Original Article

Pharmacokinetics of First-Line Antituberculosis Drugs Using WHO Revised Dosage in Children With Tuberculosis With and Without HIV Coinfection

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Background. Pharmacokinetic data on the first-line antituberculosis drugs using the World Health Organization (WHO) revised dosages for children are limited. We investigated the pharmacokinetics of these drugs in children who were mostly treated with revised dosages.

Methods. Children with tuberculosis on first-line therapy for at least 4 weeks had blood samples collected at predose, 1, 2, 4, and 8 hours postdose. Drug concentrations were determined by validated liquid chromatography mass spectrometry methods, and pharmacokinetic parameters were calculated using noncompartmental analysis. Factors associated with plasma peak concentration (C_{max}) and the area under the time–concentration curve 0–8 hours (AUC_{0-8h}) of each drug was examined using univariate and multivariate analysis.

Results. Of the 62 children, 32 (51.6%) were male, 29 (46.8%) were younger than 5 years old, and 28 (45.2%) had human immunodeficiency virus (HIV) coinfection. Three patients had undetectable pyrazinamide and ethambutol concentrations. The median (interquartile range) AUC_{0-8h} for isoniazid was 17.7 (10.2–23.4) μg·h mL^{-1}, rifampin was 26.0 (15.3–36.1) μg·h mL^{-1}, pyrazinamide was 144.6 (111.5–201.2) μg·h mL^{-1}, and ethambutol was 6.7 (3.8–10.4) μg·h mL^{-1}. Of the children who received recommended weight-band dosages, 44/51 (86.3%), 46/56 (82.1%), 27/56 (48.2%), and 21/51 (41.2%) achieved target C_{max} for isoniazid, pyrazinamide, ethambutol, and rifampin, respectively. In multivariate analysis, age, sex, HIV coinfection status, and drug dosage in milligrams per kilogram were associated with the drugs' plasma drug C_{max} or AUC_{0-8h}.

Conclusions. The revised dosages appeared to be adequate for isoniazid and pyrazinamide, but not for rifampin or ethambutol in this population. Higher dosages of rifampin and ethambutol than currently recommended may be required in most children.

Key words. children; first-line antituberculosis drugs; pharmacokinetics; revised WHO dosage; tuberculosis.

Tuberculosis (TB) is a major cause of morbidity and mortality in children. In 2013, an estimated 550,000 children became ill with TB, resulting in about 80,000 deaths in human immunodeficiency virus (HIV) uninfected children [1]. The global estimates of TB deaths in HIV-infected children are unknown. However, observational studies show poorer TB treatment outcomes in HIV coinfected children [2–5]. An analysis of mortality in 13 African and Asian countries reported a 2.6 times higher risk of death in HIV-infected compared to uninfected children [6], while another study in a primary care setting reported TB case-fatality to be 4.3 times higher in HIV-infected than in uninfected children [7]. The poor TB treatment outcome in children may in part be due ineffective therapy, as low plasma concentrations of the first-line antituberculosis drugs in children are common [8–14]. Interindividual differences in

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drug disposition could also put some children at higher risk for lower drug concentrations when dosage is based on weight only.

Previously, dosage recommendation for the antituberculosis drugs in children was the same in milligrams per kilogram of body weight as for adults. Given the concern that the low concentrations of the first-line antituberculosis drugs are due to underdosing in children [8–14], the World Health Organization (WHO) recommended increased dosages of these drugs aimed at optimizing treatment outcomes in children [15]. In 2010, WHO recommended revised dosages (range) as follows: isoniazid 10 (10–15) mg/kg, rifampin 15 (10–20) mg/kg, pyrazinamide 35 (30–40) mg/kg, and ethambutol at the previously recommended dosage (range) of 20 (15–25) mg/kg [15]. The updated guidelines in 2014 recommended a change in isoniazid dosage range to 7–15 mg/kg, with the higher end of the dosage aimed at younger children [16]. To date, few studies have evaluated the pharmacokinetics of the revised dosages in children. One study showed that South African children aged <2 years old (N = 20) given revised dosages achieved target concentrations of isoniazid, rifampin, and pyrazinamide [17]. However, another study found a high frequency of subtherapeutic maximum concentration (C_{max}) of the first-line drugs among 31 South African children aged ≤10 years old [18]. The current study is the largest study to date (to the best of our knowledge) that describes the pharmacokinetics and safety of the first-line antituberculosis drugs in children who were predominantly treated with WHO revised dosages. We also investigated the demographic and clinical factors influencing the pharmacokinetics of antituberculosis drugs in children.

METHODS

Study Population and Design

A prospective, observational, intensive pharmacokinetic study was performed at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. Eligible children were aged 3 months to 14 years old, had a diagnosis of TB, with or with HIV coinfection, and were starting 4-drug first-line therapy for TB. Children with acute hepatitis or bacterial infections requiring intravenous antibiotics, persistent vomiting, or diarrhea were excluded from the study. The Institutional Review Board of KATH, Ghana and Lifespan Hospitals, Providence, Rhode Island reviewed and approved the study. All parents and guardians provided signed informed consent. The study was registered with ClinicalTrials.gov, number NCT01687504.

