Suboptimal Antituberculosis Drug Concentrations and Outcomes in Small and HIV-Coinfected Children in India: Recommendations for Dose Modifications

Benjamin Guiastrennec¹, Geetha Ramachandran², Mats O. Karlsson¹, A.K. Hemanth Kumar², Perumal Kannabiran Bhavani², N. Poorana Gangadevi², Soumya Swaminathan², Amita Gupta³, Kelly E. Dooley³ and Radojka M. Savic⁴

This work aimed to evaluate the once-daily antituberculosis treatment as recommended by the new Indian pediatric guidelines. Isoniazid, rifampin, and pyrazinamide concentration–time profiles and treatment outcome were obtained from 161 Indian children with drug-sensitive tuberculosis undergoing thrice-weekly dosing as per previous Indian pediatric guidelines. The exposure–response relationships were established using a population pharmacokinetic-pharmacodynamic approach. Rifampin exposure was identified as the unique predictor of treatment outcome. Consequently, children with low body weight (4–7 kg) and/or HIV infection, who displayed the lowest rifampin exposure, were associated with the highest probability of unfavorable treatment (therapy failure, death) outcome ($P_{\text{unfavorable}}$). Model-based simulation of optimized ($P_{\text{unfavorable}} \leq 5\%$) rifampin once-daily doses were suggested per treatment weight band and HIV coinfection status (33% and 190% dose increase, respectively, from the new Indian guidelines). The established dose-exposure–response relationship could be pivotal in the development of future pediatric tuberculosis treatment guidelines.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✓ India recently updated its national tuberculosis treatment guidelines and is now moving toward a once-daily dosing regimen. The optimal doses of first-line drugs for all children, in India or elsewhere, has not been firmly established.

WHAT QUESTION DID THIS STUDY ADDRESS?
✓ What are the subpopulations at risk of unfavorable treatment outcomes (therapy failure, death), and what modifications can be made to the new once-daily Indian pediatric tuberculosis treatment guidelines to reduce this risk in these subgroups?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✓ Low rifampin exposures were linked to an increased probability of unfavorable treatment outcome. Rifampin exposure was the lowest in children with low body weight or HIV coinfection.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✓ Clinical practice in India is evolving and this work provides vital information to inform dosing guidelines of the future. More specifically, our simulations suggest that higher antituberculosis drug dose levels in Indian children, especially those with low body weight or HIV coinfection, can potentially prevent treatment failure or death.

The World Health Organization (WHO) estimates that among the 10.4 million new incident cases of tuberculosis (TB) in 2015, 1 million occurred in children.¹ India has the highest TB burden in the world, accounting for 27% of total incident cases.¹ TB risk in India is increased with HIV coinfection, young age, malnutrition, and other comorbidities that can intensify disease severity and lower the overall exposure of anti-TB drugs.²,³

The introduction of India’s Revised National TB Control Programme (RNTCP) has reduced the incidence of TB by providing free diagnosis and treatment, and by using the Directly Observed Treatment Short-course (DOTS) strategy.⁴ Studies demonstrated that the previous thrice-weekly dosing of first-line anti-TB in RNTCP guidelines could lead to suboptimal anti-TB drug concentrations in both adults and children.⁵,⁶ Consequently, the RNTCP pediatric guidelines were revised in 2012, refining the dosing weight bands and increasing the rifampin (RIF) dose from 10 to 15 mg/kg, although still recommending thrice-weekly dosing.⁷ In 2016, new RNTCP guidelines were issued to initiate the
transition toward a once-daily dosing regimen in an effort to align with WHO recommendations. These new guidelines are currently being evaluated via pilot studies and once-daily dosing is anticipated to be rolled out throughout India by the end of 2017. Herein, the pre-2012 and 2016 guidelines will be respectively being referred to as “previous” and “new” RNTCP guidelines.

Few studies have attempted to link the pharmacokinetic (PK) of anti-TB drug to treatment outcomes in children, owing to challenges in the assessment of microbiologic treatment response, and this has resulted in a lack of exposure–response (or pharmacodynamic [PD]) data to inform dosing recommendations for children. Population PK-PD modeling can be used to relate the time-course of drug exposure to drug effects (e.g., clinical outcome) while investigating sources of variability among individuals and to evaluate alternative dosing regimens through simulations.

Using a population PK-PD approach, we evaluated the impact of individual-level factors associated with an increased probability of unfavorable treatment (therapy failure, death) outcome (P) in children with drug-sensitive TB treated according to the previous thrice-weekly Indian RNTCP pediatric guidelines. We then evaluated the new once-daily Indian dosing recommendations through model-based simulations and provide suggested dose revisions.

RESULTS
Drug concentrations analysis
Model development was performed on the data from 161 Indian children with TB monoinfection or TB-HIV coinfection pooled from two noninterventional studies; population characteristics are summarized in Table 1. In total, 805 plasma concentrations were collected for isoniazid (INH), 794 for rifampin (RIF), and 720 for pyrazinamide (PZA). Samples below the detection limit (INH: 148, RIF: 174, PZA: 75) were excluded from the analysis. Trough concentrations (24–72 h post previous dose) represented most of the excluded samples (INH: 125, RIF: 105, PZA: 67). Time concentration profiles are presented in Figure 1.

