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Pharmacokinetics of Second-Line Antituberculosis Drugs after Multiple Administrations in Healthy Volunteers

Sang-In Park,[a] Jaeseong Oh,[a] Kyungho Jang,[a] Jangsoo Yoon,[a] Seol Ju Moon,[a] Jong Sun Park,[b] Jae Ho Lee,[b] Junghan Song,[c] In-Jin Jang,[a] Kyung-Sang Yu,[a] Jae-Yong Chung[d]

Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul, Republic of Korea[a]; Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea[b]; Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea[c]; Department of Clinical Pharmacology and Therapeutics, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea[d]

Therapeutic drug monitoring (TDM) of second-line antituberculosis drugs would allow for optimal individualized dosage adjustments and improve drug safety and therapeutic outcomes. To evaluate the pharmacokinetic (PK) characteristics of clinically relevant, multidrug treatment regimens and to improve the feasibility of TDM, we conducted an open-label, multiple-dosing study with 16 healthy subjects who were divided into two groups. Cycloserine (250 mg), *p*-aminosalicylic acid (PAS) (5.28 g), and prothionamide (250 mg) twice daily and pyrazinamide (1,500 mg) once daily were administered to both groups. Additionally, levofloxacin (750 mg) and streptomycin (1 g) once daily were administered to group 1 and moxifloxacin (400 mg) and kanamycin (1 g) once daily were administered to group 2. Blood samples for PK analysis were collected up to 24 h following the 5 days of drug administration. The PK parameters, including the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve during a dosing interval at steady state (AUC_{τ}), were evaluated. The correlations between the PK parameters and the concentrations at each time point were analyzed. The mean C_{max} and AUC_{τ}, respectively, for each drug were as follows: cycloserine, 24.9 mg/liter and 242.3 mg · h/liter; PAS, 65.9 mg/liter and 326.5 mg · h/liter; prothionamide, 5.3 mg/liter and 22.1 mg · h/liter; levofloxacin, 6.6 mg/liter and 64.4 mg · h/liter; moxifloxacin, 4.7 mg/liter and 54.2 mg · h/liter; streptomycin, 42.0 mg/liter and 196.7 mg · h/liter; kanamycin, 34.5 mg/liter and 153.5 mg · h/liter. The results indicated that sampling at 1, 2.5, and 6 h postdosing is needed for TDM when all seven drugs are administered concomitantly. This study indicates that PK characteristics must be considered when prescribing optimal treatments for patients. (This study has been registered at ClinicalTrials.gov under registration no. NCT02128308.)

Tuberculosis (TB) that is resistant to isoniazid and rifampin is considered to be multidrug resistant (MDR) regardless of whether it is resistant to other first-line drugs. MDR TB has become a major global health concern and, according to the Global Project on Anti-Tuberculosis Drug Resistance Surveillance, MDR TB accounts for more than 3% of new TB cases in at least one country in every one of the six World Health Organization (WHO) regions (1).

WHO guidelines recommend the use of at least four different drugs to treat MDR TB; it is recommended that regimens include pyrazinamide along with several second-line drugs, specifically, a later-generation fluoroquinolone, ethionamide, or prothionamide, an injectable parenteral agent (kanamycin, amikacin, or capreomycin), and either cycloserine or *p*-aminosalicylic acid (PAS) (2). However, these treatment regimens are less effective, cause more-frequent side effects, and have a narrower therapeutic effect/toxic effect ratio than first-line anti-TB drugs (3). Fortunately, therapeutic drug monitoring (TDM) can be used to overcome variations in patient responses and to improve the success of second-line therapeutic agents by allowing individualized therapies to be designed (4).

When TDM of second-line anti-TB drugs in a clinical setting is being planned, ease of use and availability, as well as clinical and bacteriological data, should be considered. For instance, for anti-TB drugs such as moxifloxacin and levofloxacin, the area under the concentration-time curve from time zero to 24 h (AUC_{0-24})/MIC is one of the most important pharmacodynamic parameters, along with the maximum plasma concentration (C_{max})/MIC (5). However, estimation of AUC values requires a minimum of six or seven blood samples and therefore is impractical as a routine measure (4). A more practical alternative to full pharmacokinetic (PK) sampling is the use of only a few sampling time points that are strongly correlated with C_{max} or AUC. Furthermore, since second-line anti-TB drugs are administered concomitantly in most cases, the possibility of multidrug interactions should always be considered. For this reason, it would be more useful to estimate the PK characteristics of second-line anti-TB drugs in the context of a therapeutically relevant dosage regimen.

