsmlx (R Speaks 'Monolix') R package (http://rsmlx.webpopix.org), in order to evaluate the robustness of the final joint model.

2.7 | Secondary pharmacokinetic parameters

The other pharmacokinetic parameters for terizidone and cycloserine were calculated using MLXTRAN coded formulae in Monolix as shown in Appendix A. The area under the concentration–time curve up to 24 hours ($AUC_{0\text{-}24h}$) and half-life were calculated by integration of the concentrations predicted from the final joint pharmacokinetic model without covariates and formulae, respectively. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained from the output file of the predicted individual concentrations. Finally, the clearance of terizidone resulting from biotransformation into cycloserine was calculated from the individual predicted estimates of terizidone apparent volume of distribution (V_p/F), *Fm* and biotransformation rate constant. Model-based simulations of the distributions of $AUC_{0\text{-}24h}$ and C_{max} were performed across 3 weight bands specified in the local treatment guideline[17] using current dose (750 mg daily). A dosing schedule was proposed that would normalise exposure across weight bands using Monte-Carlo simulations.

3 | RESULTS

Thirty-nine patients including 27 HIV infected and 20 females participated in this study. Thirty-eight patients received a daily dose of 750 mg of terizidone while 1 patient received 500 mg. In total, they provided 571 plasma concentrations, of which 272 were for terizidone. The average number of plasma samples per patient was 7 and 8 for terizidone and cycloserine, respectively. The summary of patients' demographic characteristics are displayed in Table 1. The plot of the concentration *vs* time of the original data for terizidone and cycloserine is shown in Figure 1.

3.1 | Terizidone pharmacokinetic model

The base model consisted of a 1-compartment pharmacokinetic model with first-order absorption and linear elimination. A combined additive and proportional error model best modelled the residual unexplained variability in terizidone concentration. A lag-time parameter was added to describe absorption delay but did not improve the model fit (OFV = +4). When a transit compartment model was used instead, resulted in worse fit (OFV = +8.52) than the base model. Additionally, the *ka* was overestimated and thus not plausible besides %RSE could not be estimated. The transit compartment model was then modified by setting *Ktr* equal to *ka*. This modification resulted in improved model fit (OFV = −39.1) and good parameter precision (Table 2). Therefore, the final terizidone model had the following parameters: mean transit time (*Mtt*), *ka*, *Vp/F* and apparent total clearance (Cl_{tot}/F).

TABLE 1 Summary of patients' demographic characteristics

Variable	Value
Sample size (*n*)	39
Sex	
Female (*n*)	20
Male (*n*)	19
Age (years)	32 (17–56)[a]
Total body weight–TBW (kg)	51.4 (32.4–71)[a]
Free fat mass–FFM (kg)	39.8 (24.8–51)[a]
Body mass index (kg m^{-2})	18.4 (12.4–26.1)[a]
Alanine aminotransferase (IU L^{-1})	11 (4–46)[a]
Aspartate transaminase (IU L^{-1})	33 (17–109)[a]
Albumin (g L^{-1})	32 (15–48)[a]
TBW creatinine clearance (ml min^{-1})	83 (34.5–128)[a]
FFM creatinine clearance (ml min^{-1})	60.4 (26.8–106)[a]
Total bilirubin (μmol L^{-1})	7 (2–24)[a]
Human immunodeficiency virus status	
Infected (n)	27
Uninfected (n)	12
CD4 count (cells μl^{-3})	227 (9–1243)[a]
Viral load (copies mL^{-1})	3279 (42–4 331 310)[b]

[a]Median and range.

[b]Median and range from 19 patients who had their viral load above 40 copies mL^{-1} while the viral load from 8 patients was below 40 copies mL^{-1}.

