Linezolid Pharmacokinetics in South African Patients with Drug-Resistant Tuberculosis and a High Prevalence of HIV Coinfection

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ABSTRACT The World Health Organization (WHO) recently recommended that linezolid be prioritized in treatment regimens for drug-resistant tuberculosis (TB), but there are limited data on its pharmacokinetics (PK) in patients with this disease. We conducted an observational study to explore covariate effects on linezolid PK and to estimate the probability of PK/pharmacodynamic target attainment in South African patients with drug-resistant TB. Consecutive adults on linezolid-based regimens were recruited in Cape Town and underwent intensive PK sampling at steady state. Non-compartmental analysis was performed. Thirty participants were included: 15 HIV positive, 26 on the initial dose of 600 mg daily, and 4 participants on 300 mg daily after dose reduction for linezolid-related toxicity. There was a negative correlation between body weight and exposure, with 17.4% (95% confidence interval [CI], 0.1 to 31.7) decrease in area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) per 10-kg weight increment after adjustment for other covariates. Age was an independent predictor of trough concentration, with an estimated 43.4% (95% CI, 5.9 to 94.2) increase per 10-year increment in age. The standard 600-mg dose achieved the efficacy target of free AUC/MIC of >119 at wild-type MIC values (≤ 0.5 mg/liter), but the probability of target attainment dropped to 61.5% (95% CI, 40.6 to 79.8) at the critical concentration of 1 mg/liter. When dosed at 600 mg daily, trough concentrations were above the toxicity threshold of 2 mg/liter in 57.7% (95% CI, 36.9 to 76.6). This confirms the narrow therapeutic index of linezolid, and alternative dosing strategies should be explored.

KEYWORDS drug-resistant tuberculosis, linezolid

Drug-resistant tuberculosis (TB) is an ongoing global public health crisis; there were over half a million incident cases in 2017, with a case fatality ratio of approximately 40%, more than double that of drug-sensitive TB (1). New and repurposed drugs offer the hope of improved outcomes. One such agent, the oxazolidinone linezolid, has an impressive impact on treatment outcomes when added to multidrug regimens for

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multidrug-resistant and extensively drug-resistant TB (MDR-TB and XDR-TB) (2, 3). As a result, linezolid has been promoted to the list of priority “Group A medicines” in the new WHO antituberculosis drug categorization (4) and is included in the experimental arms of multiple trials of novel regimens for drug-resistant TB. However, linezolid use is limited by dose- and duration-related toxicity, and the optimal dosing strategy that balances efficacy and toxicity is unknown (5).

The pharmacokinetics (PK) that underpins linezolid dosing is poorly defined in patients with TB, particularly at the most commonly used dose of 600 mg daily and among patients in sub-Saharan Africa, where there is a high burden of HIV coinfection (6). Understanding linezolid PK is important for several reasons. First, PK variability of antituberculosis agents has been associated with unsuccessful treatment outcomes (7), which may also lead to treatment-emergent drug resistance where drug exposure falls below PK/pharmacodynamic (PD) targets (8). Population-specific factors, including genetic polymorphisms, may influence drug disposition and drug effects (9), and it is therefore essential to perform PK studies in diverse populations. Second, the myelosuppression and neuropathy associated with linezolid use, which are often treatment limiting (3), correlate with dose and trough concentrations (10). Linezolid toxicity may be increased among HIV-positive patients (11), which is especially relevant in sub-Saharan Africa, where up to 60% of patients with drug-resistant TB are coinfecting with HIV. Third, linezolid has limited selectivity for its ribosomal target in bacteria and binds to a homologous site in human mitochondria (12). Because of these shared linezolid targets in the pathogen and host, there is a narrow therapeutic window for which the optimal PK targets and dose have not been defined (5) but which is likely to be sensitive to PK variability. Finally, efficacy targets of antituberculosis drugs are influenced by MIC distributions for *Mycobacterium tuberculosis*, but there are limited data on linezolid MICs in populations with drug-resistant TB (13). Applying observed linezolid drug exposures to putative PK/PD parameters for efficacy and toxicity may inform policy decisions around dose optimization until more robust clinical targets are defined.

We aimed to describe the PK of linezolid in a population of patients with drug-resistant TB and a high burden of HIV in South Africa. We also explored the effect of key covariates on PK parameters and estimated the probability of PK/PD target attainment corrected for the *M. tuberculosis* MIC distribution in this cohort.

RESULTS

Study population. Thirty-eight participants were screened between June 2016 and April 2018, and 30 underwent intensive PK sampling. Reasons for exclusion were discontinuation of linezolid prior to the sampling visit ($n = 4$), withdrawal of consent ($n = 2$), loss to follow-up ($n = 1$), and failed intravenous access ($n = 1$). The demographic and clinical characteristics at the time of linezolid sampling are summarized in Table 1. All participants were ambulant at the time of evaluation. Five participants were on lopinavir-ritonavir-based antiretroviral therapy (ART). Four participants were on 300 mg daily after undergoing dose reduction for suspected linezolid-related toxicity, one of whom was switched to the 300-mg dose on the day of the study visit and therefore was not at steady state.

