AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

PHARMACOLOGY

Pharmacokinetics of Second-Line Antituberculosis Drugs in Children with Multidrug-Resistant Tuberculosis in India

Agibothu Kupparam Hemanth Kumar,[a] Alok Kumar,[b] Thiruvengadam Kannan,[a] Rakesh Bhatia,[b] Dipti Agarwal,[b] Santosh Kumar,[b] Rajeshwar Dayal,[b] Sheo Pratap Singh,[b] Geetha Ramachandran[a]

[a]National Institute for Research in Tuberculosis (ICMR), Chennai, India
[b]Sarojini Naidu Medical College, Agra, India

ABSTRACT We studied the pharmacokinetics of levofloxacin (LFX), pyrazinamide (PZA), ethionamide (ETH), and cycloserine (CS) in children with multidrug-resistant tuberculosis (MDR-TB) who were being treated according to the Revised National TB Control Programme (RNTCP) guidelines in India. This observational, pharmacokinetic study was conducted in 25 children with MDR-TB at the Sarojini Naidu Medical College, Agra, India, who were being treated with a 24-month daily regimen. Serial blood samples were collected after directly observed administration of drugs. Estimations of plasma LFX, PZA, ETH, and CS were undertaken according to validated methods by high-performance liquid chromatography. Adverse events were noted at 6 months of treatment. The peak concentration (C_{max}) of LFX was significantly higher in female than male children (11.5 μg/ml versus 7.3 μg/ml; $P = 0.017$). Children below 12 years of age had significantly higher ETH exposure (area under the concentration-time curve from 0 to 8 h [AUC_{0-8}]) than those above 12 years of age (17.5 μg/ml · h versus 9.4 μg/ml; $P = 0.030$). Multiple linear regression analysis showed significant influence of gender on C_{max} of ETH and age on C_{max} and AUC_{0-8} of CS. This is the first and only study from India reporting on the pharmacokinetics of LFX, ETH, PZA, and CS in children with MDR-TB treated in the Government of India program. More studies on the safety and pharmacokinetics of second-line anti-TB drugs in children with MDR-TB from different settings are required.

KEYWORDS MDR-TB, children, India, pharmacokinetics

The World Health Organization (WHO) estimates for 2016 are that one million children (<15 years) suffer from tuberculosis (TB) worldwide, and that more than 210,000 children die each year (1). Drug-resistant TB (DR-TB) is a continuing threat and is also an issue in children. It was estimated that more than 30,000 children become sick every year with strains of multidrug-resistant TB (MDR-TB). Also, a survey by the Revised National TB Control Programmes (RNTCP) in India found that 9% of children with TB were already resistant to rifampin, which is an important first-line anti-TB drug, before they started treatment. This indicates that they had become infected with DR-TB (https://www.tbfacts.org/tb-children/).

According to RNTCP guidelines, children with MDR-TB are treated for 24 months with a daily regimen consisting of kanamycin, levofloxacin (LFX), ethionamide (ETH), cycloserine (CS), pyrazinamide (PZA), and ethambutol (EMB) for 6 months, followed by LFX, ETH, CS, and EMB for the remaining 18 months (2). In general, the pharmacokinetics of most of these drugs have been studied. Levofloxacin is well absorbed, with a bioavailability of >99% (3, 4), and has good penetration into body fluids (5), including cerebrospinal fluid (CSF) (6). It is excreted unchanged in the urine, with <5% metabolized in the liver (3). Ethionamide is rapidly absorbed after oral administration and has a short half-life of about 2 h (7). It has good tissue and CSF penetration (6, 8). Its main

Received 23 November 2017 **Returned for modification** 3 January 2018 **Accepted** 8 February 2018

Accepted manuscript posted online 20 February 2018

Citation Hemanth Kumar AK, Kumar A, Kannan T, Bhatia R, Agarwal D, Kumar S, Dayal R, Singh SP, Ramachandran G. 2018. Pharmacokinetics of second-line antituberculosis drugs in children with multidrug-resistant tuberculosis in India. Antimicrob Agents Chemother 62:e02410-17. https://doi.org/10.1128/AAC.02410-17.

Address correspondence to Geetha Ramachandran, geetha202@rediffmail.com.

TABLE 1 Patient details ($n = 25$)

Patient parameter[a]	No. (%) of patients	Median (range) value
Gender		
Female	16 (64)	
Male	9 (36)	
Age	25	16 yr (5–18 yr)
Wt	25	32.7 kg (15.0–58.3 kg)
Height	25	155 cm (104–174 cm)
MAC	25	17 cm (11–25 cm)
HAZ	25	−1.4 cm (−4.9–0.8 cm)
Stunted (≤−2.0 cm)	16 (64)	
Normal (>−2.0 cm)	9 (36)	
WAZ	25	−2.2 kg (−3.9–0.0 kg)
Underweight (≤−2.0 kg)	10 (40)	
Normal (>−2.0 kg)	15 (60)	
ATT duration	24	3.5 mo (1–18 mo)
Drug dose[b]		
Cycloserine	25	14.3 (10.0–18.0)
Ethionamide	25	15.3 (11.4–25.0)
Levofloxacin	25	20.4 (10.0–27.0)
Ethambutol	25	22.9 (16.0–28.8)
Pyrazinamide	20	33.8 (20.0–45.0)
Kanamycin	20	16.1 (11.4–33.3)

[a]MAC, mid-arm circumference; ATT, antituberculosis treatment; HAZ, height for age; WAZ, weight for age.
[b]Drug doses are given as mg/kg.

metabolite, sulfoxide, also has antimycobacterial properties. It has been reported that younger children had more rapid absorption and elimination than older children (9). Cycloserine is also well absorbed after oral administration and distributes widely to most body fluids and tissues, including CSF (6). It is primarily excreted unchanged in the urine (10). Absorption was delayed by consumption of a high-fat meal.