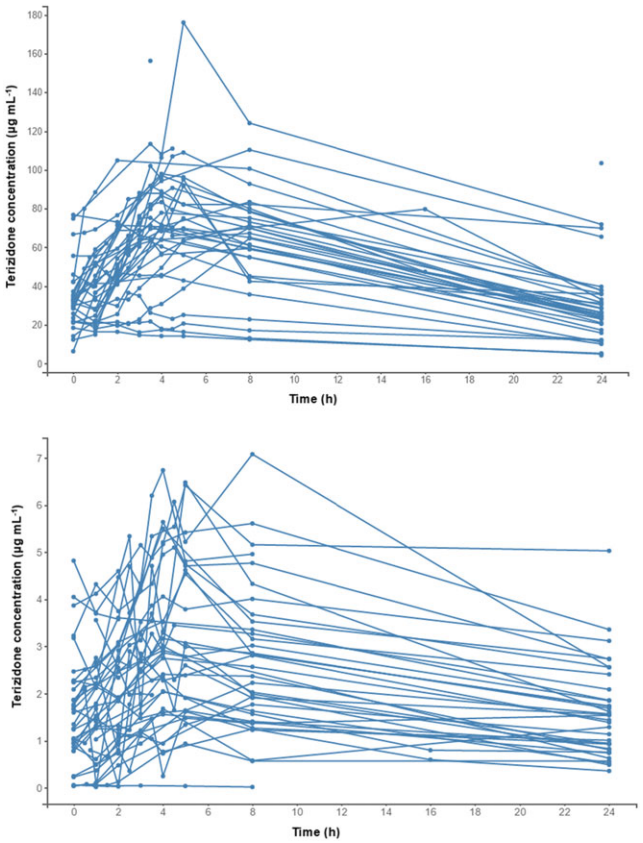

FIGURE 1 Observed concentration–time profiles for terizidone and cycloserine

TABLE 2 Population pharmacokinetic parameters from terizidone only model

Parameter	Estimate	%RSE
Mtt (h)	1.6	17.1
ka (h^{-1})	2.97	19.1
Vp/F [a] (L)	13.4	4.8
Cl_{tot}/F [a] (L h^{-1})	0.51	10.9
Coefficient (effect) of albumin on *Vp/F*	−0.61	30.3
Between-subject variability (CV%)[b]		
Mtt	82	18.3
ka	36.1	53.1
Vp/F	16	35
Cl_{tot}/F	64	13
Residual error		
Additive (µmol L^{-1})	13.6	31
Proportional	0.13	20

[a]Allometrically scaled parameters using FFM by fixing exponents to 1 and 0.75 on *Vp/F* and Cl_{tot}/F, respectively.

[b]Coefficient of variation percentage calculated as $\left(\sqrt{e^{(SD)^2}-1}\right)*100$ where SD is the estimated standard deviation.

%RSE, percentage relative standard error; *Mtt*, mean transit time; ka, absorption rate constant; *Vp/F*, apparent volume of distribution; Cl_{tot}/F, apparent total clearance of terizidone.

Allometric scaling using FFM, TBW and body mass index was performed on *Vp/F* and Cl_{tot}/F. The FFM was found to be the best descriptor of body size as it was associated with the lowest OFV. Allometric scaling using FFM after fixing exponent to 1 and 0.75 on *Vp/F* and Cl_{tot}/F resulted in model improvement (OFV = −90.04) and this explained 13.5 and 5% of the variation in *Vp/F* and Cl_{tot}/F, respectively. The covariates, sex, FFM creatinine clearance and HIV status had significant effect on Cl_{tot}/F, while albumin had significant effect on *Vp/F*. However, when all significant covariates were included in the model, only albumin remained significant on *Vp/F* and led to improved model fit (OFV = −11.9; $p = 0.0062$). The variation explained by albumin in *Vp/F* was 43.2%. There were no significant correlations found among parameter random effects. The summary of the population pharmacokinetic parameters of terizidone are shown in Table 2.

3.2 | Joint terizidone and cycloserine model

Terizidone final model was modified in order to incorporate a cycloserine compartment as illustrated in Figure 2. The Cl_{tot}/F was divided into apparent clearance of terizidone due to biotransformation into cycloserine (*Clpm* = Cl_{tot}/Fm) and apparent clearance of terizidone via other routes (*Clp* = $Cl_{tot}/(1\text{-}Fm)$). A 1-compartment model with first-order elimination best characterised cycloserine disposition. The combined additive and proportional error model best described the

