Effect of First-Line Antituberculosis Therapy on Nevirapine Pharmacokinetics in Children Younger than Three Years Old

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ABSTRACT Nevirapine-based antiretroviral therapy (ART) is one of the limited options in HIV-infected children younger than 3 years old (young children) with tuberculosis (TB) coinfection. To date, there are insufficient data to recommend nevirapine-based therapy during first-line antituberculosis (anti-TB) therapy in young children. We compared nevirapine pharmacokinetics (PK) in HIV-infected young children with and without TB coinfection. In the coinfected group, nevirapine PK was evaluated while on anti-TB therapy and after completing an anti-TB therapy regimen. Of 53 participants, 23 (43%) had TB-HIV coinfection. While the mean difference in nevirapine PK parameters between the two groups was not significant (P > 0.05), 14/23 (61%) of the children with TB-HIV coinfection and 9/30 (30%) with HIV infection had a nevirapine minimum concentration (C_{min}) below the proposed target of 3.0 mg/liter (P = 0.03). In multivariate analysis, anti-TB therapy and the CYP2B6 516G>T genotype were joint predictors of nevirapine PK parameters. Differences in nevirapine PK parameters between the two groups were significant in children with CYP2B6 516GG but not the GT or TT genotype. Among 14 TB-HIV-coinfected participants with paired data, the geometric mean C_{min} and area under the drug concentration-time curve from time zero to 12 h (AUC_{0-12}) were about 34% lower when patients were taking anti-TB therapy, while the nevirapine apparent oral clearance (CL/F) was about 45% higher. While the induction effect of anti-TB therapy on nevirapine PK in our study was modest, the CYP2B6 genotype-dependent variability in the TB drug regimen effect would complicate any dose adjustment strategy in young children with TB-HIV coinfection. Alternate ART regimens that are more compatible with TB treatment in this age group are needed. (This study has been registered at ClinicalTrials.gov under identifier NCT01699633.)

KEYWORDS CYP2B6 genotype, children, coinfection, human immunodeficiency virus, nevirapine, pharmacokinetics, tuberculosis
Tuberculosis (TB) is a common cause of morbidity and mortality in children with human immunodeficiency virus (HIV) infection, especially those younger than 5 years old (1, 2). Children with HIV infection have up to a 20-fold-increased incidence of TB (3, 4) and up to a 6-fold-greater risk of dying from TB than children with TB alone (5, 6). It was estimated that 17% of childhood TB deaths in 2015 occurred in children with HIV infection (1). While TB deaths in HIV-negative children occur predominantly in those not on TB treatment (1, 7), the risk of death in HIV-positive children is high even when receiving TB treatment (2, 7). The combination of TB and HIV is thought to be more deadly in young children because of high viral loads after acquiring HIV through vertical transmission and rapid progression of TB, underscoring the need for early antiretroviral therapy (ART) and preventive TB therapy in infected infants (2). Although data are limited in children, concurrent ART during TB treatment markedly reduces TB deaths in adults with HIV-associated TB (8–11). In HIV-infected children with suspected TB, early ART was associated with reduced mortality, with a hazard ratio of 0.08 (12). However, a key challenge to early ART is the limited number of regimen options for infants and children younger than 3 years old (young children), as the appropriate dosages for efavirenz or integrase inhibitors during first-line antituberculosis (anti-TB) therapy have not been established for this age group (13, 14).

Nevirapine-based ART remains one of the limited options for neonates and young children with TB coinfection. To date, there is insufficient evidence to continue recommending nevirapine-based ART in the setting of first-line anti-TB therapy in young children. Among HIV-infected children under 3 years old with or without TB coinfection in Zambia, the area under the drug concentration-time curve from time zero to 6 h (AUC_{0-6}) of nevirapine was 41% lower in children with TB-HIV coinfection than in children with only HIV, and trough concentrations were subtherapeutic in 11/21 (53%) of the TB-HIV-coinfected children, compared to none of the 16 children without TB (15). While these findings are yet to be replicated, increased dosages of rifampin and isoniazid recommended for children by the World Health Organization (WHO) (16) could further affect the severity of nevirapine pharmacokinetic (PK) interactions in young children. In addition, drug-gene interactions may affect nevirapine PK during anti-TB therapy, but this has not been previously explored. Nevirapine undergoes extensive metabolism by hepatic CYP3A and CYP2B6 enzymes to form 2- and 12-hydroxy nevirapine and 3- and 8-hydroxy nevirapine, respectively (17–19). The pharmacokinetic interactions between first-line anti-TB therapy and the CYP2B6 516G>T genotype led to paradoxically higher concentrations of efavirenz (a CYP2B6 substrate) in subjects with the CYP2B6 516TT genotype (20). In the present study, we compared nevirapine PK in young children with TB-HIV coinfection on first-line anti-TB therapy to those in young children with HIV infection without TB. In addition, we examined the predictors of nevirapine PK using a multivariate model that included the CYP2B6 516G>T polymorphism in all participants, and nevirapine PK during (on) and after stopping (off) anti-TB therapy in TB-HIV-coinfected participants with paired samples were compared.

