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Pharmacokinetic Modeling, Simulation, and Development of a Limited Sampling Strategy of Cycloserine in Patients with Multidrug-/Extensively Drug-Resistant Tuberculosis

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Abstract

Background and Objective Multidrug-resistant tuberculosis has much poorer treatment outcomes compared with drug-susceptible tuberculosis because second-line drugs for treating multidrug resistant tuberculosis are less effective and are frequently associated with side effects. Optimization of drug treatment is urgently needed. Cycloserine is a second-line tuberculosis drug with variable pharmacokinetics and thus variable exposure when programmatic doses are used. The objective of this study was to develop a population pharmacokinetic model of cycloserine to assess drug exposure and to develop a limited sampling strategy for cycloserine exposure monitoring.

Material and Methods Patients with multidrug-/extensively drug-resistant tuberculosis who were treated for > 7 days with cycloserine were eligible for inclusion. Patients received cycloserine 500 mg (body weight ≤ 50 kg) or 750 mg (body weight > 50 kg) once daily. MW/Pharm 3.83 (Mediware, Groningen, The Netherlands) was used to parameterize the population pharmacokinetic model. The model was compared with pharmacokinetic values from the literature and evaluated with a bootstrap analysis, Monte Carlo simulation, and an external dataset. Monte Carlo simulations were used to develop a limited sampling strategy.

Results Cycloserine plasma concentration vs time curves were obtained from 15 hospitalized patients (nine male, six female, median age 35 years). Mean dose/kg body weight was 11.5 mg/kg (standard deviation 2.04 mg/kg). Median area under the concentration–time curve over 24 h ($AUC_{0-24\text{ h}}$) of cycloserine was 888 h mg/L (interquartile range 728–1252 h mg/L) and median maximum concentration of cycloserine was 23.31 mg/L (interquartile range 20.14–33.30 mg/L). The final population pharmacokinetic model consisted of the following pharmacokinetic parameters [mean (standard deviation)]: absorption constant K_{a_po} of 0.39 (0.31) h^{-1} , distribution over the central compartment (V_d) of 0.54 (0.26) L/kg LBM, renal clearance as fraction of the estimated glomerular filtration rate of 0.092 (0.038), and metabolic clearance of 1.05 (0.75) L/h. The population pharmacokinetic model was successfully evaluated with a bootstrap analysis, Monte Carlo simulation, and an external dataset of Chinese patients (difference of 14.6% and 19.5% in measured and calculated concentrations and $AUC_{0-24\text{ h}}$, respectively). Root-mean-squared-errors found in predicting the $AUC_{0-24\text{ h}}$ using a one- (4 h) and a two- (2 h and 7 h) limited sampling strategy were 1.60% and 0.14%, respectively.

Conclusions This developed population pharmacokinetic model can be used to calculate cycloserine concentrations and exposure in patients with multidrug-/extensively drug-resistant tuberculosis. This model was successfully validated by internal and external validation methods. This study showed that the $AUC_{0-24\text{ h}}$ of cycloserine can be estimated in patients with multidrug-/extensively drug-resistant tuberculosis using a 1- or 2-point limited sampling strategy in combination with the developed population pharmacokinetic model. This strategy can be used in studies to correlate drug exposure with clinical outcome. This study also showed that good target attainment rates, expressed by time above the minimal inhibitory concentration, were obtained for cycloserine with a minimal inhibitory concentration of 5 and 10 mg/L, but low rates with a minimal inhibitory concentration of 20 and 32.5 mg/L.

Extended author information available on the last page of the article

Key Points

Cycloserine pharmacokinetics in patients with multi-drug-/extensively drug-resistant tuberculosis is highly variable

Target attainment can be improved with personalized dose adjustment, especially at higher minimal inhibitory concentration values near the breakpoint

Area under the concentration–time curve over 24 hours of cycloserine can be estimated using a 1- or 2-point limited sampling strategy in combination with a population pharmacokinetic model

1 Introduction

Rifampicin-resistant and multidrug resistant tuberculosis (MDR-TB), caused by *Mycobacterium tuberculosis* resistant to at least rifampicin and isoniazid, remains a continuing global problem with approximately 558,000 new patients in 2017 [1, 2]. Additionally, a success rate up to 85% has been reported in situations using a more personalized treatment strategy [3].

