British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2019) **85** 644–654 644

ORIGINAL ARTICLE

Optimization of dosing regimens of isoniazid and rifampicin in children with tuberculosis in India

Correspondence Binu Susan Mathew, Department of Pharmacology & Clinical Pharmacology, Christian Medical College, Vellore, Tamil Nadu, India. Tel.: +91 416 228 2721; Fax: +91 416 228 4237; E-mail: binusphilip@gmail.com

Received 19 June 2018; **Revised** 28 November 2018; **Accepted** 14 December 2018

Blessed Winston Aruldhas[1], Richard M. Hoglund[4,5], Jaya Ranjalkar[1,*], Joel Tarning[4,5], Sumith K. Mathew[1], Valsan Philip Verghese[2], Anuradha Bose[3] and Binu Susan Mathew[1]

[1]Department of Pharmacology & Clinical Pharmacology, Christian Medical College, Vellore, Tamil Nadu, India, [2]Department of Paediatrics, Christian Medical College and Hospital, Vellore, Tamil Nadu, India, [3]Department of Community Health, Christian Medical College, Vellore, Tamil Nadu, India, [4]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and [5]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

*The authors confirm that the PI for this paper is Dr Jaya Ranjalkar and that she had direct clinical responsibility for patients.

Keywords dose adequacy, isoniazid, paediatric, population pharmacokinetic, rifampicin, tuberculosis

AIMS

Pharmacokinetic studies in the past have shown inadequate antituberculosis drug levels in children with the currently available dosing regimens. This study attempted to investigate the pharmacokinetics of isoniazid and rifampicin, when used in children, and to optimize their dosing regimens.

METHODS

Data were collected from 41 children, aged 2–16 years, who were being treated with antituberculosis drugs for at least 2 months. Concentration measurements were done for 6 h and analysed using a nonlinear, mixed-effects model.

RESULTS

Isoniazid pharmacokinetics were described by a one-compartment disposition model with a transit absorption model (fixed, $n = 5$). A mixture model was used to identify the slow and fast acetylator subgroups. Rifampicin was described by a one-compartment disposition model with a transit absorption model (fixed, $n = 9$). Body weight was added to the clearance and volume of distribution of both the drugs using an allometric function. Simulations with the isoniazid model showed that 84.9% of the population achieved therapeutic peak serum concentration with the planned fixed-dose combination regimen. Simulations with the rifampicin model showed that only about 28.8% of the simulated population achieve the therapeutic peak serum concentration with the fixed-dose combination regimen. A novel regimen for rifampicin, with an average dose of 35 mg kg^{-1}, was found to provide adequate drug exposure in most children.

CONCLUSIONS

The exposure to isoniazid is adequate with present regimens. For rifampicin, a novel dosing regimen was developed to ensure adequate drug concentrations in children. However, further studies are required to assess the dose–effect relationship of higher doses of rifampicin.

DOI:10.1111/bcp.13846

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Therapeutic targets for plasma concentration of isoniazid and rifampicin are already known in the treatment of tuberculosis.
- Exposure to antituberculosis drugs is generally known to be lower in children compared to the therapeutic targets using standard dosing regimens.

WHAT THIS STUDY ADDS

- This study demonstrates that the exposure of isoniazid is adequate while that of rifampicin is inadequate using standard dosing regimens.
- The pharmacometric analysis of data presented here, suggests that a dose of 35 mg kg^{-1} is required to achieve adequate peak concentrations of rifampicin in most children.

Introduction

Tuberculosis is a global health problem, with an estimated annual global incidence of 10.4 million cases in 2016. With an annual incidence of 2.79 million cases, India accounts for 25% of the total global burden and has been found to have the largest number of cases per country [1]. Of these, 230 000 cases were in children younger than 14 years [1]. Disease burden in children is likely to be underestimated due to difficulties in accurate diagnosis, as paediatric pulmonary tuberculosis is most often smear-negative and culture confirmation is not widely available in resource-poor settings [2].

The standard treatment regimens of tuberculosis have minor variations in drug doses between the recommendations from the World Health Organization (WHO) and those of various national health programmes. The total duration of therapy includes 2 months of an intensive phase followed by 4 months of a maintenance phase in most regimens. Isoniazid and rifampicin are the two first-line drugs used in the treatment of tuberculosis throughout the treatment period, with additional pyrazinamide and ethambutol given in the initial intensive phase [3].

The Revised National Tuberculosis Control Programme (RNTCP) in India has provided free treatment since 1993, as directly observed treatment short course (Regimen 1 in Table 1). The initial regimen was changed in 2012 (Regimen 2, Table 1) to follow the WHO Rapid Advice from 2010 [4]. With the trend shifting to fixed-dose combinations (FDC) in the treatment of tuberculosis, a newer FDC-based regimen is being implemented (Regimen 3, Table 1) [5]. Regimen 3 is a daily regimen with different doses of isoniazid (7.1–12.5 mg kg^{-1}) and rifampicin (10.7–18.8 mg kg^{-1}), which is closer to the currently recommended dosing by WHO [6]. Although the national programme follows a unified approach to therapy, the choice of the regimen in the private sector is often based on the clinician's discretion.

Even with improvements in the dosing regimen, pharmacokinetic studies have shown that the currently recommended dosage regimens result in inadequate drug levels in children aged 2 months to 12 years [7–9]. Similar results have also been observed with earlier regimens in studies from India [10, 11]. Suboptimal blood levels could lead to treatment failure and the emergence of drug resistance [2]. This emphasizes the need for further pharmacokinetic studies from which new dose regimens can be proposed.

The pharmacokinetics of isoniazid and rifampicin are complex due to their unique properties. Isoniazid metabolism is dependent on the genotype of the N-acetyl transferase enzyme, which explains the large variability in its clearance [8]. Rifampicin induces multiple cytochrome P450 enzymes and glucuronidation enzymes, including those responsible for its own metabolism, causing metabolic autoinduction of rifampicin, which might lead to drug interactions when combined with other drugs [9]. The clinical efficacy of these drugs is known to correlate with the concentration attained in the patient [12]. For example, a peak isoniazid concentration (C_{max}) of <3 μg ml^{-1} after a daily regimen, or <9 μg ml^{-1} after a twice-weekly regimen are considered ineffective [12]. There are no well-defined optimal plasma concentrations available for thrice-weekly therapy. However, a study performed in children found a trend to unfavourable outcomes associated with peak concentrations <6 μg ml^{-1}, which can therefore, be considered a cut-off for clinical efficacy in the absence of more evidence [13]. Also for isoniazid, based on *in vitro* studies and *in vivo* studies, a minimum exposure after one dose ($AUC_{0–24\ h\ after\ dose}$) of 10.52 μg × h ml^{-1} is considered adequate in terms of early bactericidal activity and therefore a marker for clinical efficacy [14, 15]. For rifampicin, a peak concentration of 8–24 μg ml^{-1} is considered optimal for good clinical efficacy, and concentrations <8 μg ml^{-1} and <4 μg ml^{-1} are considered low and very low, respectively [12].

