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Pharmacokinetics of Levofloxacin in Multidrug- and Extensively Drug-Resistant Tuberculosis Patients

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ABSTRACT Pharmacodynamics are especially important in the treatment of multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB). The free area under the concentration time curve in relation to MIC (*f*AUC/MIC) is the most relevant pharmacokinetic (PK)-pharmacodynamic (PD) parameter for predicting the efficacy of levofloxacin (LFX). The objective of our study was to assess LFX PK variability in M/XDR-TB patients and its potential consequence for *f*AUC/MIC ratios. Patients with pulmonary M/XDR-TB received LFX as part of the treatment regimen at a dose of 15 mg/kg administered once daily. Blood samples obtained at steady state before and 1, 2, 3, 4, 7, and 12 h after drug administration were measured by validated liquid chromatography-tandem mass spectrometry. The MIC values of LFX were determined by the agar dilution method on Middlebrook 7H10 and the MGIT960 system. Twenty patients with a mean age of 31 years (interquartile range [IQR] = 27 to 35 years) were enrolled in this study. The median AUC_{0-24} was 98.8 mg/h/liter (IQR = 84.8 to 159.6 mg/h/liter). The MIC median value for LFX was 0.5 mg/liter with a range of 0.25 to 2.0 mg/liter, and the median $fAUC_{0-24}$/MIC ratio was 109.5 (IQR = 48.5 to 399.4). In 4 of the 20 patients, the value was below the target value of ≥100. When MICs of 0.25, 0.5, 1.0, and 2.0 mg/liter were applicable, 19, 18, 3, and no patients, respectively, had an *f*AUC/MIC ratio that exceeded 100. We observed a large variability in AUC. An $fAUC_{0-24}$/MIC of ≥100 was only observed when the MIC values for LFX were 0.25 to 0.5 mg/liter. Dosages exceeding 15 mg/kg should be considered for target attainment if exposures are assumed to be safe. (This study has been registered at ClinicalTrials.gov under registration no. NCT02169141.)

KEYWORDS levofloxacin, MDR-TB, pharmacodynamics, pharmacokinetics, pharmacology, treatment, tuberculosis, XDR-TB

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In 2015, a total of 580,000 new cases with multidrug-resistant tuberculosis (MDR-TB) and rifampin-resistant tuberculosis were estimated to occur, 45% of whom lived in the Russian Federation, India, and China. Only 20% of these cases were detected and initiated on treatment, and only 52% of the MDR-TB patients initiated on treatment in 2013 were successfully treated (1). For the European Union, the former Soviet Union is one of the major sources of imported MDR-TB and extensively drug-resistant TB

(XDR-TB). TB in Belarus, as in most countries of the former Soviet Union, still represents an issue of serious public health concern (2, 3). Among newly treatment-naive TB patients diagnosed between November 2009 and December 2010 in the city of Minsk, 35.3% had MDR-TB and 2.0% had XDR-TB; among previously treated TB patients, 76.5% had MDR-TB and 19.4% had XDR-TB (4). Treatment can take up to 2 years or more with less effective, more toxic, and more expensive drugs compared to first-line treatment. Successful treatment of TB requires not only new drugs and new regimens but at the first instance also optimization of current treatment (5, 6).

Fluoroquinolones (FQs) are the cornerstone of MDR-TB treatment. Levofloxacin (LFX) has a high *in vitro* and *in vivo* bactericidal activity against *Mycobacterium tuberculosis* and is more affordable and available in high-burden and resource-limited countries than is moxifloxacin (MFX) (7, 8). The safety profile of LFX is considered excellent (9). The efficacy of LFX has been shown to be predicted by ratio of the free area under the concentration time curve (*f*AUC) to MIC with a target of *f*AUC/MIC $\geq$ 100 (10–12). LFX has a favorable pharmacokinetic (PK) profile with a very rapid and complete (>95%) oral absorption, which is hardly influenced by food intake (13). More importantly, LFX is widely distributed throughout the body and showed high penetration into lung tissue, cerebrospinal fluid, and cavitary lesions (14–17). Despite the general preference for MFX to LFX, earlier studies showed no difference in sputum culture conversion after 3 months of treatment (18, 19). Because LFX has no effect on the QT period, it may be safer than MFX in combinations with newer anti-TB drugs such as bedaquiline and delamanid (20). Furthermore, recent studies showed the emergence of drug resistance to FQs during MDR-TB treatment (20). It was suggested that this could be explained by low FQ drug exposure (21). Unfortunately, drug concentrations were not measured in patients in whom acquired drug resistance was observed. Therefore, the objective of the present study was to explore LFX PK variability in M/XDR-TB patients in a high-burden setting and explore its potential effect on *f*AUC/MIC ratios.