Antituberculosis Treatment Regimen and Drugs

The antituberculosis treatment regimen consisted of isoniazid, rifampin, pyrazinamide, and ethambutol daily for 2 months, then isoniazid and rifampin daily for 4 months using revised recommended dosages [15, 16]. All the medications were supplied through the Global TB drug facility and were dosed according to WHO guidelines for using available dispersible fixed-dose combination (FDC) TB medicines for children [19]. For children who weighed up to 20 kg, dispersible FDC isoniazid/rifampin/pyrazinamide (30/60/150 mg) and isoniazid/rifampin (60/60 mg) tablets manufactured by MaCleods Pharmaceuticals, Mumbai, India and single ethambutol (100 mg) tablets manufactured by Riemser Pharma GmbH, Schiffweiler, Germany were prescribed in the induction phase. In the continuation phase, isoniazid/rifampin (60/60 mg) and isoniazid/rifampin (30/60 mg) tablets were used. For children weighing ≥21 kg, the FDC tablets containing isoniazid/rifampin/pyrazinamide/ethambutol (75/150/400/275 mg) manufactured by Lupin Ltd., Chkalthana, Aurangabad, India were used in the intensive phase and isoniazid/rifampin (75/150 mg and 60/60 mg) in the continuation phase. The dosing schedule based on weight using FDC tablets is shown in Table 1. Therapy was observed by a healthcare worker when the child was hospitalized and by a family member when the child was at home. Tablets were either swallowed or dispersed in water in a plastic cup and ingested. All study participants followed up at 2 weeks after starting therapy, and then monthly until treatment completion to assess for adverse events and clinical response.

Pharmacokinetic Sampling

Pharmacokinetic sampling was performed at or after 4 weeks of antituberculosis treatment. Seven days prior to pharmacokinetic sampling, parents or caregivers of patients were called on the phone to verify that medications

Table 1. Dosing for Intensive and Continuation Phase Using Dispersible Fixed-Dose Combination Tablets

	Intensive Phase			Continuation Phase	
Body Weight (kg)	HRZ (30/60/150 mg)	HR (60/60 mg)	E (100 mg)	HR (30/60 mg)	HR (60/60/mg)
5–7	1	1	1	1	1
8–14	2	1	2	2	1
15–20	3	2	3	3	2
21–30	2 tabs HRZE (75/150/400/275 mg) + 2 tabs HR (60/60 mg)			2 tabs HR (75/150 mg) + 2 tabs HR (60/60 mg)	

Abbreviations: HRZ, isoniazid/rifampin/pyrazinamide HR, isoniazid/rifampin; E, ethambutol; HRZE, isoniazid/rifampin/pyrazinamide/ethambutol

were administered and time of ingestion was documented. Children were admitted to the hospital overnight for sampling. 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 minutes after dosing. Once the 2-hour sample was obtained, children were allowed to eat without restrictions. Blood samples were collected at times 0 (predose), 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 C_{max} and area under the time–concentration curve (AUC) [17]. The samples collected in EDTA-coated tubes were placed immediately on ice and centrifuged within 30 minutes at 3000 *g* for 10 minutes. Plasma was stored at –80°C until shipment on dry ice to University of Cape Town, Cape Town, South Africa for drug concentrations assays.

Pharmacokinetic Analysis

Drug concentrations were determined using validated liquid chromatography tandem with mass spectrometry methods. The methods were validated over the concentration ranges 0.0977–26 µg/mL (isoniazid), 0.117–30 µg/mL (rifampin), 0.20–80 µg/mL (pyrazinamide), and 0.0844–5.46 µg/mL (ethambutol). The accuracy and coefficient of variation for isoniazid, rifampin, pyrazinamide, and ethambutol during sample analysis were 100.2–105.1% and 6.5–9.3% at low, medium, and high quality control concentrations. 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 $AUC_{0–8h}$, apparent oral clearance, and volume of distribution was performed using noncompartmental analysis (Phoenix Software; Pharsight Corporation, Mountain View, CA). The following target reference ranges of C_{max} were used to define low and high concentrations of each drug: isoniazid 3–6 µg/mL, rifampin 8–24 µg/mL, pyrazinamide 20–50 µg/mL, and ethambutol 2 to 6 µg/mL [20]. Some investigators consider pyrazinamide C_{max} <35 µg/mL to be low, as it was associated with poor treatment outcome of pulmonary TB among adults [21].

