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Plasma Drug Activity in Patients on Treatment for Multidrug-Resistant Tuberculosis

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Little is known about plasma drug concentrations relative to quantitative susceptibility in patients with multidrug-resistant tuberculosis (MDR-TB). We previously described a TB drug activity (TDA) assay that determines the ratio of the time to detection of plasma-cocultured *Mycobacterium tuberculosis* versus control growth in a Bactec MGIT system. Here, we assess the activity of individual drugs in a typical MDR-TB regimen using the TDA assay. We also examined the relationship of the TDA to the drug concentration at 2 h (C_2) and the MICs among adults on a MDR-TB regimen in Tanzania. These parameters were also compared to the treatment outcome of sputum culture conversion. Individually, moxifloxacin yielded superior TDA results versus ofloxacin, and only moxifloxacin and amikacin yielded TDAs equivalent to a −2-log killing. In the 25 patients enrolled on a regimen of kanamycin, levofloxacin, ethionamide, pyrazinamide, and cycloserine, the C_2 values were found to be below the expected range for levofloxacin in 13 (52%) and kanamycin in 10 (40%). Three subjects with the lowest TDA result (<1.5, a finding indicative of poor killing) had significantly lower kanamycin C_2/MIC ratios than subjects with a TDA of ≥1.5 (9.8 ± 8.7 versus 27.0 ± 19.1; $P = 0.04$). The mean TDAs were 2.52 ± 0.76 in subjects converting to negative in ≤2 months and 1.88 ± 0.57 in subjects converting to negative in >2 months ($P = 0.08$). In Tanzania, MDR-TB drug concentrations were frequently low, and a wide concentration/MIC range was observed that affected plasma drug activity *ex vivo*. An opportunity exists for pharmacokinetic optimization in current MDR-TB regimens, which may improve treatment response.

Treatment outcomes for multidrug-resistant tuberculosis (MDR-TB), defined as resistance to isoniazid and rifampin, remain inferior to drug-susceptible TB largely because second-line medications used in the treatment of MDR-TB are less potent, may require an extended treatment duration, and are associated with a greater number of side effects (1). Therefore, understanding the relative activity of an individual patient's MDR-TB regimen may inform treatment decisions, particularly in the presence of a poor response to therapy. Both murine models and human experience have demonstrated the importance of optimized drug exposure, leading to greater bacterial killing and better treatment outcomes (2–4). However, pharmacokinetic tools, such as plasma drug concentration monitoring and MIC testing, are not readily available in most areas where TB is endemic, and it remains unclear how such measurements are best utilized.

We have previously described the role of a plasma TB drug activity (TDA) assay in patients treated for drug-susceptible TB in Tanzania, which has been designated a high-burden TB country by the World Health Organization (5). Based on the original Schlichter test for bactericidal endocarditis (6, 7) and prior work in whole-blood culture of *Mycobacterium tuberculosis* (8), the TDA assay uses a patient's plasma collected during TB treatment and the patient's own *M. tuberculosis* isolate and measures the time to positivity of plasma-cocultured *M. tuberculosis*. We use the automated Bactec MGIT system (Becton Dickinson, Sparks, MD) as a replacement for conventional colony counting and normalized the time to positivity to an identical inoculum of the isolate alone in liquid culture, thereby expressing the TDA as a ratio. The use of plasma without leukocytes constrains analysis to drug exposure, and the standard pH of MGIT media negates the activity of pyrazinamide. Thus, in a typical drug-susceptible regimen, the TDA correlates with the drug concentration/MIC for isoniazid and rifampin, and a TDA of ≤1.0 indicates the presence of MDR-TB. At Kibong'oto National Tuberculosis Hospital (KNTH), a low TDA (<2.0) was significantly associated with lower isoniazid and rifampin concentrations at 2 h (C_2; i.e., the time of the estimated C_{max}), and a very low TDA (<1.5) corresponded to a trend toward lack of cure (5).