RESULTS

Study subjects. A total of 20 patients were enrolled, 8 (40%) of whom were female. An overview of study participant characteristics is provided in Table 1. All patients were diagnosed with pulmonary TB: 14 (70%) with MDR-TB and 6 (30%) with XDR-TB, 13 (65%) of whom had a cavitary lung lesion. All XDR-TB patients and 3 (15%) patients with MDR-TB had been treated for TB previously. Table 2 provides information about medicines used for the TB treatment in the groups of patients studied. Before starting chemotherapy 10 (50%) patients had sputum smear-positive TB, and all patients had sputum culture-confirmed TB. The mean time for sputum smear conversion was 1.5 months. Sputum culture converted within a median period of 3.1 months.

Assessment of treatment outcomes revealed that 13 (65%) patients eventually had a favorable outcome. The treatment outcomes of 3 (15%) patients are not assigned. One patient interrupted the treatment for two consecutive months because of comorbidity (gouty arthritis and myocardial infarction). One of the patients previously declared cured started MDR treatment again because of a new episode of bacteriologically confirmed TB, underwent resection surgery (lobectomy), and 1 year later completed the treatment. One patient died of massive pulmonary hemorrhage, though his sputum smear and culture results confirmed active TB.

Levofloxacin pharmacokinetics. The median LFX dose was 15.8 mg/kg (IQR = 13.3 to 19.6 mg/kg). Blood samples from 14 (60%) patients were obtained within 3 months of TB treatment by a median time of blood collection of 52.2 days (IQR = 22 to 110 days) after the start of LFX treatment. The median observed AUC_{0-24} and C_{max} were 98.8 mg·h/liter (IQR = 84.4 to 159.6 mg·h/liter) and 10.05 mg/liter (IQR = 8.4 to 16.2 mg/liter), respectively. One-fourth (25%) of the patients had a C_{max} below the expected minimum C_{max} of 8 mg/liter (22). Figure 1 shows the mean plasma concentration-time curve of LFX. Absorption was fast, as expected (13/20 T_{max} = 1 h), but some showed more gradual (6/20 T_{max} = 2 h) and even delayed (1/20 T_{max} = 7 h) absorption.

TABLE 1 Patient characteristics at baseline ($n = 20$)[a]

Characteristic	No. (%) of patients
Female	8 (40)
Median (IQR)	
Age (yr)	31 (27–35)
Wt (kg)	63.35 (55.25–77)
Length (cm)	174 (167–182)
BMI (kg/m^2)	20.1 (18.9–23.4)
Diagnosis	
New, treatment naive	12 (60)
Previously treated	8 (40)
Pulmonary tuberculosis	
Cavitary	13 (65)
Noncavitary	7 (35)
Sputum	
Smear positive	10 (50)
Culture positive	20 (100)
Resistance pattern	
MDR	14 (70)
XDR	6 (30)
Comorbidity	
Alcohol abuse	2 (10)
Smoking	11 (55)
Renal insufficiency	0 (0)
HIV positive	0 (0)

[a]Data are presented as the number (%) of patients except as noted otherwise in column 1 (median and interquartile range). BMI, body mass index.

No significant correlations were observed between AUC_{0-24} and C_{max} in relation to variables describing demography and TB disease. There was a significant correlation between LFX C_{max} and the following variables: LFX AUC_{0-24} ($\rho = 0.775$, $P = 0.001$), creatinine values ($\rho = -0.475$, $P = 0.034$), estimated dosage of LFX per kg of body weight ($\rho = 0.456$, $P = 0.043$), *V/F* ($\rho = -0.604$, $P = 0.005$), CL/F ($\rho = -0.714$, $P = 0.001$), AUC_{0-24} significantly correlated only with C_{max}, *V/F*, and CL/*F* ($\rho = 0.775$, $\rho = -0.583$, and $\rho = -0.947$; $P < 0.05$).