Statistical Analysis

Statistical analyses were performed using SAS 9.3 software (SAS Institute Inc., Cary, NC). Weight-for-age *Z* score (WAZ) and height-for-age *Z* score (HAZ) were calculated based on the United States National Center for Health Statistics reference median values using statistical macros for children ages <5 years old and 5–19 years old provided by the WHO [22]. WAZ < –2 is considered underweight or malnourished and ≥ –2 is considered normal, and HAZ < –2 is considered stunted and ≥ –2 is considered normal. Bivariate analyses of association between patient factors and antituberculosis drug C_{max} and $AUC_{0–8h}$ were assessed by Mann–Whitney *U* test for continuous variables or χ^2 test for categorical variables. Multivariate analysis with variable selection by the Smoothly Clipped Absolute Deviation method [23, 24] was used to find the joint effect of patient factors (sex, age, HIV status, body weight, height, form of drug administration, midarm circumference, head circumference, drug dosage) on drug pharmacokinetic parameters (C_{max} and $AUC_{0–8h}$). For all analysis, a *P* value < .05 was considered significant.

RESULTS

Study Population

Between October 2012 and May 2014, a total of 68 children with a diagnosis of TB were enrolled. Two children were lost to follow-up, 2 died, and parents withdrew consent for 2 children. Of the 62 patients in the final analysis study, 32 (51.6%) were male, 29 (46.8%) were younger than 5 years old, and 48 (77.4%) had pulmonary TB (Table 2). There were no significant differences in the baseline characteristics between the patients with and without HIV coinfection, except that the HIV coinfected patients had lower median WAZ and HAZ scores as well as were less likely to have extrapulmonary disease (Table 2). Of the HIV-infected patients with data available, the median (IQR) baseline CD4 count was 645 (109–935) cells/µL (N = 19), CD4 percent was 12.0 (6.5–16.5)% (N = 15), and viral load was 312,000 (131,789–1,177,000) copies/mL (N = 15).

Pharmacokinetics Parameters and Covariates

A summary of the pharmacokinetic parameters is shown in Table 3. Of the 62 patients, 3 had undetectable concentrations of pyrazinamide and ethambutol. The median (interquartile range [IQR]) C_{max} for isoniazid was 4.8 (3.7–6.4) µg/mL, rifampin was 6.3 (3.5–8.8) µg/mL, pyrazinamide was 28.6 (21.8–35.6) µg/mL, and ethambutol was 1.9 (0.9–3.1) µg/mL. The median (IQR) $AUC_{0–8h}$ for isoniazid was 17.7 (10.2–23.4) µg·h mL^{-1}, rifampin was 26.0 (15.3–36.1) µg·h mL^{-1}, pyrazinamide was 144.6 (111.5–201.2) µg·h mL^{-1}, and ethambutol was 6.7 (3.8–10.4) µg·h mL^{-1}.

Of the patients who received revised dosages, 44/51 (86.2%) achieved target isoniazid C_{max}, 21/51 (41.2%) achieved target rifampin C_{max}, 46/56 (82.1%) achieved target pyrazinamide C_{max} (≥20 µg/mL), and 27/56 (48.2%) achieved target ethambutol C_{max}. Overall, 18/51 (35.3%) had isoniazid C_{max} > 6 µg/mL but no child had rifampin or ethambutol C_{max} greater than the upper limit of

Table 2. Baseline Characteristics of Children With Tuberculosis (TB) and TB/Human Immunodeficiency Virus (HIV) Coinfection

Characteristic	All (N = 62)	TB (N = 34)	TB/HIV (N = 28)	*P*-value
Median (IQR) age (y)	5.0 (2.8–8.9)	5.3 (2.3–10.7)	4.9 (3.3–8.0)	.554
Age (y)				
<2	10 (16.1%)	5 (14.7%)	5 (17.9%)	.737
≥2	52 (83.9%)	29 (85.3%)	23 (82.1%)	
Age (y)				
<5	29 (46.8%)	15 (44.1%)	14 (50.0%)	.644
≥5	33 (53.2%)	19 (55.9%)	14 (50.0%)	
Male sex	32 (51.6%)	18 (52.9%)	14 (50.0%)	.818
Median (IQR) body weight (kg)	14.0 (8.8–19.7)	15.0 (9.1–22.2)	13.0 (7.5–16.9)	.118
Median (IQR) height (cm)	98.0 (84.0–120.0)	104.0 (84.0–131.0)	96.0 (74.0, 113.0)	.109
Nutritional status				
Median (IQR) weight-for-age *Z* score[a]	−2.7 (−4.0– −1.6)	−2.3 (−3.0– −1.4)	−2.9 (−4.3– −2.0)	.048
Median (IQR) height-for-age *Z* score	−2.0 (−3.0– −1.5)	−1.8 (−2.4– −1.2)	−2.9 (−4.2– −1.8)	.001
Median (IQR) midarm circumference	14. 0 (11.9–16.5)	15.0 (13.0–16.5)	13.0 (11.2–16.0)	.207
Median (IQR) head circumference	48.0 (46.0–50.0)	48.5 (47.0, 51.0)	47.0 (46.0–49.0)	.080
Median (IQR) drug dose (mg/kg)				
Isoniazid	11.1 (9.0–13.2)	11.4 (9.6–1 3.2)	10.3 (7.1–12.9)	.296
Rifampin	16.3 (13.8–19.8)	17.0 (14.9–19.8)	15.7 (13.4–18.7)	.250
Pyrazinamide	26.6 (23.7–32.0)	27.2 (24.7–33.0)	26.1 (23.0–30.1)	.336
Ethambutol	18.4 (15.8–22.0)	19.1 (16.5–22.7)	17.4 (15.3–20.2)	.220
TB disease classification				.001
Pulmonary	48 (77.4%)	21 (61.8%)	27 (96.4%)	
Extrapulmonary	14 (22.6%)	13 (38.2%)	1 (3.6%)	
Outcome[b]				.279
Completed treatment	54 (88.5%)	31 (93.34%)	23 (82.1%)	
Died	2 (3.3%)	0 (0.0%)	2 (7.1%)	
Lost to follow-up	5 (8.2%)	2 (6.1%)	3 (10.7%)	