The new World Health Organization (WHO) consolidated guidelines on drug-resistant tuberculosis treatment revised the grouping of tuberculosis drugs, based on an individual patient data meta-analysis relating the association of treatment success and death with the use of individual drugs [4]. Cycloserine, discovered in 1954 and on the WHO's List of Essential Medicines, is now recommended to be added in the treatment of patients with rifampicin-resistant/MDR-TB on longer regimens (group B drug). Cycloserine, an amino acid derivative [5, 6], inhibits the enzymes involved in the synthesis of peptidoglycan, which results in a diminished stability and rigidity to the *Mycobacterium tuberculosis* cell membrane [7]. It penetrates well into tissue and cerebrospinal fluid, and (cross-) resistance rates are low [8–10].

Currently, the lack of a target concentration and a limited sampling strategy (LSS) make individual therapy by means of therapeutic drug monitoring (TDM) challenging [11–13]. Zhou et al. [14] reported that the maximum concentration ($C_{\max}$) of cycloserine in 12 patients receiving a single dose of cycloserine varied from 19 to 85 mg/L, while the $AUC_{0-72\text{ h}}$ varied from 264 to 1153 mg h/L. Court et al. reported that the steady-state concentrations of cycloserine in patients treated with terizidone/cycloserine were higher than those reported with cycloserine [15, 16]. In addition, the limited availability of reliable

assays for cycloserine has hindered widespread research for pharmacokinetic/pharmacodynamic (PK/PD) outcomes. Currently, the WHO systematically examines the evidence for dosing second-line tuberculosis drugs, which may identify knowledge gaps [17, 18]. Deshpande et al. [18] reported that efficacy was driven by the percentage of time concentration that persisted above the minimal inhibitory concentration ($T > MIC\%$), with a desired target of 1.0 log₁₀ colony-forming units/mL kill achieved by $T > MIC\%$ of 30%. The proposed clinical susceptibility breakpoint of cycloserine was 64 mg/L at a dose of 750 mg twice daily in Monte Carlo experiments. In these Monte Carlo experiments, in 92% of patients receiving 750 mg of cycloserine twice daily, a target exposure ($T > MIC$) of at least 30% was achieved. However, high doses of cycloserine, necessary to achieve cidal activity in patients with tuberculosis, could also be neurotoxic [18]. Neurotoxicity of most drugs is related to drug exposure, either $C_{\max}$ or total drug concentration [area under the concentration–time curve (AUC)]. Unfortunately, limited data are available regarding the correlation between individual PK parameters and cycloserine neurotoxicity.

Additionally, low cycloserine concentrations may lead to suboptimal treatment response and development of resistance [19, 20]. Because of the small therapeutic window of cycloserine, from 12 to 26 µg/mL [20], TDM is of importance to reach this target concentration range.

The objective of this study was to examine the variability in PK parameters of cycloserine in patients with multidrug-/extensively drug-resistant tuberculosis (M-/XDR-TB) and to develop a population pharmacokinetic (PPK) model. The secondary aim was to develop a LSS based on the developed PPK model to estimate cycloserine drug exposure that can be used in studies to explore the PK/PD relationship of cycloserine and to optimize the dose regimen of cycloserine in patients with M-/XDR-TB.

2 Materials and Methods

2.1 Study Population

We performed a post-hoc analysis with data from a previously published pharmacokinetic study [21]. This previously published PK study was approved by the Medical Ethical Review Committee of the Republican Scientific and Practical Center for Tuberculosis and Pulmonology in Minsk, Republic of Belarus and was registered at clinicaltrials.gov (identifier number NCT 02169141).

2.2 Study Design

Treatment for MDR and XDR-TB was based on the WHO guideline and Clinical Recommendation for Tuberculosis Treatment of the Republic of Belarus. All patients received cycloserine as part of their treatment at a dose of 500 mg (body weight ≤ 50 kg) or 750 mg (body weight > 50 kg) taken orally once a day. Because of the daily supervision of medication intake by a nurse, adherence to the treatment regimen was considered to be 100%. Full plasma PK curves with seven blood samples of cycloserine (4 mL, EDTA anticoagulant) were obtained during steady state before and 1, 2, 3, 4, 7, and 12 h after drug administration of cycloserine. Demographic and medical data such as age, weight, and kidney function (plasma creatinine concentration) were collected from the medical records [21].