PK parameters. Trough concentrations were imputed for 6 participants due to extreme outlying results from presumed unobserved dosing prior to the 24-h sample. The predose concentration was below the limit of assay quantification (BLQ) in 4 participants. The full data set showing original and imputed linezolid concentrations is available in the supplementary material (Table S1), along with the respective concentration-time profiles for each subject (Fig. S1a and b).

As shown in Fig. 1, concentration-time profiles demonstrated high interindividual variations in plasma concentrations, with an overall coefficient of variation (CV) of 40.1%. There was a rapid attainment of peak concentrations, which were similar for both doses, but concentrations at early time points appeared to be highly variable. Table 2 summarizes the estimated PK parameters from observed linezolid concentra-

TABLE 1 Demographic and clinical characteristics of participants in this study^a

Variable	Value for participants (n = 30)
Age, yrs	33 (27–44)
Male sex	19 (63)
Weight, kg	58.5 (49.8–67.6)
Height, cm	164.5 (158–172)
BMI, kg/m ²	20.2 (18.1–25.5)
Ethnicity	
Black	14 (47)
Mixed	16 (53)
Baseline resistance pattern	
MDR-TB	9 (30)
XDR-TB	21 (60)
HIV positive	15 (50)
Current ART	15 (100)
Current LPV/r	5 (33)
Creatinine, μ mol/liter	65 (53–71)
Creatinine clearance, ml/min	116 (103–139)
Duration on linezolid, days	59 (55–63), range (20–95)
Daily dose of 600 mg	26 (87)
Dose, mg/kg	10.0 (8.3–11.5)

^aData are expressed as median (IQR) or number (percent). BMI, body mass index; ART, antiretroviral therapy; LPV/r, lopinavir-ritonavir.

tions, disaggregated by linezolid dose. Clearance was significantly lower among subjects who had undergone dose reduction to 300 mg daily {1.8 liters/h (interquartile range [IQR], 1.7 to 21) versus 3.1 liters/h (IQR 2.4 to 4.3) in those remaining on 600 mg daily; $P = 0.012$ }, which resulted in a longer half-life in the 300-mg group. There was a linear correlation between linezolid trough concentrations and area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) ($\rho = 0.5$; $P = 0.005$) (Fig. S2).

Covariate effects on PK parameters. Linear regression included only participants receiving the 600-mg dose ($n = 26$) since the sample size of those receiving 300 mg ($n = 4$) was too small to allow for a meaningful evaluation at that dose. There was no association between HIV infection or the use of lopinavir-ritonavir and linezolid exposure on univariable or multivariable analysis. The final multivariable model described

FIG 1 Plasma free concentration-time data for 30 subjects on linezolid. The gray lines represent concentration-time profiles for individual subjects, the green dotted line shows the median for the 600-mg dose, and the blue dotted line shows the median for the 300-mg dose. The horizontal red line on the y axis represents the critical concentration of linezolid for *M. tuberculosis* (1 mg/liter).

TABLE 2 PK parameters^a

Variable	Value		
	600-mg dose (n = 26)	300-mg dose (n = 4)	Overall (n = 30)
AUC _{0–24} , mg·h/liter (CV [%])	200.2 (139.9–250.8) (41.0)	165.8 (144.3–173.7) (13.2)	178.9 (139.9–244.4) (40.1)
k _{el} , h ^{–1}	0.08 (0.07–0.11)	0.06 (0.06–0.09)	0.08 (0.07–0.11)
t _{1/2} , h	8.4 (6.3–9.8)	11.2 (8.6–11.9)	9.1 (6.3–10.3)
C _{max} , mg/liter	14.6 (13.4–18.1)	8.4 (8.2–9.8)	14.0 (12.0–17.4)
T _{max} , h	3 (2–4)	2 (2–2)	3 (2–4)
Trough, mg/liter	3.4 (1.6–5.1) 74.0	2.4 (1.9–2.6) 47.7	2.9 (1.6–5.1) 73.0
CL/F, liters/h ^b CV (%)	3.1 (2.4–4.3) 69.4	1.8 (1.7–2.1) 14.7	2.6 (2.3–4.1) 71.4
V/F, liters ^c	37.8 (24.4–54.8)	31.2 (21.6–35.9)	36.8 (25.4–45.3)

^aData are expressed as median (IQR). AUC_{0–24}, area under the 24-h concentration-time curve; k_{el}, elimination rate constant; t_{1/2}, elimination half-life; C_{max}, maximum concentration; T_{max}, time of maximum concentration; trough, 24-h/predose concentration; CL/F, clearance; V/F, volume of distribution; CV, coefficient of variation.