The aim of this study was to investigate the pharmacokinetic properties of isoniazid and rifampicin in children in southern India and to evaluate the exposure associated with the dose regimens of these drugs in the current study.

Methods

Study design

The present study was conducted at the Clinical Pharmacology Unit and the Departments of Paediatrics and Community Health at Christian Medical College, Vellore, Tamil Nadu, India. The overall clinical efficacy and safety results from this study have been published previously [10]. Children and adolescents aged 2–16 years (hereafter referred to as children) who were newly diagnosed with pulmonary or lymph node tuberculosis with a minimum blood haemoglobin of 10 g dl^{-1} were recruited (demographic characteristics in

Table 1

Regimens in the treatment of tuberculosis

Regimen 1 Past: National programme before 2012		Regimen 2 Present: National; programme after 2012		Regimen 3 Future: Planned to be implemented in future		Regimen 4 Optimized: Simulated to attain required therapeutic range	
Weight in kg	**Dose in mg (mg kg^{-1})**	**Weight in kg**	**Dose in mg (mg kg^{-1})**	**Weight in kg**	**Dose in mg (mg kg^{-1})**	**Weight in kg**	**Dose in mg (mg kg^{-1})**
Isoniazid							
6–10	75 (12.5–7.5)	6–7	75 (12.5–10.7)	4–7	50 (12.5–7.14)		
11–17	150 (13.6–8.8)	8–11	112.5 (14.06–10.2)	8–11	100 (12.5–9.1)		
18–25	225 (12.5–9)	12–15	150 (12.5–10)	12–15	150 (12.5–10)		
26–30	300 (11.5–10)	16–17	187.5 (11.7–11.1)	16–24	200 (12.5–8.3)		
		18–22	225 (12.5–10.2)	25–29	225 (9–7.7)		
		23–30	300 (13.6–10)	30–39	250 (8.3–6.4)		
Rifampicin							
6–10	75 (12.5–7.5)	6–7	75 (12.5–10.7)	4–7	75 (18.7–10.7)	6–11	35 mg kg^{-1}[a] (suspension)
11–17	150 (13.6–8.8)	8–11	112.5 (14.06–10.2)	8–11	150 (18.7–13.6)	12–14	450 (37.5–32.1)
18–25	225 (12.5–9)	12–15	150 (12.5–10)	12–15	225 (18.7–15)	15–19	600 (31.5–40)
26–30	300 (11.5–10)	16–17	187.5 (11.7–11.1)	16–24	300 (18.7–12.5)	20–23	750 (37.5–32.6)
		18–22	225 (12.5–10.2)	25–29	375 (15–12.9)	24–27	900 (37.5–33.3)
		23–30	300 (13.6–10)	30–39	450 (15–11.5)	28–30	1050(37.5–35)

Regimen 1 is a thrice-weekly regimen. Regimen 2 is given as either daily (World Health Organization recommended) or thrice weekly (earlier recommendation by Revised National Tuberculosis Control Programme). However, the dose given at an occasion in both daily and thrice-weekly regimen are the same. Regimen 3 is an fixed-dose combination-based daily regimen

In the proposed regimen 4, an average dose of 35 mg kg^{-1} body weight was maintained in all weight bands using the already available tablet strengths. A high dose of 35 mg kg^{-1} was easily accessible for younger children (6–11 kg) by using an already available suspension formulation ([a]) of the drug

Table 2). Children with disease relapse, multidrug resistant tuberculosis and co-existing human immunodeficiency infection were excluded from the study. Isoniazid and rifampicin were given along with pyrazinamide and ethambutol to all the children in the intensive phase (first 8 weeks) of therapy either thrice weekly or daily. All the children received isoniazid and rifampicin for a minimum period of 6 months. The pharmacokinetic samples were collected during the last 2 weeks of the intensive therapy, ensuring that steady-state levels had been achieved [9].

Table 2

Demographics characteristics of the enrolled study children (n = 41)

Characteristic	Value
Age (years)	7 (3.5–13)
Sex (male/female)	29/12
Body weight (kg)	19.5 (13.7–33.7)
Height (cm)	118 (97–154)
Body mass index	14.8 (13.9–15.6)
Z score	−1.22 (−2.41 to −0.74)
Diagnosis	
Pulmonary tuberculosis	36
Lymph node tuberculosis	5

All results are expressed as median (interquartile range) unless otherwise specified

The Department of Paediatrics prescribed the daily WHO regimen as unobserved treatment [4], while the children from the peripheral care centre of the Department of Community Health were prescribed the thrice-weekly directly observed treatment according to the RNTCP recommendation [16]. Both regimens are similar in terms of the actual dose given in a day but differ with regards to the frequency i.e. either daily or thrice weekly. Target dosage (mg kg^{-1} dose) is available in Table 1 (Regimen 2). On the day of sampling, participating children were kept fasting (overnight) with no restriction on water. Ethical approval for the study was given by the Institutional Review Board (IRB Min No. 8892, Christian Medical College, Vellore). The study was explained in their own language and informed consent was obtained from the parents or guardians, and whenever possible, consent was also taken from the child. All procedures were performed according to the revised version of the Declaration of Helsinki.

Specimen collection and analytical methods

Information on age, sex, body mass index, weight and comedication history was recorded for all the children during the initial visit and on the day of pharmacokinetic study. On the day of the study, blood samples were collected before dose administration (trough concentration) and at 0.5, 1, 1.5, 2, 2.5, 4 and 6 h post-dose. The samples were collected in EDTA-containing vacutainers and were transported to the laboratory in a Styrofoam box filled with ice packs. The plasma was then immediately separated by centrifugation. Isoniazid concentration measurement was performed within 5 h from the time of collection using a validated LC–MS/MS. Rifampicin was measured using a validated HPLC-UV assay within 72 h of collection. The validation of these assays has been described previously [10]. The lower limit of quantification (LLOQ) for isoniazid and rifampicin were 0.01 μg ml^{-1} and 0.04 μg ml^{-1}, respectively. The precision (coefficient of variation) of the isoniazid assay was 6.25% and 1.70% for concentrations 0.1 μg ml^{-1} and 10 μg ml^{-1}, respectively. The precision of the rifampicin assay was 3.95% and 1.62% for concentrations of 0.24 μg ml^{-1} and 10 μg ml^{-1}, respectively.

