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Pharmacokinetics of the First-Line Antituberculosis Drugs in Ghanaian Children with Tuberculosis with or without HIV Coinfection

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ABSTRACT Although human immunodeficiency virus (HIV) coinfection is the most important risk factor for a poor antituberculosis (anti-TB) treatment response, its effect on the pharmacokinetics of the first-line drugs in children is understudied. This study examined the pharmacokinetics of the four first-line anti-TB drugs in children with TB with and without HIV coinfection. Ghanaian children with TB on isoniazid, rifampin, pyrazinamide, and ethambutol for at least 4 weeks had blood samples collected predose and at 1, 2, 4, and 8 hours postdose. Drug concentrations were determined by validated liquid chromatography-mass spectrometry methods and pharmacokinetic parameters calculated using noncompartmental analysis. The area under the concentration-time curve from 0 to 8 h (AUC_{0-8}), maximum concentration (C_{max}), and apparent oral clearance divided by bioavailability (CL/F) for each drug were compared between children with and without HIV coinfection. Of 113 participants, 59 (52.2%) had HIV coinfection. The baseline characteristics were similar except that the coinfected patients were more likely to have lower weight-for-age and height-for-age Z scores ($P < 0.05$). Rifampin, pyrazinamide, and ethambutol median body weight-normalized CL/F values were significantly higher, whereas the plasma AUC_{0-8} values were lower, in the coinfected children than in those with TB alone. In the multivariate analysis, drug dose and HIV coinfection jointly influenced the apparent oral clearance and AUC_{0-8} for rifampin, pyrazinamide, and ethambutol. Isoniazid pharmacokinetics were not different by HIV coinfection status. HIV coinfection was associated with lower plasma exposure of three of the four first-line anti-TB drugs in children. Whether TB/HIV-coinfected children need higher dosages of rifampin, pyrazinamide, and ethambutol requires further investigation. (This study has been registered at ClinicalTrials.gov under identifier NCT01687504.)

KEYWORDS children, first-line anti-TB drugs, human immunodeficiency virus, pharmacokinetics, tuberculosis

Human immunodeficiency virus (HIV) infection in children increases the risk of developing active tuberculosis (TB) (1–4) and is associated with a 2- to 6-fold-greater risk of dying from TB (3, 5–8). The poor TB treatment outcome in HIV-infected children is in part due to ongoing immunosuppression and delay in initiation of

antiretroviral therapy (9). However, ineffective drug therapy due to suboptimal drug dosages likely contributes to the high risk of death (6, 7, 10) or relapse after treatment (5, 11). Clinical studies in adults with TB have shown that low plasma antituberculosis (anti-TB) drug concentrations are associated with a delayed treatment response (12, 13) or risk of treatment failure (14–16). In particular, low plasma exposure to the rifamycins in the setting of intermittent therapy in HIV-infected patients with TB has been associated with treatment failure and emergence of rifampin resistance (16–18). While there are limited data on the relationship between pharmacokinetic (PK) parameters and pharmacodynamics in children, one study from India reported an association between low plasma maximum concentrations (C_{max}) of rifampin and pyrazinamide and unfavorable clinical outcomes in HIV-infected children (19).

Whether HIV infection adversely affects the pharmacokinetics of anti-TB drugs in children is not well studied. Several studies in adults suggest that HIV infection is associated with low plasma concentrations of rifampin and ethambutol (20–23), while others have reported no significant effect of HIV infection (24–26). Studies in children, which were limited by small sample sizes, have reported no significant effect of HIV infection on plasma concentrations of the first-line anti-TB drugs studied (27, 28). A recent comparative study that included 24 HIV-infected and 32 non-HIV-infected children given previously recommended lower drug dosages for children found significantly lower areas under the concentration-time curve from 0 to 4 h (AUC_{0-4}) of ethambutol but no differences in the pharmacokinetics of the other first-line drugs between the two groups (29). Given concerns about inadequate anti-TB drug exposures in children and poor treatment response, particularly in children with HIV coinfection (30), the World Health Organization (WHO) recommended revised higher dosages of the anti-TB drugs in all children in 2010 (31). This study examined whether the pharmacokinetics of isoniazid, rifampin, pyrazinamide, and ethambutol in children with TB/HIV coinfection were different from those in children with TB alone.

RESULTS

Study population. Between October 2012 and August 2015, a total of 168 children with a clinical diagnosis of TB were screened, of whom 131 (78.0%) were enrolled. Of the 37 excluded patients, 27 lived too far from the study site or could not return for study visits, 8 were in the continuation phase of anti-TB treatment, and 2 were older than 14 years old. Of the 131 enrolled patients, 113 patients had plasma drug assay data available; 5 had samples collected but did not meet the shipping deadline to be included in the analysis. Thirteen patients did not complete sampling (6 withdrew or discontinued the study, 4 were lost to follow-up, 2 died, and 1 was found to be ineligible).