RESULTS

Study population. During the study period, 74 HIV-infected eligible children were enrolled, of whom 8 were lost to follow-up and 6 withdrew from the study prior to PK sampling. Of the 60 participants who completed PK sampling after 4 weeks of ART (PK1), 7 were excluded from the final analysis because of the following reasons: 6 had nevirapine peak concentration at 12 h postdose, and 1 had very low concentrations throughout the sampling period, suspicious for poor medication adherence. The final study population included 30 children with HIV and 23 with TB-HIV coinfection. The demographic and clinical characteristics of all participants and by TB coinfection status are shown in Table 1. Baseline characteristics between the two groups were similar except that children with TB-HIV coinfection were significantly more likely to be shorter and to have a lower median height-for-age Z score (HAZ) and lower median hemoglobin and albumin levels than those with HIV infection. Of the 23 children with TB-HIV
TABLE 1 Baseline characteristics of study participants

| Characteristic | Value for group | P value |
|----------------|-----------------|---------|
|                | All (n = 53)    | HIV (n = 30) | TB/HIV (n = 23) |
| Median age (yr) (IQR) | 1.6 (1.1 to 2.0) | 1.5 (1.0 to 2.0) | 1.7 (1.1 to 2.0) | 0.936 |
| Median body wt (kg) (IQR) | 7.3 (6.0 to 9.0) | 7.5 (6.0 to 9.8) | 6.8 (5.9 to 8.7) | 0.282 |
| Median ht (cm) (IQR) | 73.0 (67.0 to 77.0) | 74.5 (68.0 to 80.0) | 69.0 (65.0 to 75.0) | 0.034 |
| No. (%) of participants of age (yr) | | | | 0.934 |
| ≤1 | 13 (24.5) | 8 (26.7) | 5 (21.7) |
| >1 to 2 | 28 (52.8) | 15 (50.0) | 13 (56.5) |
| >2 to 3 | 12 (22.6) | 7 (23.3) | 5 (21.7) |
| No. (%) of participants of sex | | | | 0.415 |
| Male | 31 (58.5) | 16 (53.3) | 15 (65.2) |
| Female | 22 (41.5) | 14 (46.7) | 8 (34.8) |
| No. (%) of participants with CYP2B6 516G>T genotype (n = 52) | | | | 0.725 |
| GG | 11 (20.8) | 11 (33.3) | 10 (21.7) |
| GT | 8 (15.1) | 7 (21.3) | 5 (10.6) |
| TT | 1 (2.4) | 7 (21.3) | 5 (10.6) |
| Nutritional status | | | | |
| Median wt-for-age Z score (IQR) | -3.3 (-4.0 to -1.7) | -3.0 (-3.8 to -1.1) | -3.6 (-4.6 to -2.9) | 0.068 |
| Median ht-for-age Z score (IQR) | 1.6 (-1.7 to 2.8) | 1.6 (-1.7 to 2.8) | 1.6 (-1.7 to 2.8) | 0.478 |
| Median BMI-for-age Z score (IQR) | 2.0 (1.2 to 3.0) | 2.1 (1.3 to 3.1) | 2.0 (1.2 to 3.0) | 0.478 |
| Median white blood cell count (10⁹/liter) (n = 36) | 9.1 (7.5 to 11.8) | 8.8 (8.0 to 9.9) | 9.7 (7.1 to 12.1) | 0.664 |
| Median absolute neutrophil count (n = 30) (IQR) | 2.0 (1.2 to 3.4) | 2.0 (1.4 to 3.4) | 1.9 (1.1 to 3.2) | 0.949 |
| Median hemoglobin level (g/dl) (n = 37) (IQR) | 8.9 (7.9 to 10.2) | 9.7 (8.3 to 10.7) | 8.6 (6.8 to 9.4) | 0.047 |
| Median hemocrit (%) (n = 36) (IQR) | 28.2 (24.8 to 30.9) | 30.5 (25.5 to 31.1) | 26.4 (24.2 to 29.5) | 0.167 |
| Median platelet count (10⁹/liter) (n = 36) (IQR) | 318.5 (213.5 to 420.5) | 337.5 (262.0 to 453.0) | 263.5 (199.0 to 390.0) | 0.167 |
| Median blood urea nitrogen level (mmol/liter) (n = 36) (IQR) | 2.0 (1.6 to 3.0) | 2.3 (1.7 to 3.1) | 1.9 (1.5 to 2.8) | 0.478 |
| Median serum creatinine level (μmol/liter) (n = 36 (IQR) | 25.0 (17.0 to 35.0) | 31.5 (21.0 to 37.0) | 24.0 (18.0 to 30.5) | 0.138 |
| Median calculated eGFR (ml/min/1.73 m²) (n = 36) (IQR) | 94.5 (75.4 to 136.8) | 86.7 (69.0 to 125.8) | 105.3 (81.1 to 138.6) | 0.404 |
| Median aspartate transferase level (U/liter) (n = 37) (IQR) | 50.0 (43.4 to 75.0) | 53.5 (42.5 to 76.5) | 48.0 (45.0 to 75.0) | 0.880 |
| Median alanine transferase level (U/liter) (n = 38) (IQR) | 28.5 (19.0 to 46.0) | 32.5 (21.0 to 58.0) | 25.6 (18.0 to 36.0) | 0.169 |
| Median alkaline phosphatase level (U/liter) (n = 37) (IQR) | 462.0 (269.0 to 550.0) | 533.0 (359.4 to 590.5) | 296.5 (257.0 to 487.0) | 0.073 |
| Median total bilirubin level (μmol/liter) (n = 39) (IQR) | 5.0 (4.0 to 7.0) | 5.0 (4.0 to 6.0) | 5.0 (3.0 to 8.0) | 0.622 |
| Median albumin level (g/liter) (n = 38) | 38.0 (35.0 to 42.2) | 40.4 (36.0 to 45.0) | 36.0 (31.2 to 40.0) | 0.018 |
| Median CD4 cell count (cells/μl) (n = 34) (IQR) | 992 (769 to 1,536) | 1,093 (677 to 1,784) | 923 (769 to 1,455) | 0.314 |
| Median % CD4 cells (n = 29) (IQR) | 19.0 (13.0 to 26.0) | 21.0 (17.0 to 30.0) | 13.5 (10.0 to 21.0) | 0.109 |
| Median log₁₀ HIV-1 RNA level (n = 32) (IQR) | 5.2 (4.2 to 6.2) | 5.0 (4.1 to 5.3) | 5.9 (4.4 to 6.5) | 0.101 |
| Median nevirapine dose (mg/kg) (IQR) | 10.1 (9.6 to 11.0) | 10.0 (9.6 to 10.7) | 10.3 (9.2 to 11.3) | 0.568 |
| Median nevirapine dose (mg/m²) (IQR) | 72.0 (70.0 to 90.0) | 77.5 (66.0 to 94.0) | 72.0 (70.0 to 80.0) | 0.281 |