There is a paucity of pharmacokinetic data of anti-TB drugs in children with MDR-TB. A review of literature showed that most studies were from South Africa (11–13). Not much is known about the pharmacokinetics of second-line drugs in children with MDR-TB in India. We undertook a prospective, observational study to determine the pharmacokinetics of LFX, PZA, ETH, and CS in children with MDR-TB being treated according to RNTCP guidelines.

RESULTS

A total of 25 children were included in the study (details shown in Table 1). A large proportion of children (92%) had a body mass index (BMI) below 18.5 kg/m^2. Females constituted 64% of the study population. Five and 20 children were aged below and above 12 years, respectively. Three children had extrapulmonary TB.

Shapiro-Wilks test showed that the pharmacokinetic data were not normally distributed. In view of some missing values for drug concentrations at certain time points, pharmacokinetic data were calculated in 24, 22, 25, and 25 children, respectively, for LFX, PZA, CS, and ETH. LFX estimation could not be undertaken in one child due to inadequate blood volume. There were 3 children in the continuation phase of treatment, hence they did not receive PZA. The median (range) values of the pharmacokinetic variables of drugs and proportions of children having peak concentration (C_{max}) within/above/below the therapeutic range of drugs are shown in Table 2. Peak concentrations were attained at 4 h for all drugs. Table 3 gives group-wise comparison of C_{max} and area under the concentration-time curve from 0 to 8 h (AUC_{0-8}) of drugs. The C_{max} of PZA was significantly lower in underweight children than in children with normal weight (8.7 μg/ml versus 31.2 μg/ml; $P = 0.029$), females had significantly higher LFX C_{max} than males (11.5 μg/ml versus 7.3 μg/ml; $P = 0.017$), and children

TABLE 2 Pharmacokinetics of drugs and therapeutic ranges

Variable	Value(s) for[a]:			
	Levofloxacin (n = 24)	Pyrazinamide (n = 22)	Cycloserine (n = 25)	Ethionamide (n = 25)
C_{max} (μg/ml)	8.9 (2.9–29.0)	21.4 (2.2–99.4)	31.8 (10.6–63.0)	2.4 (1.1–5.0)
T_{max} (h)	4 (0–6)	4 (2–6)	4 (2–6)	4 (2–6)
$AUC_{(0-8)}$ (μg/ml · h)	43.1 (14.4–172.0)	80.2 (16.0–501.4)	192.1 (68.7–440.8)	11.6 (5.2–24.0)
CL (ml/min)	0.15 (0.02–0.35)	0.59 (0.04–2.56)	0.65 (0.18–3.02)	0.03 (0.01–0.10)
Half-life (h)	7.9 (0.9–24.0)	17.2 (1.3–235.2)	10.5 (2.1–36.2)	3.37 (1.0–24.8)
Therapeutic range level [no. (%)]				
Within	9 (37)	7 (32)	11 (44)	18 (72)
Below	10 (42)	10 (45)	4 (16)	6 (24)
Above	5 (21)	5 (23)	10 (40)	1 (4)
Therapeutic range (μg/ml)	8–13	20–60	20–35	2–5

[a]Except for therapeutic range levels, values are presented as medians (ranges).

below 12 years of age had significantly higher AUC_{0-8} of ETH (17.5 μg/ml · h versus 9.4 μg/ml · h; P = 0.030). None of the other differences were statistically significant.

Multiple linear regression analysis by stepwise method was carried out to identify factors (age, gender, weight for age [WAZ], height for age [HAZ], and drug dose in milligrams per kilogram) that significantly influenced C_{max} and AUC_{0-8} of LFX, PZA, ETH, and CS. The factors that emerged as significant were gender for C_{max} of ETH and age for C_{max} and AUC_{0-8} of CS. Male gender was likely to decrease C_{max} of ETH by 4.92 μg/ml, and an increase in age of 1 year was likely to reduce C_{max} of CS by 1.12 μg/ml and AUC_{0-8} by 6.37 μg/ml · h (Table 4).

DISCUSSION

This prospective, observational study describes the pharmacokinetics of LFX, PZA, ETH, and CS in children with MDR-TB treated in the RNTCP in India. There are limited pediatric pharmacokinetic data available for most of these drugs.

We compared the pharmacokinetic data obtained in the present study with that reported from other studies, mostly from South Africa. The C_{max} and AUC_{0-8} of ETH observed in Indian children (2.45 μg/ml and 14.78 μg/ml · h) were quite similar to that reported by Thee et al. in South African children, the corresponding values being 3.62 μg/ml and 15.04 μg/ml · h, respectively. The time at which C_{max} was attained (T_{max}) (4 h versus 1.97 h) and half-life ($t_{1/2}$; 3.24 h versus 1.32 h) values were different (9). While the South African children received ETH at a dose range of 15 to 20 mg/kg of body weight, this dose range was 14.2 to 16.0 mg/kg in the present study. This was reflected in the former group having relatively higher C_{max} and AUC_{0-8} for ETH.