2.3 Pharmacokinetics

The concentrations of cycloserine in human plasma were measured at the Infectious Diseases Pharmacokinetics Laboratory of the College of Pharmacy (University of Florida, Gainesville, FL, USA) by a validated liquid chromatography-tandem mass spectrometry method. Pharmacokinetic calculations were performed using MW\Pharm 3.83 (Mediware, Groningen, The Netherlands). The KinPop module of MW\Pharm was used to build a one-compartment model, whereas previous studies applied a non-compartmental analysis [14, 22]. The KinPop module uses an iterative, two-stage, Bayesian PPK analysis procedure [23]. Pharmacokinetic parameters were assumed to be log-normally distributed. For parameterizing the PPK, first, an ‘a priori’ model was built based on literature data [13, 14, 24]. The ‘a priori’ structural model consisted of oral administration with an absorption constant K_a , distribution over a central compartment, and clearance from that central compartment. Volume of distribution was assumed to parallel body weight ($V_d = V/BW$). Total body clearance was calculated with CL (L/h) = CL_m (metabolic clearance) (L/h) $\times$ body surface area (m^2)/1.85 + $fr \times eGFR$ (estimated glomerular filtration rate) (L/h). The CKD-EPI equation was used to calculate eGFR values (mL/min) [25]. Because no data on intravenous administration of cycloserine were available, a fixed estimated oral bioavailability (F_{po}) of 1 was used to parameterize the PK model and thus V_d and CL_m were reported as V_d/F_{po} and CL_m/F_{po} . A visual inspection of each pharmacokinetic plasma concentration–time curve in MWPharm was performed before inclusion of these data for the parametrization of the PPK model. Non-compartmental parameters (C_{max} and time to C_{max}) were also calculated using MWPharm.

2.4 Parameterization of the Population Pharmacokinetic Model

We constructed a PPK model to predict the pharmacokinetics of cycloserine as follows: first, we fixed all PK parameters ($K_{a,po}$, V_d/F_{po} , CL_m/F_{po} , and fr) and evaluated the statistics of the model. The Akaike Information Criterion (AIC) was used as a statistic [26, 27]. Second, one by one, each of the parameters was set to Bayesian and the outcomes were statistically evaluated. A PPK model with a decrease of the AIC with two units was considered statistically significant ($p < 0.05$) [28]. The statistically best-fitting parameter setting was chosen and, in the next step, a second parameter of the remaining parameters was added one by one to the fit. Again, the parameter setting with the best statistic, based on the lowest AIC, was chosen and after this step, the third PK parameter was added to the fit and the last PK parameter was fixed. After statistical evaluation of these models, the last parameter was added to the fit. The statistically best-performing model, based on the lowest AIC, was chosen as the final PPK model for further evaluation. Finally, the effect of adding lag time (T_{lag}) into the PPK model was statistically evaluated.

2.5 Validation of the Pharmacokinetic Model

The final PPK model was validated using Monte Carlo simulations (MW\Pharm 3.83, Mediware) as well as bootstrap analyses. Using the Monte Carlo technique, 1000 sets of patients were simulated, starting from a representative patient with almost average weight and height (patient number 16; weight = 61.6 kg, height = 1.73 m, age = 25 years) to simulate plasma concentrations of cycloserine at seven time points. The variability in the PK parameters in the simulated population was calculated using a bootstrap analysis ($n = 1000$). External validation of the model was performed using data from a PK study performed by Yew et al. [29] in which eight patients were assigned to cycloserine 500 or 750 mg once daily. From this study, measured concentrations of individual patients assigned to cycloserine were compared with calculated concentrations of these individual patients at the same times with our PPK model in MWPharm using their age, eGFR, height, and weight of patients. Differences of $< 20\%$ between calculated and measured concentrations were allowed [30].

2.6 Evaluation of the Pharmacokinetic Model

Cycloserine concentration values calculated with the model were compared with measured cycloserine concentration values with a Bland–Altman plot. To test the predictive performance of the model, individual cycloserine concentration values were calculated with the model and individual patient parameters and compared with measured concentration values.

2.7 Limited Sampling Strategies

Parameterized PPK values for the absorption rate constant ($K_{a_{po}}$), volume of distribution per kilogram of body-weight (V_d), T_{lag} , Cl_m , and renal clearance as a fraction of the eGFR (fr) were subsequently used to calculate the optimal sample points for the LSS. Using Monte Carlo simulations performed by MWPharm, plasma concentrations for an average patient at seven time points with 1-h intervals within a time span of 12 h were simulated for 1000 sets of patients. The AUC was the primary outcome parameter. Practical sampling strategies were evaluated with a minimum time span between two sample points of 1 h and to a maximum of 8 h after cycloserine administration for logistical reasons. Exclusive strategies with a root-mean-squared-error (RMSE) less than 10% and an adjusted r squared (R^2) > 0.95 were considered. The precision in the prediction of the $AUC_{0-24\ h}$ is represented by the RMSE. By entering the actual measured concentrations at the selected LSS time points, the ability of the model to predict the AUC could be assessed by comparing the predicted AUC with the full AUC.