No. (%) of participants receiving nucleoside backbone | 0.415 |
| Zidovudine + lamivudine | 22 (41.5) | 14 (46.7) | 8 (34.8) |
| Abacavir + lamivudine | 31 (58.5) | 16 (53.3) | 15 (65.2) |

Anti-TB drug dose (mg/kg) (IQR) | |
| Isoniazid | 14.1 (12.2 to 16.0) |
| Rifampin | 18.8 (17.6 to 22.8) |
| Pyrazinamide | 25.8 (23.1 to 32.3) |
| Ethambutol | 17.2 (15.4 to 21.5) |

*Numbers (percentages) are reported for categorical data, and medians and interquartile ranges (IQR) are reported for continuous data. BMI, body mass index; eGFR, estimated glomerular filtration rate.

*Numbers in parentheses in the left column indicate the number of participants with available data.

coinfection, 21 (91%) had pulmonary TB. The median isoniazid and rifampin doses were 14.1 mg/kg of body weight (range, 3.3 to 24.3 mg/kg) and 18.8 mg/kg (range, 6.6 to 32.4 mg/kg), respectively.

Effect of anti-TB therapy on nevirapine pharmacokinetics. Pharmacokinetic sampling was performed at a median (range) of 4.6 (2.9 to 9.6) weeks after starting ART in all participants and at a median (range) of 4.5 (1.7 to 23.4) weeks after stopping anti-TB therapy in the TB-HIV-coinfected group. Nevirapine plasma time-concentration profiles in children with HIV infection (n = 30) and those with TB-HIV coinfection on...
anti-TB therapy (n = 23) and off anti-TB therapy (n = 16) are shown in Fig. 1. Of the three groups, children with TB-HIV coinfection on anti-TB therapy had the lowest mean nevirapine concentrations at each sampling point and the highest concentrations after stopping anti-TB therapy (Fig. 1A). In the 14 TB-HIV-coinfected children with paired samples, the mean concentrations of nevirapine at each point were lower on than off anti-TB therapy (Fig. 1B).