Median LFX C_{max} and AUC_{0-8} of 7 μg/ml and 32.5 μg/ml · h, respectively, have been reported from a study undertaken in 11 children with MDR-TB in South Africa (13). The corresponding values observed in the present study were 9.83 μg/ml and 49.84 μg/ml · h, respectively. This difference was probably because of differences in the drug doses received by children, being 15 mg/kg and 20 mg/kg in South Africa and India, respectively. However, C_{max} and AUC_{0-8} for ETH and LFX were similar between Indian and South African children when adjusted for dose. Thus, there did not seem to be geographical variations in these drug concentrations in children with MDR-TB. More studies from different settings are needed to substantiate this statement.

Very limited data on the pharmacokinetics of CS in both adults and children are available in the literature. Rough estimates for the C_{max} of CS in adults with TB at doses of 250 mg, 500 mg, and 750 mg have been reported as 8 to 9 μg/ml, 14 to 15 μg/ml, and 16 to 17 μg/ml, respectively (14). These values differ from that reported in healthy adult volunteers, the C_{max} being 12 to 30 μg/ml at a single oral dose of 500 mg (10). In a single child with MDR-TB meningitis, serum concentration of CS at 9 h postdosing was reported to be 16.5 μg/ml (15). The dose received by this child was 15 mg/kg, which is similar to the median CS dose received by children in this study. Since the time

TABLE 3 Comparison of peak concentration and exposure of drugs among different groups

Drug and parameter	No. of patients	C_{max} (μg/ml)		AUC_{0-8} (μg/ml · h)	
		Median (range)	*P* value[a]	Median (range)	*P* value[a]
Pyrazinamide					
Age					
≤12 yr	4	43.8 (30.5–63.9)	0.179	208.9 (80.2–326.5)	0.325
>12 yr	17	10.9 (2.2–99.4)		76.4 (16.0–501.4)	
Gender					
Female	12	37.3 (2.6–99.4)	0.356	195.6 (17.7–473.6)	0.320
Male	9	15.5 (2.2–88.7)		76.4 (16.0–501.4)	
HAZ					
Stunted (≤−2.0 cm)	14	20.7 (4.1–88.7)	1.000	79.8 (27.5–501.4)	0.654
Normal (>−2.0 cm)	7	21.4 (2.2–99.4)		156.2 (16.0–473.6)	
WAZ					
Underweight (≤−2.0 kg)	10	8.7 (2.2–56.4)	0.029	48.1 (16.0–344.4)	0.057
Normal (>−2.0 kg)	11	31.2 (4.5–99.4)		182.8 (28.5–501.4)	
Levofloxacin					
Age					
≤12 yr	5	5.7 (4.2–13.4)	0.155	32.7 (17.7–41.3)	0.082
>12 yr	19	10.2 (2.9–29.0)		60.7 (14.4–172.0)	
Gender					
Female	15	11.5 (5.0–29.0)	0.017	65.3 (17.7–172.0)	0.069
Male	9	7.3 (2.9–11.3)		37.5 (14.4–61.1)	
HAZ					
Stunted (≤−2.0)	16	10.7 (2.9–20.2)	0.603	60.9 (14.4–126.5)	0.198
Normal (>−2.0)	8	7.8 (4.1–29.0)		28.5 (24.1–172.0)	
WAZ					
Underweight (≤−2.0)	10	8.3 (4.1–20.2)	0.977	49.8 (24.1–126.5)	0.682
Normal (>−2.0)	14	9.8 (2.9–29.0)		39.4 (14.4–172.0)	
Ethionamide					
Age					
≤12	5	3.2 (2.1–5.0)	0.077	17.5 (11.8–24.0)	0.030
>12	20	2.3 (1.1–3.8)		9.4 (5.2–20.5)	
Gender					
Female	16	2.5 (1.1–4.3)	0.571	11.6 (6.9–21.6)	0.734
Male	9	2.1 (1.1–5.0)		11.6 (5.2–24.0)	
HAZ					
Stunted (≤−2.0)	16	2.5 (1.1–5.0)	0.799	13.3 (5.7–24.0)	0.462
Normal (>−2.0)	9	2.4 (1.8–3.2)		9.9 (5.2–21.6)	
WAZ					
Underweight (≤−2.0)	10	2.5 (1.7–4.3)	0.677	12.7 (5.2–20.5)	0.782
Normal (>−2.0)	15	2.4 (1.1–5.0)		11.5 (5.7–24.0)	
Cycloserine					
Age					
≤12	5	26.9 (23.5–46.4)	0.684	179.0 (158.4–283.4)	0.839
>12	20	33.0 (10.6–63.0)		197.1 (68.7–440.8)	
Gender					
Female	16	35.2 (10.6–63.0)	0.141	209.8 (68.7–440.8)	0.282
Male	9	24.6 (11.1–44.6)		179.0 (73.1–318.9)	
HAZ					
Stunted (≤−2.0)	16	30.1 (11.1–63.0)	0.610	201.1 (73.1–348.6)	0.336
Normal (>−2.0)	9	31.9 (10.6–60.6)		176.5 (68.7–440.8)	
WAZ					
Underweight (≤−2.0)	10	31.7 (10.6–63.0)	0.824	198.6 (68.7–348.6)	0.824
Normal (>−2.0)	15	31.8 (13.4–60.6)		192.1 (73.2–440.8)	

[a]Mann-Whitney test was performed at 5% level of significance.

points of drug estimation were not the same between the two studies, the CS concentrations cannot be compared. There is a clear need to generate more pharmacokinetic data for CS in children.