Of the 113 study participants, 59 (52.2%) had HIV coinfection, 63 (55.8%) were male, 24 (21.2%) were <2 years old, and 54 (47.8%) were <5 years old (Table 1). Except for pyrazinamide, the median drug dosages were within the revised WHO-recommended ranges. The median dosage of pyrazinamide of 24.8 mg/kg was below the new recommended range of 30 to 40 mg/kg because of the low ratio of the drug in the fixed-dose combination (FDC) tablet. There were no significant differences in the demographic and clinical characteristics of the patients with TB alone and those with TB/HIV coinfection except that the coinfected patients had lower median weight-for-age Z score (WAZ) (−2.7 versus −2.1; $P = 0.023$) and height-for-age Z score (HAZ) (−2.8 versus −1.6; $P < 0.001$) and were more likely to have pulmonary TB (94.9% versus 52.8%; $P < 0.001$). In addition, the median values for platelet count, serum sodium, alkaline phosphatase, and albumin were lower but that for aspartate transaminase (AST) was higher in the coinfected patients than in those with TB alone (Table 2).

Among the HIV-infected patients, the median (interquartile range [IQR]) baseline CD4 count was 460.0 (77.0 to 925.0) cells/µl ($n = 46$), percentage of CD4 cells was 10.0% (6.0 to 18.0%) ($n = 42$), and viral load was 267,912 (70,300 to 886,059) copies/ml ($n = 34$). Overall, 25 (73.5%) of the 34 patients with viral load data available had HIV RNA levels of >100,000 copies/ml.

TABLE 1 Baseline characteristics of children with TB with and without HIV coinfection

Characteristic	Value for group			P value
	All (n = 113)	TB (n = 54)	TB/HIV (n = 59)	
Median (IQR) age, yr	5.0 (2.2–8.3)	4.8 (2.0–8.3)	5.0 (2.9–8.9)	0.642
No. (%) with age, yr				0.822
<2	24 (21.2)	12 (22.2)	12 (20.3)	
≥2	89 (78.8)	42 (77.8)	47 (79.7)	
No. (%) with age, yr				0.708
<5	54 (47.8)	27 (50.0)	27 (45.8)	
≥5	59 (52.2)	27 (50.0)	32 (54.2)	
No. (%) male	63 (55.8)	32 (59.3)	31 (52.5)	0.570
Median (IQR) body wt, kg	14.0 (8.8–19.5)	14.1 (8.8–21.8)	14.0 (8.5–19.0)	0.406
Median (IQR) height, cm	99.0 (82.0–119.0)	99.0 (82.0–122.0)	98.0 (82.0–117.0)	0.553
Nutritional status				
Median (IQR) wt-for-age Z score[a]	−2.5 (−3.8 to −1.4)	−2.1 (−3.1 to −0.9)	−2.7 (−4.0 to −2.0)	0.023
Median (IQR) height-for-age Z score	−2.0 (−3.2 to −1.1)	−1.6 (−2.4 to −0.6)	−2.8 (−3.9 to −1.8)	<0.001
Median (IQR) BMI-for-age Z score	−1.8 (−2.9 to −0.5)	−1.4 (−2.9 to −0.6)	−2.0 (−3.4 to −0.4)	0.547
Median (IQR) midarm circumference, cm	14.0 (12.0–16.0)	14 (13.0–16.5)	13 (11.2–15.5)	0.131
Median (IQR) head circumference, cm	48.0 (46.0–50.0)	48.0 (46.0–51.0)	48 (45.0–49.0)	0.143
Median (IQR) drug dose, mg/kg				
Isoniazid	11.2 (9.1–12.8)	11.4 (9.5–12.8)	10.4 (9.0–12.9)	0.314
Rifampin	15.8 (13.6–18.8)	16.7 (14.2–18.8)	15.2 (13.5–18.8)	0.262
Pyrazinamide	24.8 (22.6–30.0)	26.5 (23.5–32.3)	24.7 (22.3–29.0)	0.228
Ethambutol	16.9 (15.0–20.6)	18.0 (15.8–22.7)	16.5 (14.9–19.4)	0.167
No. (%) with TB disease classification:				<0.001
Pulmonary	85 (75.2)	29 (52.8)	56 (94.9)	
Extrapulmonary	28 (24.8)	25 (47.2)	3 (5.1)	
No. (%) with outcome:				0.513
Completed treatment	99 (87.6)	47 (85.2)	52 (88.1)	
Lost to follow-up	8 (7.1)	4 (7.4)	4 (6.8)	
Discontinued study	4 (3.5)	3 (5.6)	1 (1.7)	
Died	2 (1.8)	0 (0.0)	2 (3.4)	

[a]The software could not calculate weight-for-age Z score for participants who were over 10 years old.