^bDose/AUC.

^cCL/k_{el}.

33% of the variability associated with AUC_{0–24} (Table 3). After adjustment for age, sex, race, and HIV status, there was a negative correlation between body weight and linezolid exposure, with an estimated 17.4% (95% confidence interval [CI], 0.1 to 31.7) decrease in AUC_{0–24} per 10-kg increment. Age was significantly associated with higher trough concentrations and remained an independent predictor on multivariable analysis, with an estimated 43.4% (95% CI, 5.9 to 94.2) increase in trough concentrations per 10-year increment in age (Table 4).

Probability of PK/PD target attainment. MIC results were available for the baseline isolates of 16 participants. The median MIC was 0.5 mg/liter (range, 0.25 to 0.5 mg/liter). At this MIC distribution, the probability of efficacy target attainment, defined as an AUC for the free, unbound fraction (fAUC)/MIC of 119, was 100% (95% CI, 87 to 100) for the 600-mg dose of linezolid. This finding was consistent after performing a sensitivity analysis using the original outlier trough concentrations. The fAUC distributions across four MIC strata are shown in Fig. 2. Although the PK/PD target would be achieved in almost all subjects at the epidemiological cutoff (ECOFF) value of 0.5 mg/liter, only 61.5% (95% CI, 40.6 to 79.8) of patients would exceed an fAUC/MIC of 119 at the critical concentration of 1.0 mg/liter (13). Trough concentrations exceeded the toxicity threshold of 2 mg/liter in 57.7% (95% CI, 36.9 to 76.6) of those on 600 mg daily and in 75% (95% CI, 19.4 to 99.4) of those who had undergone dose reduction to 300 mg daily. In a sensitivity analysis the proportions exceeding the toxicity threshold were similar when original trough concentration data were used: 67.7% (95% CI, 47.1 to 82.7) versus 60% (95% CI, 40.6 to 77.3) with imputed data at all doses.

DISCUSSION

We characterized the PK of linezolid in 30 South African patients with drug-resistant TB and a high prevalence of HIV coinfection. We showed that age and weight were the

TABLE 3 Univariable and multivariable linear regression models describing associations between the AUC_{0–24} for linezolid at 600 mg daily and selected covariates

Parameter for patients (n = 26)	Univariable		Multivariable	
	AUC _{0–24} change, % (95% CI) ^a	P value	AUC _{0–24} change, % (95% CI) ^a	P value
Male sex	–13.0 (–42.2 to 30.9)	0.488	–24.9 (–49.9 to 12.6)	0.156
Age/10-yr increase	7.3 (–11.2 to 29.6)	0.452	18.7 (–2.1 to 43.9)	0.078
Black African	9.6 (–26.5 to 63.5)	0.641	–17.3 (–33.1 to 2.2)	0.075
Wt/10-kg increase	–11.9 (–25.8 to 4.4)	0.136	–17.4 (–0.1 to –31.7)	0.049
BMI, kg/m ²	–1.6 (–5.9 to 2.9)	0.458		
HIV positive	–14.3 (–42.3 to 27.5)	0.430	–27.2 (–53.5 to 13.8)	0.154
Current LPV/r	–18.9 (–50.9 to 43.1)	0.399		
Dose, mg/kg	7.5 (–2.3 to 18.2)	0.132		
Creatinine clearance, ml/min	–0.5 (–1.1 to 0.1)	0.108		

^aPercentage change in AUC_{0–24} was calculated as follows: (e^B – 1) × 100. Variables were excluded from the final multivariable model due to collinearity or as a result of backward elimination after exceeding the P value inclusion threshold.

TABLE 4 Univariable and multivariable linear regression models describing associations between linezolid (600 mg daily) trough concentrations and selected covariates

Parameter for patients (<i>n</i> = 26)	Univariable		Multivariable	
	Trough change, % (95% CI) ^a	<i>P</i> value	Trough change, % (95% CI) ^a	<i>P</i> value
Male sex	10.7 (−41.5 to 109.9)	0.744		
Age/10-yr increase	37.4 (5.4 to 79.2)	0.021	43.4 (5.9 to 94.2)	0.022
Black African	26.1 (−31.9 to 133.3)	0.445	−13.8 (−37.4 to 18.7)	0.346
Wt/10-kg increase	9.3 (−17.1 to 43.9)	0.514		
BMI, kg/m ²	1.0 (−5.8 to 8.4)	0.770		
HIV positive	16.1 (−37.7 to 116.2)	0.625	−27.9 (−66.9 to 56.4)	0.389
Current LPV/r	11.5 (−49.4 to 145.5)	0.778	37.1 (−42.9 to 229.6)	0.463
Dose, mg/kg	−4.3 (−17.9 to 11.6)	0.560		
Creatinine clearance, ml/min	−0.2 (−1.2 to 0.8)	0.650		

^aPercentage change in trough concentrations calculated as follows: $(e^{\beta} - 1) \times 100$. Variables were excluded from the final multivariable model due to collinearity or as a result of backward elimination after exceeding the *P* value inclusion threshold.

most important predictors of linezolid exposure. A major finding was that the standard 600-mg dose resulted in exposures that reached efficacy targets, but a substantial proportion of individuals were exposed to concentrations exceeding the known toxicity threshold. Of concern, at the critical concentration (1 mg/ml) efficacy targets would be achieved in only 61.5%, which has implications for the programmatic use of linezolid as resistance is expected to increase as implementation is scaled up.