It is known that PZA is an effective first-line anti-TB drug and a key component of anti-TB treatment. It is also part of the MDR-TB treatment regimen in India and is given during the intensive phase of treatment. In an earlier study conducted in children with

TABLE 4 Factors influencing peak concentration and exposure of drugs

Drug and parameter	Peak concn				Exposure			
	Unadjusted β (95% CI[a])	*P* value	Adjusted β (95% CI)	*P* value	Unadjusted β (95% CI)	*P* value	Adjusted β (95% CI)	*P* value
Levofloxacin								
Age	−0.62 (−2.60 to 1.36)	0.523	1.41 (−13.32 to 16.14)	0.843	−3.03 (−15.87 to 9.81)	0.630	−3.59 (−99.52 to 92.34)	0.938
Gender (male)	−8.50 (−20.33 to 3.34)	0.151	−8.07 (−19.86 to 3.72)	0.168	−37.14 (−115.52 to 41.24)	0.337	−33.48 (−110.28 to 43.31)	0.373
HAZ	2.88 (−1.32 to 7.09)	0.169	3.43 (−0.73 to 7.59)	0.101	19.43 (−7.63 to 46.49)	0.151	22.87 (−4.21 to 49.95)	0.093
WAZ	−4.25 (−11.21 to 2.71)	0.219	−3.83 (−10.74 to 3.08)	0.260	−31.05 (−75.56 to 13.46)	0.162	−28.01 (−73.01 to 16.98)	0.208
Dose (mg/kg)	1.53 (−0.98 to 4.03)	0.221	1.22 (−1.36 to 3.79)	0.336	10.54 (−5.57 to 26.64)	0.189	9.55 (−7.24 to 26.35)	0.249
Pyrazinamide								
Age	−5.35 (−9.22 to −1.48)	0.009	−14.75 (−52.1 to 22.61)	0.415	−26.25 (−47.15 to −5.36)	0.016	−37.82 (−235.95 to 160.31)	0.691
Gender (male)	−11.30 (−39.64 to 17.04)	0.415	−12.58 (−41.57 to 16.40)	0.371	−64.21 (−212.67 to 84.25)	0.377	−70.17 (−223.91 to 83.57)	0.348
HAZ	2.09 (−9.35 to 13.53)	0.706	3.35 (−8.30 to 15.00)	0.551	23.10 (−36.20 to 82.40)	0.425	27.71 (−34.08 to 89.50)	0.356
WAZ	−10.68 (−26.39 to 5.02)	0.171	−9.83 (−26.55 to 6.88)	0.230	−48.55 (−132.18 to 35.08)	0.239	−47.37 (−136.04 to 41.3)	0.274
Dose (mg/kg)	1.32 (−1.04 to 3.68)	0.251	Excluded[b]		8.98 (−3.18 to 21.15)	0.136	Excluded	
Cycloserine								
Age	−0.10 (−0.23 to 0.03)	0.119	−1.12 (−2.08 to −0.16)	0.024	−0.36 (−1.15 to 0.43)	0.356	−6.37 (−12.19 to −0.56)	0.033
Gender (male)	−0.08 (−0.92 to 0.76)	0.843	−0.15 (−0.95 to 0.65)	0.702	−0.21 (−5.20 to 4.79)	0.933	−0.50 (−5.34 to 4.34)	0.831
HAZ	−0.03 (−0.32 to 0.27)	0.848	−0.01 (−0.29 to 0.28)	0.971	−0.30 (−2.07 to 1.46)	0.726	−0.17 (−1.89 to 1.54)	0.833
WAZ	0.12 (−0.36 to 0.61)	0.608	0.20 (−0.27 to 0.66)	0.383	0.39 (−2.50 to 3.29)	0.781	0.83 (−1.99 to 36.65)	0.546
Dose (mg/kg)	0.10 (−0.03 to 0.22)	0.127	Excluded		0.56 (−0.19 to 1.31)	0.136	Excluded	
Ethionamide								
Age	0.19 (−0.64 to 1.03)	0.636	4.19 (−1.23 to 9.61)	0.122	1.86 (−3.30 to 7.01)	0.463	31.45 (−2.85 to 65.74)	0.070
Gender (male)	−5.16 (−9.88 to −0.44)	0.034	−4.92 (−9.43 to −0.41)	0.034	−24.26 (−55.10 to 6.58)	0.117	−23.50 (−52.02 to 5.03)	0.101
HAZ	0.32 (−1.51 to 2.16)	0.720	0.44 (−1.15 to 2.02)	0.571	5.23 (−6.00 to 16.46)	0.345	5.56 (−4.47 to 15.59)	0.260
WAZ	−2.28 (−5.17 to 0.62)	0.117	−2.44 (−5.09 to 0.22)	0.070	−12.38 (−30.66 to 5.9)	0.174	−14.22 (−31.01 to 2.56)	0.092
Dose (mg/kg)	0.36 (−0.13 to 0.85)	0.142	Excluded		2.39 (−0.63 to 5.40)	0.115	Excluded	

[a]CI, confidence interval.