PK of the anti-TB drugs. The plasma concentration-time profiles of each drug by HIV infection status are shown in Fig. 1. The concentrations of isoniazid at all the sampling points appeared to be similar in the children with and without HIV coinfection (Fig. 1A). The concentrations of rifampin, pyrazinamide, and ethambutol after the 1-hour postdose time point were numerically lower in the patients with TB/HIV coinfection than in those with TB alone (Fig. 1B, C, and D). In fact, the median rifampin and ethambutol C_{max} were significantly lower in the HIV/TB-coinfected patients than in those with TB alone ($P < 0.05$).

The pharmacokinetic (PK) parameters of the drugs in all participants and by HIV infection status are shown in Table 3. Except for time to C_{max} (T_{max}), there were no significant differences in the isoniazid pharmacokinetic parameters between the two groups ($P > 0.05$). Rifampin, pyrazinamide, and ethambutol median AUC_{0-8} were significantly lower, while median body weight-normalized predicted apparent oral clearance as a function of bioavailability (CL/F) was significantly higher, in the children with TB/HIV coinfection than in those with TB alone (Table 3). For rifampin and ethambutol, the median C_{max} were significantly lower in the HIV/TB-coinfected patients than in those with TB alone (Table 3). The proportion of patients with median C_{max} of ethambutol of <2 µg/ml (the lower limit of the therapeutic target range) was significantly higher in the TB/HIV-coinfected patients than in those with TB alone (75.4% versus 42.3%; $P = 0.0017$). There was a trend toward a higher proportion of TB/HIV-coinfected patients than those with TB alone having median rifampin C_{max} of <8 µg/ml (72.9% versus 55.6%; $P = 0.076$).

TABLE 2 Baseline laboratory test results for children with TB with and without HIV coinfection

Characteristic (n)	Median value (IQR) for group			P value
	All (n = 113)	TB (n = 54)	TB/HIV (n = 59)	
White blood cell count, 10⁹/liter (72)	8.6 (6.0–11.9)	9.6 (6.4–11.9)	7.7 (5.2–11.7)	0.215
Absolute neutrophil count, 1,000 (65)	3.2 (1.9–4.8)	3.4 (2.2–5.7)	2.8 (1.9–3.6)	0.101
Hemoglobin, g/dl (73)	9.5 (8.7–10.7)	9.8 (9.3–11.0)	9.3 (8.4–10.4)	0.073
Hematocrit, % (70)	29.2 (26.6–32.9)	30.3 (28.4–34.0)	28.2 (25.5–32.9)	0.052
Platelets, 10⁹/liter (72)	316 (206.5–508.0)	450.0 (275.0–587.0)	276.0 (190.0–350.0)	0.001
Sodium, mmol/liter (48)	132 (127.5–135.5)	133.0 (128.5–136.0)	130.0 (127.0–133.5)	0.007
Potassium, mmol/liter (46)	4.1 (3.8–4.5)	4.2 (3.9–4.5)	4.1 (3.8–4.5)	0.491
Blood urea nitrogen, mmol/liter 79	2.8 (1.9–3.7)	2.6 (1.9–3.7)	2.9 (1.9–3.6)	0.632
Serum creatinine, μmol/liter (79)	36.0 (27.0–50.0)	35.3 (28.0–51.0)	37.0 (23.0–47.0)	0.199
Glomerular filtration rate, ml/min/1.73 m² (77)	102.4 (85.9–126.7)	98.7 (85.9–115.3)	103.4 (84.1–138.4)	0.659
AST, U/liter (790)	37.0 (29.0–51.0)	31.0 (25.0–40.0)	43.6 (36.0–56.1)	<0.001
ALT, U/liter (75)	22.0 (15.2–36.0)	19.0 (14.0–40.0)	22.6 (18.0–34.0)	0.062
Alkaline phosphatase, U/liter (77)	386.0 (292.0–563.0)	510.0 (340.0–637.0)	370.0 (160.8–474.0)	<0.001
Total bilirubin, μmol/liter (76)	6.0 (4.3–9.0)	7.0 (5.0–9.0)	5.0 (4.0–8.0)	0.412
Albumin, g/liter (79)	38.0 (32.0–43.0)	42.0 (38.0–43.0)	34.0 (30.8–38.0)	<0.001
HIV-related laboratory tests				
CD4 cells				
Count, cells/μl (46)			460.0 (77.0–925.0)	
% (42)			10.0 (6.0–18.0)	
HIV-1 RNA, copies/ml (34)			267,912 (70,300–886,059)	
Log₁₀ HIV-1 RNA (34)			5.4 (4.8–5.9)	
HIV-1 RNA >100,000 copies/ml			25 (73.5%)	