Despite its growing importance as a key drug for the treatment of drug-resistant TB, the optimal dose and duration of linezolid for this indication are unknown. There are very limited published PK data for linezolid in TB patients to help inform an effective dosing strategy that minimizes both mitochondrial toxicity and the emergence of resistance. Eight clinical studies reporting linezolid PK in TB treatment were identified in a recent systematic review (6), but these studies had four different dosing strategies and mostly used sparse sampling PK schedules, limiting their generalizability. Only two studies (*n* = 48) (2, 14) have evaluated linezolid PK at the standard dose for TB of 600 mg daily; all participants were HIV negative, and full PK profiles were available for only 10 participants (14). Our study provides a comprehensive description of plasma linezolid concentrations at the recommended dose of 600 mg daily for drug-resistant TB and is the first to include HIV-positive patients.

FIG 2 Probability density distributions for efficacy target attainment of linezolid for subjects on 600 mg daily. The solid vertical line on the x axis represents the experimentally derived efficacy target $fAUC/MIC_{0-24}$ of 119. Note the log scale on the x axis.

We found high interindividual PK variability, as has been observed in patients with Gram-positive infections (15), particularly at early sampling time points, suggesting variable absorption delay. Most of the PK variability was unexplained by the covariates included in the regression model and was likely due to stochastic effects; however, this needs to be quantified with formal population PK modeling, possibly incorporating an absorption lag phase. Linezolid clearance was lower among participants who underwent dose reduction to 300 mg, which could be explained by channeling bias, as patients with lower linezolid clearance would have higher exposure and be more susceptible to toxicity, necessitating a dose reduction. Although the sample size was small, the median trough concentration with the reduced 300 mg daily doses exceeded the toxicity threshold of 2 mg/liter in three of four participants. This finding emphasizes the need for toxicity monitoring with linezolid therapy, even after dose reduction for adverse events.

The median trough concentrations were higher in our cohort compared with the two previous studies of linezolid 600 mg daily in TB therapy (2, 14). Although there is substantial interstudy heterogeneity in linezolid PK parameters (6), our finding may suggest a longer terminal half-life with an attendant increased risk of toxicity in our population. A small clinical study found a trend toward an association between HIV infection and higher rates of linezolid toxicity (11); if this association is confirmed in larger prospective cohorts, it is likely to be explained by predisposition to the high prevalence of neuropathy and limited bone marrow reserve in people with advanced HIV disease rather than higher linezolid exposure, which we did not find. We explored the potential PK drug-drug interaction between linezolid and lopinavir-ritonavir as an additional contributing factor to increased linezolid exposures and toxicity in HIV. An association between the use of lopinavir-ritonavir and linezolid trough concentrations was not detected in our cohort, but this needs confirmation with a larger sample size.

In a previous study, increasing age accounted for a small reduction (2%) in linezolid clearance in patients with Gram-positive infection (16) but did not contribute to the development of a population PK model of linezolid in TB (17) and did not influence linezolid exposures in a study of healthy volunteers (18). In contrast, we showed a significant correlation between increasing age and linezolid trough concentrations, where every 10-year increment in age was associated with 43% higher trough concentrations; this finding needs to be validated in similar populations. We also found a significant association between weight and lower linezolid exposure in the multivariable model, an association previously reported (19). These observations have implications for dose selection and could inform therapeutic drug monitoring (TDM) strategies for linezolid, for example, by targeting TDM to older patients and those with lower weights to prevent toxicity.