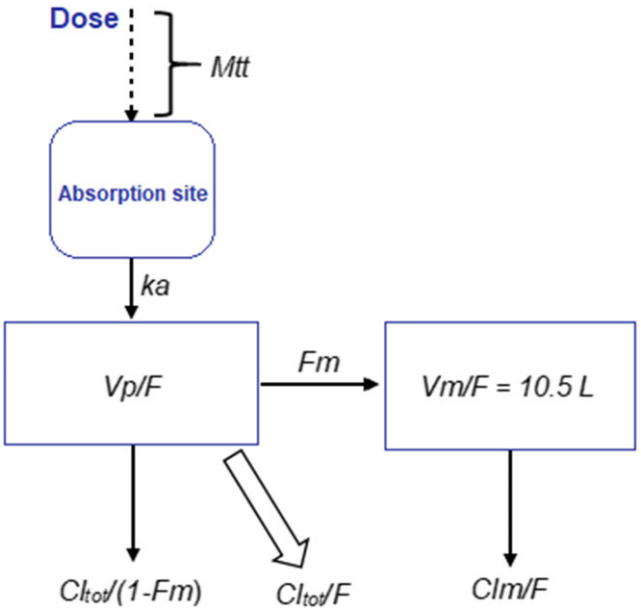

FIGURE 2 Schematic diagram for the joint pharmacokinetics model of terizidone and cycloserine. *Mtt*, mean transit time of terizidone from ingestion to its absorption; *Ad*, amount of terizidone at the absorption site; *ka*, absorption rate constant of terizidone; *Vp/F*, apparent volume of distribution of terizidone; *Clp* = $Cl_{tot}/(1\text{-}Fm)$, clearance of terizidone via other routes; *Fm*, apparent fraction of terizidone converted to cycloserine; Cl_{tot}/F, apparent total clearance of terizidone (*Clp* + *Clpm*), *Vm/F* (10.5 L), apparent volume of distribution of cycloserine; *Clm/F*, apparent clearance of cycloserine

residual unexplained variability in cycloserine concentration. Allometric scaling on apparent clearance of cycloserine (*Clm/F*) using FFM and fixing the exponent to 0.75 slightly improved the joint model with OFV change of −0.82. The variation in *Clm/F* explained by this scaling was 17%. After scaling of *Clm/F* with FFM, no covariates were found significant on *Fm*, *Clp* and *Clm/F*. The values for *Mtt*, *ka*, and *Vp/F* in the terizidone and joint model were not exactly the same but similar.

The joint model was described by the following system of ordinary differential equations 3–5:

$$dAd/dt = -ka * Ad \tag{3}$$

$$dApc/dt = ka * Ad - (Cl_{tot}/(1 - Fm))/Vp * Apc - (Cl_{tot}/Fm)/Vp * Apc \tag{4}$$

$$dAm/dt = ((Cl_{tot}/Fm)/Vp) * Apc - (Clm/F)/10.5 * Am \tag{5}$$

The amount of terizidone at the absorption site and central compartment was denoted by *Ad* and *Apc*, respectively, with *ka* as the absorption rate constant. The amount of cycloserine formed from terizidone metabolism was denoted as *Am*.

The final joint model had the parameters: *Mtt*, *ka*, *Vp/F*, *Clp*, *Fm and Clm/F* (Table 3). The final individual models of *Vp/F*, *Clp* and *Clm/F* belonging to an *i*-th individual were described as follows 6–8:

$$\frac{Vp}{F_i} = 14 * \left(\frac{FFM_i}{39.8}\right)^{1} * \left(\frac{Albumin_i}{32}\right)^{-0.51} * e^{\eta_i} \tag{6}$$

$$Clp = 0.1 * \left(\frac{FFM_i}{39.8}\right)^{0.75} * e^{\eta_i} \tag{7}$$

$$\frac{Clm}{F_i} = 2.94 * \left(\frac{FFM_i}{39.8}\right)^{0.75} * e^{\eta_i} \tag{8}$$

3.3 | Model evaluation

The plots of individual predicted *vs* observed concentration for both terizidone and cycloserine indicated that there was a good agreement