Abbreviation: IQR, interquartile range.

[a]The software could not calculate weight-for-age *Z* score for 15 participants who were >10 years old.

[b]Parent withdrew consent for 1 child after pharmacokinetic sampling.

Table 3. Median (Interquartile Range) Steady-State Pharmacokinetic Parameter Estimates of Antituberculosis Drugs in Children With Tuberculosis With or Without Human Immunodeficiency Virus Coinfection

Parameter	Isoniazid (n = 62)	Rifampin (n = 62)	Pyrazinamide (n = 59)	Ethambutol (n = 59)
C_{max} (μg/mL)	4.8 (3.7–6.4)	6.3 (3.5–8.8)	28.6 (21.8–35.6)	1.9 (0.9–3.1)
T_{max} (h)	1.1 (1.0–1.2)	2.0 (1.1–2.1)	1.2 (1.0–2.1)	2.0 (1.4–2.3)
C_{last} (μg /mL)	0.5 (0.3–1.1)	1.1 (0.4–1.7)	11.9 (7.6–17.4)	0.3 (0.2–0.4)
T_{last} (h)	8.0 (7.9–8.1)	8.0 (7.9–8.1)	8.0 (8.0–8.1)	8.0 (7.9–8.1)
$AUC_{0–8h}$ (μg·h mL^{-1})	17.7 (10.2–23.4)	26.0 (15.2–36.1)	144.6 (111.5–201.2)	6.7 (3.8–10.4)
$AUC_{0–12h}$ (μg·h mL^{-1})	18.8 (10.8–26.3)	28.6 (16.5–39.6)	179.0 (135.4–258.2)	7.3 (4.4–11.7)
$AUC_{0–\infty}$ (μg·h mL^{-1})	19.0 (11.0–27.9)	31.2 (16.9–43.6)	246.7 (159.3–364.0)	7.8 (4.9–12.2)
Vz/F (L)	22.6 (18.9–31.4)	23.5 (14.7–40.0)	12.2 (9.2–17.7)	128.5 (92.4–215.0)
NVz/F (L/kg)	1.6 (1.3–2.1)	1.6 (1.2–2.5)	0.9 (0.7–1.1)	8.6 (6.1–18.7)
CL/F (L/h)	7.7 (4.5–12.6)	7.7 (5.2–11.4)	1.5 (1.0–2.2)	34.1 (21.5–45.9)
NCL/F (L·h kg^{-1})	0.50 (0.4–1.0)	0.5 (0.4–0.8)	0.1 (0.1–0.2)	2.3 (1.6–3.5)

Abbreviations: C_{max}, peak concentration; T_{max}, time to C_{max}; C_{last}, last detectable concentration; $AUC_{0–8h}$, total area under the curve from time 0–8 h; $AUC_{0–\infty}$, total area under the curve from time 0 hours to last detectable; CL/F, apparent oral clearance; Vz/F, apparent volume of distribution; NVz/F, apparent volume of distribution adjusted for body weight, NCL/F, apparent oral clearance adjusted for body weight.

target range. For pyrazinamide, 13 (23.2%) and 2 (3.6%) of the 56 patients had pyrazinamide C_{max} > 35 and 50 μg/mL, respectively.

The C_{max} and $AUC_{0–8h}$ were compared against demographic and clinical characteristics for each drug (Tables 4 and 5, respectively). An isoniazid dose of <7 mg/kg was associated with a lower C_{max} and $AUC_{0–8h}$. Males exhibited a lower C_{max}. For rifampin, HIV coinfection, pulmonary TB, and HAZ < –2.0 were associated with lower rifampin C_{max} and $AUC_{0–8h}$. Rifampin dosage <10 mg/kg was associated with a lower C_{max} and a trend towards a lower $AUC_{0–8h}$. For pyrazinamide, age <2 years old, HAZ < –2.0, and pyrazinamide dosage <30 mg/kg were associated with lower pyrazinamide C_{max} and $AUC_{0–8h}$. For ethambutol, age <5 years, HIV coinfection, and HAZ < –2.0, and mid-upper-arm circumference <12.5 cm were significantly associated with lower ethambutol C_{max} and $AUC_{0–8h}$.