Among the tested candidate models, a two-compartment model best described INH disposition, whereas one-compartment models adequately described RIF and PZA data. Other aspects of the PK models were similar for all three drugs as highlighted hereafter. Absorption delays were described by chains of transit compartments, where significant between subject variability (INH 49.8%, RIF 64.4%, PZA 42.5% coefficient of variation (CV)) was identified in the mean transit time. Substantial between subject variability was also identified in apparent clearance (INH 49.8%, RIF 64.4%, PZA 42.5% CV) parameters; both ces (INH 74.2%, RIF 45.4%, PZA 37.4% CV) and volumes between subject variability was also identified in apparent clearan-

A covariate search was conducted to evaluate the effect of individual-level factors on the treatment outcome. Throughout this search, effects of subjects’ characteristics and INH, RIF, and PZA total weekly drug exposures at steady state were tested. Area under the concentration–time curves (AUC) was preferred over peak concentration as a marker of drug exposure; both metrics were, however, highly correlated (INH: 78%; RIF: 91% and PZA: 77%). Among all tested covariates, RIF exposure (9.81–231 μg.h/mL range) was the only independent predictor of unfavorable outcome. No statistically significant effect of INH (P = 0.74) or PZA (P = 0.81) exposures could be detected on within the ranges seen in this study (INH: 3.89–346 μg.h/mL and PZA: 351–2780 μg.h/mL). In addition to their effects on drug PK, HIV status and body weight were also tested on unfavorable. There was a trend towards HIV status having an independent effect on outcomes (P = 0.075), but weight had no impact on outcomes independent of its effect on RIF PK (P = 0.91). Owing to differences in weekly RIF exposure, the model-predicted odds ratio was 2.27 (95% confidence interval: 1.27–4.05) for TB-HIV coinfection (median AUC: 36.8 μg/mL) in reference to TB monoinfection (median AUC: 93.6 μg/mL) and 1.68 (95% confidence interval: 1.16–2.41) for children in the 6–10 kg weight band (median AUC: 60.7 μg/mL) in reference to the 26–30 kg weight band (median AUC: 96.5 μg/mL).

A model-derived weekly RIF exposure (185 μg/mL) to achieve a target of 5% or less was used for dose regimen evaluation. The relationship between RIF exposure and unfavorable is illustrated in Figure 3. Equations, parameter estimates, and predictive performances of the selected model are provided in Supplementary Material S3.
RNTCP dosing regimen evaluation

The simulation of treatment outcome under previous and new RNTCP dosing regimens (Figure 4, Table 2) revealed an increased $P_{\text{unfavorable}}$ for children with low body weight (6–10 kg for previous and 4–7 kg for new RNTCP guidelines) due to suboptimal mg/kg rifampin dose in this group or TB-HIV coinfection. Among those with HIV-TB, the median $P_{\text{unfavorable}}$ was as high as 35%. $P_{\text{unfavorable}}$, however, is expected to be reasonable ($\leq 5\%$) in most children with TB monoinfection under the new once-daily RNTCP dosing regimen.

Model-based optimized once-daily doses of RIF (i.e., based on the target RIF exposure to achieve $P_{\text{unfavorable}} \leq 5\%$) were calculated for children based on their body weight and HIV status. These doses are summarized in Table 3 and organized by the new RNTCP treatment weight bands and by TB-HIV coinfection status. The predicted optimal doses were increased for children with low body weight (4–7 kg) and especially for TB-HIV coinfected children, with doses up to 43.4 mg/kg. However, for children with TB monoinfection or TB-HIV coinfection with a body weight greater than 25 kg, the model predicted that low rates of $P_{\text{unfavorable}}$ could be achieved, even with once-daily doses as low as 5.2 mg/kg.

DISCUSSION

The study presented herein extends the work from Swaminathan et al., who exposed a link between low antituberculosis exposure and unfavorable treatment outcome in Indian children treated according to the previous RNTCP thrice-weekly dosing recommendations. This study was tailored to evaluate the new Indian dosing recommendations, identify subgroups for whom the new recommendations will result in suboptimal drug exposures, and use modeling to suggest dosing revisions to consider. A population PK-PD modeling approach was selected to enable the establishment of the dose-exposure–response relationships, the identification of subgroups “at risk,” and the simulations of alternate dosing regimens.

Our main finding was that the previously recommended thrice-weekly RNTCP dosing along with constant mg/kg dosing across all pediatric weight bands resulted in an increased $P_{\text{unfavorable}}$ in children with low body weight (6–10 kg) and/or TB-HIV coinfection due to suboptimal weekly RIF exposure. Furthermore, model-based simulations indicated that suboptimal RIF concentrations could also be expected in these subpopulations for children treated according to the new once-daily RNTCP dosing recommendations.