Treatment for MDR-TB in Tanzania became available in 2009 and is referred to as a single location, KNTH, in the Kilimanjaro region (9). Second-line susceptibility testing for agents within the MDR-TB regimen is not routinely available. Despite a high proportion of MDR-TB patients treated at KNTH successfully completing the injectable phase of inpatient chemotherapy, the time to sputum culture conversion to negative can be highly variable (10), which can prolong infectiousness and total treatment duration, and presents a considerable programmatic and patient burden. Thus, we sought here first to examine the activity within TDA of individual MDR-TB drugs at the expected C_2 range against several stock MDR and one extensively drug-resistant TB (XDR-TB) strain and then to define among adults with pulmonary MDR-TB in Tanzania the relationship of TDA to the C_2 drug concentration/

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MIC. Second, these parameters were compared to sputum culture conversion.

MATERIALS AND METHODS

Second-line drug activity in the TDA assay. Concentrations within the expected C_{max} range for pyrazinamide, ethionamide, cycloserine, ofloxacin, moxifloxacin, kanamycin, and amikacin (Sigma-Aldrich, St. Louis, MO) were spiked into plasma of a healthy, tuberculin skin test-negative volunteer. In anticipation of being able to draw plasma only at a single time point within the dosing interval in Tanzania, C_{max} estimates were used despite that the area under the time curve (AUC)/MIC is the preferred predictor of efficacy for drugs such as the fluoroquinolones. *M. tuberculosis* isolates included clinical samples at the University of Virginia with known resistance patterns, including MDR-TB and XDR-TB with further resistance to the fluoroquinolones and injectable agents (amikacin, kanamycin, or capreomycin). Susceptibility testing was repeated with a 1.0 McFarland suspension of newly cultured preparation according to the 1% proportion method using established critical concentrations for all drugs except pyrazinamide for which susceptibility testing was performed in pyrazinamide-specific Bactec MGIT media. MIC testing was performed for all drugs except pyrazinamide on MYCOTB Sensititre plates (TREK Diagnostics, Cleveland, OH) according to the manufacturer's protocol (11).

For TDA assays, a suspension of each *M. tuberculosis* isolate was prepared from Middlebrook 7H10 growth adjusted to a 0.5 McFarland turbidity and diluted 1:10 with Middlebrook 7H9 liquid media. Portions (500 μl) of the suspension was centrifuged in a 2-ml screw-cap tube at 12,000 rpm for 10 min at room temperature, and the supernatant was discarded. The pellet was resuspended in 300 μl of phosphate-buffered saline, and 300 μl of plasma was added. Tubes were incubated for 72 h at 37°C and centrifuged at 12,000 rpm for 5 min, and then the supernatant was discarded. The pellet was resuspended in 1 ml of sterile-distilled water, vortexed to obtain a smooth suspension, and then centrifuged again at 12,000 rpm for 10 min. The supernatant was discarded, 500 μl of Middlebrook 7H9–10% oleic acid-albumin-dextrose-catalase was added to the pellet, and the mixture was vortexed and then transferred to a prefilled 7-ml MGIT tube. For control tubes, two plasma-free suspensions of the identical 500-μl inocula were added for each isolate to MGIT tubes. A second set of control tubes were prepared for each isolate by further diluting the suspension 1:100 and adding 500 μl to MGIT tubes. The plasma-containing tube and the two inoculum control tubes were incubated in a MGIT 320 until the time to detection. The TDA was reported as the ratio of the time to detection of plasma cocultured TB in hours to the time to detection of control (identical 1:10 suspension), whereby a TDA of <1.0 represented growth, a TDA of 1.0 represented stasis, and a TDA of >1.0 represented killing. The ratio of the time to detection of the 1:1,000 suspension to the 1:10 suspension was recorded to establish the TDA ratio equivalent to −2-log killing. All experiments were approved by the Institutional Biosafety Committee of the University of Virginia.

Plasma drug concentrations, MIC, and TDA in subjects treated for MDR-TB in Tanzania. Subjects beginning MDR-TB therapy at KNTH for suspected pulmonary MDR-TB were eligible for analysis. The inclusion criteria were an age of ≥18 years, chest X-ray findings consistent with TB, and sputum culture positivity for *M. tuberculosis* later confirmed to be resistant to isoniazid and rifampin. Mycobacterial cultures prior to treatment were performed at the Kilimanjaro Clinical Research Institute (KCRI) in Bactec MGIT 960 system. Speciation of *M. tuberculosis* was confirmed by using a DNA probe for *M. tuberculosis* complex (Gen-Probe, San Diego, CA), and susceptibility testing was performed for isoniazid, rifampin, ethambutol, and streptomycin in MGIT with a SIRE kit (12). Except for pyrazinamide, the MICs for the remaining drugs used in the MDR-TB regimen were determined by using a MYCOTB Sensititre plate.