[b]Excluded indicates the variance inflation factor (VIF) was computed, and collinearity was ruled out in the model.

drug-susceptible TB in India, we observed the C_{max} and AUC_{0-8} of PZA to be 36.95 μg/ml and 214.6 μg/ml · h, respectively (16). These values are higher than those observed in the present study, which were 25.95 μg/ml and 93.52 μg/ml · h. In both studies, children received TB treatment in the Government of India program and received similar PZA doses, 32.9 mg/kg in the previous and 33.8 mg/kg in the present study. The only difference between the two studies was that one was conducted in children with drug-susceptible TB and other in children with drug-resistant TB, which was probably the contributing factor.

A relatively higher proportion of children had their peak concentrations of ETH maintained within the therapeutic range compared to other drugs. However, significant proportions of children having peak concentrations of PZA, LFX, and CS either above or below the therapeutic range were observed in this study. Since 40% of children had CS concentrations above the therapeutic range, it would be interesting to obtain information on adverse drug events and relate them to drug levels. The therapeutic ranges of drugs monitored in this study, however, were not validated but were based on several clinical studies. Detailed pharmacokinetic-pharmacodynamic data from human studies are emerging (17, 18). Better drug targets for the C_{max}/AUC relative to the MICs are being defined (19–22). For most drugs, therapeutic ranges derived from clinical studies of both healthy volunteers and patients with TB have been predicted after standard drug doses. It is known that the standard doses are generally effective, hence the concentrations obtained from such doses should also be effective (23).

According to the RNTCP, the recommended drug doses are 7.5 to 10 mg/kg for LFX, 30 to 35 mg/kg for PZA, and 15 to 20 mg/kg for ETH and CS. However, in this study, children seemed to have been underdosed for CS but overdosed for LFX. In spite of being underdosed, 40% of the children had peak concentrations of CS above the therapeutic range. Likewise, despite having received a higher dose than recommended, 42% of the children had their LFX peak concentration below the lower limit of the therapeutic range. Denti et al. reported that LFX dosed at 15 or 20 mg/kg in South African children with MDR-TB resulted in considerably lower exposures than those for adults (11). Using a pediatric population pharmacokinetic model, Savic et al. suggested oral LFX doses of 19 to 33 mg/kg that would attain exposure targets (24). In some cases, tablets had to be broken and administered to children to achieve the required dose in

a particular weight band, which probably caused variations in drug concentrations. Use of liquid formulations could be considered. The wide range of weight bands could also have contributed to inaccurate dosing. Furthermore, we did not observe a significant correlation between drug doses and peak concentration of drugs, and this relationship remains unclear.

This is probably the first study to report that female children had higher drug concentrations than male children, despite receiving similar drug doses in milligrams per kilogram. Although the mechanism of sex-related differences in drug pharmacokinetics is poorly understood, these findings are similar to those reported for first-line anti-TB drugs (25–27). Of course, the number of children in this study was quite small. We observed peak concentrations and exposure of LFX and CS were lower in children less than 12 years old. However, the reverse was observed with respect to ETH and PZA. This finding differs from the pharmacokinetic study of ETH conducted in South African children, which demonstrated that younger children (<2 years) had lower ETH exposure than older children (9). While the South African study had children ranging from 3 months to 13 years, children recruited to our study were aged 5 to 17 years; there was only one child who was 5 years old. A study by Mase et al. reported higher LFX peak concentrations in children less than 2 years old (28). It is known that younger children tend to eliminate drugs more rapidly than older children (29). A single-dose pharmacokinetic study of LFX in 85 children in the age range of 6 months to 12 years showed that LFX absorption, as indicated by C_{max}, T_{max}, and distribution, were not age dependent, but LFX elimination was age dependent. Children less than 5 years of age cleared LFX twice as fast as adults (4). It therefore appears that not all drugs follow similar patterns of elimination, and that age-related variations in drug pharmacokinetics across populations also exist. In this study, there was only one child aged 5 years, with the remaining ranging from 10 to 18 years. It would have been ideal to have had children across all ages, with more belonging to the younger age group. In multivariate regression analysis by a stepwise method, we observed that age (>12 years) significantly influenced CS drug concentrations. Male gender was likely to reduce ETH concentrations significantly.

Although a systematic assessment of the occurrence of adverse events was not undertaken, no serious adverse events that required hospitalization were observed. All children were continuing treatment.

The study was limited by the modest sample size; there were small numbers in different subgroups. The therapeutic ranges used for drugs in this study have not been validated or related with treatment outcome or occurrence of adverse events. Crushing or splitting of tablets could have caused variations in drug levels. Blood draws were stopped at 8 h postdosing and limited to a few time points only. Hence, clearance and half-life were calculated by extrapolating the terminal phase of the curve. Since the majority of the patients attained C_{max} at 4 h, it would have been ideal to have obtained drug concentrations beyond 8 h. Nonetheless, this is first and only study from India reporting on the pharmacokinetics of LFX, ETH, PZA, and CS in children with MDR-TB treated in the Government of India program. Additional studies on the safety and pharmacokinetics of second-line anti-TB drugs in children with MDR-TB and their impact on treatment outcomes and occurrence of adverse events should be considered.