Population pharmacokinetic modelling

Isoniazid and rifampicin concentration–time data were characterized separately using nonlinear mixed-effects modelling, in the software NONMEM 7.3. The first-order conditional estimation method with η–ε interaction (FOCE-I) was used throughout modelling. The pharmacokinetic parameters were estimated using the natural-log transformed plasma concentrations [17, 18]. Perl-Speaks-NONMEM v.4.6.0 [19], R v.3.3.0 (R Foundation for Statistical Computing) [20] with the Xpose4 package v.4.6.0 [21], RStudio [22] and Pirana v.2.9.6 [23] were used for data exploration, diagnostics, graphics and automation throughout the modelling process. Measured plasma concentrations below the LLOQ were imputed with half of the LLOQ (Beal's M5-method) [24]. Several disposition models (e.g. one-, two- and three-compartment disposition models) and absorption models [first-order absorption with and without lag, and transit compartment models with a fixed number of transit compartments (1–15) with k_a and k_{tr} assumed equal or estimated separately] were evaluated [25]. Relative bioavailability (F) was fixed to unity for the population but with an estimated interindividual variability. An exponential error model was used to describe the interindividual variability in the pharmacokinetic parameters and the residual unknown variability was described by an additive error on the individually predicted logarithmic concentrations (i.e. essentially equivalent to an exponential error on the arithmetic scale).

Effect of both body weight and fat-free mass were evaluated with allometric scaling on all clearances and volumes parameters with an exponent of 0.75 and 1, respectively [26]. In addition, the exponents were also estimated. Biologically plausible covariates (e.g. age, sex, body mass index, weight, height and albumin) were tested with a step-wise covariate approach using a forward selection (P = 0.05) and a more stringent backward elimination step (P = 0.01) [27]. For isoniazid, a mixture model was used evaluated to describe the different clearances in patients with slow and fast acetylator status (since the genotype was not known).

Visual inspection of the goodness of fit of observed versus predicted concentration–time profile and the objective function value (OFV), proportional to −2 times the log-likelihood of data, were used for model discrimination. A reduction in OFV of 3.84 was considered a significant improvement in model fit ($P < 0.05$) between two nested models, with one degree of freedom difference. The predictive performance of the models was evaluated using visual predictive checks (n = 1000). Shrinkage of both η and ε were calculated to determine the reliability of diagnostic plots [28]. The robustness of the final parameter estimates was determined using bootstrapping by generating 1000 resampled datasets. The *posthoc* individual pharmacokinetic parameter estimates were used to calculate individual C_{max} and exposures ($AUC_{0–24}$).

Dose optimization

The developed final population pharmacokinetic models were used to evaluate the exposure of isoniazid and rifampicin through stochastic simulations in NONMEM. A total of 1000 individuals were simulated per kg of body weight from 6 to 30 kg for every currently recommended dose regimen and, if necessary, for an improved dose regimen. Steady-state peak concentrations and exposures were evaluated as a proxy for the efficacy of the different regimens.

Results

A total of 41 children completed the study, of whom 27 were on the thrice-weekly intermittent regimen recommended by RNTCP and 14 were on the daily regimen recommended by the WHO. For isoniazid, there were a total of 290 concentration–time measurements from 39 children, as samples from two children could not be measured due to an LC–MS/MS breakdown on the day of sampling. For rifampicin, there were 284 concentration–time measurements from 39 children, with two children excluded from the modelling process as the concentration–time profile showed an unreasonable delay in absorption of around 2 h, which could have been due to an error in recording sampling time or due to measurement errors. A total of 20 (7%) out of 290 observations and 45 (16%) out of 284 observations were below the LLOQ for isoniazid and rifampicin, respectively. All the observations below LLOQ were part of the elimination phase and almost exclusively trough concentrations for both the drugs.

Pharmacokinetic properties of isoniazid

Isoniazid concentration–time data were most adequately described by a one-compartment disposition model. A two-compartmental disposition model resulted in a significantly improved model fit compared to a one-compartmental model (ΔOFV = −6.8; 2df) but produced a very high interindividual variability on the peripheral volume of distribution and showed a low precision in this parameter estimate (RSE = 66% CV). The absorption phase was best described by a transit compartment model with five fixed transit compartments (k_A and k_{TR} were set to be equal since estimating them separately did not improve the model fit). The transit absorption model proved superior to the first order absorption model with lag (ΔOFV = −19.2). The addition of relative bioavailability improved the model fit significantly (ΔOFV = −16.6).

Body weight added allometrically to clearance and volume parameters improved the model significantly (ΔOFV = −37.3). Estimating the exponent of the allometric scaling did not improve the model fit and was therefore not pursued further (ΔOFV = −3.2). A mixture model was successfully implemented (ΔOFV = −11.9) to identify the two subgroups with different elimination clearances, resulting in an estimated 31% of children having rapid clearance (i.e. fast acetylator status). No other covariates could be retained in the stepwise covariate approach. Primary parameter estimates and secondary parameters derived from the final model are presented in Table 3.

A bootstrapping procedure found the parameter estimates to be reliable with acceptable relative standard errors (Table 3). The η shrinkages and ε shrinkage were found to be low (Table 3). Goodness-of-fit plots and the visual predictive check indicated that the model described the observed data well and had good predictive performance (Figure 1).

Pharmacokinetic properties of rifampicin

The pharmacokinetic properties of rifampicin were best described by a one-compartment disposition model. A two-compartmental disposition model resulted in a significantly improved model fit compared to a one-compartmental model (ΔOFV = −34.0). However, this resulted in a negligible improvement in visual predictive check and goodness of fit diagnostics. The absorption phase was best described by a transit compartment model with nine transit compartments (ΔOFV = −156). k_A and k_{TR} were set to be equal, since estimating them separately did not improve the model fit. The addition of relative bioavailability improved the model fit significantly (ΔOFV = −48.2). Body weight was added allometrically to clearance and volume parameters resulted in a significant improvement in model fit (ΔOFV = −7.3). Estimating the allometric scaling did not improve the model fit further (ΔOFV =3.4). The autoinduction of metabolizing enzymes could not be estimated since only steady-state samples were collected. No other covariates could be retained in the stepwise covariate approach. Primary parameter estimates and secondary parameters derived from the final model are presented in Table 4.