Demographic and clinical data were abstracted from the medical chart. The majority of patients had sputum collected monthly to determine the time to culture negativity (conversion) per KNTH protocol. At 2 weeks after treatment initiation, venous blood was collected from all subjects at 2 h after administration of the standardized weight-based daily regimen of levofloxacin, kanamycin (intramuscular), ethionamide, pyrazinamide, and cycloserine in a fasting state. Blood was transported on ice to KCRI, where plasma was separated for the TDA assay or immediately stored at −80°C.

An aliquot of plasma was shipped on dry ice to the Infectious Diseases Pharmacokinetics Laboratory at the University of Florida. Plasma drug concentrations were measured by using validated high-performance liquid chromatography (ethionamide and levofloxacin), gas chromatography with mass spectrometry (pyrazinamide), or colorimetric assays (cycloserine) according to established protocols. The kanamycin concentrations were later assayed by validated liquid-chromatography-tandem mass spectrometry at the University of Groningen Medical Center, Groningen, Netherlands.

For all analyses, means were compared using a Student *t* test, and the standard deviations reported unless otherwise specified or medians were compared by the Mann-Whitney test when appropriate. The correlation of continuous variables was assessed by using the Pearson coefficient. All *M. tuberculosis* TDA experiments were performed in duplicate at KCRI. Written consent was provided by all subjects, and the protocols were approved by the institutional review boards at Tumaini University in Moshi, Tanzania, the University of Virginia, the University of Florida, and the National Institute for Medical Research in Dar es Saalam, Tanzania.

RESULTS

Second-line drug activity in the TDA assay. Three MDR isolates from clinical samples at the University of Virginia were studied for their range of susceptibility to the following drugs: ethionamide (MIC ≤ 0.03 to 2.5 μg/ml), cycloserine (4 to 8 μg/ml), kanamycin (≤0.06 to 1.2 μg/ml), amikacin (all 0.25 μg/ml), ofloxacin (0.5 to 1.0 μg/ml), moxifloxacin (0.12 to 0.25 μg/ml), and pyrazinamide (no MIC available), as well as one XDR isolate with resistance to all drugs. For all MDR isolates, the ratio of 1:100/1:10 control suspension equivalent to the 2-log decrement did not vary (1.75 ± 0.08).

For both the control plasma without drug and the plasma containing pyrazinamide, the TDA values were <1.0, a finding indicative of growth (Fig. 1). There was only modest killing observed for ethionamide (TDA range, 1.11 to 1.32) or cycloserine (1.09 to 1.48), with the lowest TDA values occurring with isolates that had the highest MICs (2.5 and 8.0 μg/ml for ethionamide and cycloserine, respectively). The TDA values for kanamycin (TDA range, 1.41 to 1.53) and amikacin (1.53 to 1.71) were observed despite aminoglycoside concentrations at the lower end of the expected C_{max} range. Ofloxacin had worse killing (TDA range, 0.93 to 1.35) than moxifloxacin, which alone had the highest TDA values of individual drugs and exceeded a −2-log killing in both isolates for which the moxifloxacin concentration/MIC was 25.

An additive dose-response relationship in TDA was observed at the lowest concentrations of moxifloxacin and kanamycin with an MDR strain but not with the XDR strain (Fig. 2). For a regimen used in the standard MDR-TB treatment at KNTH (kanamycin, ofloxacin, ethionamide, pyrazinamide, and cycloserine), the mean TDA was only 1.57 ± 0.17 and did not clearly exceed the killing of the aminoglycoside or moxifloxacin alone (Fig. 1). However, when moxifloxacin was substituted for ofloxacin, the mean TDA increased to 1.80 ± 0.17 ($P = 0.03$).