---

Table 1 Population characteristics and treatment outcomes

| Covariate | TB monoinfection study | TB-HIV coinfection study | P value* | Total |
|-----------|------------------------|--------------------------|----------|-------|
| Number of participants | 84 | 77 | NA | 161 |
| Sex (male/female) | 41/43 | 50/27 | 0.040 | 91/70 |
| Age (years) | 8 (5–11) | 9 (7–11) | 0.008 | 8 (6–11) |
| Weight (kg) | 17.8 (12.9–22.8) | 17.0 (14.2–22.4) | 0.734 | 17.5 (13.9–22.5) |
| BMI (kg/m²) | 14.1 (12.7–15.2) | 14.4 (13.4–15.5) | 0.240 | 14.2 (13.3–15.3) |
| INH acetylator (slow/rapid) | 57/27 | 52/25 | 0.967 | 109/52 |
| ART usage | 0 | 45 | NA | 45 |
| CD4 cell count (%) | — | 11.0 (5.0–19.5) | NA | — |
| Z-scores | | | | |
| HAZ | $-1.2 (-2.1$ to $-0.29)$ | $-3.0 (-4.1$ to $-2.0)$ | $<0.001$ | $-2.0 (-3.2$ to $-0.93)$ |
| WAZ | $-1.8 (-2.4$ to $-1.1)$ | $-2.7 (-3.4$ to $-1.9)$ | $<0.001$ | $-2.2 (-2.9$ to $-1.4)$ |
| Tuberculosis type | | | | $<0.001$ |
| Pulmonary | 19 | 49 | | 68 |
| Extra pulmonary | 63 | 28 | | 91 |
| Both | 2 | 0 | | 2 |
| Treatment outcome | | | | $0.443$ |
| Favorable | 55 | 54 | | 109 |
| Unfavorable | 15 | 18 | | 33 |
| Unknown | 14 | 5 | | 19 |

ART, antiretroviral treatment; BMI, body mass index; HAZ, height for age Z-score; HIV, human immunodeficiency virus; INH, isoniazid; NA: not applicable; TB, tuberculosis; WAZ, weight for age Z-score.

*Statistical testing performed using a Mann–Whitney U test at 5% level of significance. **Z-scores calculated using the EPI-INFO 2002 software package (v. 3.4.3): Centers for Disease Control and Prevention, Atlanta, GA. *n* or Median (Interquartile Range).
Interestingly, published INH, RIF, and PZA population PK models in TB infected South African children did not adequately fit the data from Indian children. Overall, published models in South African children indicated higher apparent clearance, shorter absorption delays, and smaller apparent volume for all three drugs compared to the Indian children studied herein. The origin of these differences (i.e., analytical, formulation, genetic or nutritional factors, magnitude of autoinduction) could not be investigated with the current study design. Herein, relative bioavailability was found to be nonlinearly correlated with total body weight resulting in lower drug exposures in children with low body weight. Given the dosing protocol, body weight, however, was highly correlated with age and dose. Thus, beyond the effect of body size, this finding may partly be explained by differences in tablet formulation used for the 6–10 kg weight band. Furthermore, as similar trends were observed for INH, RIF, and PZA, other factors such as malabsorption due to disease severity or malnutrition could also have contributed to the effect. Whether or not TB-HIV coinfection impacts the PK of anti-TB drugs is debated. Herein, HIV status had a pronounced and clinically relevant impact on TB drug pharmacokinetics observed as lower INH (−19.5%) and RIF (−41.5%) relative bioavailability, higher RIF clearance (+31.6%), and higher between-subject variability on clearance (+74%) and volume of distribution (+106%). These effects were associated with TB-HIV coinfection rather than the use of antiretroviral treatment (ART). Children in the TB-HIV coinfection study were more severely affected by malnutrition (median height for age Z-score (HAZ) = −3.0 and weight for age Z-score (WAZ) = −2.7) than children with TB monoinfection (median HAZ = −1.2 and WAZ = −1.8). Thus, the observed effects of TB-HIV coinfection on apparent clearance and relative bioavailability were likely due to a complex combination of factors including; drug–drug interactions, malabsorption, and malnutrition, which are not independently distinguishable with the current study design.

Treatment outcome in Indian children has herein been linked to the PK of INH, RIF, and PZA through a population PK-PD approach. The in vitro activities of INH, RIF, and PZA have been shown to correlate well with both AUC and peak concentration. In order to facilitate the translation from thrice-weekly to daily dosing, AUC was selected as a marker of drug exposure over peak concentration. AUC and peak concentrations were, however, highly correlated for all three drugs (INH 78%, RIF 91%, and PZA 77%). Weekly RIF exposure at steady state was the only independent predictor of treatment outcomes. This should not be interpreted as RIF being the only active drug in TB treatment, but rather that despite similar exposure trends of the INH and PZA across individuals (e.g., low body weight), RIF was likely the most severely underdosed anti-TB drug. Even though no additional covariate effect was supported by the data on the treatment outcomes, body weight and HIV coinfection substantially affected $P_{\text{unfavorable}}$ through their impact on RIF exposure. Despite differences in the studied populations, dosing regimen, and methodology, others have also reported an increased rate of low RIF exposure or unfavorable treatment outcome in adults and children with low body weight or with TB-HIV coinfection.