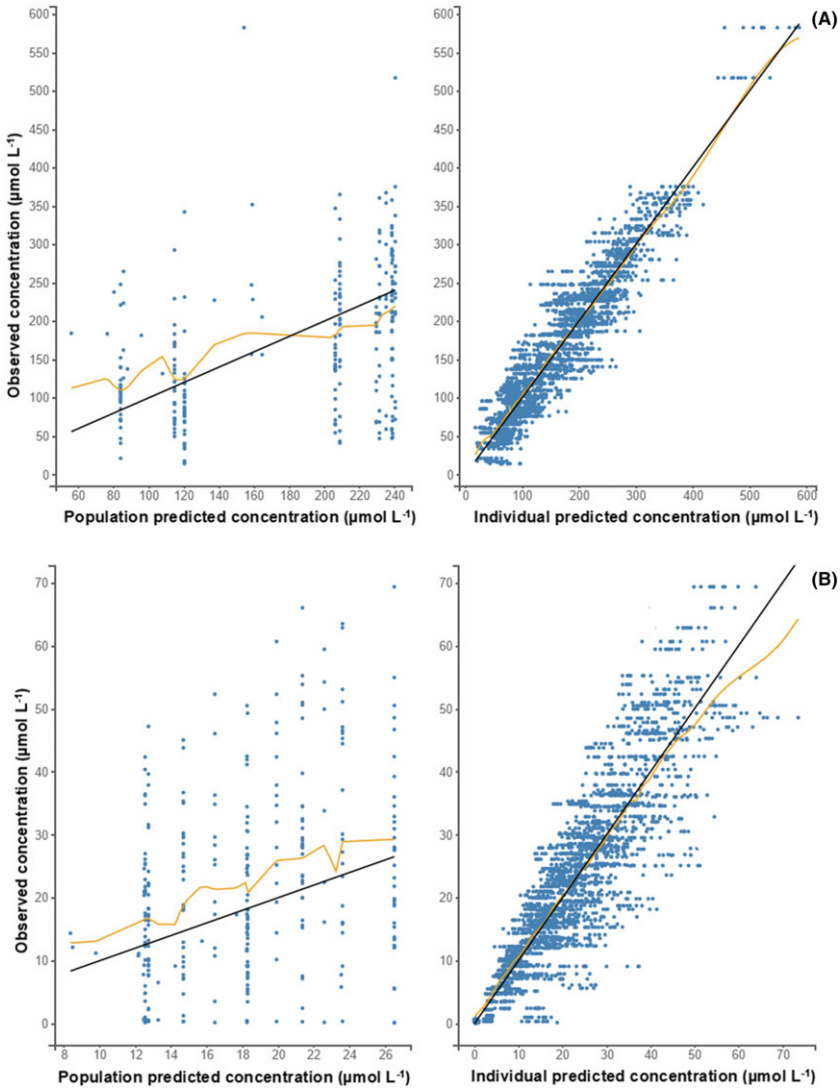

FIGURE 3 Population and individual predicted *vs* observed concentrations for terizidone A, and cycloserine B

between predicted and observed concentration (Figure 3). Similarly, Figure 4 showed that there was no bias observed in the plots of individual-weighted residuals *vs* time and predicted concentration. The visual predictive checks for the joint model are shown in Figure 5. Most of the observed data points lay within 90% prediction interval (generated from 1000 simulations) except for the 95th percentile of cycloserine visual predictive checks that showed some over prediction in variability. The bootstrap parameters in Table 3 were similar to the ones estimated from the original data set. Therefore, the developed joint model described fairly the observed plasma concentration–time profiles for terizidone and cycloserine.

3.4 | Secondary pharmacokinetic parameters

The secondary pharmacokinetic parameters for terizidone were C_{max}, T_{max}, $AUC_{0\text{-}24h}$, half-life and *Clpm* and for cycloserine were C_{max}, T_{max}, $AUC_{0\text{-}24h}$ and half-life (Table 4). The MLXTRAN model file for Monolix in Appendix A gives the code of how these parameters were calculated. The simulated distributions of $AUC_{0\text{-}24h}$ and C_{max} (Figure 6) show a decreasing trend in the median value across the 3 weight bands, 33–50 kg, 51–70 kg and > 70 kg, respectively. Monte-Carlo simulations showed that a terizidone daily (every 24 hours) dose of 750, 900 and 1200 mg achieved similar exposure across the weight bands 33–50, 51–70 and > 70 kg, respectively. The proposed dosing schedule is shown in Table 5.

4 | DISCUSSION

There is scarce information in the literature on pharmacokinetics of terizidone although it is an old drug. In this study, we, for the first, time developed a population pharmacokinetic model of terizidone and a joint model (with cycloserine) at steady state in patients with drug-resistant tuberculosis. A 1-compartment model with first-order absorption and elimination best described terizidone pharmacokinetics. A modified transit compartment model described better the absorption delay than the lag time, as the precision in the former