PK targets for efficacy have not been established for linezolid in TB treatment. Although maximum concentration (C_{max})/MIC (20) and trough/MIC (21) have been associated with bacterial killing using *ex vivo* and *in vitro* models, the PK/PD index most consistently linked to linezolid activity in *M. tuberculosis* is the $fAUC_{0-24}/MIC$ ratio (22–24). A hollow-fiber infection model, which recapitulates human drug exposure, showed that optimal mycobacterial kill was achieved at an $fAUC_{0-24}/MIC$ ratio of 119 (22); this was used as the PK/PD parameter in a recent simulation of published linezolid PK data to determine the probability of efficacy target attainment at wild-type MIC values (6). Using data from 10 patients with full PK profiles, with an estimated median AUC_{0-24} of 98.6 mg·h/liter (17), those simulations predicted that 45% would fail to achieve the target at a daily dose of 600 mg. Reassuringly, in our participants linezolid exposures were higher (median AUC_{0-24} , 200.2 mg·h/liter), translating into probability of target attainment of 100% across the MIC distribution in baseline isolates and 96% at the population wild-type MIC cutoff of 0.5 mg/kg, supporting the efficacy of the 600-mg daily dose. However, linezolid exposures did not exceed the putative efficacy threshold at the critical concentration of 1 mg/liter in 38% of our subjects. With the expanding use of linezolid for TB treatment it will be essential to monitor for evidence of “MIC creep” in the population.

Unlike the PK/PD parameter for efficacy, the linezolid toxicity threshold is relatively well defined as a trough concentration of 2 mg/liter, supported by clinical evidence (10) as well as data from preclinical models showing that mitochondrial toxicity is related to trough concentrations (21). Although a 600-mg daily dose was likely to reach the efficacy target in our cohort, almost 58% also exceeded this threshold concentration for linezolid toxicity, clearly illustrating the narrow therapeutic window of linezolid. In murine models, linezolid's sterilizing ability is dose related and can occur within 2 months of effective combination therapy (25, 26). In TB patients, neurological toxicity tends to occur late, usually after 2 months of therapy (27). Based on these observations, an appealing dosing strategy could be to provide higher linezolid doses (1,200 mg daily) for an initial intensive phase of treatment, followed by either discontinuation, dose reduction, or intermittent dosing (28) that allows longer periods within the PK safety window. This strategy needs to be evaluated in prospective studies.

We acknowledge a number of limitations of our study, including the inability of noncompartmental analysis to assess intraindividual PK variability, evaluation at only a single time point during treatment, an incomplete PK profile and non-steady-state dosing for one participant each, and small numbers of participants receiving the reduced 300-mg dose. Importantly, we had to impute the trough concentrations for six participants due to extremely high values after suspected unobserved dosing prior to the 24-h sample. If anything, inclusion of the original data would have biased the results toward higher trough concentrations and overall exposures. Thus, our reported findings may represent a conservative estimate of both efficacy and toxicity target attainment.

In conclusion, we found substantial variability in linezolid drug concentrations in this cohort of patients with drug-resistant TB and a high prevalence of HIV infection. Much of this variability was unexplained, but age and weight were identified as predictors of trough concentrations and exposure, respectively. The standard 600-mg dose is likely to achieve efficacy targets for *M. tuberculosis* isolates with linezolid wild-type MICs. The clinical impact of this needs to be evaluated by linking linezolid PK to toxicity and efficacy endpoints. In the meantime, the expanding use of linezolid at 600 mg daily for drug-resistant TB should be supported by programmatic surveillance of MICs and adverse events. Alternative dosing strategies and TDM should be explored to optimize the use of this important but toxic antituberculosis agent.

MATERIALS AND METHODS

Study population. We conducted a prospective observational PK/PD study of linezolid in adults treated with linezolid-containing regimens for drug-resistant TB in South Africa. We enrolled participants from two studies: an observational cohort study of patients with pre-XDR and XDR-TB on bedaquiline containing regimens (PROBeX) and the intervention arm of an open-label clinical trial examining a shortened injection-free regimen for MDR-TB (NExT; ClinicalTrials.gov NCT02454205). The initial dose of linezolid used in both studies was 600 mg daily but was reduced to 300 mg daily in the event of toxicity at the discretion of local clinicians or trial staff. Consecutive participants enrolled in the intervention arm of the NExT trial and those receiving linezolid as part of standard of care in PROBeX were approached to provide informed consent for intensive PK sampling. Eligible participants were over the age of 18 years, had a known HIV test result, and had culture-confirmed drug-resistant TB. Most of the participants in PROBeX were inpatients at the time of the intensive sampling visit, and all of the NExT participants attended as outpatients.

The study was approved by the ethics committees at the University of Cape Town (references 264/2015 and 920/2015) and Albert Einstein College of Medicine (references 2014 to 4348).

Data collection. Participants underwent PK sampling on a single occasion predose and at 1, 2, 3, 4, 5, 6, and 24 h after a standardized meal and observed linezolid administration. Some participants in the PROBeX cohort had an additional sample taken at 8 and 48 h as part of other study procedures. The sampling visit was scheduled at month 2 of linezolid treatment and was thus performed at steady state. Blood draws were done through a peripheral intravenous catheter placed for the duration of the first day of the visit. Samples were collected into 10-ml K3EDTA Vacutainer tubes and centrifuged ($1,500 \times g$ for 10 min) within 30 min of collection. At least 1.5 ml of plasma was pipetted into polypropylene tubes and immediately frozen at -80°C . Linezolid concentrations were measured at the Division of Clinical Pharmacology at the University of Cape Town using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Using a deuterated internal standard, the LC-MS/MS method for linezolid was validated over a calibration range of 0.100 mg/liter to 30 mg/liter. Over the period of sample analysis ($n = 8$ batches), a mean accuracy of 98.8% was achieved, with a mean precision of 5.93% (CV).