The treatment outcome under thrice-weekly vs. once-daily dosing in children has been the topic of a long debate, leading to conflicting conclusions. The population PK-PD approach used in this analysis allowed us to predict treatment outcomes under different scenarios while integrating the effect of covariates and...
the variability in PK parameters. The model-based simulations showed a clear trend toward higher \( P_{unfavorable} \) under previous thrice-weekly dosing compared to new once-daily dosing RNTCP recommendations.\(^7,9\) The new RNTCP guidelines, similar to guidelines in other settings, still assumes similar mg/kg dose levels across weight bands, disregarding maturation processes occurring throughout childhood.\(^32\) Thus, under previous and new RNTCP dosing recommendations, \( P_{unfavorable} \) was at the highest in the first (previous: 6–10 kg and new: 4–7 kg) weight band due to a low RIF exposure when compared to the older children. On the basis of the simulations, children with TB-HIV coinfection treated with the new RNTCP-recommended doses also displayed higher \( P_{unfavorable} \) than children with TB monoinfection across all weight bands. Although no TB treatment guidelines currently recommend an increase of RIF doses for subjects with TB-HIV coinfection, there is accumulating evidence that higher RIF doses along with nutritional supplementation may significantly improve outcomes in this population.\(^5,22,24,33,34\)

Optimized RIF doses based on a target weekly exposure criteria associated with a \( P_{unfavorable} \) of 5% or less are herein proposed to overcome poor treatment outcomes in some subgroups. This work represents the first step in the definition of a target exposure derived from pediatric data. Additional studies should be conducted to refine this target or extend to additional endpoints such as a target daily peak concentration. Moreover, the tolerability of dose increases in children should be carefully evaluated in appropriate clinical studies. Encouragingly, RIF has lately been tested in adults at doses up to 35 mg/kg without a significant increase in the incidence of side effects, and children are generally recognized to be more tolerant than adults to anti-TB treatment at similar mg/kg.\(^35–37\)

The present study has several limitations. First, the PK sampling times at 0, 2, 4, 6, and 8 h postdose were not optimal for...
estimation of absorption-related parameters and required the use of literature priors to support the estimation of the absorption rate constant of INH, RIF, and PZA.

The predictive performances of the models were, however, carefully evaluated (Supplementary Materials S2). Second, the developed model may not be generalizable to other populations, as differences in the apparent clearances and volume of distribution were observed in our Indian cohort compared with South African children (Figure 2), possibly due to poor nutritional status in our study population.

Third, TB-HIV coinfection was associated with

![Figure 4](https://example.com/figure4.png)

**Figure 4** Predicted probability of unfavorable treatment outcome ($P_{\text{unfavorable}}$) under previous (left panel), new (central panel), and optimized (right panel) revised national TB control program (RNTCP) dosing recommendations. Rifampin weekly exposures at steady state were simulated ($n = 1,000$) for children within the pediatric RNTCP weight range (i.e., 6–30 kg for previous and 4–39 kg for new and optimized dosing recommendations); $P_{\text{unfavorable}}$ distributions were computed for each weight band, and TB-HIV coinfection status. [Color figure can be viewed at cpt-journal.com]

Table 2 Intensive phase pediatric dosing as recommended by the Revised National Tuberculosis Control Program (RNTCP)

| Weight band | Isoniazid dose | Rifampin dose | Pyrazinamide dose | Ethambutol dose | $P_{\text{unfavorable}}$ | $P_{\text{unfavorable}}$ |
|-------------|----------------|---------------|-------------------|-----------------|-----------------------|-----------------------|
|             | mg (mg/kg)$^a$| mg (mg/kg)$^a$| mg (mg/kg)$^a$    | mg (mg/kg)$^a$  | median (CI$^{95}$)     | median (CI$^{95}$)     |
| Previous thrice-weekly RNTCP recommendations$^b$ |             |               |                   |                  |                       |                       |
| 6–10        | 75 (9.4)      | 75 (9.4)      | 250 (31.3)        | 200 (25.0)      | 0.272 (0.196–0.330)   | 0.352 (0.261–0.395)   |
| 11–17       | 150 (10.7)    | 150 (10.7)    | 500 (35.7)        | 400 (28.6)      | 0.184 (0.104–0.251)   | 0.304 (0.202–0.367)   |
| 18–25       | 225 (10.5)    | 225 (10.5)    | 750 (34.9)        | 600 (27.9)      | 0.127 (0.0698–0.196)  | 0.268 (0.158–0.34)    |
| 26–30       | 300 (10.7)    | 300 (10.7)    | 1,000 (35.7)      | 800 (28.6)      | 0.092 (0.0304–0.177)  | 0.242 (0.0836–0.336)  |
| New once-daily RNTCP recommendations$^c$ |             |               |                   |                  |                       |                       |
| 4–7         | 50 (9.1)      | 75 (13.6)     | 150 (27.3)        | 100 (18.2)      | 0.105 (0.0331–0.191)  | 0.241 (0.114–0.345)   |
| 8–11        | 100 (10.5)    | 150 (15.8)    | 300 (31.6)        | 200 (21.1)      | 0.034 (<0.01–0.0932)  | 0.154 (0.039–0.288)   |
| 12–15       | 150 (11.1)    | 225 (16.7)    | 450 (33.3)        | 300 (22.2)      | 0.0113 (<0.01–0.0519) | 0.103 (0.0116–0.235)  |
| 16–24       | 200 (10.0)    | 300 (15.0)    | 600 (30.0)        | 400 (20.0)      | <0.01 (<0.01–0.0178)  | 0.0659 (0.0102–0.181) |
| 25–29       | 225 (8.3)     | 375 (13.9)    | 850 (31.5)        | 575 (21.3)      | <0.01 (<0.01–0.0175)  | 0.0521 (<0.01–0.195)  |
| 30–39       | 250 (7.2)     | 450 (13.0)    | 1,100 (31.9)      | 750 (21.7)      | <0.01 (<0.01–0.001)   | 0.0396 (<0.01–0.123)  |