The objective of this study was to evaluate the PK characteristics of second-line anti-TB drugs being used among patients with MDR TB, with a view to applying the results to clinical practice,

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Address correspondence to Jae-Yong Chung, jychung@snubh.org.

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TABLE 1 Dosing regimen for each study group from day 1 morning dose to day 5 morning dose[a]

Group	Regimen common to both groups	Part of regimen different between groups
1 ($n = 7$)	Cycloserine (250 mg) p.o. twice daily, PAS (5.28 g) p.o. twice daily	Levofloxacin (750 mg) p.o. once daily, streptomycin (1 g) i.m. once daily
2 ($n = 9$)	Prothionamide (250 mg) p.o. twice daily, pyrazinamide (1,500 mg) p.o. once daily	Moxifloxacin (400 mg) p.o. once daily, kanamycin (1 g) i.m. once daily

[a] PAS, *p*-aminosalicylic acid; i.m., intramuscular; p.o., *per os*.

including prescribing. In addition, to reduce the number of sampling time points required to estimate AUC or C_{max}, the most reliable sampling time points were determined.

MATERIALS AND METHODS

Study subjects and study design. Healthy male volunteers who had no history of renal or hepatic impairment and who passed a rigorous on-site physical examination and clinical laboratory tests, including urine screening and a 12-lead electrocardiogram, were enrolled in the study. Written informed consent was obtained prior to the start of the procedures described in this study. This study was approved (protocol number B-1309/219-003) by the institutional review board of the Seoul National University Bundang Hospital (Seongnam, Republic of Korea) and was conducted in accordance with the principles of the Declaration of Helsinki and good clinical practice guidelines (ClinicalTrials.gov registration no. NCT02128308).

A randomized, open-label, parallel-group, multiple-dosing study was conducted in the Seoul National University Bundang Hospital Clinical Trials Center. The patients were divided into two groups so that as many second-line anti-TB drugs as possible could be studied with two different dosing regimens. The two regimens were chosen to reflect treatments that are currently used clinically. Group 1 received cycloserine, *p*-aminosalicylic acid (PAS), and prothionamide *per os* (p.o.) twice daily, pyrazinamide and levofloxacin p.o. once daily, and streptomycin intramuscularly (i.m.) once daily from day 1 to day 5. For group 2, the same regimen was applied except that levofloxacin and streptomycin were replaced with moxifloxacin and kanamycin, respectively (Table 1).

Pharmacokinetic assessment. Serial blood samples were collected in heparin-containing tubes at 0 (i.e., predosing), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after dosing on day 5. Blood samples were centrifuged for 10 min at approximately 1,910 × *g* at 4°C. Plasma (1 ml) was then aliquoted into polypropylene tubes and stored at −70°C until PK analysis.

The plasma concentrations of cycloserine, PAS, prothionamide, pyrazinamide, levofloxacin, moxifloxacin, streptomycin, and kanamycin were determined using ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). This analytical method was developed previously for simultaneous measurement of the blood concentrations of nine second-line anti-TB drugs (6). In brief, the analytes were separated on a high-strength silica (HSS) T3 column (50.0 by 2.1 mm, 1.8-μm particles; Waters, Watford, United Kingdom) at a flow rate of 200 μl/min. The mobile phases were 10 mM ammonium formate in 0.1% formic acid (mobile phase A) and acetonitrile in 0.1% formic acid (mobile phase B). Separation was achieved using a gradient program. Detection was performed using MS/MS. The interassay calibration variability was evaluated using concentrations of 5.0 to 100 μg/ml for streptomycin, kanamycin, cycloserine, and PAS, 0.5 to 10 μg/ml for prothionamide, and 1.0 to 20 μg/ml for moxifloxacin and levofloxacin. The values for the lower limit of quantification (LLOQ), defined as the lowest concentration with precision of <20% and accuracy within ±20% for each drug, were as follows: 5.0 μg/ml for cycloserine, 5.0 μg/ml for PAS, 0.5 μg/ml for prothionamide, 1.0 μg/ml for levofloxacin, 2.5 μg/ml for streptomycin, 0.5 μg/ml for moxifloxacin, and 2.5 μg/ml for kanamycin.