Multiple linear regression analysis with LFX C_{max} as an independent variable using a backward elimination strategy excluded creatinine, *V/F*, and CL/*F* from the model with an adjusted R^2 of 0.886. Multiple linear regression analysis with LFX AUC_{0-24} as independent variable using backward elimination strategy showed CL/*F* and C_{max} as the two best predictors with an adjusted R^2 of 0.714.

TABLE 2 Antituberculosis medication according to WHO criteria ($n = 20$)[a]

Treatment group	Medicine	No. (%) of patients
Fluoroquinolones	Levofloxacin	20 (100)
Injectable anti-TB medication	Capreomycin	18 (90)
	Kanamycin	2 (10)
Other core second-line agents	Etionamide	2 (10)
	Protionamide	16 (80)
	Cycloserine	19 (95)
Add-on agents	Pyrazinamide	11 (55)
	Ethambutol	3 (15)
	p-Aminosalicylic acid	15 (75)
	Amoxicillin-clavulanate	5 (25)

[a]See reference 31.

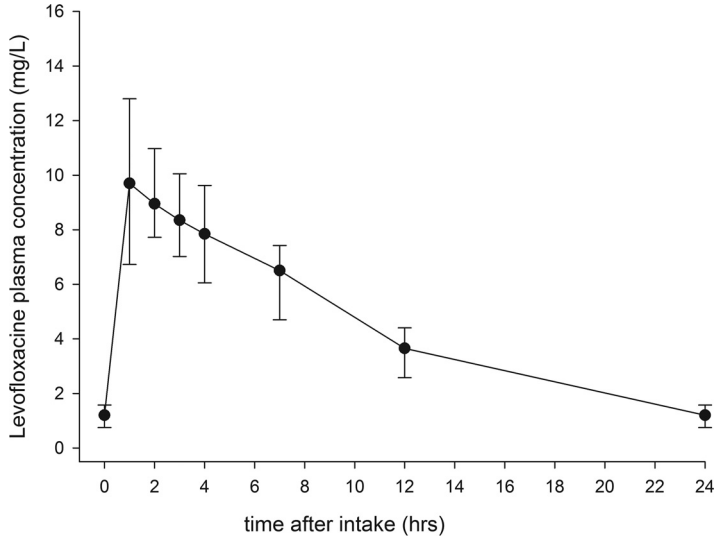

FIG 1 Mean plasma concentration time curve of LVX with one standard deviation ($n = 20$).

Bacteriologic assessment. All included patients were resistant to at least isoniazid and rifampin. Table 3 presents an overview of the susceptibilities and resistances to the TB medicines used in M/XDR-TB regimens.

The LFX MIC values for *M. tuberculosis* ranged from <0.25 to 2 mg/liter, with median ranges of 0.5 (IQR = 0.25 to 0.5 mg/liter). Thirteen (65%) patients had MIC values ranging from 0.25 to 0.5 mg/liter. In 3 (15%) patients, *M. tuberculosis* showed an MIC value of >2 mg/liter, and these isolates were therefore considered LFX resistant. The MIC results obtained for LFX using the 7H10 agar method did not differ from the data obtained using the MGIT method.

Pharmacokinetics/pharmacodynamics. The median $fAUC_{0-24}$/MIC ratio was 118.7 (IQR = 89.4 to 399.4) using a given plasma protein unbound fraction of 65% and 143.3 (IQR = 63.5 to 522.2) by a free fraction of 85%. The $fAUC_{0-24}$/MIC value was below the target ratio of 100 in 5 patients by using 0.65 as the free fraction and in 4 patients if free fraction was estimated at 0.85. At an MIC of 0.25 mg/liter, 19 of 20 patients with an unbound fraction of 0.65 and all patients with the estimated free fraction of 0.85 exceeded the target PK *f*AUC/MIC ratio of 100. If the MIC were set at 0.5, 18 patients would reach the target; if the MIC were 1.0, 3 patients would reach the target; at an MIC

TABLE 3 Susceptibility and resistance to TB medicines according to WHO criteria ($n = 20$)[a]