$^a$Doses in mg/kg reported for the average total body weight of each weight band. $^b$Doses administered thrice-weekly using single drug formulation. $^c$Doses administered once-daily using fixed dose combination (FDC) tablets. $^d$Reported as median and the 95% confidence interval (CI$^{95}$) around the simulated medians ($n = 1,000$). Simulations performed using the developed population PK-PD model.
important changes in the RIF exposure, although all subjects with TB-HIV coinfection were recruited as part of one study\textsuperscript{11} and all subjects with TB monoinfection as part of another study.\textsuperscript{12} This design could have possibly contributed to the magnitude of the observed effect. Thus, caution should be used in the interpretation of the differences between subjects with TB alone vs. TB-HIV coinfection. Fourth, treatment outcome was modeled as favorable in the case of treatment completion or cure and unfavorable in the case of treatment failure or death. Therefore, the treatment outcomes model could not distinguish between death and treatment failure. This strategy, however, was selected due to the relatively small incidence of deaths (nine cases) in the studied population.\textsuperscript{11,12} Furthermore, children with missing outcome (19 cases) had to be excluded from the treatment outcome analysis. While the treatment outcome in these children could not be inferred, having a treatment outcome missing was not linked with any specific characteristics associated with either favorable or unfavorable outcome, and thus were not expected to impact the conclusions. Fifth, the predicted optimized doses did not account for possible RIF PK nonlinearity in the extrapolation to high dose levels.\textsuperscript{36} RIF is also known to induce its own metabolism and this should be considered when shifting from thrice-weekly to once-daily dosing and/or increasing dose levels.\textsuperscript{38} Given the design of the current study, development of an RIF autoinduction model was not possible. Integration of the RIF autoinduction model from Smythe et al. in the treatment outcome suggested that the proposed optimized doses in Table 3 might need to be increased by 20% on average to accommodate the expected autoinduction (data not shown).\textsuperscript{39} Since the effect of maturation on RIF autoinduction has not properly been evaluated in children, the proposed doses should be confirmed clinically prior to their implementation.

In conclusion, the new Indian pediatric dosing recommendations could be evaluated through a population PK-PD approach linking the anti-TB exposure to the probability of unfavorable outcome. The main findings were an increased risk of poor treatment outcomes in children with low body weight and with TB-HIV coinfection under the previous thrice-weekly RNTCP dosing regimen. While the new once-daily RNTCP dosing regimen showed an overall improvement of predicted treatment outcome, children with low body weight and TB-HIV coinfection are still expected to display higher than expected rates of poor outcomes due to the use of constant mg/kg doses across weight bands. Optimized RIF doses based on a weekly RIF target exposure are provided. Clinical practice in India is rapidly evolving and the work presented herein may represent a cornerstone in the use of higher RIF pediatric doses in subpopulations at risk in the future.

METHODS

Study population

Data were obtained from two noninterventional studies in treatment-naive Indian children aged 1–15 years, diagnosed with drug-sensitive TB monoinfection \((n = 84)\) or TB-HIV coinfection \((n = 77)\).\textsuperscript{11,12} Clinical examination and phenotypic INH acetylator status determination were performed upon recruitment (Table 1). Children were recruited from six study sites located in India, including Agra, Bengaluru, Chennai, and Madurai. Studies were approved by the Institutional Ethics Committees of all study sites.\textsuperscript{11,12} Subjects’ parent/guardian provided written informed consent; children aged 7 or older provided assent.

Study design

Children received a thrice-weekly course of anti-TB treatment according to previous RNTCP guidelines for 6 months.\textsuperscript{7} Treatment was provided in patient-wise boxes available in four different weight bands (Table 2). Children eligible for ART were treated according to the National AIDS Control Organization guidelines.\textsuperscript{40} At least two weeks after treatment initiation, PK profiles of INH, RIF, and PZA were evaluated and anthropometrics measurements were recorded. Doses were administered by the study team after an overnight fast. Children continued anti-TB treatment and ART whenever required according to guideline recommendations. At the end of the study, TB treatment outcomes were reported as unfavorable (i.e., treatment failure or death), favorable (i.e., treatment completion or cure) or unknown (i.e., lost during follow-up).\textsuperscript{11,12}

Data collection

On the PK study day, 2-mL blood samples were drawn at trough and 2, 4, 6, and 8 h postdose. INH, RIF, and PZA concentrations were determined by validated high-performance liquid chromatography methods.\textsuperscript{31,42} Assays were linear within the following ranges of plasma concentration: 0.25–10.0 \(\mu\)g/mL for INH, 0.25–15.0 \(\mu\)g/mL for RIF and 1.25–50.0 \(\mu\)g/mL for PZA. Between- and within-run variation were