Table 4. Effect of Patient Factors on Plasma Peak Concentration (C_{max}) of the First-Line Antituberculosis Drugs in Ghanaian Children With Tuberculosis

	Isoniazid			Rifampin			Pyrazinamide			Ethambutol		
Characteristic	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value
Age (y)			.812			.324			.024			.052
<2	10	4.8 (2.0–6.4)		10	4.7 (2.3–8.7)		10	18.3 (8.7–32.0)		8	1.0 (0.4–2.0)	
≥2	52	4.8 (3.7–6.4)		52	6.4 (3.9–8.8)		49	29.1 (23.5–35.6)		51	2.1 (1.0–3.1)	
Age (y)			.689			.061			.235			.010
<5	29	4.8 (3.7–6.1)		29	5.5 (3.2–8.2)		28	26.5 (19.8–33.2)		27	1.5 (0.7–2.1)	
≥5	33	4.8 (3.7–6.5)		33	7.7 (5.1–9.2)		31	29.1 (22.8–36.7)		32	2.6 (1.4–3.4)	
Sex			.021			.386			.092			.542
Female	30	5.8 (4.2–6.5)		30	6.7 (3.5–8.9)		29	32.3 (23.6–39.1)		30	2.3 (0.9–3.1)	
Male	32	4.2 (3.3–5.6)		32	5.8 (3.5–8.4)		30	25.5 (20.6–32.5)		29	1.6 (0.9–2.8)	
HIV infection			.102			.021			.289			.011
Negative	34	5.4 (4.2–6.4)		34	7.4 (4.9–9.3)		32	30.4 (25.0–35.4)		33	2.4 (1.5–3.3)	
Positive	28	4.2 (3.5–6.0)		28	5.5 (3.0–8.2)		27	23.2 (19.2–36.5)		26	1.1 (0.7–2.4)	
TB site			.737			.024			.358			.553
Pulmonary	48	4.7 (3.6–6.5)		48	5.9 (3.4–8.4)		45	26.4 (20.7–35.6)		45	1.9 (0.8–3.1)	
Extrapulmonary	14	5.0 (4.2–6.2)		14	8.4 (6.6–9.7)		14	30.4 (25.6–34.1)		14	2.0 (1.5–2.7)	
Weight-for-age *Z* score			.374			.297			.469			.175
≥–2.0	17	5.0 (4.5–6.5)		17	8.2 (4.7–9.3)		14	24.9 (22.0–29.7)		16	2.3 (1.1–3.1)	
<–2.0	35	4.5 (3.6–6.3)		35	5.9 (3.3–8.7)		35	26.4 (20.3–36.5)		33	1.5 (0.8–2.4)	
Height-for-age *Z* score			.175			.021			.013			.004
≥–2.0	29	4.9 (4.0–6.4)		29	7.7 (5.1–9.2)		29	32.0 (25.6–37.2)		29	2.8 (1.5–3.3)	
<–2.0	32	4.4 (2.9–6.2)		32	5.5 (2.5–7.9)		30	22.8 (17.3–33.4)		29	1.4 (0.7–2.0)	
MUAC (cm)			.672			.114			.249			.006
≥12.5	40	4.8 (3.6–6.4)		40	6.7 (3.5–9.0)		38	29.0 (23.5–35.6)		39	2.4 (1.4–3.3)	
<12.5	16	5.7 (3.1–6.6)		16	5.2 (2.5–7.3)		16	22.4 (17.7–33.5)		14	1.0 (0.6–1.7)	
Dose in new range[a]			.007			.028			.001			.229
No	9	3.4 (2.5–4.0)		5	3.0 (2.8–3.4)		38	23.8 (20.6–31.1)		11	1.7 (0.8–2.4)	
Yes	53	5.1 (4.0–6.5)		57	6.6 (4.5–8.8)		21	33.8 (31.4–39.2)		48	2.0 (0.9–3.1)	
New weight-band dose[b]			.104			.250			.297			.619
Yes	51	5.0 (3.7–6.4)		51	6.5 (3.7–8.9)		56	27.7 (21.6–34.6)		56	1.9 (0.9–3.1)	
No	11	4.0 (2.5–5.7)		11	5.9 (2.8–7.7)		3	35.6 (24.2–40.9)		3	2.4 (1.7–2.8)	

Abbreviations: IQR, interquartile range; HIV, human immunodeficiency virus; TB, tuberculosis; MUAC, mid-upper-arm circumference.

[a]New World Health Organization (WHO) recommended dosage range (isoniazid 7–15 mg/kg, rifampin 10–20 mg/kg, pyrazinamide 30–40 mg/kg, ethambutol 15–25 mg/kg).

[b]Some patients received old weight-band dosage prior to adoption of new WHO guidelines.