Group	Medicine	No. of patients	
		Resistant	Susceptible
Fluoroquinolones	Ofloxacin	8	12
	Levofloxacin (Minsk vs RIVM)[b]	2/3	18/17
Injectable anti-TB medication	Amikacin	5	15
	Capreomycin	5	15
	Kanamycin	7	13
	Streptomycin	20	0
Other core second-line agents	Etionamide	1	19
	Protionamide	1	19
	Cycloserine	1	19
Add-on agents	Pyrazinamide	5	15
	Ethambutol	16	4

[a]See reference 31.
[b]RIVM, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

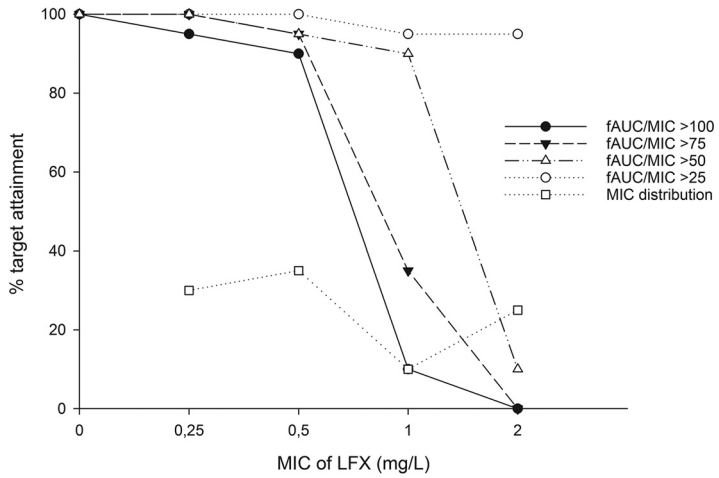

FIG 2 Percent target attainment at different *f*AUC/MIC ratios. The *f*AUC/MIC values were calculated using the observed AUCs in patients assuming a 65% unbound drug concentration. Target attainment was considered 100% if all individual *f*AUC/MIC values were greater than the predefined target *f*AUC/MIC values 100, 75, 50, and 25, respectively. The open squares show the observed MIC distribution expressed as a percentage.

of 2.0 mg/liter, no patients would reach the target if the free fraction value were 65%; and 19, 7, and no patients would reach the PK/PD target if free drug fraction were 85%. Figure 2 displays the target attainment (%) using the observed AUC/MIC values by 65% of the unbound concentration. The $fAUC_{0-24}$/MIC and C_{max}/MIC ratios and the MICs of LFX for each patient are shown in Table 4. The median C_{max}/MIC ratio was 21.6 (IQR = 13.6 to 40.8).

DISCUSSION

The objective of this study was to determine LFX concentrations and assess variability in PK/PD in M/XDR-TB patients. We observed a nearly 6-fold difference in the AUC values and 4-fold differences in the C_{max} values. In our study, 25% of the patients had a C_{max} below the reference range of 8 to 12 mg/liter, meaning that they are at risk for a poor response to treatment (22). One patient even showed a very low AUC of 31.2

TABLE 4 Ratio of unbound protein ($fAUC_{0-24}$) relative to the MIC of LFX (n = 20)

Patient	C_{max} (mg/liter)	MIC (mg/liter)	C_{max}/MIC	AUC_{0-24} (mg·h/liter)	$fAUC_{0-24}$/MIC[a]	$fAUC_{0-24}$/MIC[b]
1	16.2	0.25	64.8	139.6	362.96	474.64
2	13.1	0.5	26.2	159.6	207.48	271.32
3	14	0.25	56	153.6	399.36	522.24
4	8.7	0.25	34.8	114.4	297.44	388.96
5	9.2	1	9.2	99.82	64.88	84.85
6	13.1	0.5	26.2	119.1	154.83	202.47
7	10.2	0.25	40.8	82.66	214.92	281.04
8	4.3	1	4.3	31.35	20.38	26.65
9	10.8	0.5	21.6	85.38	110.99	145.15
10	6.8	0.5	13.6	79.77	103.70	135.61
11	11.1	0.25	44.4	111.3	289.38	378.96
12	9.8	0.5	19.6	90.85	118.10	154.45
13	7.6	0.5	15.2	83.16	108.11	141.37
14	12.3	2	6.15	126.5	41.11	53.76
15	7.1	0.5	14.2	68.76	89.39	116.89
16	9.9	2	4.95	156.9	50.99	66.68
17	12.5	0.25	50	97.81	254.31	332.55
18	7.6	≥2	–[c]	85.44	–	–
19	9.2	≥2	–	97.78	–	–
20	12	≥2	–	123.2	–	–