Demographics and culture conversion in subjects on a standard MDR-TB regimen in Tanzania. A total of 25 subjects were enrolled with the mean age of 34.2 ± 14.7, 18 (72%) of whom were male. All patients had pulmonary TB only, and all had a history of treatment for drug-susceptible TB. Four (16%) subjects were HIV

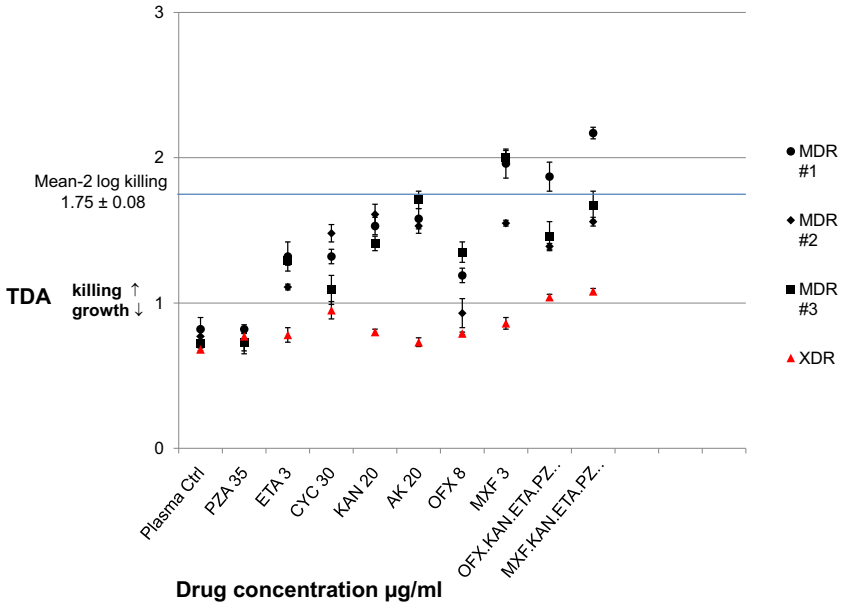

FIG 1 TDA assays were performed using volunteer plasma alone or spiked with a concentration at the lower end of the expected C_{max} range for pyrazinamide (PZA), ethionamide (ETA), cycloserine (CYC), kanamycin (KAN), amikacin (AK), ofloxacin (OFX), or moxifloxacin (MXF) and in combination against three MDR isolates susceptible to those medications (in black) and one XDR isolate resistant to all medications (in red). The TDA is reported as a ratio of the time to detection of plasma-cocultured *M. tuberculosis* versus the time to detection of *M. tuberculosis* alone (control), whereby a TDA of <1.0 indicates growth, a TDA of 1.0 indicates stasis, and a TDA of >1.0 indicates killing. The TDA ratio equivalent to −2-log killing (the time to the detection of 10^{-3} control inoculum/the time to the detection of 10^{-1} control inoculum) was expressed as an average ± the standard deviation for all MDR isolates (blue line).

infected (median CD4 count, 360 cells/μl; interquartile range [IQR], 267 to 393 cells/μl), and all were on antiretroviral therapy. The pretreatment body mass index was available for 19 (76%), with a mean of 18.4 ± 4.8. All subjects converted their sputum culture to negative and at a median time of 2 months (IQR, 1 to 3). There were no deaths reported.

***C*$_2$ drug concentrations.** All 25 subjects had blood collected for plasma C_2 drug concentrations after 14 days of a standardized MDR-TB regimen (Table 1). The concentrations of levofloxacin (mean, 8.0 ± 2.8 μg/ml) were lower than the expected range for 13 subjects (52%). Nine subjects (36%) had kanamycin concentrations below the expected range (mean, 26.9 ± 9.0 μg/ml [including two subjects with trace concentrations]). Seven subjects (28%) had concentrations of both levofloxacin and kanamycin below the expected range. In contrast to all other medications, where concentrations that were higher than the expected range were rare, 12 (52%) cycloserine concentrations exceeded the upper limit of the C_2 range. Pyrazinamide was not lower than the expected range for any subject. For subjects with either a levofloxacin, kanamycin, or cycloserine concentration below the expected C_2 range, the mean age, gender distribution, or HIV status did not significantly differ compared to those that had a drug concentration within or above the expected range (Student *t* test for age and Fisher exact for gender and HIV status).