Pharmacokinetic data analyses. We used the linear-up and log-down trapezoidal method to estimate the AUC_{τ}, which is the AUC during a dosing interval when the concentrations had reached steady state on day 5 of the dosing administration. The observed values were used to estimate the steady-state C_{max} for each drug. The apparent clearance at steady state (CL/*F*) was calculated as the dose divided by the AUC from time zero to infinity at steady state (AUC_{inf}). The terminal elimination rate constant (λ_z) was estimated from the regression line of the logarithmically transformed plasma concentrations versus time over the terminal log-linear portion. The terminal half-life at steady state ($t_{1/2}$) was calculated as the natural logarithm of 2 divided by λ_z. Noncompartmental analysis using Phoenix version 1.3 (Certara, St. Louis, MO) was used to calculate the PK parameters.

Statistical analyses. The PK parameters of second-line anti-TB drugs were summarized using descriptive statistics. Possible differences in drug exposure were determined based on the differences in C_{max} and AUC_{τ} values for cycloserine, PAS, and prothionamide between group 1 and group 2, and the geometric mean ratio (GMR) and its 90% confidence interval (CI) for group 2 versus group 1 were estimated. Unpaired *t* tests were used to compare the CL/*F* differences between group 1 and group 2. Analysis was performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC). Statistical significance was confirmed with *P* values of less than 0.05.

Selection of sampling time points for limited-sampling TDM strategy. After full PK profiles were obtained, the most reliable sampling time point for each second-line anti-TB drug was determined by analysis of correlations between C_{max} or AUC_{τ} and the concentrations of the drugs measured in the samples collected at each time point after dosing on day 5. Pearson's correlation analyses were repeated for all sampling time points from 0 to 24 h for each of the studied second-line anti-TB drugs, using R version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). The optimal sampling time points were determined based on the adjusted r^2 and *P* values, using the correlation analyses.

RESULTS

Demographic characteristics. A total of 16 subjects were enrolled and completed the study, including seven subjects in group 1 and nine subjects in group 2. As the purpose of this study was to explore and to describe the PK characteristics of second-line anti-TB drugs, it was desirable to set the minimum number of subjects based on experience from previous studies, rather than on calculations required for a hypothesis study. Thus, the required number of subjects for this study was determined to be at least six for each dose group. The demographic characteristics of the subjects are presented in Table 2. There were no significant differences in demographic characteristics, such as age, height, weight, and body mass index (BMI), between the two randomized treatment groups (all *P* values of >0.05, Student's *t* test).

PK analysis. The PK characteristics of each second-line anti-TB drug regimen after concomitant administration are presented in Table 3 and were generally consistent with the previously reported values for separate administration. C_{max} and AUC_{τ} values were estimated after the 5-day dosing of each second-line anti-TB drug according to the regimens presented in Table 1. Relatively larger interindividual variations were observed for cycloserine, PAS, and prothionamide than for the other second-line anti-TB drugs (Table 3 and Fig. 1). The observed C_{max} values for cycloserine, moxifloxacin, streptomycin, and kanamycin were consistent with the available literature data (Table 4). Prothionamide and levofloxacin showed $t_{1/2}$ values similar to the reported $t_{1/2}$ values for each drug. The time to C_{max} (T_{max}) and $t_{1/2}$ for

TABLE 2 Subject characteristics

Characteristic[a]	All subjects ($n = 16$)	Group 1 ($n = 7$)	Group 2 ($n = 9$)
Age (median [range]) (yr)	26 (21–40)	25 (22–32)	28 (21–40)
Weight (mean [range]) (kg)	66.3 (57.9–81.0)	69.3 (61.0–81.0)	63.9 (57.9–68.7)
Height (mean [range]) (cm)	171.3 (161.0–178.0)	173.0 (167.0–178.0)	170.0 (161.0–177.0)
BMI (mean [range]) (kg/m^2)	22.5 (19.6–25.9)	23.2 (20.6–25.9)	22.0 (19.6–24.0)

[a] Means are arithmetic means. BMI, body mass index.

streptomycin and kanamycin were consistent with previously reported data, showing rapid absorption after i.m. injection (Table 3 and Fig. 2).

Subgroup analysis showed higher mean concentrations of cycloserine and prothionamide in group 2 than in group 1 (Fig. 1A and C). The GMR was greater than 1.0 (90% CI) for the C_{max} and AUC_{τ} values in group 2 versus group 1 for cycloserine and prothionamide (Table 5). For cycloserine, the mean CL/*F* values for the two groups were significantly different, with ($P = 0.010$) or without ($P = 0.026$) normalization for body weight. For prothionamide, the CL/*F* values for the two groups were significantly different ($P = 0.046$), but the difference was not significant after normalization for body weight ($P = 0.100$). On the other hand, for PAS, although the mean concentrations were higher in group 2 than in group 1 until 3 h after dosing, the 90% CI of the GMR included 1.0, indicating no significant differences between the two groups (Table 5 and Fig. 1B). For PAS, there was no significant difference between the two groups with respect to the CL/*F* values with ($P = 0.873$) or without ($P = 0.925$) normalization for body weight.