2.8 Target Attainment

To evaluate the effect of different MIC values on the PK/PD index, $T > MIC$ ratios were calculated. This ratio was chosen because it is the key driver for cycloserine efficacy [18]. $T > MIC$ ratios were defined for MIC values of 5, 10, 20, and 32.5 mg/L, as recommended by the WHO [5]. Drug $T > MIC$ ratios were calculated per patient as time (%) of the 24-h dosing interval at steady state (generated with MWPharm) that the cycloserine concentration was above MIC. The time the PK curves exceeded the MIC ($T > MIC$ %) were also calculated by performing Monte Carlo simulation of 1000 patients. Finally, mean and standard deviation (SD) values were calculated per PK/PD index in the total population.

2.9 Statistics

All statistics were performed using both MWPharm and IBM SPSS Statistics for Windows version 22.0. When normally distributed, mean (SD) values are presented. Covariate analysis for the PPK parameters was performed using MWPharm. Correlations between PK and demographic data were tested for significance using Mann–Whitney U tests and two-tailed independent t tests for categorical and continuous variables. Univariable and multivariable linear regression analyses were performed using AUC as a dependent variable and patient characteristics (body weight, age) as independent variables. Standardized coefficients

(beta) and p values were reported for each independent variable.

3 Results

3.1 Pharmacokinetics and Demographic Data

In total, 17 plasma concentration–time curves were obtained from 17 hospitalized patients in the Republican Research and Practical Centre for Pulmonology and Tuberculosis in Minsk. From these 17 curves, 15 were included for the PPK model building after visual inspection; the PK curves of two patients (4 and 10) were excluded from further analyses because the measured values for the cycloserine concentrations of these patients seemed incorrect. Demographic data are shown in Table 1. Fifteen patients received cycloserine 750 mg once daily and two patients received cycloserine 500 mg once daily orally. The mean dose per kilogram of body weight was 11.5 mg/kg (SD 2.04 mg/kg). The median $AUC_{0-24\ h}$ was 888 h mg/L (interquartile range 728–1252 h mg/L). The median time to C_{max} was 3.84 h (interquartile range 3.12–6.64 h) and median C_{max} was 23.31 mg/L (interquartile range 20.14–33.30). The coefficient of variance of the $AUC_{0-24\ h}$ was 46.5%, indicating that the number of patients included in the model is sufficient to achieve a power level of > 80% [31].

We also tested the statistical power of each parameter, by comparing our mean PK parameters with our literature model. A statistical power of 100%, 76.4%, 84.6%, and 100% was found for respectively, Cl_m , V_d , $K_{a_{po}}$, and fr . The mean absorption half-life was 2.39 h (SD 2.27 h) and the mean elimination half-life was 0.55 h (SD 0.33 h). The PK parameters of the model based on literature data and the parameterized model are displayed in Table 2. A brief summary of the parameterization of the PPK model is displayed in the Table 1 of the Electronic Supplementary Material. A plot of the mean cycloserine concentration–time curve and a scatter plot of measured cycloserine values are shown in Fig. 1.

The $AUC_{0-24\ h}$ was correlated with C_{max} (median time to $C_{max} = 3.84\ h$) computed by: $AUC_{0-24\ h} = 18.349 \times C_{max} - 1.382$ with a beta coefficient of 0.979 ($p < 0.001$) using linear regression. The $AUC_{0-24\ h}$ was also significantly correlated with $C_{t=4}$ of cycloserine, computed by: $AUC_{0-24\ h} = 37,868 \times C_{(t=4)} - 16,875$ with a beta coefficient of 0.957 ($p < 0.001$) (Fig. 2). After uni- and multivariable linear regression analyses, $AUC_{0-24\ h}$ was not correlated with sex, age, weight, body mass index, length, diagnosis, body surface area, creatinine clearance, absorption time, use of alcohol, or smoking.