TDA distribution and correlation with *C*$_2$/MIC. A TDA assay was performed for 21 patients, since 4 did not have pretreatment *M. tuberculosis* isolates available. The mean time to detection for control tubes among all isolates was 104.8 h ± 16.0. The mean TDA for −2-log killing was 1.9 ± 0.48, which did not statistically differ compared to the MDR isolates in the *in vitro* experiments. The mean TDA of subjects while on the multidrug regimen was 2.25 ± 0.55, with considerable variability between patients (range, 1.42 to 4.9). *M. tuberculosis* MICs were determined for 18 subjects, including 3 subjects with very low TDAs of <1.5 (5). The mean kanamycin C_2/MIC values in subjects with a TDA of <1.5 and in those with a TDA of ≥1.5 were 9.8 ± 8.7 and 27.0 ± 19.1, respectively ($P = 0.04$). For levofloxacin the C_2/MIC values were 9.6 ± 5.9 and 17.0 ± 15.0 ($P = 0.19$), for ethionamide the C_2/MIC values were 3.0 ± 1.8 and 1.55 ± 1.4 ($P = 0.29$), and for cycloserine the C_2/MIC values were 2.0 ± 0.84 and 4.65 ± 3.0 ($P = 0.04$), respectively (Fig. 3). Among subjects with a TDA of ≥1.5, there was no correlation of any individual drug concentration/MIC with TDA.

Prediction of culture conversion. Except for two subjects that had culture converted prior to the initiation of MDR-TB therapy, for subjects that achieved sputum culture conversion within ≤2 months ($n = 14$) the mean TDA was 2.53 ± 0.76, while in those with a culture conversion within >2 months, the mean TDA was 1.88 ± 0.57 ($n = 5$) ($P = 0.08$) (Table 2). Subjects with a culture conversion within ≤2 months were younger, but age did not independently correlate with the TDA ($R^2 = 0.004$, $P = 0.84$) or with C_2/MIC for kanamycin ($R^2 = 0.001$, $P = 0.91$), levofloxacin ($R^2 = 0.008$, $P = 0.74$), ethionamide ($R^2 = 0.04$, $P = 0.45$), or cycloserine ($R^2 = 0.02$, $P = 0.60$). Furthermore, 13 (93%) subjects undergoing culture conversion within ≤2 months had a TDA value greater than −2-log killing compared to only 1 (20%) of those with a conversion within >2 months ($P = 0.02$).

DISCUSSION

In this study of plasma drug activity, the concentrations of levofloxacin and kanamycin were frequently below the expected range in Tanzanian subjects initiating MDR-TB treatment, and consequently a wide range of concentration/MIC values was observed. We believe this to be a critical observation since quantitative sus-