Sampling time points for TDM of second-line anti-TB drugs. Table 6 shows the most reliable sampling time points for each second-line anti-TB drug; these represent the time points at which the drug concentrations were found to have the strongest correlations with C_{max} or AUC_{τ} values. Based on the adjusted r^2 and *P* values, the concentrations measured in samples collected 2 to 3 h after dosing on day 5 correlated most strongly with the C_{max} values for cycloserine, PAS, prothionamide, and levofloxacin, compared with samples collected at other time points. For levofloxacin, the concentrations sampled 2 h and 1.5 h after dosing were most strongly correlated with C_{max} and AUC_{τ}, respectively. In the case of moxifloxacin, the concentration in the sample collected 6 h after dosing was most strongly correlated with both C_{max} and AUC_{τ}. For streptomycin and kanamycin, the concentrations in samples collected 1 h after dosing were most strongly correlated with the C_{max} values for both drugs.

DISCUSSION

This study shows that the measured PK characteristics of the tested second-line anti-TB drugs after concomitant administration were consistent with those reported for separate administration (4, 7, 8). Analyses of correlations between the PK parameters and the observed concentrations at each time point suggested that the time points 1, 2.5, and 6 h postdosing are required for accurate limited-sampling TDM when all seven drugs are administered concomitantly. To the best of our knowledge, the present study is the first clinical study to evaluate the PK characteristics of second-line anti-TB drugs in the context of the commonly used regimens for MDR TB with multiple doses.

In general, the results of this study correspond with the PK characteristics reported previously for single drug administrations (4, 7, 8). However, compared to single drug administration, some differences in PK characteristics were observed when the drugs were administered concomitantly. For example, although the observed mean C_{max} for cycloserine corresponded with the literature data (9), the median T_{max} was 2 h longer than the known range of T_{max} values (4). For PAS, the mean C_{max} value for all subjects was ~28% greater than the reported mean C_{max} (10), which is thought to be due to the 32% larger dose used in this study; however, the mean AUC_{τ} was ~11% smaller than the reported value of AUC_{0-12} after the administration of 4 g twice daily (10). In the case of prothionamide, the observed mean C_{max} and AUC_{τ} values for all subjects were ~2-fold greater than the previously reported values of each parameter, although the mean dose used in the previous studies was 55.3% larger than that in the present study (11). For levofloxacin, the mean C_{max} was more than 17.5% smaller than the reference C_{max} (4); for moxifloxacin, the mean C_{max} was consistent with previous data (4). The mean C_{max}, T_{max},

TABLE 3 Summary of pharmacokinetic parameters of second-line antituberculosis drugs[a]

Drug	T_{max} (median [range]) (h)	C_{max} (mean ± SD) (mg/liter)	CV (%) for C_{max}	AUC_{τ} (mean ± SD) (mg · h/liter)	CV (%) for AUC_{τ}	CL/*F* (mean ± SD) (liters/h)	CV (%) for CL/*F*	$t_{1/2}$ (mean ± SD) (h)	*V*/F (mean ± SD) (liters)
Cycloserine	4.0 (2.0–6.0)	24.9 ± 9.9	39.8	242.3 ± 99.8	41.2	1.2 ± 0.5	41.6	20.3 ± 13.9	10.7 ± 4.4
PAS	2.5 (0.5–6.0)	65.9 ± 32.2	48.9	326.5 ± 152.5	46.7	20.7 ± 11.9	57.6	1.8 ± 0.5	16.6 ± 13.9
Prothionamide	3.0 (1.5–6.0)	5.3 ± 1.9	35.9	22.1 ± 7.9	35.8	12.8 ± 4.7	36.7	2.4 ± 0.5	41.0 ± 14.6
Levofloxacin	4.0 (3.0–6.0)	6.6 ± 1.0	15.2	64.4 ± 19.0	29.6	12.3 ± 2.8	22.8	8.2 ± 3.3	117.1 ± 23.9
Moxifloxacin	4.0 (1.0–6.0)	4.7 ± 1.5	31.7	54.2 ± 14.0	25.9	7.8 ± 1.9	23.9	13.7 ± 2.6	103.8 ± 25.7
Streptomycin	1.0 (0.5–1.5)	42.0 ± 10.8	25.8	196.7 ± 25.6	13.0	5.2 ± 0.7	12.9	3.1 ± 0.4	22.6 ± 2.8
Kanamycin	1.0 (0.5–2.5)	34.5 ± 4.3	12.5	153.5 ± 25.7	16.7	6.7 ± 0.9	14.2	3.2 ± 1.0	30.4 ± 9.8