A bootstrapping procedure found the parameter estimates to be reliable with acceptable relative standard errors (Table 4). The η shrinkages and ε shrinkage were found to be low (Table 4). Goodness-of-fit plots and the visual predictive check indicated that the model described the observed data well and had good predictive performance (Figure 1).

Dose optimization

The final population pharmacokinetic models were used to simulate the isoniazid and rifampicin concentration–time profiles for a population of children weighing 6–30 kg (n = 1000 at each bodyweight). Drug exposures (i.e. C_{max} and AUC_{0-24}) after administration of the investigated regimens were derived from the *posthoc* estimates from NONMEM. The percentage of children obtaining the target concentration with the different dosage regimens is summarized in Table 5.

For isoniazid, when given as Regimen 2 (current regimen), the simulations suggest that only 59% of all patients had a $C_{max} > 6$ μg ml^{-1} when given as the thrice weekly regimen while 96% had a $C_{max} > 3$ μg ml^{-1} when given as the daily

Table 3

Population pharmacokinetic parameters of isoniazid

Parameter	Population estimates (%RSE)	95% CI	%CV for BSV [%RSE]	95% CI	Shrinkage (%)
CL_{SLOW}/F (l h^{-1})	2.59 (9.95)	2.12–3.17	-	-	-
CL_{FAST}/F (l h^{-1})	7.79 (7.38)	6.71–9.01	-	-	-
Vc/F (l)	29.7 (7.66)	25.3–34.6	23.4 (14.1)	16.9–30.2	25.4
MTT(h)	0.547 (12.6)	0.411–0.695	68.2 (13.9)	49.3–94.3	13.8
No. of trans comp	5 fix	-	-	-	-
F (%)	100 fix	-	41.8 (12.1)	30.1–51.9	23.5
RUV	0.0967 (9.79)	0.0639–0.141	-	-	29.46
Secondary parameters					
$AUC_{(0-24)}$ (mg × h l^{-1})	34.04 (23.29–44.23)[a]				
C_{max} (mg l^{-1})	5.90 (4.12–7.73)[a]				
T_{max} (h)	1.10 (0.82–1.43)[a]				

Population estimates are given for a typical child weighing 19.4 kg with tuberculosis. CL_{FAST} is the apparent elimination clearance in patients with fast acetylator status (seen in 31% of children) and CL_{SLOW} is the apparent elimination clearance in patients with slow acetylator status (seen in 69% of children), as derived by a mixture model. Vc/F is the apparent volume of distribution of the central compartment. MTT is the mean transit time. No. of trans comp is the number of transit compartments used in the absorption model. F is the relative bioavailabilty. BSV is between subject variability. RSE is relative standard error and is calculated as 100 × (standard deviation/mean). RUV is the residual unexplained variability. C_{max} is the maximum concentration. T_{max} is the time after dose to reach C_{max}. AUC is the area under the concentration–time curve from time 0 to 24 h
[a]values are presented as median (interquartile range)

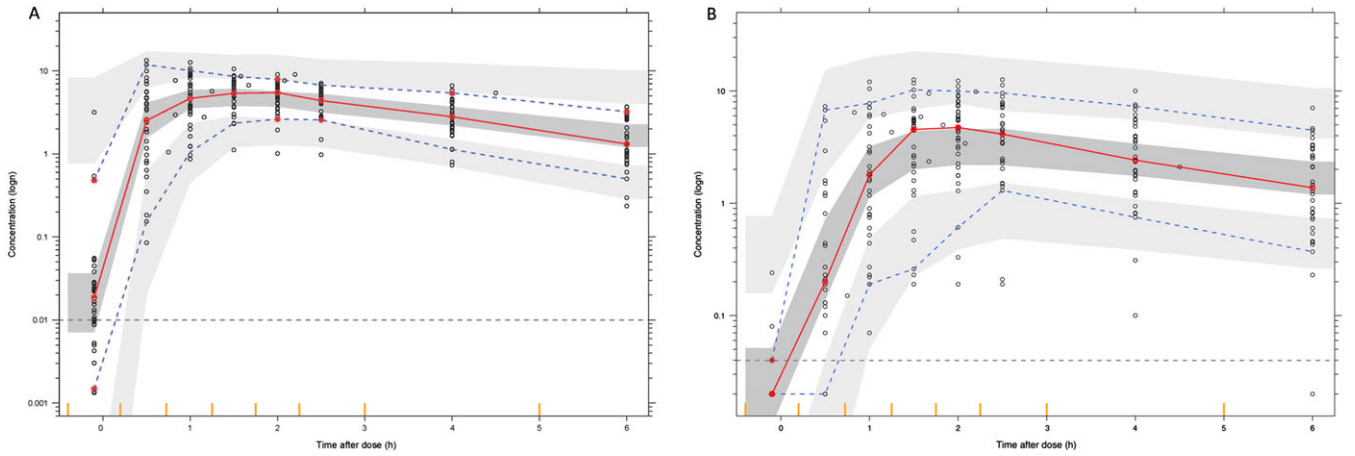

Figure 1

Visual predictive checks of the final models. (A) Prediction corrected visual predictive check of the final population pharmacokinetic model of isoniazid. (B) Visual predictive check of the final population pharmacokinetic model of rifampicin. Both A and B are based on 1000 stochastic simulations. Open circles represent the observations, and solid lines represent 50th percentile while the dotted lines represent the 5th and 95th percentiles of the observed data. The shaded areas represent the 95% confidence intervals around the simulated 5th, 50th, and 95th percentiles. The horizontal dotted line represents the lower limit of quantification

Table 4

Population pharmacokinetic parameters of rifampicin

Rifampicin	Population Estimates (%RSE)	95% CI	%CV for BSV [%RSE]	95% CI	Shrinkage (%)
CL/F (l h^{-1})	8.11 (10.9)	6.62–10.1	-	-	-
Vc/F (l)	44.7 (14.6)	34.2–59.5	42 (12.6)	31.1–53.9	3.40
MTT (h)	0.932 (9.92)	0.743–1.12	52.2 (12.7)	39.7–68.0	5.06
No. of trans comp	9 fix	-	-	-	-
F (%)	100 fix	-	68.0 (16.6)	40.0–95.6	10.71
RUV	0.271 (8.96)	0.191–0.379	-	-	19.61
Secondary parameters					
AUC $_{(0-24)}$ (mg × h l^{-1})	25.19 (15.69–34.85)[a]				
C_{max} (mg l^{-1})	4.73 (2.74–6.23)[a]				
T_{max} (h)	1.59 (1.31–2.12)[a]				