The geometric mean (GM) values for the nevirapine PK parameters were similar between the two groups (Table 2). However, there was a trend toward lower mean nevirapine maximum concentration (C_{max}) and AUC_{0–12} in children with TB-HIV coinfection while on anti-TB therapy than in those with HIV infection. A similar trend was observed when we included the 7 participants who were excluded from our final analysis (see Table S1 in the supplemental material). A nevirapine minimum concentration (C_{min}) of <3.0 mg/liter, which is considered to be subtherapeutic (21), occurred in 14/23 (60.9%) of the children with TB-HIV coinfection during anti-TB therapy and in 9/30 (30.0%) of those with HIV infection (P = 0.03). After stopping anti-TB therapy in the TB-HIV-coinfected children, mean nevirapine PK parameters were higher than but not statistically different from those of the children with HIV infection (Table 2). Only 3/16 (18.8%) of the children with TB-HIV coinfection had a nevirapine C_{min} of <3.0 mg/liter off anti-TB therapy.

In the multivariate model that included all participants with PK1 data, the CYP2B6 516G>T single nucleotide polymorphism (SNP) and TB coinfection were significantly associated with nevirapine PK (Table 3). CYP2B6 516GG or GT genotype compared to TT genotype status was associated with decreased nevirapine exposure, and TB-HIV

### TABLE 2 Nevirapine pharmacokinetic parameters in HIV-infected children with and without TB coinfection and on and off anti-TB therapy

| Parameter               | Value for group | TB/HIV | P value for HIV vs TB-HIV groups on ATT (PK1) | P value for HIV vs TB-HIV groups off ATT (PK2) |
|-------------------------|-----------------|-------|---------------------------------------------|---------------------------------------------|
| GM T_{max} (h) (95% CI) | 3.5 (2.9–4.1)   | 3.3 (2.6–4.2) | 3.7 (2.9–4.7) | 3.0 (2.2–4.0) | 0.529 | 0.574 |
| GM C_{max} (mg/liter) (95% CI) | 5.7 (4.9–6.6) | 6.4 (5.3–7.7) | 4.8 (3.8–6.2) | 7.6 (6.4–9.1) | 0.066 | 0.209 |
| GM C_{min} (mg/liter) (95% CI) | 3.1 (2.4–4.1) | 3.3 (2.2–5.0) | 2.8 (2.0–3.9) | 4.8 (3.8–6.1) | 0.579 | 0.117 |
| GM AUC_{0–12} (mg · h/liter) (95% CI) | 56.0 (47.8–65.7) | 64.1 (52.8–77.7) | 47.0 (36.0–61.4) | 75.2 (62.2–91.0) | 0.053 | 0.274 |
| GM CL/F (l/h) (95% CI) | 1.3 (1.1–1.6) | 1.2 (1.0–1.5) | 1.5 (1.2–2.0) | 0.8 (0.8–1.2) | 0.113 | 0.133 |
| No. (%) of participants with C_{min} < 3 mg/liter | 23 (43.4) | 9 (30.0) | 14 (60.9) | 3 (18.8) | 0.030 | 0.498 |

*ATT, antituberculosis therapy; T_{max}, time to peak concentration; C_{max}, peak concentration; C_{min}, minimum concentration; AUC_{0–12}, area under the curve from time zero to 12 h; CL/F, apparent clearance; CI, confidence interval. T_{max} could not be calculated for 3 HIV-infected children.
coinfection status was associated with decreased nevirapine exposure. There was a significant interaction between anti-TB therapy and the CYP2B6 516G/T genotype on nevirapine exposure and clearance. Among children with the CYP2B6 516GG genotype, those with TB-HIV coinfection had lower median nevirapine C max and AUC 0–12 and a higher apparent oral clearance (CL/F) than those with HIV infection (Fig. 2). Among children with the CYP2B6 516GT or TT genotype, median values of nevirapine PK parameters were similar between the two groups (Fig. 2). Other factors, such as sex, baseline weight, and height, were not associated with nevirapine PK parameters in the model. Overall, 6/10 (60%) participants with CYP2B6 516GG, 15/32 (47%) with GT, and 2/10 (20%) with TT genotypes had a nevirapine C min of <3.0 mg/liter.

Among 14 TB-HIV-coinfected participants with paired samples on and off anti-TB therapy, the GM values of nevirapine C max, C min, and AUC 0–12 were significantly lower.