MATERIALS AND METHODS

Patients. The study population consisted of children aged 5 to 18 years who were attending the pediatrics department/TB chest department at the Sarojini Naidu Medical College, Agra, north India, during April to August 2017. All of the children were bacteriologically confirmed to have MDR-TB based on drug susceptibility tests. Diagnosis and treatment were in accordance with the RNTCP guidelines (2). Table 5 provides details of drugs and doses for different weight bands. All of the children received drugs from the RNTCP, procured through the Global TB drug facility. All of the drugs were available as tablets (Macleods Pharmaceuticals Ltd.). The tablets were available as LFX at 250 mg and 500 mg, PZA at 500 mg and 750 mg, ETH at 250 mg, and CS at 250 mg.

Consecutive children who were HIV seronegative and willing to undergo hospitalization and blood draws were included in the study. Children who were too sick or moribund were not included. The

TABLE 5 Weight band recommendations followed from the RNTCP

No.	Drug	Dose (mg) by wt band		
		16–25 kg	26–45 kg	46–70 kg
1	Kanamycin	500	500	750
2	Levofloxacin	250	750	1,000
3	Ethionamide	375	500	750
4	Ethambutol	400	800	1,200
5	Pyrazinamide	500	1,250	1,500
6	Cycloserine	250	500	750
7	Pyridoxine	50	100	100

parent/guardian of the child gave informed written consent, and children aged seven and above gave assent. The study commenced after obtaining Institutional Ethics Committee approval.

Study procedures. Children found eligible for the study underwent a detailed clinical examination. Details regarding demographics, anthropometric measurements, type of TB, previous anti-TB treatment (ATT), etc., were noted from the child's records.

The pharmacokinetic study was conducted after the children had taken ATT regularly for at least 1 month. On the study day, serial blood samples at predosing and at 2, 4, 6, and 8 h after directly observed drug administration were collected in heparinized Vacutainer tubes. All drugs were available as tablets; the doses were according the weight bands and were administered after breakfast with water. All children received drugs from the same manufacturing sources as those used by the RNTCP. None of them were on any concomitant medications other than anti-TB drugs. The blood samples were centrifuged immediately, and plasma samples were separated and stored at −20°C. Plasma samples were transported to the National Institute for Research in Tuberculosis, Chennai, south India, in dry ice for drug estimations.

Treatment and follow-up. All children continued to receive ATT under direct observation per RNTCP guidelines at the respective centers. Adherence was monitored by the medical officers at the centers according to the existing program guidelines.

Drug estimations. Plasma concentrations of LFX, ETH, CS, and PZA were estimated by high-performance liquid chromatography (HPLC) according to validated methods described elsewhere. In brief, the method to estimate LFX involved deproteinization of the sample with perchloric acid and analysis of the supernatant using a reversed-phase C_{18} column (150 mm) using a fluorescence detector set at an excitation wavelength of 290 nm and an emission wavelength of 460 nm. The mobile phase consisted of a mixture of phosphate buffer and acetonitrile. The retention time of LFX was 1.8 min (30). The method for estimation of PZA involved deproteinization of plasma with parahydroxy benzaldehyde and trifluoroacetic acid and was analysis using a reversed-phase C_8 column and UV detector set at 267 nm. The mobile phase consisted of water-methanol containing perchloric acid and tetrabutyl *n*-ammonium hydroxide. The retention time of PZA was 2.5 min (31). The method for estimation of CS involved extraction of the drug using solid-phase extraction cartridges. The analytical column was an Atlantis T3, and the mobile phase was a mixture of phosphate buffer, acetonitrile, and isopropyl alcohol. The retention time of CS was 4.8 min (32). The method for estimation of ETH involved deproteinization of the sample with perchloric acid and analysis of the supernatant using a reversed-phase CN column (150 mm) and UV detector set at 267 nm. The mobile phase consisted of Milli-Q water and methanol containing 0.05% perchloric acid and 0.1% tetrabutyl *n*-ammonium hydroxide. The retention time of ETH was 4.9 min (33).

Calculation of pharmacokinetic variables. Certain pharmacokinetic variables, such as peak concentration (C_{max}), time at which C_{max} was attained (T_{max}), area under the concentration-time curve from 0 to 8 h (AUC_{0-8}), half-life ($t_{1/2}$), and clearance (CL) were calculated based on noncompartmental analysis using Phoenix WinNonlin 6.4 software (Certara LP). AUC_{0-8} was calculated using the linear trapezoidal rule.

Assessment of nutritional status. The z scores for weight and height were computed based on the child's age and gender using the EPI-NUT component of the EPI-INFO 2002 software package (version 3.4.3) from the CDC (based on NCHS reference median values) (34).

Statistical evaluation. Data were analyzed using STATA 15.0 (StataCorp, College Station, TX). Shapiro-Wilks test was used to assess normality of the pharmacokinetic data. Values were expressed as medians and ranges. Nonparametric Mann-Whitney U test and Kruskal-Wallis test with Bonferroni correction were used to compare subgroups. Proportion of patients having C_{max} within the therapeutic ranges (8 to 13 μg/ml for LFX, 20 to 60 μg/ml for PZA, 2 to 5 μg/ml for ETH, and 20 to 35 μg/ml for CS) (23) were calculated. Multiple linear regression analysis by a stepwise method was carried out to identify factors that influenced C_{max} and AUC_{0-8} of drugs. Age, HAZ, WAZ, and drug doses were taken as continuous variables, and gender was taken as a binary variable. The variance inflation factor (VIF) was computed, and collinearity was ruled out in the model. A P value of ≤ 0.05 was considered statistically significant.