Population estimates are given for a typical child weighing 19.4 kg with tuberculosis. CL/F is the apparent elimination clearance, Vc/F is the apparent volume of distribution of the central compartment, MTT is the mean transit time. No. of trans comp is the number of transit compartments used in the absorption model. F is the relative bioavailabilty. BSV is between subject variability. RSE is relative standard error and is calculated as 100 × (standard deviation/mean). RUV is the residual unexplained variability. C_{max} is the maximum concentration. T_{max} is the time after dose to reach C_{max}. AUC is the area under the concentration–time curve from time 0 to 24 h

[a]values are presented as median (interquartile range)

regimen. However, 99.9% of all the patients had an AUC_{0-24} > 10.5 μg × h ml^{-1} for both daily and thrice-weekly regimens (Figure 2), which should be considered adequate treatment. The simulated concentrations with the planned Regimen 3 resulted in a C_{max} > 3 μg ml^{-1} in about 88% of patients and simulated AUC_{0-24} > 10.5 μg × h ml^{-1} in 96% of all patients. The percentage of patients attaining the target in each of the acetylator statuses is elaborated in Table 5. Fast acetylators are generally at higher risk of underexposure, but 85% of the patients had the required C_{max} > 3 μg ml^{-1} and 94% of patients had the required AUC > 10.5 μg × h ml^{-1} with regimen 3. Thus, there was no need to improve the dosing regimen further for isoniazid.

For rifampicin, all the regimens were found to show a low exposure (C_{max}) compared to the therapeutic level (Figure 3). Even when simulated with the planned regimen 3, only 29% of patients had a C_{max} > 8 μg ml^{-1} while 34% had a C_{max} < 4 μg ml^{-1}. To improve the treatment with rifampicin, multiple novel regimens were tried using available formulations and a new and improved dose regimen was derived, as

Table 5

Target attainment in a simulated population with adequate exposure with various regimens

Drug	Target	Percentage of simulated population who attained target			
		Regimen 1	Regimen 2	Regimen 3	Regimen 4
Isoniazid					
Fast acetylators	$C_{max} > 6$ μg ml^{-1}	41.6	47.6		
	$C_{max} > 3$ μg ml^{-1}	88.6	90.6	84.9	
	$AUC_{0-24} > 10.5$ μg × h ml^{-1}	95.9	97.1	94.1	
Slow acetylators	$C_{max} > 6$ μg ml^{-1}	60.6	73.1		
	$C_{max} > 3$ μg ml^{-1}	95.9	98.6	94.4	
	$AUC_{0-24} > 10.5$ μg × h ml^{-1}	99.9	100	99.9	
Rifampicin					
	$C_{max} > 8$ μg ml^{-1}	15.1	17.8	28.8	74.2
	$C_{max} < 4$ μg ml^{-1}	53.7	48.9	34.3	5.2

Isoniazid: $C_{max} > 6$ μg ml^{-1} and > 3 μg ml^{-1} are considered as therapeutic targets in thrice-daily and daily therapy respectively. An $AUC_{0-24} > 10.5$ μg × h ml^{-1} is considered adequate in terms of early bactericidal activity
Rifampicin: $C_{max} > 8$ μg ml^{-1} is considered as therapeutic target and < 4 μg ml^{-1} is considered very low
All regimens are as described in Table 1. Regimen 3 is only daily therapy. Regimen 4 was simulated only for rifampicin as exposure for isoniazid with present regimen was deemed adequate

presented in Table 1. Using this regimen, 74% of patients attained a $C_{max} > 8$ μg ml^{-1} with an average dose of 35 mg kg^{-1} (Figure 4).

Discussion

This study developed a population pharmacokinetic models for the antituberculosis drugs isoniazid and rifampicin. The developed model was used to evaluate the current dosing regimens and to derive a novel regimen that would reach the recommended therapeutic target concentration.

Isoniazid pharmacokinetics were described by a one-compartment disposition model. Apparent elimination clearances and apparent volume of distribution of the central compartment reported earlier [7, 29], differs slightly compared to the values estimated with the present model but this difference could potentially be attributed to ethnicity, differences in age distribution of the children, and the different methods of classification of acetylator status. In contrast to adults, data on isoniazid elimination clearance in children are scarce. The median simulated AUC_{0-8} obtained in this study was 25.53 [interquartile range (IQR) 18.22–31.76], which is much lower compared to the median AUC_{0-8} of 41.1 (IQR 33.0–59.9) obtained in an adult population in a study from India [30]. One of the limitations of the present study is that the acetylator status of the children is not known. If it was known, it could have been included as a categorical covariate in the present model. However, this was handled by introducing the mixture model, which adequately described the population size of the two groups. In the present model, 30.8% were found to be fast acetylators and 69.2% to be slow acetylators. This finding is supported by results from another study on the phenotype of acetylation status in India where 66% were found to be slow acetylators [31]. An interesting observation with the model was that the maximum concentration of isoniazid was reached at 1.1 h, with an IQR of 0.8–1.4 h. This information could help in designing future studies in children as this is in contrast with most studies, which assumed that maximum concentrations would be reached at 2 h.

Rifampicin pharmacokinetics were described by a one-compartment disposition model. Although the two-compartment disposition model provided a better OFV did not improve in terms of VPC. Moreover, the two-compartment model also produced an implausible terminal elimination half-life of 1220 h compared to published literature (2–3 h). The children in the present study, although densely sampled, were only sampled for a period of 6 h to minimize inconvenience for them, which a prolonged sampling schedule would have caused. This lack of late samples might limit the information about the elimination phase, which could have resulted in this effect. Most published literature also describe rifampicin by a one-compartment disposition model [7, 9]. Thus, a one-compartment model was carried forward in the modelling process. The hepatic clearance of rifampicin nearly doubles over the course of the therapy due to auto-induction and reached a steady state after 22–40 days [9, 32]. Since all the children in this study had around 60 days of rifampicin therapy before pharmacokinetic samples were collected, the clearance could be assumed to remain constant over time and an auto-induction model was not used. The parameter estimates obtained from the model developed was similar to that reported earlier [7, 29].