In the multivariate analysis of factors associated with the anti-TB drug pharmacokinetics (Table 4), we assessed model fittings by r^2, proportion of the variance in pharmacokinetic parameters explained by selected models, and model significance. Dose and N-acetyl transferase 2 (NAT2) slow metabolizer genotype status influenced
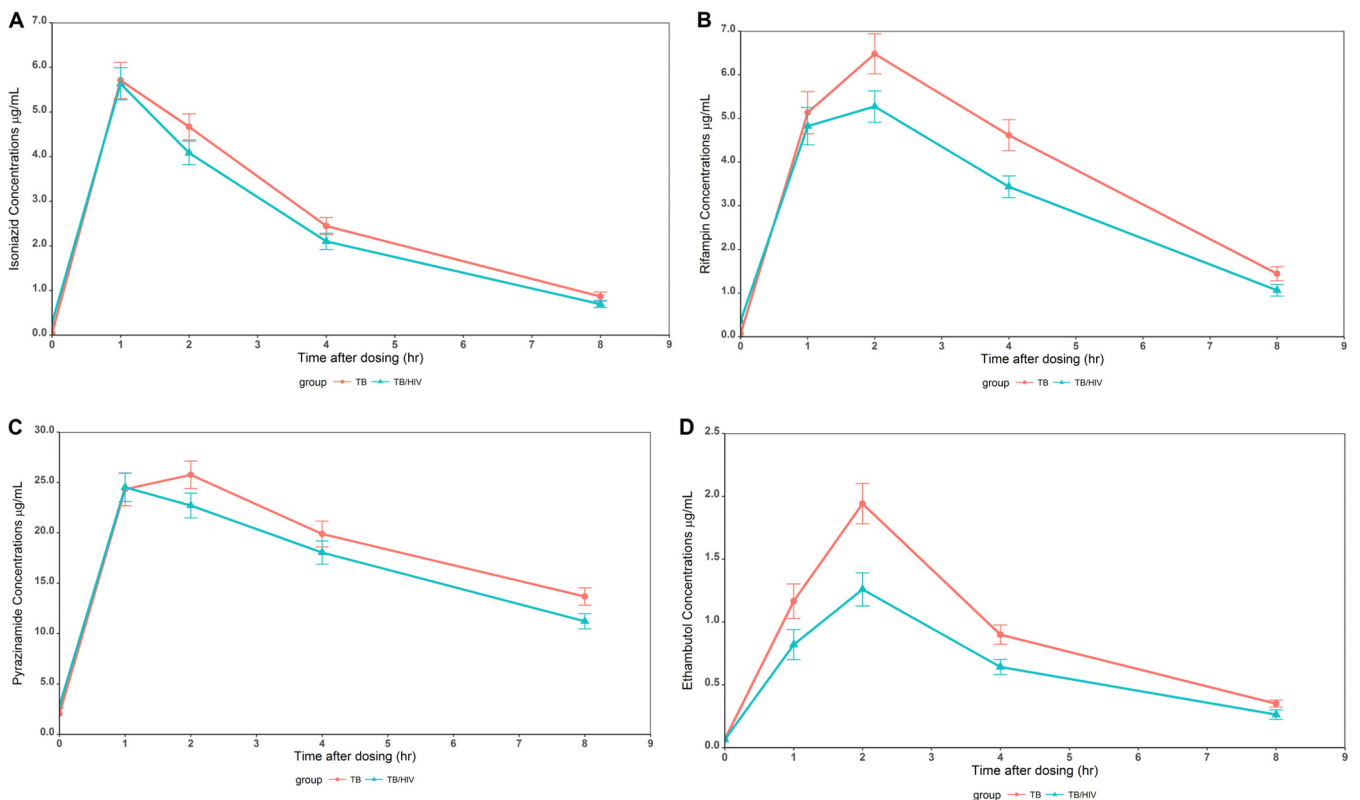

FIG 1 Plasma concentration-time profiles of isoniazid (A), rifampin (B), pyrazinamide (C), and ethambutol (D) in children with TB and HIV/TB coinfection.

TABLE 3 Steady-state pharmacokinetic parameter estimates for antituberculosis drugs by HIV coinfection status