---

Table 3 Optimized once-daily dosing regimen of rifampin

| Weight band | TB monoinfection | TB-HIV coinfection |
|-------------|------------------|--------------------|
| kg          | Dose\textsuperscript{b} | \(P_{\text{unfavorable}}\) | Dose\textsuperscript{b} | \(P_{\text{unfavorable}}\) |
|             | mg (mg/kg)       | median (CI\textsubscript{95}) | mg (mg/kg) | median (CI\textsubscript{95}) |
| 4–7         | 109 (19.9)       | 0.0535 (0.0107–0.129) | 239 (43.4) | 0.0606 (<0.01–0.202) |
| 8–11        | 126 (13.3)       | 0.0554 (0.0129–0.126) | 275 (28.9) | 0.0591 (<0.01–0.196) |
| 12–15       | 139 (10.3)       | 0.0506 (0.0107–0.124) | 311 (23.0) | 0.057 (<0.01–0.18) |
| 16–24       | 154 (7.7)        | 0.048 (0.0162–0.101)  | 346 (17.3) | 0.0464 (<0.01–0.149) |
| 25–29       | 166 (6.2)        | 0.0484 (0.0106–0.124) | 383 (14.2) | 0.0492 (<0.01–0.188) |
| 30–39       | 180 (5.2)        | 0.0478 (0.0173–0.0966) | 404 (11.7) | 0.0509 (<0.01–0.142) |

\textsuperscript{a}Optimized doses given for single drug formulations. \textsuperscript{b}Doses in mg/kg calculated using the average total body weight of each weight band. \textsuperscript{c}Reported as median and the 95% confidence interval (CI\textsubscript{95}) around the simulated medians \((n = 1,000)\). Simulations performed using the developed population PK-PD model.
Drug concentrations analysis

The concentration–time profiles of INH, RIF, and PZA were modeled separately. For each drug, one- and two-compartment models with linear/saturable elimination and constant/saturable relative bioavailability were evaluated. Herein, the relative bioavailability was defined as the difference of bioavailability of a given individual in relation to the typical individual (i.e., 17.8 kg individual with TB monoinfection). Due to the sparseness of the data during the absorption phase, estimation of first-order oral absorption rate constants was supported by use of prior information from published models for INH, RIF, and PZA. Delays in drug absorption were tested using chains of transit compartments. Between-subject variability was investigated on structural model parameters and was assumed to follow a log-normal distribution. Proportional, additive, and combined (i.e., proportional and additive) error models were evaluated to describe the residual unexplained variability (e.g., measurement error).

Allometric scaling based on total body weight (normalized by the population median: 17.8 kg) was applied to apparent clearances and volumes using exponents of \( \frac{3}{4} \) and 1, respectively. Age-related maturation parameters for INH and RIF clearances and RIF absorption delay were set to literature values. The effects of rapid INH acetylator genotype were implemented on clearance and the relative bioavailability based on prior information. A covariate search was performed to identify the effect of influential factors on drugs exposure. As part of this process, effects of age, total body weight, dose, sex, nutritional status, TB-HIV coinfection, and ART intake were investigated on apparent clearances, volumes, and relative bioavailabilities. All covariate-parameter relationships were sequentially tested using forward selection (\( P < 0.05 \)) followed by backward deletion steps (\( P \geq 0.01 \)) to account for multiple testing. The effect of categorical covariates was implemented as fractional change in the typical parameter value in relation to the reference (i.e., most common) category. Continuous covariates were first implemented using linear relationships; nonlinear relationships were tested for upon their inclusion in the forward selection step.

Treatment outcomes analysis

The treatment outcome was reported as either favorable for cure (based on bacteriological evidence) and therapy completion (based on clinical evaluation) or unfavorable for therapy failure (based on clinical, microbiological, or radiographic failure) and death. Treatment outcome (favorable vs. unfavorable) was modeled using a logistic regression model, where \( P_{\text{unfavorable}} \) was expressed as a function of predictors. Odds ratios were calculated using the exponent of the estimated effect slope. Effects of INH, RIF, and PZA drug exposures implemented as the total weekly area under the concentration–time curve and subject characteristics were tested as predictors through forward selection (\( P < 0.05 \)) and backward deletion steps (\( P \geq 0.01 \)).

Parameter estimation and model selection

Population PK-PD analyses were performed in NONMEM (v. 7.3; Icon Development Solutions, Ellicott City, MD) and aided by functionalities of the PsN toolkit (v. 4.5.2). PK data were fitted using a first-order conditional estimation method with interaction. Parameter uncertainty was reported as relative standard error (RSE) was obtained from the NONMEM sandwich estimator computed with an importance sampling step. Model selection was guided by evaluation of goodness-of-fit plots, parameter estimates values, and comparison of the objective function values (−2log likelihood) with a significance level of \( P < 0.05 \) (2-sided) for nested models. Throughout their development, model predictions were also evaluated with simulation-based diagnostics. Finally, the capacity of the selected PK models to properly predict drug exposure was assessed using performance predictive checks plots where the observed population drug exposure median was compared to the distribution of 1,000 simulated population medians of drug exposure.

RNTCP dosing regimen evaluation

The selected PK-PD model was used to predict \( P_{\text{unfavorable}} \) under previous and new RNTCP dosing regimens. Accordingly, drug exposures were simulated 1,000 times for children within the RNTCP pediatric weight range (i.e., 6–30 kg for previous and 4–39 kg for new dosing recommendations). \( P_{\text{unfavorable}} \) were then individually computed and summarized for previous and new RNTCP weight bands and for each significant covariate of treatment outcomes. In line with WHO’s suggested target efficacy for drug-sensitive TB, the drug exposure associated with a target \( P_{\text{unfavorable}} \) of 5% or less was used to compute the optimized dose for each simulated subject. These optimized doses were then summarized for each of the new RNTCP treatment weight bands.

Additional Supporting Information may be found in the online version of this article.