[a] T_{max}, time to reach the maximum blood concentration at steady state; SD, standard deviation; C_{max}, maximum plasma concentration at steady state; CV, coefficient of variation; AUC_{τ}, area under the plasma concentration-time curve within a dosing interval at steady state; CL/*F*, apparent clearance at steady state; $t_{1/2}$, terminal elimination half-life; *V*/F, apparent volume of distribution; PAS, *p*-aminosalicylic acid. Means are arithmetic means.

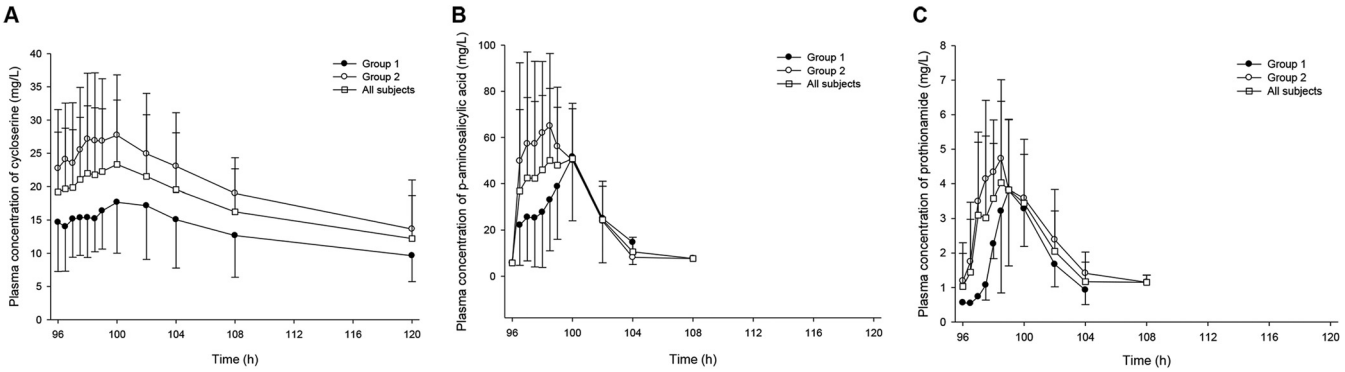

FIG 1 Mean plasma concentration-time profiles for cycloserine (A), *p*-aminosalicylic acid (B), and prothionamide (C) after multiple administrations in group 1, in group 2, and in all subjects on day 5. Error bars, standard deviations.

and $t_{1/2}$ values for streptomycin and kanamycin were within the previously reported ranges (4, 8).

Several factors might have contributed to the fact that the mean concentrations of cycloserine and prothionamide and the GMRs (90% CI) of the C_{max} and AUC_{τ} values for cycloserine and prothionamide were higher in group 2 than in group 1 (Table 5 and Fig. 1). For example, the estimated CL/*F* (oral clearance) for cycloserine in group 1 was 2-fold larger than that for group 2; because the elimination of cycloserine is dependent on renal clearance (8), differences in bioavailability could be a possible explanation, considering that both groups consisted of healthy subjects without any abnormalities in renal function. Additionally, the apparent volume of distribution (*V*/*F*) for cycloserine was 1.6-fold larger in group 1 than in group 2. Similarly, the CL/*F* and *V* values for prothionamide in group 1 were 1.5- and 1.4-fold larger, respectively, than those in group 2. Therefore, the difference in the bioavailabilities of cycloserine and prothionamide was most likely caused by the different drugs in each group. In the case of PAS, the mean concentrations in group 2 were higher than those in group 1 until 3 h after dosing, although the 90% CIs for C_{max} and AUC_{τ} suggested no significant differences between the two groups. The absorption of PAS granules, which have enteric coating and which are designed for sustained release, might have been affected by the concomitantly administered drugs (8), although further studies with a larger number of subjects are needed for precise comparisons.