Table 1 Demographic data study population

Patient characteristics cycloserine (<i>n</i> = 15)	Modeling population	Range (minimum–maximum value)
Patients, <i>N</i>	15 (9 male)	–
Age (years)	35.0 (29.0–37.5) ^a	25.0–53.0
Height (m*)	1.74 (1.69–1.81) ^a	1.60–1.88
Weight (kg)	64.0 (54.5–77) ^a	47.0–84.0
Dose/kg body weight (mg/kg)	11.2 (9.75–12.8) ^a	9.1–14.7
BMI (kg/m ²)	20.6 (18.9–23.5) ^a	17.6–28.7
Diagnosis		
1. MDR-TB, <i>n</i> (%)	10 (66.7)	–
2. XDR-TB, <i>n</i> (%)	5 (33.3)	–
SCr, μmol/L	76.0 (70.5–82.5) ^a	64.0–102
Smokers, <i>n</i> (%)	9 (60.0)	–
Alcohol abuse, <i>n</i> (%)	1 (6.67)	–
AUC _{0–24 h} (h*mg/L)	888 (728–1252) ^a	345–1967
<i>C</i> _{max} (mg/L)	23.31 (20.14–33.30) ^a	9.37–48.7
Time to <i>C</i> _{max} (h)	3.84 (3.12–6.64) ^a	1.44–8.16
Elimination rate constant (<i>K</i> _e)	1.76 (1.33–2.41) ^a	1.10–4.65

AUC_{0–24 h} area under the plasma concentration–time curve over 24 h, BMI body mass index, *C*_{max} maximum concentration, *h* hours, MDR-TB multidrug-resistant tuberculosis, SCr serum creatinine, XDR-TB extensively drug-resistant tuberculosis

^aMedian data (represented with 25th and 75th percentiles), except for sex, smokers, and alcohol usage

Table 2 Pharmacokinetic parameters of the literature and parameterized pharmacokinetic (PK) model, Monte Carlo simulation, and bootstrap validation

PK parameter	Literature model mean (SD)	Parameterized model mean (SD)	PK model Monte Carlo population mean (SD)	Bootstrap analysis mean (95% CI)
<i>V</i> _d / <i>F</i> _{po} (L/kg LBM)	0.31 (0.10) ⁽¹⁴⁾	0.54 (0.26)	0.33 (0.092)	0.54 (0.40–0.66)
<i>CL</i> _m / <i>F</i> _{po} (L/h)	1.11 (0.53) ⁽¹⁴⁾	1.05 (0.75)	0.91 (0.035)	1.05 (0.001–1.13)
<i>K</i> _{a,po} (h ^{−1})	0.35 (0.052) ⁽¹³⁾	0.39 (0.31)	0.37 (0.065)	0.39 (0.22–0.65)
Fr	0.65 (0.098) ⁽²⁴⁾	0.092 (0.038)	0.70 (0.13)	0.092 (0.031–0.27)

CI confidence interval, *CL*_m metabolic clearance, *F*_{po} fixed estimated oral bioavailability, *h* hours, *K*_{a,po} absorption constant, LBM lean body mass, *V*_d/*F*_{po} distribution over the central compartment, SD standard deviation

3.2 Validation of the Pharmacokinetic Model

The PK model based on a Monte Carlo simulation of 1000 patients is displayed in Table 2. The PK model based on the bootstrap analysis is shown in Table 2. External validation was performed using measured cycloserine concentrations of eight Chinese patients (Table 3). We observed a mean difference of − 14.6% of the measured cycloserine concentrations by Yew et al. [29] compared with the calculated cycloserine concentrations for these patients with our PPK model. We observed a mean difference of − 19.5% of the measured AUC_{0–24 h} by Yew et al. [29] compared with the calculated cycloserine AUC_{0–24 h} for these patients with our PPK model. Our PPK model generally underestimated cycloserine concentrations and AUC in these eight patients.

3.3 Evaluation of the Pharmacokinetic Model

A Bland–Altman plot showing the AUC_{0–24 h} and predicted cycloserine concentration with our PPK model is displayed in Fig. 3, presenting the internal validation of our PPK model.