[a]As determined using a protein unbound fraction of 65%.
[b]As determined using a protein unbound fraction of 85%.
[c]–, three patients had MICs of >2 mg/ml and were not included.

mg·h/liter and a C_{max} of 4.3 mg/liter. He was previously treated for MDR-TB and later diagnosed with XDR-TB, showing an important relation to acquired drug resistance. Our median AUC and C_{max} values for LFX were slightly lower than the results of the PK study that Peloquin et al. performed in Brazilian patients and that Thwaites et al. performed in TB patients from Vietnam (12, 15), but the results are similar to those reported in previous studies by Piscitelli et al. and Chien et al. (23, 24). These differences may be the result of the severity of the disease, but they may also have been influenced by individual factors in the TB patients, such as renal function, body mass index (BMI), drug interaction, comorbidity, and polymorphism of the drug resistance-related mutation in MDR isolates from diverse geographical regions (14, 25–28). We found a good correlation between C_{max} and AUC_{0-24}. The relevance of C_{max}, in addition to the AUC, was shown earlier in a randomized controlled trial by Thwaites et al. (15).

More than half of the patients (65%) demonstrated quick absorption of LFX and reached C_{max} 1 h after administration of the drug. These results support the previous suggestion to use blood sampling earlier than 1 h in the future studies (12). One patient had extremely slow absorption with a T_{max} of 7 h. Apparently, this is not uncommon, as Peloquin et al. described one outlier in their study as well (12).

The study of Rosales-Klintz et al. demonstrated geographical differences in the frequency and distribution of resistance-related mutations, including Belarus, with the highest level of FQ resistance-related mutations (28). Many studies addressed the molecular epidemiology and description of MDR isolates. Studies on whole-genome sequencing (WGS) in combination with PK/PD data will probably play an important role in the design of clinical treatment regimens.

The proposed PK targets for fluoroquinolones against *M. tuberculosis* of an AUC_{0-24}/MIC of $>$100 and C_{max}/MIC of $>$10, were achieved in only 12 (70%) patients with MIC values of 0.25 to 0.5 mg/liter using a median LFX dose of 15.8 mg/kg. The analysis of target attainment by Alsultan et al. showed that higher doses of LFX in the range of 17 to 20 mg/kg might be needed to improve its activity and reduce development of drug resistance (29, 30). Our data confirm that the current dosing may be too low to reach the proposed targets in all patients.

At an MIC of 2, poor target attainment was observed across all doses. Just three patients in our study, using a MIC of 1.0 mg/liter, exceeded an *f*AUC/MIC ratio of 100, and none of them did so if the ratio was calculated using an MIC of 2.0 mg/liter. Even if we recalculated target attainment using a higher free fraction of 0.85, only 8 patients using an MIC of 1 mg/liter—and no patients using an MIC of 2 mg/liter—reached the therapeutic target. In both situations, this lack of drug exposure may contribute to the risk of acquired resistance, as shown by Cegielski et al. (20); often low values of C_{max} preceded acquired drug resistance. However, these data need to be interpreted relative to MIC values.

Personalized dose adjustment, based on measurements of drug concentration and MIC, is the way forward to ensure clinical efficacy and to avoid the emergence of drug resistance. The new World Health Organization (WHO) treatment guidelines for MDR-TB defined the dose of LFX as 750 mg/day or more and noted that the definition of a "high dose" will be the subject of discussion for another WHO guideline development group (31).

In practice, conventional drug susceptibility testing (DST) takes multiple weeks; therefore, the results may not accurately reflect the bacterial population by the time the results become available. Hopefully, WGS will relate all resistance mutations to phenotypic susceptibility, enabling rapid dose selection (32). According to a TBNET consensus statement, adoption of a standard WHO MDR-TB treatment regimen according to the local drug resistance pattern is the first step to a more individualized TB treatment in MDR-TB high-prevalence countries, especially when the prevalence of FQ resistance is high. In this context, the results for the performance of new commercially available MIC testing by a MycoTB Plate are very promising for use in resource-limited settings (33, 34). In addition, dried blood spot sampling and limited sampling strategies may provide

a helpful alternative to conventional plasma sampling, enabling optimal drug exposure during treatment (29, 35).