ACKNOWLEDGMENTS

Benjamin Guiastrennec initiated this project during his internship at UCSF. This work was supported by the National Institute of Child Health and Human Development (grant number R01HD074944), the Clinical and Translational Science Institute (grant number 8 KL2 TR000143-07) and the Swedish Research Council (grant number 521-2011-3442). The authors thank Ms. V. Sudha for drug estimations by HPLC, Mr. S. Venkatesh for patient counselling and follow-up, as well as Dr. M. Bergstrand and Dr. S. Gupta for advice.

CONFLICT OF INTEREST

There are no conflicts of interest.

FUNDING

National Institute for Child Health and Human Development: Amita Gupta, Kelly (Elise) Dooley R01HD74944; Clinical and Translational Science Institute: Radogka Savic B KL2 TR000143-07; Swedish Research Council Forms: Benjamin Guiastrennec, Mats (Olof) Karlsson 521-2011-3442. This work was kindly supported by the funders listed above.

AUTHOR CONTRIBUTIONS

B.G., G.R., M.K., A.K., P.B., N.G., S.S., A.G., K.E.D., and R.M.S. wrote the article; B.G., G.R., M.K., A.G., K.E.D., and R.M.S. designed the research; B.G., M.K., and R.M.S. performed the research; B.G., M.K., and R.M.S. analyzed the data.

© 2017 The Authors. Clinical Pharmacology & Therapeutics published by Wiley Periodicals, Inc. on behalf of American Society for Clinical Pharmacology and Therapeutics

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

1. World Health Organization (WHO). Global tuberculosis report. Geneva Contract No WHO/HTM/TB/201613. 2016.
2. Ramachandran, G., Kumar, A.K.H. & Swaminathan, S. Pharmacokinetics of anti-tuberculosis drugs in children. Indian J. Pediatr. 78, 435–442 (2011).
3. Pai, M., Daftary, A. & Satyanarayana, S. TB control: challenges and opportunities for India. Trans. R. Soc. Trop. Med. Hyg. 110, 158–60 (2015).
4. Verma, R., Khanna, P. & Mehta, B. Revised national tuberculosis control program in India: the need to strengthen. Int. J. Prev. Med. Medknow Publications; 4, 1–5 (2013).

5. Ramachandran, G. et al. Low serum concentrations of rifampicin and pyrazinamide associated with poor treatment outcomes in children with multidrug-resistant tuberculosis. Pediatr. Infect. Dis. J. 35, 1 (2016).

6. Hemanth Kumar, A.K. et al. Pharmacokinetics of thrice-weekly rifampicin, isoniazid and pyrazinamide in adult tuberculosis patients in India. Int. J. Tuberc. Lung Dis. 20, 1236–1241 (2016).

7. National guidelines on diagnosis and treatment of pediatric tuberculosis. Central TB division, Government of India, 2012.

8. World Health Organization (WHO). Guidance for national tuberculosis programmes on the management of tuberculosis in children, 2nd ed. Geneva Contract No WHO/HTM/TB/201403, 2014.

9. Central TB division. Technical and operational guidelines for tuberculosis control in India 2016: Revised national TB control programme. New Delhi: Ministry of health and family welfare, 2016.

10. Mukherjee, A. et al. Pharmacokinetics of isoniazid, rifampicin, pyrazinamide and ethambutol in Indian children. BMC Infect. Dis. 15, 126 (2015).

11. Ramachandran, G. et al. Pharmacokinetics of first-line anti-tuberculosis drugs in HIV-infected children with tuberculosis treated with intermittent regimens in India. Antimicrob. Agents Chemother. 59, 1162–1167 (2015).

12. Ramachandran, G. et al. Age, nutritional status and INH acetylator status affect pharmacokinetics of anti-tuberculosis drugs in children. Int. J. Tuberc. Lung Dis. 17, 800–806 (2013).

13. Swaminathan, S. et al. Drug concentration thresholds predictive of therapy failure and death in children with tuberculosis: bread crumb trails in random forests. Clin. Infect. Dis. 63 (Suppl 3), S63–74 (2016).

14. Jain, S.K. et al. Pediatric tuberculosis in young children in India: a prospective study. Biomed. Res. Int. (2013).

15. Swaminathan, S. & Ramachandran, G. Challenges in childhood tuberculosis. Clin. Pharmacol. Ther. 98, 240–244 (2015).

16. Upton, R.N. & Mould, D.R. Basic concepts in population modeling, simulation, and model-based drug development: part 3-introduction to pharmacodynamic modeling methods. CPT Pharmacometrics Syst. Pharmacol. 3, e88 (2014).

17. Mould, DR. & Upton, R.N. Basic concepts in population modeling, simulation, and model-based drug development. Part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometrics Syst. Pharmacol. 2, e38 (2013).

18. Zvada, S.P. et al. Population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis in sinus evaluation of currently recommended doses. J. Antimicrob. Chemother. 69, 1339–1349 (2014).

19. Milán-Segovia, R.D.C. et al. Relative bioavailability of rifampicin in a three-drug fixed-dose combination formulation. Int. J. Tuberc. Lung Dis. 14, 1454–1460 (2010).

20. Wilkins, J.J. et al. Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption. Antimicrob. Agents Chemother. 52, 2138–2148 (2008).