Simulation were performed to evaluate dose regimens for the different drugs. For isoniazid, 94% of all children on daily therapy with the present regimen had adequate simulated C_{max}. Moreover, about 99% of all children had simulated $AUC_{0-24} > 10.5$ μg h ml^{-1} irrespective of the frequency of dosing. After simulations with Regimen 3 (daily therapy with a

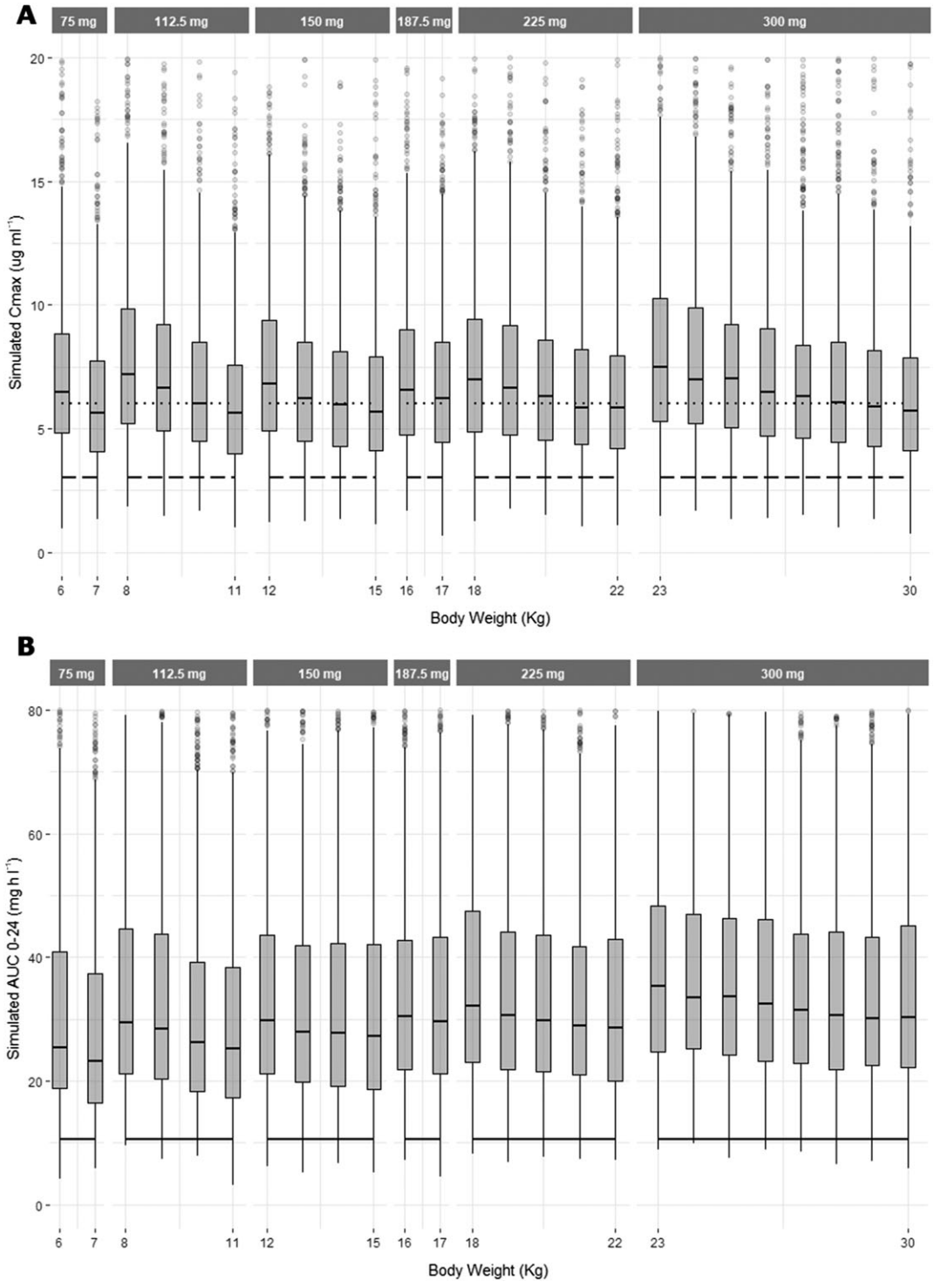

Figure 2

Stochastic simulated median, 25th and 75th percentiles of (A) maximum concentrations (C_{max}) and (B) area under the concentration–time curve (AUC) values after dosing with isoniazid according to national programme post-2012 (regimen 2). The dotted line in figure A represents the required therapeutically effective C_{max} of 6 μg ml^{-1} in an intermittent regimen, while the dashed line represents the therapeutically effective concentration of 3 μg ml^{-1} in the daily regimen. The solid line in figure B represents the AUC of 10.52 μg × h ml^{-1}, which is considered as therapeutically effective AUC

slightly lower mean per kg dose for isoniazid), adequate C_{max} and AUC were still obtained in most of the children. This was true even when simulated with fast acetylators alone. Therefore, it was decided that further optimization of the dose regimen for isoniazid was not necessary. Even if the dosing for children aged 2–16 years seems to be adequate, this