Table 5. Effect of Patient Factors on Area Under the Concentration-Time Curve ($AUC_{0–8h}$) of the First-Line Antituberculosis Drugs in Ghanaian Children With Tuberculosis

	Isoniazid			Rifampin			Pyrazinamide			Ethambutol		
Characteristic	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value	No.	Median (IQR)	*P*-Value
Age (y)			.398			.398			.032			.026
<2	10	13.6 (6.4–25.0)		10	20.3 (10.4–35.4)		10	95.9 (53.9–143.6)		8	3.7 (2.1–5.9)	
≥2	52	18.3 (11.0–23.4)		52	28.6 (15.6–36.1)		49	148.8 (125.5–201.2)		51	7.3 (4.1–10.4)	
Age (y)			.168			.027			.122			.002
<5	29	15.9 (7.8–22.8)		29	21.9 (11.7–33.8)		28	130.0 (96.0–185.8)		27	5.2 (2.9–6.8)	
≥5	33	19.0 (13.2–25.9)		33	31.5 (21.1–36.9)		31	162.4 (116.1–207.6)		32	8.4 (5.8–11.6)	
Sex			.063			.285			.133			.318
Female	30	19.1 (14.4–23.4)		30	26.4 (15.9–37.5)		29	162.4 (123.3–211.6)		30	7.5 (4.2–10.7)	
Male	32	14.9 (7.8–22.9)		32	25.7 (15.0–34.3)		30	133.1 (105.4–186.2)		29	6.4 (3.8–8.5)	
HIV infection			.060			.008			.048			.019
Negative	34	19.4 (13.4–25.9)		34	32.3 (21.1–42.3)		32	165.9 (129.7–206.4)		33	8.1 (5.3–11.0)	
Positive	28	14.3 (9.1–20.0)		28	20.3 (11.2–32.3)		27	128.6 (94.6–194.2)		26	4.9 (3.4–7.6)	
TB site			.586			.016			.318			.441
Pulmonary	48	17.4 (10.3–23.4)		48	24.1 (14.1–34.3)		45	134.1 (105.4–200.7)		45	6.7 (3.6–10.4)	
Extrapulmonary	14	18.6 (9.4–23.6)		14	36.1 (23.7–42.3)		14	157.0 (125.5–209.8)		14	6.5 (5.3–10.2)	
Weight-for-age *Z* score			.211			.161			.766			.226
≥–2.0	17	19.4 (15.8–23.4)		17	34.7 (21.8–36.9)		14	137.6 (125.5–169.4)		16	7.0 (4.5–10.1)	
<–2.0	35	14.4 (7.9–22.8)		35	24.1 (15.0–34.8)		35	129.4 (97.3–189.9)		33	5.2 (3.5–8.1)	
Height-for-age *Z* score			.052			.004			.001			.008
≥–2.0	29	19.4 (15.7–23.6)		29	33.8 (23.7–38.2)		29	181.6 (143.6–209.8)		29	8.4 (5.7–11.1)	
<–2.0	32	14.3 (8.6–20.4)		32	20.7 (10.6–30.1)		30	119.0 (94.4–162.4)		29	4.2 (3.3–8.1)	
MUAC (cm)			.807			.233			.164			.015
≥12.5	40	18.3 (11.9–23.4)		40	28.6 (16.8–35.8)		38	147.4 (128.6–205.2)		39	8.1 (4.1–11.0)	
<12.5	16	17.7 (7.9–25.2)		16	20.4 (11.4–32.7)		16	127.9 (88.8–188.1)		14	3.9 (2.4–6.1)	
Dose in new range[a]			.015			.054			.002			.214
No	9	10.4 (8.2–13.4)		5	13.6 (11.5–15.0)		38	129.6 (105.4–152.1)		11	6.6 (3.5–8.1)	
Yes	53	19.0 (13.0–23.6)		57	27.7 (16.4–36.2)		21	194.2 (169.4–217.7)		48	6.7 (4.0–10.8)	
New weight-band dose[b]			.068			.196			.267			.282
Yes	51	19.0 (9.4–25.0)		51	27.7 (15.7–36.6)		56	144.1 (110.9–197.5)		56	6.5 (3.8–10.3)	
No	11	13.0 (10.2–17.7)		11	24.2 (11.5–30.8)		3	207.6 (116.1–245.9)		3	9.2 (7.6–10.4)	

Abbreviations: IQR, interquartile range; HIV, human immunodeficiency virus; TB, tuberculosis; MUAC, mid-upper-arm circumference.

[a]New World Health Organization (WHO) recommended dosage range (isoniazid 7–15 mg/kg, rifampin 10–20 mg/kg, pyrazinamide 30–40 mg/kg, ethambutol 15–25 mg/kg).

[b]Some patients received old weight-band dosage prior to adoption of new WHO guidelines.