Because the 24-h dose was unobserved and may have been administered prior to the 24-h sample, concentration-time profiles were inspected for each subject to compare predose and 24-h concentrations. The 24-h concentration was considered highly unlikely to represent the true trough value where it exceeded the predose concentration and was $>50\%$ of the concentration at the prior sampling time point (6 or 8 h). This was based on the published elimination half-life of linezolid of ~ 6 h (29, 30) and the assumption that the 24-h concentration would therefore fall below the 6- or 8-h concentration in the absence of additional dosing. In these cases, the 24-h concentration was imputed from either the predose concentration or the mean of the predose and 48-h concentrations where available (and when the 48-h concentration satisfied the same criteria in relation to the predose value). Predose concentrations reported as below the limit of assay quantification (BLQ) were imputed as 50% of the lower limit of detection (i.e., 0.05 mg/liter) unless there was a history of missed doses prior to the PK visit, in which cases BLQ was replaced by a value of 0.

Demographic and clinical data were collected from participants at the time of the PK visit, as well as from other visits as part of the parent studies. Data included HIV status, linezolid dose and duration, concomitant antituberculosis drugs and antiretrovirals, and most recent serum creatinine. Timing of administration of linezolid and other antituberculosis drugs was recorded.

Linezolid MIC testing was performed on *M. tuberculosis* isolates collected at the time of entry into the parent studies using the mycobacterial growth indicator tube (MGIT) system and continuous growth monitoring with Epicenter software (31). Dilutions ranged from 0.25 mg/liter to 2 mg/liter based on the epidemiological cutoff (ECOFF) value of 0.5 mg/liter (32) and the critical concentration of 1 mg/liter (13).

Analysis. Demographic and clinical characteristics were summarized and compared using the Wilcoxon rank sum test for continuous variables and χ^2 test for dichotomous variables. Noncompartmental analysis was used to estimate linezolid PK parameters from observed concentrations. The area under the concentration-time curve over the 24-h dosing period (AUC_{0-24}) was computed using the cubic splines method. The trough concentration was defined as the plasma concentration 24 h after observed intake (actual or imputed as described above). The elimination rate constant (k_{el}) was assessed by linear regression analysis of the last three concentrations in the terminal log-linear period. The apparent clearance of the drug (CL/F) and the volume of distribution after oral administration (V/F) were calculated using standard equations.

We performed linear regression to explore associations between clinically relevant covariates and linezolid exposure. AUC_{0-24} and trough concentrations were log transformed and regressed versus weight, age, sex, ethnicity, HIV status, estimated creatine clearance (calculated using the Cockcroft-Gault formula), and concurrent use of ritonavir-boosted lopinavir. The last parameter was included to explore a possible drug-drug interaction with linezolid, which may be a substrate of the drug transporter P-glycoprotein (33) that is inhibited by HIV protease inhibitors. Parameters with a P value of <0.5 were retained in the multivariable model, using a backward stepwise approach. Regression coefficients were exponentiated and transformed into a value reflecting percentage change, determined by the formula $(e^{\beta} - 1) \times 100$, for ease of interpretation.

The PK/PD target for efficacy was defined as free AUC_{0-24}/MIC ($fAUC/MIC$) of 119, based on findings from a hollow-fiber infection model (22). Protein binding of 30% was used to calculate $fAUC$ (30). The PK/PD parameter for toxicity was a trough concentration of 2 mg/liter, based on clinical data showing increased mitochondrial and clinical linezolid toxicity above this threshold (10). The probability of target attainment was calculated as the proportion of subjects with PK exposures above the efficacy and toxicity targets. Probability distributions were constructed using kernel densities of PK parameters, stratified by MIC. Statistical analysis, including noncompartmental analysis, was performed using Stata version 14.2 (StataCorp).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.02164-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