21. Barroso, E.C. et al. Serum concentrations of rifampicin, isoniazid, and intestinal absorption, permeability in patients with multidrug resistant tuberculosis. Am. J. Trop. Med. Hyg. 81, 322–329 (2009).

22. Gurumurthy, P. et al. Decreased bioavailability of rifampicin and other antituberculosis drugs in patients with advanced human immunodeficiency virus disease. Society 48, 4473–4475 (2004).

23. Graham, S.M. et al. Low levels of pyrazinamide and ethambutol in children with tuberculosis and impact of age, nutritional status, and human immunodeficiency virus infection. Society 50, 407–413 (2006).

24. Bekker, A. et al. Ethambutol in infants dosed according to revised WHO-recommended treatment guidelines. Antimicrob. Agents Chemother. 60, 2171–2179 (2016).

25. Jayaram, R. et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrob. Agents Chemother. 47, 2118–2124 (2003).

26. Gumbo, T., Pasipanodya, J.G., Nuemberger, E., Romero, K. & Hanna, D. Correlations between the hollow fiber model of tuberculosis and therapeutic events in tuberculosis patients: learn and confirm. Clin. Infect. Dis. 61 (Suppl 1), S18–24 (2015).

27. Munoz-Sellart, M., Yassin, M.A., Tumato, M., Merid, Y. & Cuevas, L.E. Treatment outcome in children with tuberculosis in southern Ethiopia. Scand. J. Infect. Dis. 41, 450–455 (2009).

28. Hallu, D., Abegaz, W.E. & Belagirl, M. Childhood tuberculosis and its treatment outcomes in Addis Ababa: a 5-years retrospective study. BMC Pediatr. 16, 61 (2014).

29. McMillan, H. et al. Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight? Implications for international dosing guidelines. Antimicrob. Agents Chemother. 56, 3232–3238 (2012).

30. Antwi, S. et al. Pharmacokinetics of the first-line antituberculosis drugs in Ghanaian children with tuberculosis with or without HIV coinfection. Antimicrob. Agents Chemother. 61 (2017).

31. Mathew JL. Evidence in health-care practice! Missing the forest for the trees? Clin. Epidemiol. Global Health Elsevier; 2, 97–100 (2014).

32. Anderson, B.J. & Holford, N.H.G. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab. Pharmacokinet. 24, 25–36 (2009).

33. Jeremiah, K. et al. Nutritional supplementation increases rifampin exposure among tuberculosis patients coinfected with HIV. Antimicrob. Agents Chemother. 58, 3468–3474 (2014).

34. Merle, C.S. et al. High-dose rifampicin tuberculosis treatment regimen to reduce 12-month mortality of TB/HIV co-infected patients: The RAFA trial results. 21st Int AIDS Conf. (2017).

35. Boeree, M.J. et al. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. Lancet Infect. Dis. Published by Elsevier. This is an Open Access article under the CC BY license; 17, 39–49 (2016).

36. Chirehwa, M.T. et al. Model-based evaluation of higher doses of rifampin using a semimechanistic model incorporating autoinduction and saturation of hepatic extraction. Antimicrob. Agents Chemother. 60, 487–494 (2016).

37. Frydenberg, A.R. & Graham, S.M. Toxicity of first-line drugs for treatment of tuberculosis in children: review. Trop. Med. Int. Health. 14, 1329–1337 (2009).

38. Loos, U. et al. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. Klin Wochenschr. 63, 1206–1211 (1985).

39. Smythe, W. et al. A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients. Antimicrob. Agents Chemother. 56, 2091–2098 (2012).

40. National AIDS control organisation. Guidelines for HIV care and treatment in infants and children. November 2006.

41. Ramasamy, K.H. et al. A validated high-performance liquid chromatography method for the determination of rifampicin and desacetyl rifampicin in plasma and urine. Indian J. Pharmacol. 36, 231–233 (2004).

42. Hemanth Kumar, G.R. Simple and rapid liquid chromatography method for determination of moxifloxacin in plasma. J. Chromatogr. B 877, 1205–1208 (2009).

43. Savic, R.M., Janker, D.M., Kerbusch, T., & Karlsson, M.O. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J. Pharmacokinet. Pharmacodyn. 34, 711–726 (2007).

44. Ellard, G.A. & Gammon, P.T. Pharmacokinetics of isoniazid metabolism in man. J. Pharmacokin. Biopharm. 4, 83–113 (1976).

45. Keizer, R.J., Karlsson, M.O. & Hooker, A. Modeling and simulation workbench for NONMEM: tutorial on Pirana, PnX, and Xpose. CPT Pharmacometrics Syst. Pharmacol. 2, e50 (2013).

46. Beal, S., Sheiner, L.B., Boeckmann, A., & Bauer RJ. NONMEM User’s Guides 1989-2009 (Icon Development Solutions. Ellicott City, MD, 2009).

47. Bergstrand, M., Hooker, A.C., Wallin, J.E. & Karlsson, M.O. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 13, 143–151 (2011).

48. Acharya, C., Hooker, A.C., Türküyilmaz, G.Y., Jönsson, S. & Karlsson, M.O. A diagnostic tool for population models using non-compartmental analysis: the ncappc package for R. Comput. Methods Programs Biomed. Elsevier Ireland; 127, 83–93 (2016).

49. World Health Organization (WHO). Target Regimen Profiles for TB Treatment. Geneva Contract No WHO/HTM/TB/201616. 2016.