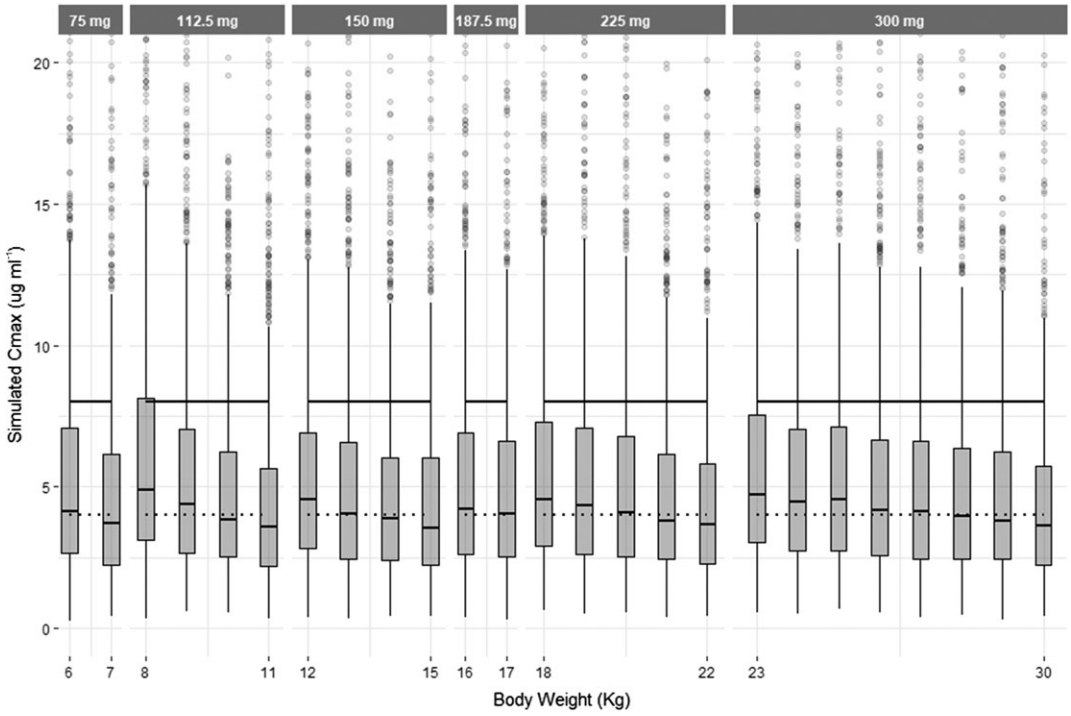

Figure 3

Stochastic simulated median, 25th and 75th percentiles of maximum concentration values after dosing with rifampicin according to national programme post-2012 (regimen 2). The solid line represents the required therapeutically effective concentration of 8 μg ml^{-1}, while the dotted line represents a concentration of 4 μg ml^{-1}, below which is considered very low

cannot be assumed to be true for younger children (age <2 years) as enzyme maturation needs to be taken into account for this group [33].

Exposure to rifampicin was generally very low in all regimens and only about 17.8% of the children had a high enough exposure with the widely used Regimen 2. Attaining the recommended C_{max} is important in rifampicin therapy to be able to cure the disease as its long postantibiotic effect is directly dependent on the peak concentration [34, 35]. The simulations performed showed that the WHO-recommended FDC-based regimen (Regimen 3, daily therapy with a slightly higher mean per kg dose for rifampicin) was better than the previous regimens, but with only 28.8% of the children getting adequate exposure. Therefore, simulations of a new optimal multiple dose regimen were investigated. Based on the simulations a dose of 35 mg kg^{-1} was found to result in adequate exposure in most patients receiving adequate treatment (74.2% above 8 μg ml^{-1} and only 5.15% below 4 μg ml^{-1}). Although this is an increased dose compared to that recommended by WHO, recent clinical trials in adults using higher doses of up to 35 mg kg^{-1} per kg of rifampicin have been shown to be safe when given daily [36, 37].

The dosing of rifampicin has been debated for a long time. The current adult dose of 600 mg was recommended in the 1970s based on the perception that toxicity was dose-related, and due to the high cost of rifampicin at that time [38]. With the current evidence of an adequate safety profile with higher doses (35 mg kg^{-1}) and lower manufacturing costs, dose revisions should be considered [36, 37]. To our knowledge, high-dose therapy has not yet been investigated in children. However, it has been shown that adverse events are generally lower in children compared to adults [39]. The flu-like symptoms initially considered to be related to high doses are now believed to be due to the intermittent nature of past therapies [40]. An ideal strategy would be to use regimens prescribed in current guidelines to initiate therapy and thereafter to individualize doses based on therapeutic drug monitoring. However, this might not be feasible in resource-limited settings. therefore, the practical approach would be to initiate therapy with higher doses of rifampicin, as has been simulated in the present study (approximately 35 mg kg^{-1}). Similar advice has been given recently by authors of other studies [29, 41]. Most current information on drug dosing is based on *in vivo* experiments and phase I trials without a clear concentration-clinical efficacy relationship [12]. Therefore, additional studies with both pharmacokinetic and pharmacodynamic data would be beneficial.

In conclusion, this study characterized the pharmacokinetic properties of isoniazid and rifampicin using nonlinear mixed-effects modelling. The current dosing schedules were adequate for isoniazid, but modelling and simulation suggested that rifampicin was under-dosed. The developed models were used to derive a new optimized dose regimen in order to attain recommended therapeutic concentrations and improve the treatment of tuberculosis in children.

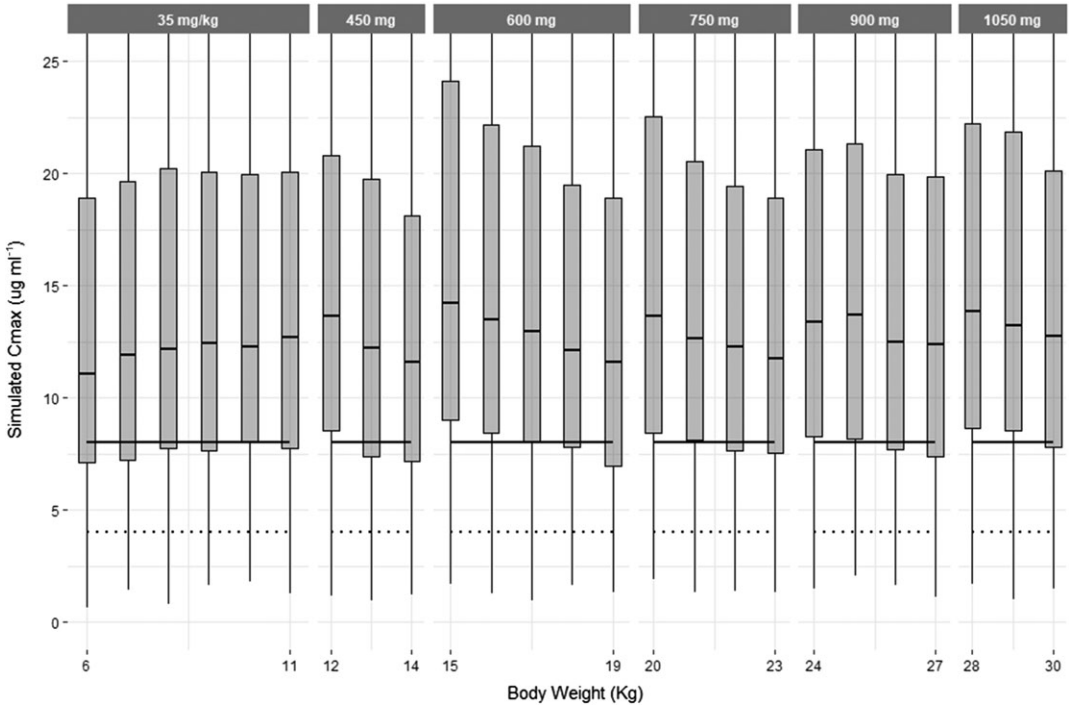

Figure 4

Stochastic simulated median, 25th and 75th percentiles of maximum concentration values after the derived optimal dose regimen for rifampicin (35 mg kg^{-1}). The band comprising of 6–11-kg children will use a suspension available commercially. Other weight bands will use regular capsules. The solid line indicates the required therapeutically effective concentration of 8 μg ml^{-1}, while the dotted line represents a concentration of 4 μg ml^{-1}, below which is considered very low

Further studies are needed to investigate the dose–effect relationship of antituberculous drugs in children, especially with higher doses of rifampicin.