3.4 Limited Sampling Strategies

Bias, RMSE, and correlation coefficient of the LSS AUC compared with the full AUC are presented in Table 4. Using a one-point LSS for cycloserine at time points 3 h and 4 h, a RMSE of respectively, 5.03 and 1.60% and an *R*² of 0.9522 and 0.9956 were generated. Using a two-point LSS for cycloserine at time points 3 h and 8 h or 2 h and 7 h, a RMSE of respectively, 0.14 and 0.16% and an *R*² of 0.9998 and 1.00

Fig. 1 Plot of mean cycloserine curve and scatter of all measured cycloserine concentrations [mean $\pm$ standard deviation (SD)]

Fig. 2 Linear regression between $C(t=4)$ and area under the concentration–time curve over 24 h ($AUC_{0-24\text{ h}}$) of cycloserine (CS)

were generated. Using a three-point LSS for cycloserine at time points 2 h, 3 h and 7 h or 1 h, 3 h, and 7 h, a RMSE of respectively, 0.05 and 0.06% and an R^2 of 1.00 and 1.00 were generated.

3.5 Target Attainment

The $T > \text{MIC}$ percentages [represented as mean (SD)], using a range of MIC values between 5 and 32.5 mg/L,

were 98.4% (4.4%), 85.6% (31.8%), 27.6% (33.9%), and 11.5% (24.2%) in the 15 included patients with M-/XDR-TB for MICs of respectively 5, 10, 20, and 32.5 mg/L. The $T > \text{MIC}$ percentages (mean) were 100%, 100%, 48%, and 0% in the Monte Carlo simulation of 1000 patients for MICs of respectively, 5, 10, 20, and 32.5 mg/L. In Fig. 4, target attainment is plotted vs a range of MICs.

Table 3 External validation: difference (%) between measured and calculated concentration cycloserine with our population pharmacokinetic model

Patient no	$t=0$	$t=1$	$t=2$	$t=4$	$t=8$
1	6.37	1.28	7.53	6.33	14.8
2	4.89	6.76	3.69	1.90	8.68
3	83.6	4.05	45.9	45.5	120
4	15.3	5.99	11.1	3.29	19.5
5	11.0	16.8	7.96	16.1	12.5
7	3.35	1.46	12.9	3.18	6.41
8	2.18	3.53	1.07	9.49	10.8
10	16.0	5.47	0.91	11.9	15.2

4 Discussion

With data from a pharmacokinetic study, we were able to parameterize a PPK model for cycloserine, perform an internal validation by bootstrap and Monte Carlo analyses of this model, and develop an LSS for future studies with less blood samples. Our PPK model was also successfully externally validated with a bias of less than 20% using a Chinese dataset.

To our knowledge, this is the first PPK model with an LSS of cycloserine developed for use in patients with M-/XDR-TB. The RMSEs found in predicting the $AUC_{0-24\text{ h}}$ from samples at 4 h (1.60%), 2 h and 7 h (0.16%), 3 h and 8 h (0.14%) and 1 h, 3 h, and 7 h (0.06%) in combination with a PPK model are very low. The advantage of this strategy is that total exposure can be predicted adequately with one, two, or three sampling points within a dose interval, with a limited burden for both the healthcare system and for the patient. In our study, both LSS and $C_{t=4}$ were strong predictors for $AUC_{0-24\text{ h}}$. To optimally and precisely predict $AUC_{0-24\text{ h}}$ in patients, both of these strategies can be applied. Our PPK model can be used in combination with measured cycloserine concentration, dose, and patient characteristics to simulate the PK profile of cycloserine in patients with M-/XDR-TB to calculate $T > \text{MIC}$. Additionally, AUCs can be calculated to reflect the possibility of adverse events.

We observed six- and five-fold inter-individual differences in $AUC_{0-24\text{ h}}$ and C_{max} , both in our study population and the Monte Carlo simulations. These differences can be due to slow or insufficient absorption or to dose-dependent pharmacokinetics. Of the patients enrolled in our study, four (26.7%) had a C_{max} below the recommended range of 20–35 mg/L, while three (20.0%) had a C_{max} above this range. Thus, 46.7% of the patients had inadequate cycloserine serum concentrations, which underlines the necessity of TDM in this population. Our median $AUC_{0-24\text{ h}}$ and C_{max} were higher compared with previous studies [13, 14, 32], which is either due to lower values for metabolic clearance

or lower values for the renally cleared fraction compared with previous studies that were mainly focusing on healthy subjects [13, 14]. As tuberculosis can cause chronic kidney disease, renal failure [33], tuberculous interstitial nephritis [34], and hyperbilirubinemia [35], a decreased renal cycloserine clearance may be a consequence. This could be due to renal parenchymal infections and insidious tuberculous interstitial nephritis, which destroys renal parenchyma.