ACKNOWLEDGMENTS

We thank all of the children who took part in the study and V. Sudha and A. Vijayakumar for drug estimations by HPLC.

G.R. and D.A. designed the study and wrote the study protocol; G.R. and R.B. obtained regulatory approvals at the respective sites; R.B. and D.A. recruited suitable study participants; A.K., S.K., and S.P.S. conducted the study; A.K.H.K. supervised drug estimations; K.T. performed statistical analysis; G.R. drafted the manuscript; and R.D. provided critical input and overall guidance.

We have no conflicts of interest to declare.

REFERENCES

1. World Health Organization. 2017. Global TB report. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/publications/global_report/en/.
2. Revised National TB Control Programme. 2012. RNTCP guidelines for programmatic management of drug resistant tuberculosis (PMDT) in India. Central TB Division, Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India, New Delhi, India. https://tbcindia.gov.in.
3. Chien SC, Rogge MC, Gisclon LG, Curtin C, Wong F, Natarajan J, Williams RR, Fowler CL, Cheung WK, Chow AT. 1997. Pharmacokinetic profile of levofloxacin following once-daily 500-milligram oral or intravenous doses. Antimicrob Agents Chemother 41:2256–2260.
4. Chien S, Wells TG, Blumer JL, Kearns GL, Bradley JS, Bocchini JA, Jr, Natarajan J, Maldonado S, Noel GJ. 2005. Levofloxacin pharmacokinetics in children. J Clin Pharmacol 45:153–160. https://doi.org/10.1177/0091270004271944.
5. Siefert HM, Kohlsdorfer C, Steinke W, Witt A. 1999. Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: tissue distribution in male rats. J Antimicrob Chemother 43(Suppl 2):61–67.
6. Donald PR. 2010. Cerebrospinal fluid concentrations of antituberculosis agents in adults and children. Tuberculosis (Edinb) 90:279–292. https://doi.org/10.1016/j.tube.2010.07.002.
7. Jenner PJ, Ellard GA, Gruer PJ, Aber VR. 1984. A comparison of the blood vessels and urinary excretion of ethionamide and prothionamide in man. J Antimicrob Chemother 13:267–277. https://doi.org/10.1093/jac/13.3.267.
8. Conte JE, Jr, Golden JA, McQuitty M, Kipps J, Lin ET, Zurliden E. 2000. Effects of AIDS and gender on steady-state plasma and intrapulmonary ethionamide concentrations. Antimicrob Agents Chemother 44:1337–1341. https://doi.org/10.1128/AAC.44.5.1337-1341.2000.
9. Thee S, Seifart HI, Rosenkranz B, Hesseling AC, Magdorf K, Donald PR, Schaaf HS. 2011. Pharmacokinetics of ethionamide in children. Antimicrob Agents Chemother 55:4594–4600. https://doi.org/10.1128/AAC.00379-11.
10. Zhu M, Nix DE, Adam RD, Childs JM, Peloquin CA. 2001. Pharmacokinetics of cylcoserine under fasting conditions and with high-fat meal, orange juice, and antacids. Pharmacotherapy 21:891–897. https://doi.org/10.1592/phco.21.11.891.34524.
11. Denti P, Garcia-Prats AJ, Draper HR, Wiesner L, Winckler J, Thee S, Dooley KE, Savic Rm McIlleron HM, Schaaf HS, Hesseling AC. 2017. Levofloxacin population pharmacokinetics in South African children treated for multidrug-resistance tuberculosis. Antimicrob Agents Chemother 62:e01521-17. https://doi.org/10.1128/AAC.01521-17.
12. Thee S, Garcia-Prats AJ, Draper HR, McIlleron HM, Wiesner L, Castel S, Schaaf HS, Hesseling AC. 2015. Pharmacokinetics and safety of moxifloxacin in children with multidrug-resistant tuberculosis. Clin Infect Dis 60:549–556. https://doi.org/10.1093/cid/ciu868.
13. Thee S, Garcia-Prats AJ, McIleron HM, Wiesner L, Castel S, Norman J, Draper HR, Van der Merwe PL, Hesseling AC, Schaaf HS. 2014. Pharmacokinetics of ofloxacin and levofloxacin for prevention and treatment of multidrug-resistant tuberculosis in children. Antimicrob Agents Chemother 58:2948–2951. https://doi.org/10.1128/AAC.02755-13.
14. Zitkova L, Tousek J. 1974. Pharmacokinetics of cycloserine and terizidone. A comparative study. Chemotherapy 20:18–28.
15. DeVincenzo JP, Berning SE, Peloquin CA, Husson RN. 1999. Multidrug-resistant tuberculosis meningitis: clinical problems and concentrations of second-line antituberculosis medications. Ann Pharmacother 33:1184–1188. https://doi.org/10.1345/aph.19008.
16. Ramachandran G, Hemanth Kumar AK, Bhavani PK, Poorana Gangadevi N, Sekar L, Vijayasekaran D, Banu Rekha VV, Ramesh Kumar S, Ravichandran N, Mathevan G, Swaminathan S. 2013. Age, nutritional status and isoniazid acetylator status influence pharmacokinetics of anti-TB drugs in children. Int J Tuberc Lung Dis 17:979–984. https://doi.org/10.5588/ijtld.12.0628.
17. Burhan E, Ruesen C, Ruslami R, Ginanjar A, Manqunnegoro H, Ascobat P, Donders R, van Crevel R, Aarnoutse R. 2013. Isoniazid, rifampin, and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. Antimicrob Agents Chemother 57:3614–3619. https://doi.org/10.1128/AAC.02468-12.
18. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis 208:1464–1473. https://doi.org/10.1093/infdis/jit352.
19. Gumbo T, Louie A, Deziel MR, Parsons LM, Salfinger M, Drusano GL. 2004. Selection of a moxifloxacin dose that suppresses drug resistance in *Mycobacterium tuberculosis*, by use of an in vitro pharmacodynamics infection model and mathematical modelling. J Infect Dis 190:1642–1651. https://doi.org/10.1086/424849.
20. Pasipanodya J, Srivastava S, Gumbo T. 2012. New susceptibility breakpoints and the regional variability of MIC distribution in *Mycobacterium tuberculosis* isolates. Antimicrob Agents Chemother 56:5428. https://doi.org/10.1128/AAC.00976-12.
21. Gumbo T, Louie A, Deziel MR, Liu W, Parsons LM, Salfinger M, Drusano GL. 2007. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob Agents Chemother 51:3781–3788. https://doi.org/10.1128/AAC.01533-06.
22. Gumbo T, Dona CS, Meek C, Leff R. 2009. Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel in vitro model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs. Antimicrob Agents Chemother 53:3194–3204. https://doi.org/10.1128/AAC.01681-08.
23. Alsultan A, Peloquin CA. 2014. Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs 74:839–854. https://doi.org/10.1007/s40265-014-0222-8.
24. Savic RM, Ruslami R, Hibma JE, Hesseling A, Ramachandran G, Ganiem AR, Swaminathan S, McIlleron H, Gupta A, Thakur K, van Crevel R, Aarnoutse R, Dooley KE. 2015. Pediatric tuberculosis meningitis: model-based approach to determining optimal doses of the anti-tuberculosis drugs rifampin and levofloxacin for children. Clin Pharmacol Ther 98:622–629. https://doi.org/10.1002/cpt.202.
25. Hemanth Kumar AK, Kannan T, Chandrasekaran V, Sudha V, Vijayakumar A, Ramesh K, Lavanya J, Soumya Swaminathan, Ramachandran G. 2016. Pharmacokinetics of thrice-weekly rifampicin, isoniazid and pyrazinamide in adult tuberculosis patients in India. Int J Tuberc Lung Dis 20:1236–1241.
26. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. 2006. Determinants of rifampin, isoniazid, pyrazinamide and ethambutol pharmacokinetics in a cohort of tuberculosis patients. Antimicrob Agents Chemother 50:1170–1177. https://doi.org/10.1128/AAC.50.4.1170-1177.2006.
27. Ray J, Gardiner I, Marriott D. 2003. Managing antituberculosis drug therapy by therapeutic drug monitoring of rifampicin and isoniazid. Intern Med J 33:229–234. https://doi.org/10.1046/j.1445-5994.2003.00390.x.
28. Mase SR, Jereb JA, Gonzalez D, Martin F, Daley CL, Fred D, Loeffler A, Menon L, Morris SB, Brostrom R, Chorba T, Peloquin CA. 2016. Pharmacokinetics and dosing of levofloxacin in children treated for active or latent multidrug-resistant tuberculosis Federated States of Micronesia and Republic of the Marshall Islands. Pediatr Infect Dis J 35:414–421. https://doi.org/10.1097/INF.0000000000001022.
29. Bartelink IH, Rademaker HC, Schobeen AF, Van den Anker JN. 2006. Guidelines on paediatric dosing on the basis of development physiology and pharmacokinetic considerations. Clin Pharmacokinet 45:1077–1097. https://doi.org/10.2165/00003088-200645110-00003.