Drug interactions among second-line anti-TB drugs are not well known, and this study was not designed to assess these interactions; nonetheless, it is possible to speculate on the possibility of drug interactions based on the reported PK mechanisms of the concomitantly administered second-line anti-TB drugs. The PK characteristics of aminoglycosides, such as streptomycin and kanamycin, are known to be similar, and more than 90% is excreted unchanged in the urine (7, 8). Therefore, it is more likely that the PK characteristics of levofloxacin and moxifloxacin were responsible for the observed differences between the two groups. However, the reported differences between moxifloxacin and levofloxacin mainly concern the mechanisms of metabolism, rather than effects on the absorption process or the bioavailability of the other drugs. Levofloxacin is primarily excreted unchanged in the urine, with <5% being metabolized in the liver (12). In contrast, moxifloxacin has multiple routes of elimination, with approximately 50% undergoing glucuronide and sulfate conjugation in the liver, 25% being excreted unchanged in the feces, and 20 to 25% being excreted unchanged in the urine (5, 13–15). Further studies on the factors that could have affected the drug interactions, focusing on the absorption process or bioavailability, are necessary, as knowledge of the mechanisms of metabolism is not sufficient to explain the differences observed here.

Finally, weight can be considered one of the factors affecting group differences. Although no statistically significant difference was observed, the mean difference in body weight between the two groups was 5.4 kg ($P = 0.057$) (Table 2). However, even after normalization of the GMRs of the C_{max} and AUC_{τ} values for cy-

TABLE 4 Summary of literature data on pharmacokinetic parameters of second-line antituberculosis drugs

Drug	Dosage[a]	T_{max} (h)	C_{max} (mg/liter)	AUC_{τ} (mg · h/liter)	$t_{1/2}$ (h)	Reference(s)
Cycloserine	250–500 mg QD or BIW	2	20–35		7	4[b]
	250 mg BID		25–30		10	9
PAS	4 g BID	5.2	51.3	368		10
Prothionamide	250 mg or 375 mg BID (mean dose, 5.9 mg/kg)	3.4	2.5	11.3	3	11
Levofloxacin	500–1,000 mg QD	1–2	8–13		9	4[b]
Moxifloxacin	400 mg QD	1–2	3–5		7	4[b]
Streptomycin	15 mg/kg QD	0.5–1.5	35–45[c]		3	4, 8[b]
Kanamycin	15 mg/kg QD	0.5–1.5	35–45[c]		3	4[b]

[a] QD, every day; BID, twice daily; BIW, twice weekly; AUC_{τ}, area under the plasma concentration-time curve within a dosing interval at steady state; C_{max}, maximum plasma concentration at steady state; $t_{1/2}$, terminal elimination half-life; T_{max}, time to reach the maximum blood concentration at steady state; PAS, *p*-aminosalicylic acid.
[b] Indicating "normal" values of the pharmacokinetic parameters for standard doses of drugs.
[c] C_{max} calculated using linear regression of data recorded to 1 h after the i.m. dose.