The new WHO M/XDR guidelines recommend LFX at 10 to 15 mg/kg once daily, and additionally for MDR-TB treatment, with the additional advice to use weight-based dosing for adults ≥45 kg of 1,000 mg once daily and 750 mg for body weights of <45 kg (31). Therapeutic drug monitoring (TDM) may help to optimize drug exposure of LFX further in patients with different body weights.

Our study has several limitations. As in any study with intensive PK sampling, the sample size was too low to assess the actual impact of suboptimal exposure on clinical endpoints. The impact of a few patients that are lost to follow-up is relatively high in a small study and makes definite conclusions less solid. Furthermore, *M. tuberculosis* isolates from three patents with MICs of >2 mg/liter appeared to be resistant when retested for LFX.

However, the strength of the study is that both drug exposures and MICs were evaluated. This is of particular importance since low drug exposure in a patient with a low MIC still resulted in clinical cure. However, low drug exposure in combination with a higher MIC value translates to a slow response and a higher risk of acquired drug resistance (36).

Clinicians should be mindful of the potential variability in drug exposure, as shown in our study. Based on this study and earlier studies, we believe now is the time to evaluate PK/PD optimized dosing of FQs in a prospective randomized controlled trial. Since actual MIC values are not readily available, one should aim at an *f*AUC/MIC of >100 for an MIC of 1 mg/liter. TDM should be performed early after start of TB treatment to ensure optimal LFX exposure. Once the actual MIC value is available, the dose can be further individualized. Combining the available LFX pharmacokinetic data from clinical studies and further dose selection using hollow-fiber infection models, population PK modeling in combination with Monte Carlo simulations will help to further optimize LFX treatment further (37, 38).

Conclusion. LFX drug concentrations were highly variable in our study population and not sufficient to reach the target exposure in the case of higher MICs. Higher dosing in combination with TDM may thus help to optimize efficacy and reduce the risk of drug resistance.

MATERIALS AND METHODS

Study subjects. Patients diagnosed with MDR-TB or XDR-TB confirmed by standard microbiological criteria (culture-based, molecular, or both), at least 18 years of age (male and female), hospitalized in the Republican Scientific and Practical Center for Pulmonology and Tuberculosis in Minsk, Republic of Belarus, with signed informed consent, and treated for more than 7 days with LFX were eligible for inclusion in this side study. Subjects were excluded from the study if they were pregnant, had diabetes mellitus, or previously had shown intolerance to LFX. The Medical Ethical Review Committee of the Republican Scientific and Practical Center for TB and Pulmonology in Minsk, Republic of Belarus, approved the study protocol. The study was registered at ClinicalTrials.gov (identifier number NCT02169141).

Study design. Treatment of MDR and XDR-TB was based on the WHO guidelines and clinical recommendations for TB treatment of the Republic of Belarus (39). All patients received LFX at the dose of 15 mg/kg once daily, which was rounded to 750 or 1,000 mg. The drug was administered on an empty stomach, but the participants were allowed to take a light breakfast after drug ingestion. The adherence to the treatment regimen was 100% since medication intake was supervised by the nurse. Blood samples (4 ml, with EDTA an anticoagulant) were obtained at steady state before (time zero) and 1, 2, 3, 4, 7, and 12 h postdosing. Plasma was separated and frozen at −20°C until samples were analyzed. For each included patient, data were collected from the medical records, including demographic data, medical history, and bacteriological and laboratory parameters. The rates of sputum smear and culture conversion were determined every month after initiating therapy.

Alcohol abuse was defined as the drinking of at least 5 U of alcohol per day for at least 5 days in the previous month. A history of smoking was defined as the use of any tobacco product on a regular basis in the past 5 years.