Drug and parameter	Value for group			P value
	All ($n = 113$)	TB ($n = 54$)	TB/HIV ($n = 59$)	
Isoniazid				
C_{max}, µg/ml	5.75 (4.27–7.47)	5.85 (4.27–7.47)	5.32 (4.03–7.61)	0.794
T_{max}, h	1.05 (1.00–1.22)	1.07 (1.00–1.45)	1.02 (1.00–1.15)	0.015
AUC_{0-8}, µg · h/ml	19.73 (13.24–26.43)	21.15 (16.48–25.92)	18.37 (12.20–26.90)	0.231
Normalized V_z/F, liters/kg	1.51 (1.20–1.90)	1.55 (1.27–1.84)	1.47 (1.15–2.00)	0.957
Normalized CL/F, liters/h/kg	0.47 (0.33–0.71)	0.44 (0.32–0.70)	0.50 (0.33–0.78)	0.405
Rifampin				
C_{max}, µg/ml	6.39 (4.92–8.77)	7.65 (5.20–9.10)	5.83 (3.71–8.26)	0.025
T_{max}, h	2.00 (1.07–2.17)	2.01 (1.13–2.28)	1.83 (1.02–2.08)	0.090
AUC_{0-8}, µg · h/ml	27.25 (20.62–36.32)	30.49 (21.93–38.44)	24.88 (15.95–35.27)	0.030
Normalized V_z/F, liters/kg	1.46 (1.11–2.10)	1.37 (1.08–2.05)	1.63 (1.21–2.22)	0.142
Normalized CL/F, liters/h/kg	0.50 (0.37–0.74)	0. 45 (0.35–0.66)	0.53 (0.41–0.86)	0.044
Pyrazinamide ($n = 110$)				
C_{max}, µg/ml	26.05 (21.70–35.10)	26.90 (23.15–34.60)	24.60 (20.60–36.50)	0.259
T_{max}, h	1.16 (1.00–2.07)	1.93 (1.05–2.10)	1.04 (1.00–1.77)	0.006
AUC_{0-8}, µg · h/ml	140.50 (114.18–186.19)	151.04 (124.64–188.81)	126.53 (105.41–182.34)	0.034
Normalized V_z/F, liters/kg	0.87 (0.74–1.08)	0.88 (0.75–1.08)	0.85 (0.73–1.19)	0.661
Normalized CL/F, liters/h/kg	0.11 (0.08–0.14)	0.10 (0.07–0.12)	0.12 (0.09–0.16)	0.010
Ethambutol ($n = 110$)				
C_{max}, µg/ml	1.67 (0.87–2.68)	2.28 (1.48–3.05)	1.33 (0.75–1.92)	<0.001
T_{max}, h	2.04 (1.70–2.23)	2.03 (1.40–2.20)	2.05 (1.83–2.33)	0.641
AUC_{0-8}, µg · h/ml	6.17 (3.71–9.15)	7.57 (5.16–10.68)	4.76 (2.98–6.76)	<0.001
Normalized V_z/F, liters/kg	8.98 (6.68–18.09)	8.12 (5.97–11.04)	11.25 (7.70–18.72)	0.008
Normalized CL/F, liters/h/kg	2.38 (1.76–3.38)	1.96 (1.51–2.73)	2.95 (2.20–3.81)	0.001

isoniazid C_{max} ($r^2 = 0.09$; $P = 0.005$), AUC_{0-8} ($r^2 = 0.21$; $P < 0.001$), and CL/F ($r^2 = 0.41$; $P < 0.001$). For rifampin, dose, sex, and HIV coinfection status jointly influenced C_{max} ($r^2 = 0.12$; $P = 0.001$), AUC_{0-8} ($r^2 = 0.17$; $P < 0.001$), and CL/F ($r^2 = 0.15$; $P < 0.001$). For pyrazinamide, dose and HIV coinfection jointly influenced AUC_{0-8} ($r^2 = 0.17$; $P < 0.001$), and CL/F ($r^2 = 0.26$; $P < 0.001$), while dose only influenced C_{max} ($r^2 = 0.10$; $P < 0.001$). For ethambutol, dose, sex, and HIV coinfection status were joint predictors of C_{max} ($r^2 = 0.32$; $P < 0.001$), AUC_{0-8} ($r^2 = 0.32$; $P < 0.001$), and CL/F ($r^2 = 0.29$; $P < 0.001$).

Safety and tolerability. Of the 113 patients included in this analysis, 99 (87.6%) completed therapy, 8 patients were lost to follow-up, 4 discontinued the study, and 2 died. The two patients who died were both HIV infected and severely malnourished. Of the patients who had liver function tests (LFTs) done at baseline and 4 weeks on anti-TB therapy, only one HIV-infected patient developed grade 3 elevation of AST. No patients developed grade 4 elevation of AST or alanine aminotransferase (ALT) at 4 weeks of anti-TB treatment or required treatment discontinuation to medication side effects.

DISCUSSION

The main finding of this study is that children with TB/HIV coinfection had significantly lower plasma exposure and a higher apparent oral clearance of rifampin, pyrazinamide, and ethambutol. In addition, the median C_{max} of rifampin and ethambutol were significantly lower in the children with TB/HIV coinfection than in those with TB alone. While our study was not designed to investigate the relationship between pharmacokinetics and TB treatment outcome, the lower median plasma exposure and C_{max} of rifampin and ethambutol in the HIV-coinfected patients is concerning and raises the question of whether inadequate drug pharmacokinetics is a contributor to the higher risk of an unfavorable treatment response. At least one group reported a

TABLE 4 Coefficient and standardized estimates of joint predictors of drug plasma pharmacokinetics from multivariate analysis using MCP modeling