Table 6. Coefficient and Standardized Estimates of Joint Predictors of Drugs Plasma Peak Concentration (C_{max}) and Area Under the Concentration–Time Curve (AUC_{0-8h})

Drug	Parameter	Predictor	Parameter Estimate	SE	Standardized Estimate[a]	*P*-value
Isoniazid	C_{max}	Dose in mg/kg	0.211	0.098	0.300	.036
		Female vs. male	1.676	0.597	0.330	.007
		TB vs. TB/HIV	0.860	0.647	0.169	.189
		Body weight in kg	0.009	0.046	0.028	.844
	AUC_{0-8h}	Age (y)	0.289	0.357	0.109	.423
		Dose in mg/kg	0.722	0.372	0.263	.057
		Female vs. male	4.767	2.411	0.240	.053
		TB vs. TB/HIV	4.112	2.478	0.206	.103
Rifampin	C_{max}	Age (y)	0.217	0.116	0.251	.066
		Dose in mg/kg	0.191	0.096	0.266	.053
		Female vs. male	0.778	0.776	0.120	.320
		TB vs. TB/HIV	1.628	0.797	0.251	.046
	AUC_{0-8h}	Age (y)	1.144	0.499	0.295	.026
		Dose in mg/kg	0.935	0.415	0.291	.028
		Female	4.538	3.339	0.156	.179
		TB vs. TB/HIV	8.585	3.432	0.294	.015
Pyrazinamide	C_{max}	Dose in mg/kg	0.605	0.187	0.383	.002
		Female vs. male	3.692	2.466	0.173	.140
		Height in cm	0.167	0.050	0.393	.002
	AUC_{0-8h}	Dose in mg/kg	2.737	1.074	0.311	.014
		Female vs. male	18.949	13.879	0.159	.178
		TB vs. TB/HIV	9.611	14.641	0.080	.514
		Height in cm	0.964	0.295	0.409	.002
Ethambutol	C_{max}	Age (y)	0.144	0.040	0.423	.001
		Dose in mg/kg	0.047	0.033	0.168	.165
		Female vs. male	0.233	0.294	0.092	.431
		TB vs. TB/HIV	0.636	0.301	0.249	.039
	AUC_{0-8h}	Age (y)	0.517	0.121	0.484	<.000
		Dose in mg/kg	0.119	0.101	0.136	.244
		Female vs. male	0.945	0.897	0.118	.297
		TB vs. TB/HIV	1.807	0.921	0.225	.055

Abbreviations: HIV, human immunodeficiency virus; TB, tuberculosis; SE, standard error.

[a]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, standardized coefficient estimate = 0.251 for age in predicting rifampin C_{max} means that 1 standard deviation increase in age leads to a 0.251 standard deviation increase in predicted rifampin C_{max}, with the other variables held constant. The predicting power of the predictors for rifampin C_{max} can be ranked as: dose > age ≈ HIV coinfection status > sex.

Multivariate Factors Associated With Plasma Anti-TB Drug Concentrations

In the multivariate model dose in milligrams per kilogram, sex, HIV status, and body weight explained 22.2% variability in isoniazid C_{max} ($P = .006$), while age, dose in mg/kg, sex, and HIV status together explained 17.1% variability in isoniazid AUC_{0-8h} ($P = .028$). Age, dose in milligrams per kilogram, sex, and HIV status together explained 18.7% variability in C_{max} ($P = .017$) and 25.3% variability in AUC_{0-8h} ($P = .002$) of rifampin. Dose in milligrams per kilogram, sex, and height together explained 27.5% variability in pyrazinamide C_{max} ($P < .001$), while dose in mg/kg, sex, HIV status, and height together explained 27.2% variability in pyrazinamide AUC_{0-8h} ($P = .002$). Age, dose in milligrams per kilogram, sex, and HIV status together explained 28.8% variability in C_{max} ($P = .001$) and 32.6% variability in AUC_{0-8h} ($P < .001$) of ethambutol. The standardized coefficient giving relative strength of each of the selected variables in predicting the drugs' C_{max} and AUC_{0-8h} in the multivariate analysis is shown in Table 6.

Clinical Outcome

Of the 62 patients included in this analysis, 54 (84%) completed therapy, 5 patients were lost to follow-up, 2 died, and 1 discontinued the study after pharmacokinetic sampling. The 2 patients who died were both HIV-infected, severely malnourished (WAZ < −3), and had low C_{max} for rifampin (0.63 and 3.33 μg/mL). Two patients developed grade 1 or 2 elevation of liver enzymes at 4 weeks of antituberculosis treatment, 7 patients developed grade 1, and 3 developed grade 2 elevation of serum alkaline phosphatase. No patients discontinued TB therapy as a result of treatment side effects.

DISCUSSION

The main finding of this study is that a majority of children who received revised WHO recommended dosages of the first-line antituberculosis drugs achieved target plasma C_{max} for isoniazid and pyrazinamide, but not for rifampin and ethambutol. Overall, 86% and 82% of children in our study who received drug dosages within the new

recommended weight-band range achieved the plasma C_{max} for isoniazid and pyrazinamide of at least 3 μg/mL and 20 μg/mL, respectively. However, only 41% and 48% of the children who received the WHO recommended dosage achieved target C_{max} target for rifampin and ethambutol of 8 μg/mL and 2 μg/mL or higher, respectively. No patients exhibited high rifampin or ethambutol C_{max} values. In addition, hepatotoxicity was rare and no treatment-limiting side effects were observed.