PK analysis. The concentrations of LFX in human plasma were analyzed in the Clinical Toxicology and Drugs Analysis Laboratory of the Department of Clinical Pharmacy and Pharmacology at the University Medical Centre Groningen by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS). All analyses were performed on a Thermo Fisher (San Jose, CA) triple quadrupole LC-MS/MS apparatus with a Finnigan Surveyor LC pump and a Finnigan Surveyor autosampler. The mobile phase consisted of an aqueous buffer (containing ammonium acetate, 10 g/liter; acetic acid, 35 mg/liter;

trifluoroacetic anhydride, 2 ml/liter of water), water, and acetonitrile and had a flow rate of 0.3 ml/min. Samples prepared by using 100 μl of serum or plasma and 750 μl of precipitation reagent (a mixture of methanol and acetonitrile [4:21, vol/vol]) containing the deuterated internal standard (for both levofloxacin and metabolite) were vortexed for 1 min and subsequently centrifuged at 11,000 $\times$ *g* for 5 min. From the clear upper layer, 2 μl was injected onto the LC-MS/MS system. The Finnigan TSQ Quantum Discovery mass selective detector was operating in electrospray positive ionization mode and performed selected reaction monitoring. The g mass parameters for LFX were 362.1.1 *m/z* to 44 *m/z*; for deslevo 348.1 *m/z* to 38 *m/z*, a scan width of 0.5 *m/z* was used. The calibration curves were linear within the concentration ranges of 0.10 to 5.00 mg/liter for LFX and 0.10 to 4.99 mg/liter for deslevo, using correlation coefficients (R^2) of 0.999 and 0.998, respectively. Dilution integrity was determined by diluting the concentrations that were greater than "high" 10 times with blank serum. This procedure was performed in quintuplicate for three consecutive days and showed that the accuracy and precision were within limits. The lower limit of quantification for LFX and deslevo was 0.10 mg/liter. This method is precise and accurate: the within-day precision ranged between 1.4 and 2.4% for LFX and between 1.5 and 5% for deslevo, and the between-day precision ranged from 3.6 to 4.1% for LFX and from 0.0 to 3.3% for deslevo. The calculated accuracy ranged from 0.1 to 12.7% for LFX and from 0.2 to 15.6% for deslevo.

The values of the PK parameters were calculated using noncompartmental analysis, using MW/Pharm (version 3.82; Medi-Ware, Netherlands). C_{max} was defined as the highest observed serum concentration. The AUC from time zero to 24 h (AUC_{0-24}) was calculated using the log-linear trapezoidal rule. In this case, the concentration of LFX at 24 h after oral administration was estimated to be equal to its concentration before administration of the dose as samples were obtained at steady state.

Based on the range of levels of LFX protein binding from approximately 20 to 40%, two separate *f*AUC_{0-24} values were estimated by multiplying the individual total AUC by unbound fractions of 0.65 and 0.85 (11, 40, 41). Free-drug AUC_{0-24}/MIC ratios were calculated by dividing the *f*AUC_{0-24} by the MIC value for LFX. Target attainment was calculated by dividing the number of patients that achieved the predefined *f*AUC/MIC target by the total number of study subjects. Since the exact *f*AUC/MIC is a debated subject, we used the target values 100, 75, 50, and 25 to be more informative.

Bacteriologic assessment. Drug susceptibility testing (DST) for clinical practice was performed at the Belarusian National Reference Laboratory in Minsk with a Bactec MGIT960 system using the critical concentration. For study purposes, the MIC values for LFX were determined at the National Mycobacterium Reference Laboratory in the Netherlands (National Institute for Public Health and the Environment [RIVM], Bilthoven, Netherlands). The MICs of LFX were determined using the agar dilution method on Middlebrook 7H10 medium and the MGIT960 system (42). The MIC was defined as the lowest drug concentration inhibiting 99% of the colony counts compared to the medium without the antibiotic.

Clinical evaluation. For clinical evaluation, we used the definitions of treatment outcome according to the WHO "definitions and reporting framework for tuberculosis" for patients treated by second-line regimens, i.e., cured, treatment completed, treatment failed, died, lost to follow-up, not evaluated, and treatment success (43).

Statistical methods. Values are expressed as medians with the interquartile range (IQR) for continuous variables and as percentages for categorical variables. Correlations between two continuous variables were calculated using the Spearman correlation coefficient. The Mann-Whitney U test was used to assess whether PK/PD distributions were different for patient subgroups by gender, age, BMI, and creatinine levels. Linear regression analysis was performed to assess which factors were associated with AUC_{0-24} and C_{max}. Variables with a P value of <0.05 in univariate analysis were included in multiple linear regression, and backward analysis was conducted to obtain the most parsimonious model, thereby removing nonsignificant variables, starting with the one with the highest P value. A P value of <0.05 was considered statistically significant. All statistical calculations were performed in SPSS 22 (SPSS, Chicago, IL).

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