Drug	Parameter	Predictor	r^{2a}	P value for model significance	Parameter estimate (SE)	P value	Standardized estimate[b]
Isoniazid	C_{max}	Dose (mg)	0.09	0.005	0.002 (0.001)	0.007	0.249
		NAT2 non-slow vs slow			0.177 (0.095)	0.063	0.171
	AUC_{0-8}	Dose (mg)	0.21	<0.001	0.030 (0.013)	0.020	0.201
		NAT2 non-slow vs slow			8.351 (1.739)	<0.001	0.408
	CL/F	Dose (mg)	0.41	<0.001	0.005 (0.001)	<0.001	0.451
		NAT2 non-slow vs slow			−0.650 (0.107)	<0.001	−0.447
Rifampin	C_{max}	Dose (mg)	0.12	<0.001	0.002 (0.001)	0.001	0.300
		TB/HIV vs TB			−0.182 (0.107)	0.092	−0.153
	AUC_{0-8}	Dose (mg)	0.17	<0.001	0.042 (0.012)	0.001	0.312
		Male vs female			−4.234 (2.473)	0.090	−0.150
		TB/HIV vs TB			−5.364 (2.474)	0.032	−0.191
	CL/F	Dose (mg)	0.15	<0.001	0.002 (0.001)	<0.001	0.367
		Male vs female			0.178 (0.120)	0.141	0.131
		TB/HIV vs TB			0.195 (0.120)	0.108	0.144
Pyrazinamide	C_{max}	Dose (mg)	0.10	<0.001	0.001 (<0.001)	0.001	0.312
	AUC_{0-8}	Dose (mg)	0.17	<0.001	0.099 (0.022)	<0.001	0.391
		TB/HIV vs TB			−10.638 (9.369)	0.259	−0.101
	CL/F	Dose (mg)	0.26	<0.001	0.002 (<0.001)	<0.001	0.509
		TB/HIV vs TB			0.195 (0.133)	0.144	0.124
Ethambutol	C_{max}	Dose (mg)	0.32	<0.001	0.002 (<0.001)	<0.001	0.439
		Male vs female			−0.154 (0.115)	0.181	−0.109
		TB/HIV vs TB			−0.396 (0.115)	0.001	−0.280
	AUC_{0-8}	Dose (mg)	0.32	<0.001	0.010 (0.002)	<0.001	0.439
		Male vs female			−1.009 (0.576)	0.083	−0.141
		TB/HIV vs TB			−2.005 (0.579)	0.001	−0.280
	CL/F	Dose (mg)	0.29	<0.001	0.002 (<0.001)	<0.001	0.491
		Male vs female			0.201 (0.106)	0.061	0.156
		TB/HIV vs TB			0.349 (0.106)	0.001	0.272

[a]Proportion of the variance in PK parameters explained by selected models.
[b]The standardized estimates are all in the same standardized units, so that the relative strength in predicting outcomes can be assessed by comparing them. For example, the standardized coefficient estimate of 0.312 for dose in predicting rifampin AUC_{0-8} means that a one-standard-deviation increase in dose leads to a 0.312-standard-deviation increase in predicted rifampin AUC_{0-8}, with the other variables held constant. The predicting power of the predictors for rifampin AUC_{0-8} can be ranked as dose > HIV infection status > sex.

relationship between lower rifampin and pyrazinamide C_{max} and unfavorable TB treatment outcome (death, failure, and default) in HIV-infected children (19) and in a combined group of HIV-infected and uninfected children in India treated with a thrice-weekly regimen (32). Thus, strategies to improve TB treatment outcomes in children, especially with HIV coinfection, should include optimized dosages of rifampin, pyrazinamide, and ethambutol. For rifampin and ethambutol, dosages higher than those currently recommended may be needed in TB/HIV-coinfected children, but for pyrazinamide, increasing the ratio of pyrazinamide in the available FDC tablet to allow for the higher recommended dosages for all children may be adequate.

Prior studies that examined the effect of HIV infection on the pharmacokinetics of anti-TB drugs in children were inconclusive, likely because of small sample size. Among Malawian children, C_{max} and AUC_{0-24} of pyrazinamide ($n = 27$) and C_{max} of ethambutol ($n = 18$) were similar in the children with and without HIV infection (27). Another study among 44 South African children (21 HIV infected) reported similar rifampin C_{max} and AUC_{0-6} irrespective of HIV infection status (28). In contrast, a recent study that enrolled 66 children with TB (24 HIV coinfected) in India who were given previously recommended daily dosages of the drugs reported significantly lower ethambutol C_{max} and AUC_{0-4} in the TB/HIV-coinfected children than in the children with TB alone, but rifampin, pyrazinamide, and isoniazid C_{max} and AUC_{0-4} were similar between the two groups (29). Another Indian study that included 84 HIV-uninfected and 77 HIV-infected children given three-times-weekly dosages based on the old treatment guidelines

found significantly lower rifampin C_{max} and AUC_{0-8} in the HIV-infected children than in those with TB alone (32). The current study found significantly lower rifampin and ethambutol C_{max} and AUC_{0-8}, as well as a lower pyrazinamide AUC_{0-8}, in the TB/HIV-coinfected children than in the children with TB alone. The strength of the current study includes a sample size adequate to detect a significant effect of HIV infection, use of the revised WHO recommended dosages for children in over 90% of the participants, and use of the available dispersible FDC tablets, which taken together make our findings relevant to current childhood TB treatment recommendations.