To the best of our knowledge, only 2 published pharmacokinetics studies have evaluated the revised dosages of the first-line TB drugs in children [17, 18]. Our findings confirm the adequacy of the revised dosages of isoniazid and pyrazinamide as reported in a study of children younger than 2 years of age in South Africa [17]. However, unlike the abovementioned study [17], we found a high prevalence (>50%) of low rifampin and ethambutol C_{max}. A recent study of 31 children aged <10 years treated predominantly with revised dosages reported that only 6%, 15%, 55%, and 65% attained target C_{max} for rifampin, ethambutol, pyrazinamide, and isoniazid, respectively [18]. Children are often excluded from pharmacokinetic–pharmacodynamic studies because they do not have microbiological-proven pulmonary TB to allow for an objective assessment of treatment outcome. However, if one assumes that the plasma therapeutic targets for these drugs are the same for children as in adults, then our data together with those of Hiruy et al. [18] suggest that the revised dosages of rifampin and ethambutol in particular are inadequate for most children, and even higher dosages than currently recommended are needed.

We found that it was not possible to give pyrazinamide at a dose of 30–40 mg/kg to most children in our study using the available WHO-approved dispersible isoniazid/rifampin/pyrazinamide (30/60/150 mg) FDC tablets. For each weigh-band, the children with higher weight received a dose below the target range. This problem was anticipated in the WHO dosing instructions for the use of currently available FDC TB medicines for children given the fixed proportions of the components of FDC tablets [19]. Overall, 38 children in our study received a pyrazinamide dose <30 mg/kg, yet a majority of them had pyrazinamide C_{max} within the reference range of 20–50 μg/mL [20], similar to findings of another study that gave pyrazinamide at a dose of 20–30 mg/kg to children [14]. If one uses pyrazinamide $C_{max} \geq 35$ μg/mL as the desired target cutoff as suggested by some investigators [21], then 77% of the children in our study who were appropriately dosed based on weight-band did not achieve this target. Thus, as previously noted [25, 26], the ratios of the components of the FDC tablets may need to vary according to a child's age and weight.

Key factors that influenced the pharmacokinetics of the drugs in our study included age, sex, HIV coinfection status, nutritional state, and drug dosage in milligrams per kilogram. It is recognized that younger children compared to older children or adults tend to have lower concentrations of the antituberculosis drugs when similar weight-based dosages are used [8–13]. Children younger than 2 years old had lower pyrazinamide and ethambutol C_{max} and AUC_{0-8h}, while those younger than 5 years old had lower rifampin and ethambutol AUC_{0-8h}. Whether younger children have higher metabolism of these drugs remains unclear, as most studies including ours did not measure metabolite concentrations. An association between nutritional status and the pharmacokinetics of the first-line antituberculosis drugs has been reported by some investigators [27], but others have found no significant relationship [9, 17, 28]. Stunted children (HAZ < –2) in our study had lower median C_{max} and AUC_{0-8h} values for all the drugs except for isoniazid. HIV coinfection is an important factor that has been associated with poor TB treatment responses [2–5], but it is unclear if this is due to low plasma drug concentrations. Some studies that included small numbers of HIV-infected children reported no association between HIV status and antituberculosis drug pharmacokinetics [9, 17]. In contrast, we found significantly lower median rifampin and ethambutol C_{max} in the HIV-coinfected patients compared to those with TB alone, as well as a lower AUC_{0-8h} of all drugs except isoniazid in the HIV-coinfected patients. Whether the lower concentrations of the antituberculosis drugs contribute to poor outcomes in HIV-coinfected children was difficult to evaluate in our study, as almost all the patients had smear-negative disease and cultures were not done. The 4 patients who died in this study (2 before pharmacokinetic sampling) were HIV infected. The 2 patients who died after sampling were both malnourished and also had low rifampin C_{max}.

We recognize that our study has some limitations. Treatment was observed by a family member at home, and variable adherence could have affected our results. We tried to reduce the effect of poor adherence by calling parents daily during the week prior to pharmacokinetic sampling to ensure that medications were administered. Secondly, we did not examine pharmacogenetic factors and could not account for the effect of genetic factors. Isoniazid is primarily metabolized by hepatic *N*-acetyltransferase type 2 (NAT-2), and genetic polymorphisms in the NAT2 gene are associated with variable pharmacokinetics [29]. Also, the *SLCO1B1* polymorphisms lead to variable rifampin exposure in adults [30–32]. We plan to perform genetic analysis in future to examine the contribution of genetic

factors to the variability in isoniazid and rifampin concentrations in children.

Notwithstanding the above limitations of our study, the results show a high prevalence of low concentrations of rifampin and ethambutol in Ghanaian children with TB despite receiving revised dosages. These findings support higher dosages than currently recommended for rifampin and ethambutol in children. In addition, HIV coinfection was an important covariate for lower plasma exposure of all the first-line drugs in the multivariate analysis. Whether HIV-infected children need higher dosages of these drugs needs to be examined carefully. In the absence of therapeutic drug monitoring in resource-poor settings, there is a need for pharmacokinetic studies of higher dosages of the first-line drugs in children to identify the optimal tolerable dosages at the population level.

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