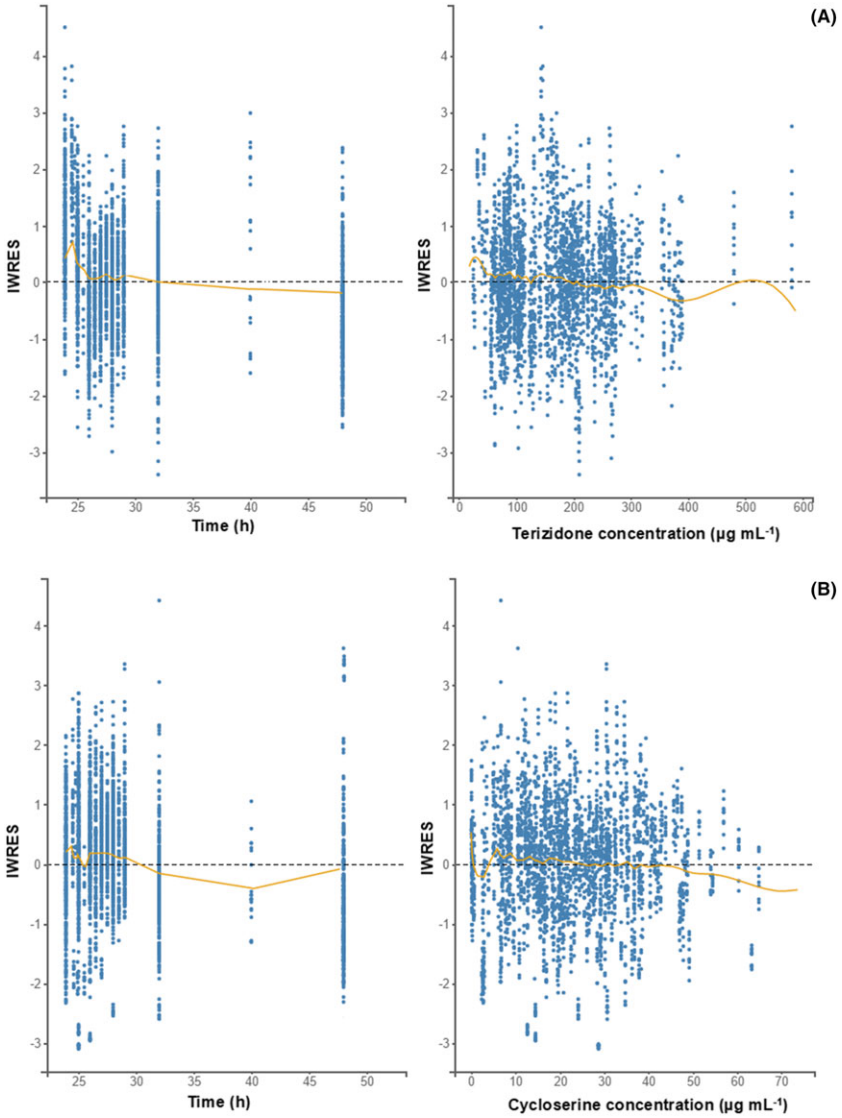

FIGURE 4 Individual-weighted residuals *vs* time and predicted concentrations for terizidone A, and cycloserine B

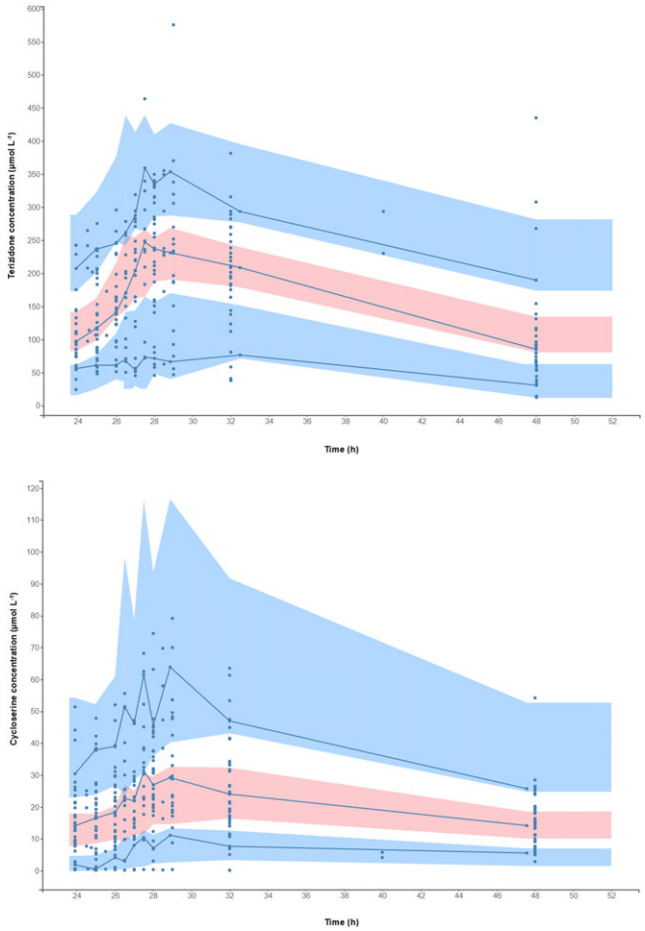

FIGURE 5 Visual predictive checks of terizidone and cycloserine generated from 1000 simulations. The shaded areas represent the prediction interval at 5th, 50th and 95th percentiles (95% confidence interval). The solid lines represent the empirical median of the 5th, 50th and 95th percentiles. The observed data is represented by dots