### TABLE 3

Multivariate analysis of the association of patient factors with nevirapine pharmacokinetic parameters in 53 HIV-infected children with and without TB coinfection

| Variable               | C max (µg/ml) | C min (µg/ml) | AUC 0–12 (µg · h/ml) | CL/F (liters/h) |
|------------------------|---------------|---------------|----------------------|----------------|
|                        | Estimate SE   | P value       | Estimate SE   | P value       | Estimate SE   | P value       |
| CYP2B6 GG vs GT        | -0.11 0.17    | 0.494         | -0.26 0.20    | 0.202         | -0.25 0.20    | 0.219         |
| CYP2B6 GG vs TT        | -0.59 0.20    | 0.005         | -0.71 0.24    | 0.005         | -0.78 0.24    | 0.002         |
| CYP2B6 GT vs TT        | -0.47 0.15    | 0.004         | -0.45 0.19    | 0.020         | -0.53 0.19    | 0.006         |
| TB-HIV vs HIV          | -0.35 0.14    | 0.019         | -0.37 0.17    | 0.035         | -0.48 0.17    | 0.007         |
| TB-HIV.GG vs HIV.GG    | -0.48 0.29    | 0.110         | -0.71 0.36    | 0.050         | -0.78 0.35    | 0.032         |
| TB-HIV.GT vs HIV.GT    | -0.15 0.15    | 0.316         | -0.02 0.18    | 0.926         | -0.18 0.18    | 0.318         |
| TB-HIV.TT vs HIV.TT    | -0.41 0.27    | 0.138         | -0.39 0.33    | 0.241         | -0.47 0.32    | 0.153         |

*Estimate is the mean difference between the groups. C max, peak concentration; C min, minimum concentration; AUC 0–12, area under the curve from time zero to 12 h; CL/F, apparent clearance; HIV.GG, HIV.GT, or HIV.TT, children with HIV infection who have the CYP2B6 516GG, GT, or TT genotype; TB-HIV.GG, TB-HIV.GT, or TB-HIV.TT, children with TB-HIV coinfection who have the CYP2B6 516GG, GT, or TT genotype.

FIG 2 Nevirapine pharmacokinetic parameters in HIV-infected children by CYP2B6 516G>T genotype. Differences in median nevirapine C max, AUC 0–12, and CL/F between HIV and TB-HIV groups were significant only for the GG genotype but not the GT or TT genotype. *, P < 0.05; **, P = 0.07.
TABLE 4 Nevirapine pharmacokinetic parameters for children on (PK1) and off (PK2) antituberculosis therapy and PK1/PK2 geometric mean ratios for 14 TB-HIV-coinfected children with paired samples.

| Parameter          | GM value for sampling (95% CI) | P value | PK1/PK2 GMR (90% CI) |
|--------------------|---------------------------------|---------|----------------------|
|                    | PK1                              | PK2     |                      |
| T<sub>max</sub> (h) | 4.5 (3.3—5.9)                   | 3.0 (2.2—4.1) | 0.016  | 1.50 (1.16—1.96) |
| C<sub>max</sub> (mg/liter) | 5.2 (3.8—7.1)            | 8.1 (6.7—9.7) | 0.003  | 0.64 (0.52—0.80) |
| C<sub>min</sub> (mg/liter) | 3.4 (2.4—4.8)               | 5.2 (4.0—6.6) | 0.012  | 0.66 (0.51—0.85) |
| AUC<sub>0—12</sub> (mg · h/liter) | 52.4 (37.8—72.6) | 79.5 (65.1—97.0) | 0.006  | 0.66 (0.53—0.82) |
| CL/F (liters/h)    | 1.4 (1.0—1.9)                 | 1.0 (0.8—1.1) | 0.012  | 1.45 (1.16—1.82) |

PK1, pharmacokinetic sampling after at least 4 weeks of therapy; PK2, pharmacokinetic sampling after 4 weeks of stopping antituberculosis therapy; GM, geometric mean; GMR, geometric mean ratio; T<sub>max</sub>, time to peak concentration; C<sub>max</sub>, peak concentration; C<sub>min</sub>, minimum concentration; AUC<sub>0—12</sub>, area under the curve from time zero to 12 h; CL/F, apparent clearance.

and CL/F was higher while on anti-TB therapy than while off anti-TB therapy (Table 4 and Fig. 3).

**Clinical outcome and virologic response by TB coinfection status.** Of the 55 children who completed at least one PK sampling, 46 completed the study, 5 were lost to follow-up, 2 discontinued the study, and 2 with TB-HIV coinfection died. There was
no discontinuation of ART due to medication side effects. On average, the aspartate aminotransferase (AST) level decreased by 16.9 and the alanine aminotransferase (ALT) decreased by 1.7 at 4 weeks of ART from baseline values. Of the 25 children who had viral load data after 6 months of ART, 11 (44%) had HIV RNA levels of $>200$ copies/ml. There was no significant difference in nevirapine PK, TB coinfection status, or $CYP2B6$ 516G/$T$ genotype frequency between the children with HIV RNA levels of $<200$ copies/ml and those with RNA levels of $\geq 200$ copies/ml (Table S2).