The underlying mechanism for the effect of HIV coinfection on the drug pharmacokinetics could be different for each drug. The lower plasma exposure of the three drugs in the TB/HIV-coinfected patients could be due to impaired absorption and/or enhanced metabolism or excretion, since the two groups were given similar dosages. With noncompartmental analysis and oral dosage forms, the two main PK parameters, CL/F and volume of distribution in the terminal phase divided by bioavailability (V_z/F), are both tied to bioavailability F. While it is possible for a dramatic increase or decrease in either CL or V_z to occur, a more likely explanation in the case of oral dosage forms is a change in F. This is especially true when both CL and V_z are seen to be similarly affected; that is, both CL and V_z get larger because F is smaller. Finally, there are several other studies in adults pointing to the same problem, i.e., malabsorption in HIV-infected patients (20–23). The HIV-infected children were not yet started on antiretroviral drugs at the time of pharmacokinetic sampling, so drug-drug interactions with antiretroviral drugs was not factor in this study. Given that we did not measure metabolite concentrations or perform intestinal absorption tests, we are not able to attribute the lower drug exposure to impaired absorption or enhance metabolism or elimination.

In the multivariate analysis, drug dose was a joint factor with HIV infection in influencing the pharmacokinetics of all three affected drugs. Sex was the other joint factor that affected rifampin and ethambutol pharmacokinetics. However, based on the model fitting results, dose is the major influential factor for pharmacokinetic parameters of all the first-line anti-TB drugs, suggesting that dose adjustment in children, especially those with HIV coinfection, will help achieve the necessary pharmacokinetic profile associated with efficacy in adults.

There were some limitations of our study. The pharmacokinetic sampling was done at five time points (time zero and 1, 2, 4, and 8 hours postdose) but not over the entire 24-hour dosing interval, and so we could not determine the trough concentrations of the drugs. However, such a sampling scheme has been used in other pediatric studies, when sampling at steady state is considered sufficient to estimate key pharmacokinetic parameters such as C_{max} and AUC up to the time of the last sample (33, 34). The factors selected in the multivariate analysis do not explain all the interindividual variability in the pharmacokinetics. While in our multivariate analysis the selected models were statistically significant, the small r^2 values suggest that there might be other significant effects for pharmacokinetics, which deserves further study.

In conclusion, our study provides evidence that HIV coinfection in children with TB adversely affects the pharmacokinetic profiles of three of the four first-line drugs. Whether this is the underlying reason for the higher risk of unfavorable TB treatment outcome in children with TB/HIV coinfection than in those with only TB requires further evaluation. Drug formulations that will allow for the upper end of the currently recommended dosage range for pyrazinamide to be administered could help optimize the pharmacokinetic profile of the drug in TB/HIV-coinfected patients. However, for rifampin and ethambutol, higher dosages than currently recommended may be needed, especially in HIV-infected children, as dose was the main factor associated with AUC and C_{max} after correcting for HIV infection status.

MATERIALS AND METHODS

Study population and design. A two-arm, parallel-assignment pharmacokinetic study in children with active TB with or without HIV coinfection was performed at Komfo Anokye Teaching Hospital
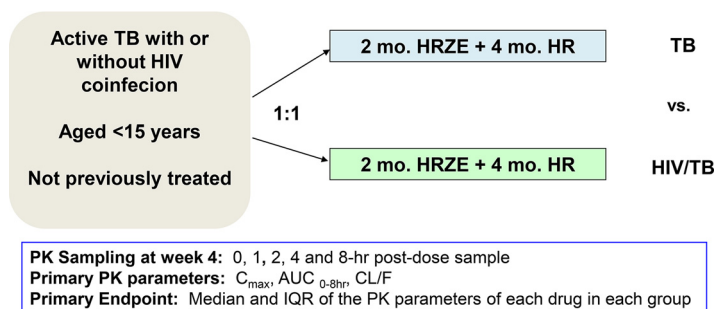
FIG 2 Study design and overview of endpoints. H, isoniazid; R, rifampin; Z, pyrazinamide; E, ethambutol.

(KATH), Kumasi, Ghana (Fig. 2). Children aged 3 months to 14 years old, for whom informed consent was provided by a parent or guardian, were recruited from outpatient clinics and inpatient wards at KATH. Children whose parents refused to provide consent or could not return for scheduled visits were excluded.