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REFERENCES

- World Health Organization. 2018. Global tuberculosis report 2018. World Health Organization, Geneva, Switzerland.
- Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H, Kwak H, Kim H, Jeon HS, Jeong I, Joh JS, Chen RY, Olivier KN, Shaw PA, Follmann D, Song SD, Lee JK, Lee D, Kim CT, Dartois V, Park SK, Cho SN, Barry CE, III. 2012. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. *N Engl J Med* 367:1508–1518. <https://doi.org/10.1056/NEJMoa1201964>.
- Zhang X, Falagas ME, Vardakas KZ, Wang R, Qin R, Wang J, Liu Y. 2015. Systematic review and meta-analysis of the efficacy and safety of therapy with linezolid containing regimens in the treatment of multidrug-resistant and extensively drug-resistant tuberculosis. *J Thorac Dis* 7:603–615. <https://doi.org/10.3978/j.issn.2072-1439.2015.03.10>.
- World Health Organization. 2018. Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). World Health Organization, Geneva, Switzerland.
- Wasserman S, Meintjes G, Maartens G. 2016. Linezolid in the treatment of drug-resistant tuberculosis: the challenge of its narrow therapeutic index. *Expert Rev Anti Infect Ther* 14:901–915. <https://doi.org/10.1080/14787210.2016.1225498>.
- Millard J, Pertinhez H, Bonnett L, Hodel EM, Dartois V, Johnson JL, Caws M, Tiberi S, Bolhuis M, Alffenaar JC, Davies G, Sloan DJ. 2018. Linezolid pharmacokinetics in MDR-TB: a systematic review, meta-analysis and Monte Carlo simulation. *J Antimicrob Chemother* 73:1755–1762. <https://doi.org/10.1093/jac/dky096>.
- Pasipanodya JG, Srivastava S, Gumbo T. 2012. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clin Infect Dis* 55:169–177. <https://doi.org/10.1093/cid/cis353>.
- Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. 2011. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J Infect Dis* 204:1951–1959. <https://doi.org/10.1093/infdis/jir658>.
- Wilkinson GR. 2005. Drug metabolism and variability among patients in drug response. *N Engl J Med* 352:2211–2221. <https://doi.org/10.1056/NEJMra032424>.
- Song T, Lee M, Jeon H-S, Park Y, Dodd LE, Dartois V, Follman D, Wang J, Cai Y, Goldfeder LC, Olivier KN, Xie Y, Via LE, Cho SN, Barry CE, Chen RY. 2015. Linezolid trough concentrations correlate with mitochondrial toxicity-related adverse events in the treatment of chronic extensively drug-resistant tuberculosis. *EBioMedicine* 2:1627–1633. <https://doi.org/10.1016/j.ebiom.2015.09.051>.
- Hughes J, Isaakidis P, Andries A, Mansoor H, Cox V, Meintjes G, Cox H. 2015. Linezolid for multidrug-resistant tuberculosis in HIV-infected and -uninfected patients. *Eur Respir J* 46:271–274. <https://doi.org/10.1183/09031936.00188114>.
- De Vriese AS, Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL, Vanopdenbosch LJ, Martin JJ, Grooten CC, Vandecasteele S, Boelaert JR. 2006. Linezolid-induced inhibition of mitochondrial protein synthesis. *Clin Infect Dis* 42:1111–1117. <https://doi.org/10.1086/501356>.
- World Health Organization. 2018. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. World Health Organization, Geneva, Switzerland.
- Dietze R, Hadad DJ, McGee B, Molino LP, Maciel EL, Peloquin CA, Johnson DF, Debanne SM, Eisenach K, Boom WH, Palaci M, Johnson JL. 2008. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med* 178:1180–1185. <https://doi.org/10.1164/rccm.200806-892OC>.
- Boak LM, Rayner CR, Grayson ML, Paterson DL, Spelman D, Khumra S, Capitano B, Forrest A, Li J, Nation RL, Bulitta JB. 2014. Clinical population pharmacokinetics and toxicodynamics of linezolid. *Antimicrob Agents Chemother* 58:2334–2343. <https://doi.org/10.1128/AAC.01885-13>.
- Tsuji Y, Holford NH, Kasai H, Ogami C, Heo YA, Higashi Y, Mizoguchi A, To H, Yamamoto Y. 2017. Population pharmacokinetics and pharmacodynamics of linezolid-induced thrombocytopenia in hospitalized patients. *Br J Clin Pharmacol* 83:1758–1772. <https://doi.org/10.1111/bcp.13262>.
- McGee B, Dietze R, Hadad DJ, Molino LP, Maciel EL, Boom WH, Palaci M, Johnson JL, Peloquin CA. 2009. Population pharmacokinetics of linezolid in adults with pulmonary tuberculosis. *Antimicrob Agents Chemother* 53:3981–3984. <https://doi.org/10.1128/AAC.01378-08>.
- Sisson TL, Jungbluth GL, Hopkins NK. 2002. Age and sex effects on the pharmacokinetics of linezolid. *Eur J Clin Pharmacol* 57:793–797.
- Abe S, Chiba K, Cirincione B, Grasela TH, Ito K, Suwa T. 2009. Population pharmacokinetic analysis of linezolid in patients with infectious disease: application to lower body weight and elderly patients. *J Clin Pharmacol* 49:1071–1078. <https://doi.org/10.1177/0091270009337947>.
- Wallis RS, Jakubiec WM, Kumar V, Silvia AM, Paige D, Dimitrova D, Li X, Ladutko L, Campbell S, Friedland G, Mitton-Fry M, Miller PF. 2010. Pharmacokinetics and whole-blood bactericidal activity against *Mycobacterium tuberculosis* of single doses of PNU-100480 in healthy volunteers. *J Infect Dis* 202:745–751. <https://doi.org/10.1086/655471>.
- Brown AN, Drusano GL, Adams JR, Rodriguez JL, Jambunathan K, Baluya DL, Brown DL, Kwara A, Mirsalis JC, Hafner R, Louie A. 2015. Preclinical evaluations to identify optimal linezolid regimens for tuberculosis therapy. *mBio* 6:e01741-15. <https://doi.org/10.1128/mBio.01741-15>.
- Srivastava S, Magombedze G, Koeuth T, Sherman C, Pasipanodya JG, Raj P, Wakeland E, Deshpande D, Gumbo T. 2017. Linezolid dose that maximizes sterilizing effect while minimizing toxicity and resistance emergence for tuberculosis. *Antimicrob Agents Chemother* 25:e00751-17. <https://doi.org/10.1128/AAC.00751-17>.
- Williams KN, Stover CK, Zhu T, Tasneen R, Tyagi S, Grosset JH, Nuermberger E. 2009. Promising antituberculosis activity of the oxazolidinone PNU-100480 relative to that of linezolid in a murine model. *Antimicrob Agents Chemother* 53:1314–1319. <https://doi.org/10.1128/AAC.01182-08>.
- Deshpande D, Srivastava S, Pasipanodya JG, Bush SJ, Nuermberger E, Swaminathan S, Gumbo T. 2016. Linezolid for infants and toddlers with disseminated tuberculosis: first steps. *Clin Infect Dis* 63:S80–S87. <https://doi.org/10.1093/cid/ciw482>.
- Zhao W, Guo Z, Zheng M, Zhang J, Wang B, Li P, Fu L, Liu S. 2014. Activity of linezolid-containing regimens against multidrug-resistant tuberculosis in mice. *Int J Antimicrob Agents* 43:148–153. <https://doi.org/10.1016/j.ijantimicag.2013.10.010>.
- Tasneen R, Betoudji F, Tyagi S, Li SY, Williams K, Converse PJ, Dartois V, Yang T, Mendel CM, Mdululi KE, Nuermberger EL. 2016. Contribution of oxazolidinones to the efficacy of novel regimens containing bedaquiline and pretomanid in a mouse model of tuberculosis. *Antimicrob Agents Chemother* 60:270–277. <https://doi.org/10.1128/AAC.01691-15>.
- Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA, Castiglia P, De Lorenzo S, Ferrara G, Koh WJ, Schecter GF, Shim TS, Singla R, Skrahina A, Spanevello A, Udwadia ZF, Villar M, Zampogna E, Zellweger JP, Zumla A, Migliori GB. 2012. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. *Eur Respir J* 40:1430–1442. <https://doi.org/10.1183/09031936.00022912>.
- Chang KC, Yew WW, Cheung SW, Leung CC, Tam CM, Chau CH, Wen PK, Chan RC. 2013. Can intermittent dosing optimize prolonged linezolid treatment of difficult multidrug-resistant tuberculosis? *Antimicrob Agents Chemother* 57:3445–3449. <https://doi.org/10.1128/AAC.00388-13>.