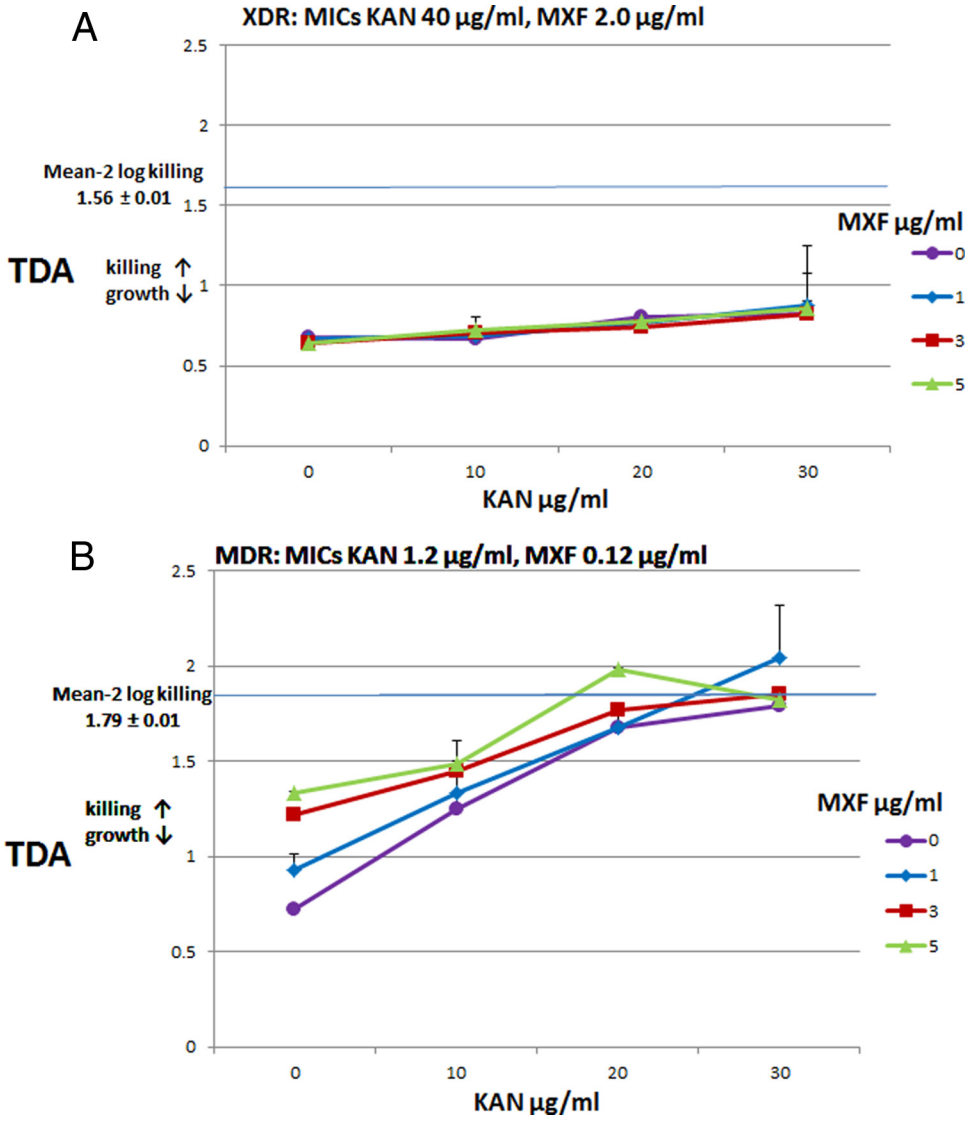

FIG 2 Checkerboard of increasing concentrations of moxifloxacin (MXF) and kanamycin (KAN) against a resistant isolate (XDR) (A) and representative susceptible isolate (MDR) (B). TDA assays were performed using concentrations below and within the expected C_{max} range for MXF (3 to 5 μg/ml) and KAN (25 to 35 μg/ml) with standard error bars.

ceptibility for *M. tuberculosis* may become increasingly available and pharmacokinetic targets for second-line drug concentrations relative to MIC are not definitely established (11, 13). While the application of individual second-line drug concentration or MIC measurement requires further prospective study, subjects from Tanzania that had a faster time to sputum culture conversion were more likely to have a TDA value in excess of −2-log killing.

Other studies of first-line anti-TB drug concentrations in similar African settings have found considerable variability that has been difficult to predict based on weight or other comorbidities (14), and the relationship to treatment response has been limited by the lack of comparison to quantitative susceptibility of the isolate (15). Even less is known about the circulating concentrations of the key drugs relative to the MIC within a MDR-TB regimen. We found low C_2 values that were not predicted by age, gender, or HIV status. Although there have been no completed randomized trials to guide treatment duration for MDR-TB (16, 17), our findings suggest an opportunity for the study of optimizing pharmacokinetics to hasten culture conversion or ultimately shorten treatment duration. For example, in a recent comparison of ofloxacin AUC/MIC values in MDR-TB patients treated in South Africa, only 45% achieved a target AUC/MIC, and in those with an isolate that had a MIC of 2.0 μg/ml, still susceptible by conventional testing, none achieved the target AUC/MIC (18). Despite increasing evidence for the role of more recent fluoroquinolones in the treatment of ofloxacin-resistant isolates (19, 20), these fluoroquinolones remain unavailable in many resource-limited settings. Furthermore, recent studies of levofloxacin demonstrate the best pharmacokinetic properties at a dose of 1,000 mg daily (21, 22). Such optimization may be favorable in Tanzania, given that no subject in the present study was on a 1,000-mg dose of levofloxacin and the C_2 for all subjects was below the median C_{max} of 15.5 μg/ml that may be expected with the higher levofloxacin dose (22). Similarly, ethionamide may be tuberculocidal at high