Our $AUC_{0-24\text{ h}}$ and C_{max} values were higher compared with the healthy volunteers despite the higher weight-based dose [13]. This is probably owing to the fact that our study focused on patients diagnosed with M-/XDR-TB instead of healthy volunteers. Therefore, a hypothetical decreased renal clearance may be the reason for our higher values for $AUC_{0-24\text{ h}}$. We decided to use a fixed bioavailability ($F=1$), similar to previous studies [13, 14, 36], this meant a direct comparison can be made with our results. We found a low value for fr in our parametrized PPK model compared with the fr found in the literature. This can be explained by a hypothetical decreased renal clearance in patients with M-/XDR-TB. We also found a higher mean cycloserine concentration compared with Hung et al. [32]. This is probably owing to the fact that Hung et al. studied the pharmacokinetics of cycloserine in a fed condition, while in our study, cycloserine was administered on an empty stomach. High-fat meals decrease cycloserine serum concentrations [37], probably explaining the higher mean cycloserine concentrations found in our study.

Our parameterized PPK model slightly underpredicts cycloserine serum concentrations at higher concentrations. This can be explained by assuming delayed absorption leading to higher serum concentrations. However, this can also indicate the presence of a second compartment. To investigate this theory, we also built a two-compartment model, but this provided no improvement in the AIC. Therefore, we do not expect that the pharmacokinetics of cycloserine can be better estimated with a two-compartment model.

As Alghamdi et al. found that inclusion of T_{lag} in their final PPK model led to a better fit during the absorption phase, we also tested this theory. After inclusion of T_{lag} in our PPK model, we observed unrealistic high values for $K_{a_{\text{po}}}$. We also did not have values for cycloserine serum concentrations in the absorption phase, which made it difficult to construct a PPK model containing T_{lag} . We therefore did not include this parameter in our final PPK model. The values from our PPK model for CL_m and V_d were almost identical compared to those from the PPK model of Alghamdi et al. This strengthens the use of our PPK model in patients with MDR-TB. To test the robustness, our PPK model was successfully validated using Monte Carlo and bootstrap validation methodologies. Our Monte Carlo validation had lower values for V_d , CL_m , and $K_{a_{\text{po}}}$, but higher values for fr compared with our parameterized PPK model.

Fig. 3 Measured and calculated cycloserine (CS) concentrations with line of identity (upper panel) and Bland–Altman graph of measured and calculated concentration (conc) CS (lower panel). *CI* confidence interval, *Std Dev* standard deviation

Extrapolation of *fr* to a greater population may explain why this value is more comparable to the literature value for *fr*. A high degree of inter-individual differences was observed in PK parameters in our study population. This variability did not alter by selecting a median patient to construct the

Monte Carlo model. Therefore, we conclude that our developed PPK model can be used to accurately estimate cycloserine exposure.

We also externally validated our dataset using data from a study conducted in China by Yew et al. [29]. Measured

Table 4 Best-performing limited sampling strategies for one, two, and three time points

Time point(s) of sampling post-steady state ($t=0 \rightarrow$ steady state), h	r (AUC)	Prediction bias (%)	RMSE (%)
0	0.2687	1.54	15.5
4	0.9956	-0.44	1.60
3	0.9522	1.08	5.03
5	0.9458	-2.63	5.95
2	0.8846	1.75	7.68
1	0.8343	2.00	9.04
3 and 8	0.9998	-0.03	0.14
2 and 7	1.00	0.06	0.16
2 and 6	1.00	-0.04	0.17
3 and 7	1.00	-0.09	0.24
1 and 5	1.00	-0.11	0.29
2, 3, and 7	1.00	-0.00	0.05
2, 5, and 7	0.9991	0.00	0.05
1, 4, and 6	1.00	0.00	0.05
2, 4, and 7	1.00	0.00	0.05
1, 4, and 7	0.9998	-0.00	0.06
1, 3, and 7	1.00	-0.00	0.06

The precision in the prediction of the AUC over 24 h is represented by the RMSE

The times displayed in bold are the optimal time points [best r (AUC), prediction bias and/or RMSE] for performing LSS

AUC area under the concentration–time curve, h hours, LSS limited sampling strategy, RMSE root-mean-square-error

Fig. 4 Target attainment for individual patients and Monte Carlo-simulated patients plotted against varying minimal inhibitory concentration values for cycloserine. *SD* standard deviation, $T > MIC$ time above the minimal inhibitory concentration

concentrations by Yew et al. [29] and calculated cycloserine concentrations in our PPK model differed by less than 20%. Our model underestimated cycloserine concentrations

in these Chinese patients, which may be owing to differences in clearance between Caucasian and Asian populations [38]. Another difference is that the patients in the study of Yew et al. [29] had lower eGFR values compared with our patients. These inter-racial differences increase the need for population- and race-specific PK models and an LSS to optimally estimate exposure.