TABLE 3 Terizidone and cycloserine population pharmacokinetic parameters from a joint model

	Model estimate		Bootstrap	
Parameter	Estimate	%RSE	Median	95% CI (lower, upper)
Mtt (h)	1.43	14	1.01	0.92, 1.75
ka (h^{-1})	3.2	10.5	3.1	2.69, 3.4
Vp/F [a] (L)	14	11	13.4	12.4, 14.6
Clp [a] (L h^{-1})	0.1	12	0.08	0.05, 0.09
Fm	0.29	10	0.24	0.19, 0.33
Clm/F [a] (L h^{-1})	2.94	20.2	2.7	2.44, 3.01
Coefficient (effect) of albumin on *Vp/F*	−0.51	54.9	−0.50	−0.73, −0.47
Between-subject variability (CV%)[b]				
Mtt	75	10	83	71.2, 96
Ka	46	28	48	43, 56
Vp/F	22	15	25	91, 27
Clp	52	16.1	46.2	44, 51
Fm	27	31.2	21.8	19, 29
Clm/F	189	11	171	162, 226
Residual error				
Additive, terizidone (µmol L^{-1})	25	17.9	21	20, 24.4
Proportional, terizidone	0.04	63.1	0.04	0.04, 0.05
Additive, cycloserine (µmol L^{-1})	0.29	33.6	0.3	0.22, 0.3
Proportional, cycloserine	0.32	6.14	0.3	0.24, 0.31

[a]Allometrically scaled parameters using FFM by fixing exponents to 1 on *Vp/F* and 0.75 on *Clp* and *Clm/F*.

[b]Coefficient of variation percentage calculated as $\left(\sqrt{e^{(SD)^2}-1}\right)*100$ where SD is the estimated standard deviation.

%RSE, percentage relative standard error; CI, confidence interval; *Mtt*, mean transit time; ka, absorption rate constant; *Vp/F*, apparent volume of distribution; Cl_{tot}/F, apparent total clearance of terizidone; *Fm*, fraction of terizidone that is converted into cycloserine; *Clm/F*, apparent clearance of cycloserine.

was better than the latter. Initially, addition of a lag time or transit compartment to model the delayed absorption did not improve the model fit. This indicated a possibility of over parameterisation for the transit compartment model. When the number of parameters were reduced from 3 to 2 by setting *ka* equal to *Ktr*, this resulted in improved model fit. The average time (*Mtt*) it took from the ingestion of terizidone to its absorption was about 102 minutes (1.7 hours). It then underwent fast absorption with *ka* of 2.97 h^{-1}. Within a median T_{max} of 4 hours, terizidone reached a C_{max} of 239 µmol L^{-1}.

Adjusting for the effect of body size (allometric scaling) on *Vp/F* and Cl_{tot}/F using FFM and fixing exponents, resulted in model improvement. The FFM was the best descriptor of body size as it was associated with the lowest OFV. As a result of this scaling, the BSV in *Vp/F* and Cl_{tot}/F decreased by 13.5 and 5%, respectively. The population estimates of the *Vp/F* and Cl_{tot}/F were thus estimated with good precision. Serum albumin had significant effect on the *Vp/F* and accounted for 43.2% of the variation in the *Vp/F*. Additionally, this improved the model fit. The effect was in such a way that high serum albumin concentration was significantly associated with low values of *Vp/F* or vice versa. This phenomenon is well known as increased drug binding resulting from increased serum albumin concentration tend to decrease the volume of distribution.[31,32] This effect of albumin on *Vp/F* may have potential clinical impact on patients with hepatic impairment.