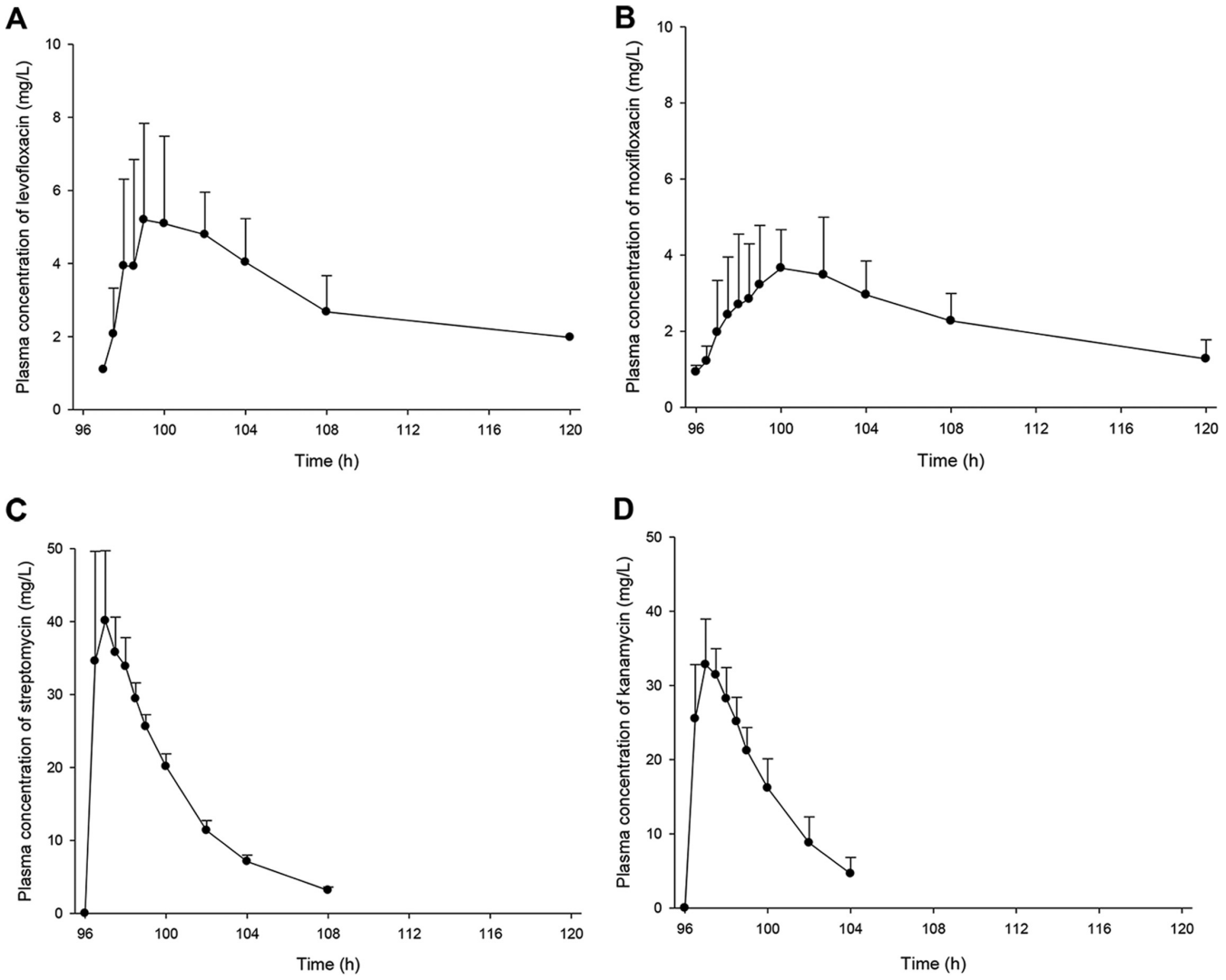

FIG 2 Mean plasma concentration-time profiles of levofloxacin (A), moxifloxacin (B), streptomycin (C), and kanamycin (D) after multiple administrations on day 5. Error bars, standard deviations.

closerine and prothionamide for weight, the values remained larger in group 2 than in group 1. This demonstrates that weight differences are less likely to be responsible for the differences in mean concentrations among the studied drugs.

Although further studies are needed to clarify the factors responsible for the differences between the two groups shown in this study, the work demonstrates that the possibility of interpatient differences in PK absorption/elimination characteristics should be considered when treating patients with MDR TB. In addition, such differences are one of the reasons why TDM should be performed to confirm the presence of PK interactions and to help prevent toxicity resulting from interactions.

Another objective of this study was to determine the optimal sampling time points for limited-sampling TDM. Among the second-line anti-TB drugs studied, cycloserine, PAS, and prothionamide had relatively larger interindividual variations than did the other drugs (Fig. 1 and Table 3). This suggests that there is a greater need for TDM of cycloserine, PAS, and prothionamide to improve the chances of safe successful therapy. For cycloserine, PAS, and prothionamide, target C_{max} values for serum levels have been proposed (5, 8). Additionally, serious central nervous system toxicity may be associated with elevated serum concentrations of cycloserine (4). The activity of aminoglycosides such as streptomycin and kanamycin relies on high C_{max}/MIC ratios due to concentration-dependent antibiotic and postantibiotic effects (7). Based on the known PK parameters and MICs of fluoroquinolones, the expected pharmacodynamic parameters of moxifloxacin and levofloxacin can be derived from C_{max}/MIC and AUC/MIC values (5). In this respect, the sampling time points can be suggested based on the adjusted r^2 and P values, as presented in Table 6. When cycloserine, PAS, and prothionamide are used together, the sampling time points should be set at 2 to 3 h after dosing, with regard to C_{max}. When aminoglycosides such as streptomycin and kanamycin are added, an additional sampling time point 1 h after dosing can be helpful for the prediction of efficacy. Adding a 6-h sampling time point after dosing is needed for TDM when moxifloxacin is used. A 6-h sampling time point is also useful for distinguishing between malabsorption and late absorption of the

TABLE 5 Comparison of pharmacokinetic parameters for cycloserine, PAS, and prothionamide in group 1 versus group 2[a]