TABLE 1 Drug concentrations (C_2) and C_2/MIC ratios in patients being treated for MDR-TB[a]

Drug (expected C_2 range [μg/ml])	Mean C_2 (μg/ml) ± SD	No. of subjects with a C_2 range below the expected level (%)	Median MIC (μg/ml) (IQR)	Mean C_2/MIC ratio ± SD
Levofloxacin (8–12)	8.0 ± 2.8	13 (52)	0.75 (0.25–1.0)	15.8 ± 14.1
Kanamycin (25–35)	26.0 ± 10.2	10 (40)	1.2 (0.6–2.5)	22.9 ± 18.7
Cycloserine (20–35)	33.9 ± 12.2	3 (13)	8.0 (8.0–16.0)	4.3 ± 3.0
Ethionamide (1–5)	3.6 ± 1.8	1 (4)	2.5 (1.2–5.0)	1.8 ± 1.5
Pyrazinamide (20–60)	43.1 ± 9.7	0	NA	NA

[a] Drug concentrations were determined for all subjects ($n = 25$), except for a limited sample that precluded analysis of cycloserine concentrations in two subjects. Drug concentrations were determined at 2 h (C_2) after medication administration for peak estimation and compared to the expected C_2 range. Levofloxacin was given as a 750-mg daily oral dose, kanamycin was given as a 15-mg/kg intramuscular dose (or 10 mg/kg if the patient age was ≥60 years), cycloserine was given as a 500-mg oral dose (as component of a 10- to 15-mg/kg daily equivalent), ethionamide was given as a 250-mg oral dose (as a component of a 15-mg/kg daily equivalent divided), and pyrazinamide was given as a 20- to 30-mg/kg daily oral dose. MICs were determined for *M. tuberculosis* isolates for 18 subjects by using a MYCOTB Sensititre plate. NA, not applicable.

enough concentrations (23), and yet only 22% of subjects had C_2/MIC ratios of >2. Although ethionamide may have a more unpredictable time to peak concentration than the other oral agents tested (13) and consequently the measured C_2 may have underestimated the peak in some subjects, other alternative second-line agents such as *para*-aminosalicylic acid may provide more consistent drug exposure.

The TDA assay was found to predominantly measure the concentration-dependent activity of the aminoglycoside and the fluoroquinolone components in a standard MDR-TB regimen in Tanzania. Operationally, therefore, a TDA value approaching 1.0 may be considered to have little plasma killing from the fluoroquinolone or the aminoglycoside, a condition akin to XDR-TB, which portends higher mortality and overall treatment failure. Such lack of drug exposure has been described as functional resistance (4, 24) and can occur with an isolate with a higher MIC still considered susceptible by conventional testing in a host when the circulating drug concentration is below the expected range. Many MDR-TB programs in resource-limited settings increasingly rely on rapid molecular susceptibilities to rifampin or other first-line drug susceptibilities in the MGIT system to commence MDR-TB treatment while awaiting second-line susceptibilities at a national or supranational reference laboratory. This process can take many months; meanwhile, the TDA assay could be performed in-house on an MGIT system and give an earlier indication of XDR-TB.

Increasingly TB programs are looking for strategies to decentralize MDR-TB care from overcrowded inpatient facilities (25). The present study suggests that, even in the absence of optimizing drug dosage or a regimen change in response to drug concentration/MIC information, such baseline parameters or TDA results may complement traditional the clinical information used to stratify a patient for early discharge from an inpatient facility. Similarly, although appeal exists for the use of plasma drug activity in the study of new drug development, such investigation is more logically accomplished with an intracellular model such as whole-blood culture that can better account for the activity of all drugs in the regimen (26).