The pharmacokinetic parameters (*Mtt*, *ka* and *Vp/F*) of the joint model, although not exactly the same, were similar to those of terizidone model. Additionally, these parameters were estimated with good precision. In the joint model, it was clearly observed that the

TABLE 4 Summary of the secondary pharmacokinetic parameters of terizidone and cycloserine

Pharmacokinetic parameters	Median and range
Terizidone	
C_{max} (μmol L^{-1})	239 (64.2–520)
T_{max} (h)	4 (2–8)
$AUC_{0\text{-}24h}$ (μmol h L^{-1})	1635 (483–8954)
Half-life (h)	17.8 (9–45)
Clpm (L h^{-1})	0.47 (0.05–1.88)
Cycloserine	
C_{max} (μmol L^{-1})	24.1 (0.54–63.5)
T_{max} (h)	8 (3–8)
$AUC_{0\text{-}24h}$ (μmol h L^{-1})	203 (3.6–99)
Half-life (h)	2.49 (0.32–11.9)

C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; $AUC_{0\text{-}24h}$, area under the concentration–time curve up to 24 hours; *Clpm*, apparent clearance of terizidone due to biotransformation into cycloserine.

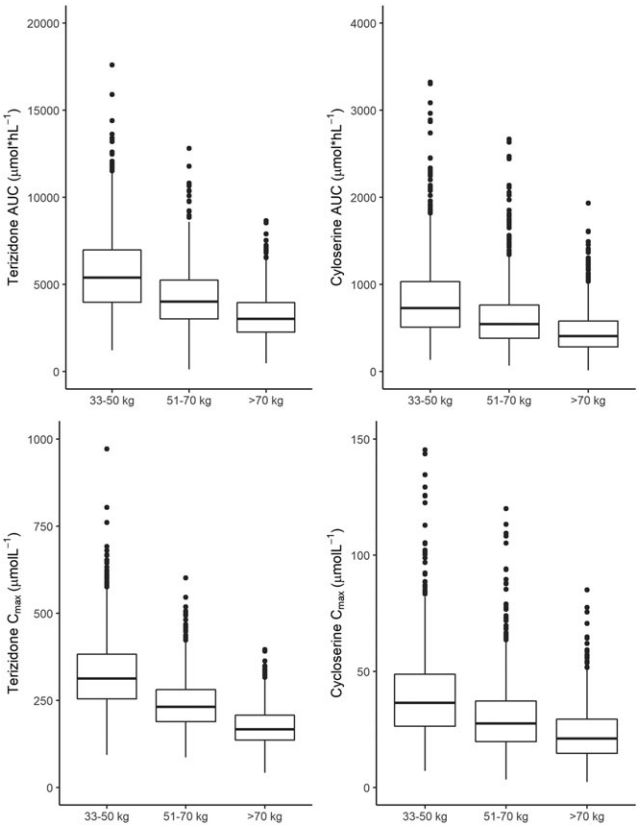

FIGURE 6 Box plots of simulated area under the concentration–time curve up to 24 hours (AUC) and maximum plasma concentration (C_{max}) for the current recommended terizidone daily dose of 750 mg stratified by weight band

estimated *Clp* (0.1 L h^{-1}) was lower than *Clpm* (0.47 L h^{-1}). This implied that terizidone clearance via biotransformation into cycloserine was higher than clearance via other routes. The closeness of the sum value

TABLE 5 Proposed dosing schedule of terizidone across 3 weight bands

Weight band (kg)	Dose (mg)[a]	Simulated exposure	
		$AUC_{0\text{-}24h}$ (μmol h L^{-1})[b]	C_{max} (μmol L^{-1})[b]
33–50	750	4929 (3694–6317)	278 (228–336)
51–70	900	5045 (3929–6392)	278 (227–335)
> 70	1200	5047 (3926–6381)	279 (228–335)

[a]Dosed every 24 hours.

[b]Values expressed as median and interquartile range.

of *Clp* and *Clpm* (0.57 L h^{-1}) and Cl_{tot}/F (0.51 L h^{-1}) indicated that the joint model was able to discriminate well between the *Clpm* driven by *Fm* and *Clp*. On average, 29% of the total amount of terizidone in the body was being converted to cycloserine, suggesting that 71% of the remaining was eliminated via other routes. However, this was not the absolute but apparent fraction, owing to the unavailability of urine data. No covariates tested had significant effect on *Clp*, *Fm* and *Clm/F*. Meanwhile, there was high BSV observed in *Clm/F* and *Mtt*, which could not be explained by the covariates tested. The pharmacokinetic parameters of terizidone in our study are different from the previously reported,[13] where blood was sampled after a single dose or before steady state. In the previous study,[13] *Vp/F* and Cl_{tot}/F were higher (245.6 L, 6.4 L h^{-1}) than in our study (13.4 L, 0.51 L h^{-1}). Meanwhile, in the study by Zitkova and Toušek,[13] the observed average C_{max} resulting from 750 mg dose of terizidone was very different from the C_{max} that we simulated using their model (approximately 19 *vs* 6.1 μg mL^{-1}, respectively). It is noteworthy that the method[33] Zitkova and Toušek[13] used to determine terizidone concentrations was validated for cycloserine and not terizidone. In their study,[13] terizidone was not determined directly but estimated based on the cycloserine concentration. Therefore, concentrations might have been inaccurately estimated and lead to incorrect estimation of terizidone pharmacokinetic parameters. These shortcomings associated with determination of terizidone in Zitkova and Toušek's study[13] may explain the differences in *Vp/F* and Cl_{tot}/F in our study.