29. Alffenaar JW, van Altena R, Harmelink IM, Filguera P, Molenaar E, Wessels AM, van Soolingen D, Kosterink JG, Uges DR, van der Werf TS. 2010. Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. *Clin Pharmacokinet* 49:559–565. <https://doi.org/10.2165/11532080-000000000-00000>.
30. Stalker DJ, Jungbluth GL. 2003. Clinical pharmacokinetics of linezolid, a novel oxazolidinone antibacterial. *Clin Pharmacokinet* 42:1129–1140. <https://doi.org/10.2165/00003088-200342130-00004>.
31. Springer B, Lucke K, Calligaris-Maibach R, Ritter C, Böttger EC. 2009. Quantitative drug susceptibility testing of *Mycobacterium tuberculosis* by use of MGIT 960 and EpiCenter instrumentation. *J Clin Microbiol* 47: 1773–1780. <https://doi.org/10.1128/JCM.02501-08>.
32. Schon T, Jureen P, Chryssanthou E, Giske CG, Sturegard E, Kahlmeter G, Hoffner S, Angeby KA. 2011. Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 15:502–509. <https://doi.org/10.5588/ijtld.10.0238>.
33. Bolhuis MS, van Altena R, van Soolingen D, de Lange WC, Uges DR, van der Werf TS, Kosterink JG, Alffenaar JW. 2013. Clarithromycin increases linezolid exposure in multidrug-resistant tuberculosis patients. *Eur Respir J* 42:1614–1621. <https://doi.org/10.1183/09031936.00001913>.