**DISCUSSION**

The main finding of this study is that first-line anti-TB therapy using WHO-recommended higher doses of rifampin and isoniazid resulted in modest decreases (34%) in nevirapine exposure and $C_{\text{min}}$ in children with TB-HIV coinfection. However, 61% of children with TB-HIV coinfection on concurrent anti-TB therapy, compared to 30% of children with HIV infection and 19% of coinfected children off anti-TB therapy, had a subtherapeutic nevirapine $C_{\text{min}}$. In multivariate analysis, coadministration of anti-TB therapy and $CYP2B6$ 516G/$T$ genotypes were joint predictors of nevirapine exposure and clearance, with a significant interaction between the two predictors. Among the participants with viral load data at 6 months of ART, 44% had unsuppressed HIV RNA, with no significant difference in virologic suppression rates between the two groups. Although we found only a modest effect of anti-TB therapy on nevirapine PK in our study population, the variability in the interactions between nevirapine and anti-TB regimen based on $CYP2B6$ genotype would likely complicate strategies to optimize nevirapine PK in young children, as one-dose adjustment would not fit all patients. Individualized nevirapine dosing during TB treatment in young children based on $CYP2B6$ genotyping would be difficult to implement in resource-limited settings.

A previous study reported a 41% reduction in nevirapine exposure in Zambian children while on TB treatment in comparison to controls of a similar age (15). While the 34% reduction in nevirapine exposure in our study seemed to be lower than that in the above-mentioned study (15), a high proportion of TB-HIV-coinfected children (61% in our study and 53% in the previous study) had subtherapeutic nevirapine trough concentrations. In contrast, no difference in the median nevirapine $AUC_{0-12}$ (85.3 versus 70.8 mg · h/liter, respectively) was observed during and after stopping rifampin-containing anti-TB treatment among older Thai children aged 4.4 to 11.7 years (22). Compared to adult studies with a design similar to ours, the 34% mean reduction in the nevirapine $AUC_{0-12}$ is consistent with the 36% reduction reported among 16 South African patients (23) and the 26% reduction in the median nevirapine $AUC_{0-12}$ on anti-TB therapy compared to off anti-TB therapy among 16 patients in Burkina Faso (24).

The modest decrease in nevirapine exposure during first-line anti-TB therapy may reflect an overall effect of the anti-TB drug combination regimen and not just rifampin. Nevirapine undergoes metabolism by hepatic CYP3A and CYP2B6 enzymes to form 2- and 12-hydroxy nevirapine and 3- and 8-hydroxy nevirapine, respectively (17–19). Rifampin is a well-known inducer of CYP enzymes and drug transporters (25–27). In a study among 13 Indian adults, the addition of rifampin alone to standard nevirapine-based ART for a week led to a 46% reduction in the mean nevirapine $AUC_{0-12}$ (28). On the other hand, a study in Spain in which 4-drug anti-TB therapy was added to nevirapine-based ART reported only a 31% reduction in the nevirapine $AUC_{0-12}$ (29). Isoniazid is a potent inhibitor of CYP3A4 *in vitro* (30) and *in vivo* (31) and could partly reduce the induction effect of rifampin on CYP3A4 during combination therapy. Among adults on nevirapine-based ART randomized to isoniazid or placebo for at least 1 month, isoniazid therapy was associated with a 24% increase in the median nevirapine $AUC_{0-12}$ (32). Using diazepam, a substrate of CYP3A4 and CYP2C19, as the victim drug, coadministration of isoniazid with diazepam led to reduced diazepam clearance, but when a combination of isoniazid, rifampin, and ethambutol was given with diazepam, there was a marked increase in diazepam clearance, confirming that the overall effect of rifampin- plus isoniazid-containing therapy on
the CYP3A substrate is induction (33). While CYP3A metabolism is the dominant pathway for nevirapine elimination, the significant effect of CYP2B6 genotype on the interactions between anti-TB therapy and nevirapine in our study also suggests that CYP2B6 plays an important role in nevirapine metabolism and drug-drug interactions.

Our study has some limitations. We had planned to initiate nevirapine at a full dose without lead-in in the children with TB-HIV coinfection, as a study in Ugandan adults reported that initiation of nevirapine at 200 mg twice daily during anti-TB therapy reduced the risk of a subtherapeutic nevirapine \( C_{\text{min}} \) (34). However, this was not possible due to the national program requiring nevirapine dose escalation for all children due to concerns related to toxicity. Thus, we were not able to examine whether omitting the lead-in dose in young children could have reduced the proportion of subtherapeutic concentrations during concomitant anti-TB therapy. As with other PK studies with long intervals between sampling periods, variability in medication adherence could have influenced the results between the two groups or two periods. It was not feasible to directly observe therapy for these types of PK studies. Another limitation is that we considered a nevirapine \( C_{\text{min}} \) of <3.0 mg/liter to be subtherapeutic based on a widely cited study in adults (21). However, this boundary has not been validated in children, and so we could have over- or underestimated the proportion of children with nevirapine subtherapeutic concentrations. Finally, only 42% of our participants had 6-month viral load data available. The small numbers of participants, in addition to the short duration of follow-up and lack of pretreatment drug resistance data, severely limited our ability to explore a relationship between PK parameters and virologic response.