A complete medical history, physical examination, and nutritional status assessment were performed at enrollment and relevant data collected using standardized forms. Baseline measurements prior to initiation of anti-TB treatment included complete blood count (CBC), blood urea nitrogen, serum creatinine, and liver function tests (LFTs), as well as CD4 cell count determination and plasma HIV-1 RNA levels in the HIV-infected children. LFTs were repeated at week 4 of therapy or when clinically indicated to evaluate for drug toxicity. Study participants were evaluated at 2 weeks of anti-TB treatment and then monthly to assess for adverse events and clinical response to therapy. The Institutional Review Boards (IRBs) of KATH, Ghana, and Lifespan Hospitals, Providence, RI, reviewed and approved the study. All parents or guardians of study participants provided signed informed consent. The study was registered with ClinicalTrials.gov under identifier NCT01687504.

Anti-TB treatment regimen and drug dosages. The treatment regimen, using revised dosage recommendations (31), consisted of isoniazid at 7 to 15 mg/kg, rifampin at 10 to 20 mg/kg, pyrazinamide at 30 to 40 mg/kg, and ethambutol at 15 to 25 mg/kg daily for 2 months and then isoniazid at 7 to 15 mg/kg and rifampin at 10 to 20 mg/kg daily for 4 months. The medications were dosed according to WHO guidelines for using available dispersible fixed-dose combination (FDC) TB medicines for children (35). At the beginning of the study and prior to the adoption of the WHO recommended elevated dosages of the drugs in Ghana, 11 children received the old recommended dosages of isoniazid and rifampin. The medications were either swallowed or dispersed in water in a plastic cup and ingested, and administration was observed by a health care worker during hospitalization and by a family member at home.

PK sampling. Pharmacokinetic (PK) sampling was performed at or after 4 weeks of anti-TB treatment as previously described (36). At the time of sampling, none of the HIV-coinfected patients were on antiretroviral therapy. On the day of sampling, medications were administered after an overnight fast in nonbreastfed children. Children on exclusive breastfeeding were allowed to breastfeed as needed throughout the study. A light standard breakfast was provided 30 min after dosing. Once the 2-hour sample was obtained, children were allowed to eat without restrictions. Blood samples were collected at time zero (predose) and at 1, 2, 4, and 8 hours postdose. This sampling scheme, when conducted at steady state, was considered sufficient to estimate key pharmacokinetic parameters such as maximum concentration (C_{max}) and area under the concentration-time curve (AUC) (33). The samples, collected in EDTA-coated tubes, were placed immediately on ice and centrifuged within 30 min at $3,000 \times g$ for 10 min. Plasma was stored at $-80°C$ until shipment on dry ice to the University of Cape Town, Cape Town, South Africa, for drug concentrations assays.

PK analysis. Drug concentrations were determined using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods as we previously described (36). The observed C_{max} and time to C_{max} (T_{max}) were determined by inspection of the serum concentration-time graphs for each drug. The calculation of AUC from time zero to 8 h (AUC_{0-8}), predicted apparent oral clearance as a function of bioavailability (CL/F), and volume of distribution in the terminal phase as a function of bioavailability (V_z/F) was performed using noncompartmental analysis (Phoenix software; Pharsight Corporation, Mountain View, CA).

Sample size and power justification. The primary endpoints were C_{max} and AUC_{0-8} in children with active TB with or without HIV coinfection. The power analysis was based on comparison of C_{max} in children with TB and in those with TB/HIV coinfection. The literature showed that C_{max} has a coefficient of variation (CV) of 27 to 68% (37). With 58 children per group (116 in total) and a 15% dropout rate, the study had 80% power to detect at least a 17% to 37% difference in pharmacokinetic parameters between the two groups. The power analysis was based on a 2-sample t test with a two-sided significance level of 0.05.

Statistical analysis. Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC). The weight-for-age Z score (WAZ), height-for-age Z score (HAZ), and body mass index (BMI)-for-age Z score were calculated based on the U.S. National Center for Health Statistics (NCHS) reference median values using statistical macros for children ages <5 years old and 5 to 19 years old

provided by the WHO (38). Bivariate analyses of association between patient factors and anti-TB drug pharmacokinetic parameters were assessed with the Wilcoxon rank sum test for continuous variables or the Fisher exact test for categorical variables. Multivariate regression was used to explore the joint effect of demographics and clinical variables on the PK parameters. The minimax concave penalty (MCP) (39, 40) variable selection method was used to select the predictors that were influential on pharmacokinetic parameters (C_{max}, AUC_{0-8}, and CL/F). The MCP variable selection has the properties of unbiasedness, sparsity, and continuity, meaning that coefficient estimators are nearly unbiased for informative covariates, automatically set to zero for noninformative covariates, and continuous in data to avoid instability in model prediction. Inference and model fitting statistics of r^2 were obtained through model refitting using the selected variables to explore the magnitude of effects and the proportion of the variance in PK parameters explained by selected models. For all analyses, a P value of <0.05 was considered significant.

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