The present study has several limitations. The narrow range of clinical response to treatment of all subjects, including a median time to culture conversion of 2 months, may limit the generalizability of plasma drug activity in predicting outcomes that otherwise would have been observed among a more diverse patient population. The cohort of patients with MDR-TB for the present study had survived a prolonged referral process (10), and all HIV-infected patients were on antiretroviral therapy prior to initiation

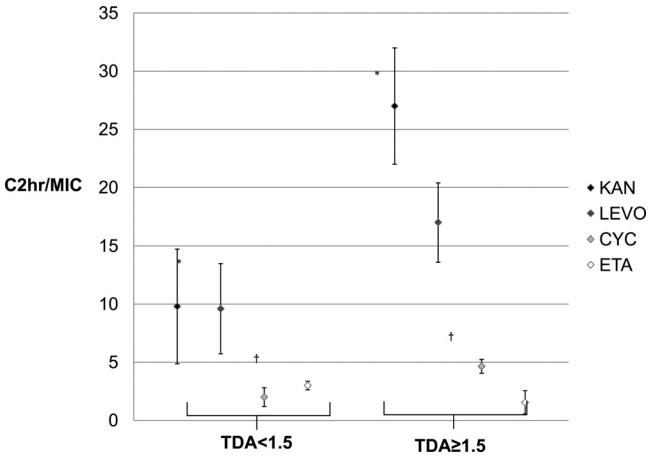

FIG 3 Comparison of mean C_2 drug concentration/MICs in patients with a very low TDA ratio. The mean C_2 drug concentration/MIC and standard errors for kanamycin (KAN), levofloxacin (LEVO), cycloserine (CYC), and ethionamide (ETA) in patients with plasma TDA ratios were determined to be <1.5 ($n = 3$) and ≥1.5 ($n = 15$) and *M. tuberculosis* isolates with available MIC. *, $P = 0.04$; †, $P = 0.04$.

TABLE 2 Predictors of sputum culture conversion to negative[a]

Characteristic	Sputum culture conversion ≤2 mos ($n = 14$)	Sputum culture conversion >2 mos ($n = 5$)	P
Mean age (yrs) ± SD	29.5 ± 13.3	46.6 ± 16.3	0.08
No. of subjects (%)			
Male	8 (57)	5 (100)	0.13
HIV infected	2 (14)	2 (40)	0.27
Chest X-ray showing cavitation	6 (43)	4 (80)	0.30
Mean TDA ratio ± SD	2.52 ± 0.76	1.88 ± 0.57	0.08
Proportion with TDA > −2-log killing (%)	13 (93)	1 (20)	0.02

[a] "Conversion" refers to sputum culture conversion to negative for two consecutive months. Sputum cultures were performed monthly. TDA, TB drug activity. Subjects with culture conversion prior to initiation of MDR-TB regimen ($n = 2$) and subjects for whom a pretreatment *M. tuberculosis* isolate was not available ($n = 4$) were excluded. Only 13 subjects had pretreatment body mass index and culture conversion data available and thus were thus excluded from the comparison.

of the MDR-TB regimen. Other factors that may stratify for treatment response, such as the initial sputum collection time to positivity (as a measure of pretreatment bacterial load), were not available for analysis in all patients. Certainly, plasma concentrations may not represent the active concentrations achieved at the predominant site of infection.

In addition, plasma was drawn only at a single time point (i.e., at 2 h) in the dosing interval and treatment schedule (2 weeks). Thus, for a drug such as the fluoroquinolone, a low C_2 level may underestimate the overall regimen efficacy when the best pharmacokinetic/pharmacodynamic parameter is the AUC/MIC (18). Therefore, while measurement of the drug concentration at the C_2 time point has been operationalized for the management of drug-susceptible TB (27, 28), comparison of plasma drug concentrations and TDA at multiple time points in the dosing interval and at a later date in the treatment schedule may be informative for cases of MDR-TB. Furthermore, no subject's *M. tuberculosis* isolate had MICs indicative of XDR-TB. Although clinically fortunate, the *in vitro* findings of a TDA of <1.0 against an isolate resistant to both fluoroquinolone and aminoglycoside could not be replicated in the field. However, as rapid molecular diagnostics for drug resistance become more widely available in Tanzania and other similar settings, more seriously ill patients that otherwise would not have survived to referral will be treated for MDR-TB, and we expect to encounter isolates with more complex patterns of resistance.

Despite the limitations of the study, we describe here an important examination of plasma drug concentrations relative to quantitative susceptibility within a standard MDR-TB regimen. Plasma drug activity, as indicated by the TDA, may allow earlier identification of XDR-TB or the functional equivalent due to inadequate circulating drug concentrations. The concept of functional drug resistance requires further study in MDR-TB to determine the effect on later treatment outcomes and in methodologies for maximizing drug exposure.

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