Drug and group	T_{max} (median [range]) (h)	C_{max} (mean ± SD) (mg/liter)	GMR for C_{max} (90% CI)[b]	AUC_{τ} (mean ± SD) (mg · h/liter)	GMR for AUC_{τ} (90% CI)[b]	CL/*F* (mean ± SD) (liters/h)	$t_{1/2}$ (mean ± SD) (h)	*V*/F (mean ± SD) (liters)
Cycloserine								
Group 1	4.0 (3.0–6.0)	18.6 ± 7.7	1.66 (1.24–2.22)	184.5 ± 81.1	1.61 (1.18–2.19)	0.6 ± 0.3	16.4 ± 5.1	13.5 ± 4.9
Group 2	2.5 (2.0–4.0)	29.9 ± 8.8		287.2 ± 92.5		0.3 ± 0.2	23.3 ± 17.9	8.6 ± 2.6
PAS								
Group 1	4.0 (2.0–6.0)	54.9 ± 28.0	1.37 (0.83–2.27)	323.6 ± 186.0	1.04 (0.52–2.06)	6.5 ± 4.5	1.7 ± 0.2	15.3 ± 10.0
Group 2	1.8 (0.5–4.0)	75.6 ± 34.3		327.6 ± 152.7		6.0 ± 3.2	1.8 ± 0.6	17.1 ± 15.7
Prothionamide								
Group 1	3.0 (2.5–4.0)	4.3 ± 1.8	1.54 (1.16–2.04)	17.8 ± 6.3	1.45 (1.08–1.95)	15.3 ± 4.8	2.2 ± 0.5	47.7 ± 14.9
Group 2	2.3 (1.5–6.0)	6.3 ± 1.5		25.7 ± 7.6		10.1 ± 3.8	2.5 ± 0.6	35.2 ± 12.5

[a] T_{max}, time to reach the maximum blood concentration at steady state; SD, standard deviation; AUC_{τ}, area under the plasma concentration-time curve within a dosing interval at steady state; CL/*F*, apparent clearance at steady state; C_{max}, maximum plasma concentration at steady state; GMR, geometric mean ratio; PAS, *p*-aminosalicylic acid; $t_{1/2}$, terminal elimination half-life; *V*/F, apparent volume of distribution.
[b] GMR of group 2 versus group 1, with 90% confidence interval (CI).

other TB agents, and an additional later time point might be considered for regimens including moxifloxacin. Therefore, when all seven drugs evaluated in this study are being administered, at least three sampling time points are needed, i.e., 1 h, 2.5 h, and 6 h after dosing.

Extrapolating the results of this study to larger MDR TB patient populations may be difficult, due to the small number of healthy, relatively young, male subjects who participated in the study. However, in conjunction with the PK characteristics described in other studies, the PK profiles of the second-line anti-TB drugs described here could be used to develop basic guidelines for TDM.

This study provides valuable information concerning the PK characteristics of second-line anti-TB drugs in commonly used multiple-dosing regimens for MDR TB. Possible drug interactions were observed when fluoroquinolones were coadministered with cycloserine, PAS, and prothionamide, and this may warrant further study in patients with MDR TB. Based on the results of this study, it is also possible to recommend sampling time points for use in reduced-sampling TDM of second-line anti-TB drugs. It is envisioned that, when treating patients in a complex clinical setting, such a procedure will enable optimal adjustments of dosing regimens in order to overcome problems such as slow responses to treatment, toxicity, or suspected drug interactions.

TABLE 6 Recommended sampling time points as estimated by analysis of correlations between C_{max} or AUC_{τ} values and concentrations of second-line antituberculosis drugs measured in samples at each time point on day 5[a]

Drug	C_{max}		AUC_{τ}	
	Sample time (h)	Adjusted r^2	Sample time (h)	Adjusted r^2
Cycloserine	2	0.939[b]	4	0.968[b]
PAS	3	0.891[b]	3	0.906[b]
Prothionamide	2.5	0.602[b]	4	0.565[b]
Levofloxacin	2	0.917[b]	1.5	0.877[b]
Moxifloxacin	6	0.523[c]	6	0.849[b]
Streptomycin	1	0.928[b]	6	0.918[b]
Kanamycin	1	0.858[b]	4	0.937[b]

[a] C_{max}, maximum plasma concentration of drug at steady state; AUC_{τ}, area under the plasma concentration-time curve within a dosing interval at steady state; PAS, *p*-aminosalicylic acid.
[b] $P \leq 0.001$.
[c] $P \leq 0.05$.

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