The precision at which pharmacokinetic parameters were estimated in the joint and terizidone model was generally good. However, the %RSE in the estimation of BSV in *ka*, proportional error estimate and coefficient of albumin on *Vp/F* were slightly above 50%. There was good agreement between observed and predicted concentrations and the model predicted well the concentrations across all time points as no bias was seen in the residual plots. The visual predictive checks indicated that the joint model fitted well the observed terizidone and cycloserine concentration–time data, as most of the observed data overlapped with the simulated percentiles. Although the model showed slight over prediction in the absorption phase, the bootstrap parameters were comparable with those estimated from the original data set. Therefore, the final joint model without covariates that was used to estimate the C_{max}, T_{max}, $AUC_{0\text{-}24h}$, half-life and *Clpm* was appropriate.

The local treatment guideline for the management of drug-resistant tuberculosis[17] recommends terizidone daily dose of 750 mg for patients in the weight bands 33–50 and 51–70 kg and 750–1000 mg for patients >70 kg. Although no information on the target $AUC_{0\text{-}24h}$ or C_{max} for terizidone is available from the literature, model-based simulations show that a 750 mg daily does not achieve similar $AUC_{0\text{-}24h}$ or C_{max} across the 3 weight bands. Therefore, we propose a terizidone daily dose of 900 and 1200 mg for patients in the weight bands 51–70 and > 70 kg, respectively. This would ensure the achievement of a similar exposure in patients weighing 33–50 kg and taking 750 mg of the drug.

In conclusion, we report, for the first time the population pharmacokinetics of terizidone and its metabolite cycloserine in patients with drug-resistant tuberculosis. We characterised the secondary pharmacokinetic parameters of terizidone and cycloserine. High serum albumin concentration was significantly associated with low *Vp/F* in this patient population. The FFM was found to be the best descriptor of body size and most ideal for body size effect adjustment. On average, 29% of the terizidone amount in the body was converted into cycloserine. The low Cl_{tot}/F or long half-life supports once daily dosing of terizidone in drug-resistant tuberculosis patients.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

M.M. did the modelling, interpreted pharmacokinetic data, and drafted the manuscript. P.M. conceived and designed the project, collected blood samples and patient information, conducted laboratory tests, and co-drafted the manuscript. Both authors approved the final draft.

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APPENDIX A

AUC computation of parent and metabolite for 1-compartment model with first-order absorption and elimination with parameters Mtt, ka, Vp, Cl, Fm and Clm using ODE.

```
[LONGITUDINAL]
input = {Mtt, ka, Vp, Cl, Fm, Clm}

PK:
depot (target = Ad, Mtt, Ktr = ka, ka)

EQUATION:
odeType = stiff
Vm = 10.5
kp = Cl/(Vp*(1-Fm))
kt = Cl/(Vp*Fm)
km = Clm/Vm

;Initial conditions
t_0 = -120
Ad_0 = 0
Apc_0 = 0
Am_0 = 0

;Differential equations
ddt_Ad = -ka*Ad
ddt_Apc = ka*Ad - kp*Apc - kt*Apc
ddt_Am = kt*Apc - km*Am
ddt_AUCp = 1/Vp * Apc
ddt_AUCm = 1/Vm * Am
if(t < 24)
   AUC24p = AUCp
end
if(t < 48)
   AUC48p = AUCp
end
AUC24_48p = AUC48p - AUC24p
if(t < 24)
   AUC24m = AUCm
end
if(t < 48)
   AUC48m = AUCm
end
AUC24_48m = AUC48m - AUC24m

;other PK parameters
T_HalfTZ = log(2)/(kp + kt)
T_HalfCS = log(2)/km
Cl_TZtrans = kt*Vp

Cp = Apc/Vp
Cm = Am/Vm

OUTPUT:
output = {Cp, Cm}
table = {AUC24_48p, AUC24_48m, T_HalfTZ, T_HalfCS,
Cl_TZtrans}
```