In summary, first-line anti-TB therapy using revised higher TB drug dosages for children led to modest decreases in nevirapine exposure and \( C_{\text{min}} \) in TB-HIV-coinfected children younger than 3 years old. However, the high proportion of children who had a subtherapeutic nevirapine \( C_{\text{min}} \) during TB treatment is concerning. The variability in the effect of the anti-TB combination regimen on nevirapine clearance based on CYP2B6 genotype suggests that nevirapine dosing may need to be individualized via therapeutic drug monitoring in young HIV-infected children with TB coinfection. Genetic testing to guide dosing of nevirapine during concurrent TB treatment in young children would be difficult to implement in countries where TB is endemic. Thus, there is an urgent need to intensify research efforts to replace nevirapine with integrase strand inhibitors for young children with TB-HIV coinfection, as was recently done for adults and older children, since the drug-drug interactions may be easier to manage at the population level (14).

MATERIALS AND METHODS

Study design. A two-arm parallel-assignment pharmacokinetic study (see Fig. S1 in the supplemental material) was performed at the Komfo Anokye Teaching Hospital (KATH) in Ghana between October 2012 and November 2017. Children aged 3 to 35 months or weighing <10 kg with HIV infection with or without TB coinfection who were ART naive and were not previously exposed to nevirapine were enrolled. Children with opportunistic infections other than TB, who had acute illness other than malnutrition, or whose parents declined to sign written consent were excluded. The Institutional Review Boards (IRBs) of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (reference number CHRPE/AP/107/11); Lifespan Hospitals in Providence, RI (CMTT/project number 204412 Pedi Cat B); and the University of Florida, Gainesville, FL (IRB number IRB201601266), reviewed and approved the study. All parents or guardians of study participants provided written informed consent. The study was registered at ClinicalTrials.gov under identifier NCT01699633.

Treatment regimens. ART consisted of nevirapine plus zidovudine and lamivudine or abacavir and lamivudine twice daily. Nevirapine was dosed at 200 mg/m\(^2\) once daily for the first 14 days and then increased to 200 mg/m\(^2\) twice daily thereafter in accordance with WHO guidelines at the time of the study (35). Antituberculosis therapy consisted of isoniazid, rifampin, pyrazinamide, and ethambutol daily for 2 months and then isoniazid and rifampin daily for 4 months. Currently recommended revised dosages (16) were prescribed, and available dispersible fixed-dose combination (FDC) tablets for children (36) were used.

Monitoring and follow-up. At enrollment, a complete medical history, physical examination, and nutritional status assessment were performed, and relevant data were collected using standardized forms. Baseline measurements prior to initiation of ART included complete blood count, blood urea

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nitrogen level determination, serum creatinine level determination, liver function tests (LFTs), CD4 cell count, and plasma HIV-1 RNA level determination. Study participants were evaluated monthly to assess adverse events and clinical response to therapy. Liver function tests were repeated at week 4 of ART, and determination of HIV-1 plasma RNA level was repeated after 12 and 24 weeks of ART. However, some participants did not complete all of the planned laboratory testing due to missed sample collection, failed laboratory testing, lack of reagents, or damaged equipment at the study site during the study period.

Pharmacokinetic sampling. All participants were admitted to the hospital for PK sampling, which was performed after 4 weeks of starting ART in all participants and after 4 weeks after completing anti-TB therapy in the TB-HIV-coinfected group. On the day of sampling, the study drugs were administered after at least a 2-h fast in nonbreastfed children; children on exclusive breastfeeding were fed as needed throughout the study. Blood samples were collected at 0, 2, 8, and 12 h postdose for determination of nevirapine concentrations. The samples collected into EDTA-coated tubes were centrifuged within 30 min at 3,000 × g for 10 min. Plasma samples were stored at −80°C and then shipped on dry ice to the University of Cape Town (Cape Town, South Africa) for drug concentration assays.