30. Hemanth Kumar AK, Sudha V, Ramachandran G. 2016. A simple and rapid liquid chromatography method for determination of levofloxacin in plasma. SAARC J TB Lung Dis HIV/AIDS 14:27–32. https://doi.org/10.3126/saarctb.v14i1.17726.
31. Hemanth Kumar AK, Sudha V, Ramachandran G. 2012. Simple and rapid liquid chromatography method for simultaneous determination of isoniazid and pyrazinamide in plasma. SAARC J TB Lung Dis HIV/AIDS 9:13–18.
32. Hemanth Kumar AK, Arun Kumar Polisetty, Sudha V, Vijayakumar A, Ramachandran G. 28 September 2017. A selective and sensitive high performance liquid chromatography assay for the determination of cycloserine in human plasma. Ind J Tuberc https://doi.org/10.1016/j.ijtb.2017.08.034.
33. Hemanth Kumar AK, Sudha V, Ramachandran G. 2014. Simple and rapid high pressure liquid chromatography method for estimation of ethionamide in plasma. Asian J Biomed Pharmaceut Sci 38:1–5.
34. World Health Organization. 1995. Physical status: the use and interpretation of anthropometry. Technical report series no 854. World Health Organization, Geneva, Switzerland. http://www.who.int/childgrowth/publications/physical_status/en/index.html.