This study also explored the ‘target attainment’ of cycloserine. We used the same PK/PD index, namely $T > MIC$, as Alghamdi et al. [36] used. The $T > MIC$ values of an individual patient with M-/XDR-TB can be calculated by simulating PK profiles using the measured cycloserine concentration with our PPK model, together with individual patient characteristics and administered doses. Good target attainment was achieved for $T > MIC$ values ranging from 5 to 10 mg/L, while target attainment decreased using a MIC from 20 to 32.5 mg/L. This is in line with the results of Alghamdi et al., who found that the current dosing of cycloserine is sufficient for MICs up to 16 mg/L. In patients with lower $T > MIC$ values, target attainment in patients with M-/XDR can be

reached with a personalized dose adjustment, based on measurements of drug concentrations and our PPK model. In patients with $T > MIC$ values lower than 64%, representing 80% of the maximal kill (EC_{80}) value, a dose increase of cycloserine from 500 to 1000 mg is needed, as suggested by Alghamdi et al. [36]. It is confirmed that the $T > MIC$ ratio determines the success of therapy and therefore TDM for cycloserine has a potential added value in patients with M-/XDR-TB.

4.1 Strengths and Limitations

Our study has several strengths and limitations. As our study measured plasma concentrations during steady state, a detailed profile of PK parameters per patient was obtained and peak serum concentrations could be observed. In addition to an internal validation, an external validation of the PPK model was performed using data from a study performed in China. As the measured cycloserine concentrations by Yew et al. [29] did not highly differ from the cycloserine concentrations calculated for the patients in our PPK model ($< 20\%$), this increases the generalizability of the study outcomes to a greater population. Furthermore, this is the first study developing an LSS for patients diagnosed with M-/XDR-TB to show that the $AUC_{0-24\text{ h}}$ of cycloserine can be reliably estimated using linear regression with a fixed sample point or an LSS with one, two, or three time points in combination with a PPK model as described. This strategy enables the possibility to perform target finding in a large-scale study.

A major limitation of our study remains the small sample size, which is a typical problem of studies using rich PK sampling [39]. Although there is a PK/PD target for cycloserine [36], linezolid [40], and fluoroquinolones [41], an evaluation of the combination of these targets is still lacking. This makes it difficult to analyze the results with respect to efficacy. Additionally, AUCs can be estimated to reflect the possibility of adverse events. Desphande et al. [18] stated that doses that are likely to achieve cidal activity in patients with tuberculosis are high, and could therefore be neurotoxic. Zhu et al. [20] stated that TDM is essential to maintain appropriate cycloserine concentrations and to be assured that cycloserine concentrations are within the target range. Therefore, we propose an estimation of cycloserine AUC, as a reflection of the possibility of adverse events and as TDM, for future studies.

Another limitation is the fact that cycloserine samples were drawn during the elimination phase, and not in the absorption and distribution phases of the PK curve. This made it hard to examine whether inclusion of T_{lag} in the final PPK model would also lead to a good fit. We recommend future studies draw cycloserine samples also during the absorption phase so that T_{lag} can be correctly measured.

5 Conclusions

This study developed a PPK model for cycloserine in patients with M-XDR-TB that was successfully validated by internal and external validation methods. This model can therefore be used in combination with patient characteristics, dose, and measured cycloserine concentrations to simulate the PK profile of cycloserine in patients with M-/XDR-TB to calculate the $T > MIC$. In addition, AUCs can be calculated as a reflection of the possibility of adverse events. This study also found a good target attainment for cycloserine at MICs of 5 and 10 mg/L but a decreasing target attainment at MICs of 20 and 32.5 mg/L. Future studies should focus on finding the target exposure that translates into better outcomes of cycloserine in multidrug regimens.

Compliance with Ethical Standards

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Conflict of Interest Ruben van der Galiën, Natasha van't Bovenind-Vrubleuskaya, Charles Peloquin, Alena Skrahina, Daan J. Touw, and Jan-Willem C. Alffenaar declare that they have no conflict of interest.

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