Nevirapine plasma concentrations were determined from 10 μl human plasma using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay developed at the Division of Clinical Pharmacology. The samples were processed with a protein precipitation extraction method using acetonitrile, followed by high-performance liquid chromatography with MS/MS detection. Nevirapine-d3 was used as the internal standard. The extraction procedure was followed by liquid chromatographic separation using a Luna PFP(2) 110-Å, 50- by 2-mm, 5-μm analytical column (Phenomenex). An A8 Scieux API 4000 mass spectrometer at unit resolution in the multiple-reaction monitoring (MRM) mode was used to monitor the transitions of the protonated precursor ions at m/z 266.9 to the product ions at m/z 198.2 and of the protonated molecular ions at m/z 270.1 to the product ions at m/z 229.1 for nevirapine and the internal standard, respectively. The assay was validated over a concentration range of 0.0195 μg/ml to 20 μg/ml. The accuracy (percent nominal) and precision (percent coefficient of variation) statistics of the lower limit of quantitation and low-, medium-, and high-quality controls were between 90.8% and 100.8% and below 9.3% for nevirapine during inter- and intraday validation. The laboratory participated in the Clinical Pharmacology Quality Assurance (CPQA) external quality control (QC) program under a contract with the Division of AIDS of the National Institute of Allergy and Infectious Diseases. This assay was CPQA approved.

Calculations of the maximum or peak concentration (Cmax), time to Cmax (Tmax), minimum concentration (Cmin), the AUC from time zero to 12 h (AUC0–12), and estimated apparent oral clearance (CL/F) were performed using noncompartmental analysis (Phoenix Software; Pharsight Corporation, Mountain View, CA). Findings were confirmed by inspection of the plasma concentration-time graphs.

Genotyping of human allelic variants. Genotyping for the CYP2B6 516G>T (rs3745274) single nucleotide polymorphism (SNP) was performed by TaqMan allelic discrimination on a QuantStudio 12K Flex system (Life Technologies, Foster City, CA). The CYP2B6 516G>T SNP was included in our analysis based on its known significant influence on nevirapine PK in adults (37, 38) and in children (38). This was the only SNP that we found to be associated with nevirapine PK among 7 functional CYP SNPs that we examined (data not shown). In addition, anti-TB therapy is known to interact with the CYP2B6 gene, leading to increased concentrations of efavirenz, a CYP2B6 substrate (20).

Sample size and power justification. A sample size of 29 children per group with a 15% attrition rate (leaving at least 25 participants per group) was determined to have at least 80% power to detect at least an 18% to 30% difference in the nevirapine AUC0–12 between the two groups based on a coefficient of variation of 24 to 44% (40) and a 2-sample t test with a one-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 U.S. National Center for Health Statistics (NCHS) reference median values, using statistical macros for children aged <5 years provided by the WHO (41). The geometric means (GMs) of nevirapine PK parameters with 95% confidence intervals (CI) were summarized by TB coinfection status and by study visit. The geometric mean ratio (GMR) with the 90% CI was calculated for nevirapine PK during and after stopping anti-TB therapy in the coinfected group; PK parameters were considered bioequivalent if the 90% CI of the GMR fell between 0.8 and 1.25 in accordance with guidance by the Food and Drug Administration. Bivariate analyses of associations between patient factors and nevirapine PK parameters were performed using the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. The signed-rank test was applied to compare within-group changes of PK parameters for the TB-HIV coinfection group on and off TB treatment. Multivariate regression was used to explore the joint effect of demographics and clinical variables on the PK parameters. Stepwise variable selection with the AICC (corrected Akaike information criterion) was used to select the predictors of nevirapine PK parameters. For all analyses, a P value of <0.05 was considered significant.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00839-19.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.
ACKNOWLEDGMENTS

We thank the study participants and the supportive staff of the TB and HIV clinics at KATH who helped with patient enrollment. We also thank Maxwell Owusu and Eugene Adu Ahwireng for their assistance in specimen handling and processing.

This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development at the National Institutes of Health (grant number HD071779). F.S.G. was supported in part by Lifespan/Tufts/Brown Center for AIDS Research (P30 AI042853). The University of Cape Town (UCT) Clinical PK Laboratory was supported in part via the Adult Clinical Trial Group (ACTG), by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award numbers UM1 AI068634 and UM1 AI068636, as well as the Infant Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT), funding provided by the National Institute of Allergy and Infectious Diseases (U01 AI068632), the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Institute of Mental Health grant AI068632. H.Y. utilized core services and support from the University of Rochester Center for AIDS Research (CFAR), an NIH-funded program (P30 AI078498). M.H.C. was supported by the National Institute of General Medical Sciences at the National Institutes of Health (grant number R01 GM102130). A.K. received additional support from the Gatorade Trust through funds distributed by the University of Florida Department of Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

A.E., S.A., H.Y., F.S.G., M.H.C., D.J.G., and A.K. were responsible for conception and design of the study. S.A., A.E., A.O., D.B., T.O., A.D., L.W., and J.N. acquired data. H.Y., W.A.A., C.A.P., T.L., and A.K. analyzed and interpreted the data. All authors were involved in drafting the paper or revising it critically for important intellectual content, and all authors approved the final version.

We report no conflicts of interest.

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