Competing Interests

There are no competing interests to declare.

The authors thank the children who participated in this research study and their parents, without whom the study would not have been possible. We would like to thank Prof Jacob Peedicayil for his constructive criticism on the manuscript. We also thank the senior technicians Daisy Rani R. and Lavanya S. for the processing of plasma samples. This project was funded by the fluid research grant of the Christian Medical College, Vellore, Tamil Nadu, India. J.T. and R.M.H were partly funded by the Bill and Melinda Gates Foundation and the Wellcome Trust of Great Britain.

Contributors

B.W.A., R.M.H., J.T., J.R. and B.S.M. were responsible for the study design. J.R., S.K.M., B.S.M., V.P.V. and A.B. were involved in the acquisition of data. B.W.A., R.M.H. and J.T. were responsible for pharmacokinetic analysis. B.W.A. and R.M.H. analysed and interpreted the data and wrote the first draft of the report, although all authors had the opportunity to review the data and contributed to the editing of the final report.

References

1 WHO. Global tuberculosis report 2017. WHO. Available at http://www.who.int/tb/publications/global_report/en/ (last accessed 20 December 2017).

2 Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. Clin Infect Dis 2010; 50 (Suppl. 3): S184–94.

3 Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children. Geneva: World Health Organization, 2015.

4 Ridge A, Grzemska M, Hill S, Gie R, World Health Organization. Rapid Advice: Treatment of Tuberculosis in Children. 2010. Available at http://whqlibdoc.who.int/publications/2010/9789241500449_eng.pdf (last accessed 19 October 2017).

5 Chaudhuri AD. Recent changes in technical and operational guidelines for tuberculosis control programme in India - 2016: a paradigm shift in tuberculosis control. J Assoc Chest Physicians 2017; 5: 1.

6 FDC_Factsheet.pdf. Available at http://www.who.int/tb/FDC_Factsheet.pdf (last accessed 20 December 2017).

7 Zvada SP, Denti P, Donald PR, Schaaf HS, Thee S, Seddon JA, *et al*. Population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis: *in silico* evaluation of currently recommended doses. J Antimicrob Chemother 2014; 69: 1339–49.

8 Seng KY, Hee KH, Soon GH, Chew N, Khoo SH, Lee LSU. Population pharmacokinetic analysis of isoniazid,

acetylisoniazid, and isonicotinic acid in healthy volunteers. Antimicrob Agents Chemother 2015; 59: 6791–9.

9 Smythe W, Khandelwal A, Merle C, Rustomjee R, Gninafon M, Bocar Lo M, *et al*. A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients. Antimicrob Agents Chemother 2012; 56: 2091–8.

10 Ranjalkar J, Mathew SK, Verghese VP, Bose A, Rose W, Gupta D, *et al*. Isoniazid and rifampicin concentrations in children with tuberculosis, on either daily or intermittent regimen – implications for the revised RNTCP 2012 doses, in India. Int J Antimicrob Agents 2017; 51: 663–9.

11 Rangari GM, Roy V, Sethi GR, Mishra TK, Khanna A. Blood levels of isoniazid in Indian children with tuberculosis. Indian J Tuberc 2015; 62: 80–5.

12 Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 2002; 62: 2169–83.

13 Ramachandran G, Hemanth Kumar AK, Bhavani PK, Poorana Gangadevi N, Sekar L, Vijayasekaran D, *et al*. Age, nutritional status and INH acetylator status affect pharmacokinetics of anti-tuberculosis drugs in children. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis 2013; 17: 800–6.

14 Donald PR, Parkin DP, Seifart HI, Schaaf HS, van Helden PD, Werely CJ, *et al*. The influence of dose and N-acetyltransferase-2 (NAT2) genotype and phenotype on the pharmacokinetics and pharmacodynamics of isoniazid. Eur J Clin Pharmacol 2007; 63: 633–9.

15 Kiser JJ, Zhu R, D'Argenio DZ, Cotton MF, Bobat R, McSherry GD, *et al*. Isoniazid pharmacokinetics, pharmacodynamics, and dosing in south African infants. Ther Drug Monit 2012; 34: 446–51.

16 National Guidelines on Diagnosis and Treatment of Pediatric Tuberculosis; 2012. Available at https://tbcindia.gov.in/showfile.php?lid=2904 (last accessed 5 December 2017)

17 Beal SL, Sheiner LB. Estimating population kinetics. Crit Rev Biomed Eng 1982; 8: 195–222.

18 Beal SL, Sheiner LB, Boeckmann AJ, Bauer RJ (eds). NONMEM 7.3.0 Users Guides. (1989–2013). Available at ftp://nonmem.iconplc.com/Public/nonmem 730/guides (last accessed 5 December 2017).

19 Lindbom L, Pihlgren P, Jonsson EN, Jonsson N. PsN-toolkit – a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed 2005; 79: 241–57.

20 R Core Team. (2016). R: A language and environment for statistical computing. Available at https://www.R-project.org/ (last accessed 5 December 2017).

21 Jonsson EN, Karlsson MO. Xpose--an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed 1999; 58: 51–64.

22 RStudio Team. (2016). RStudio: Integrated Development for R. Available at http://www.rstudio.com/ (last accessed 5 December 2017).

23 Keizer RJ, van Benten M, Beijnen JH, Schellens JHM, Huitema ADR. Piraña and PCluster: a modeling environment and cluster infrastructure for NONMEM. Comput Methods Programs Biomed 2011; 101: 72–9.

24 Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn 2001; 28: 481–504.

25 Savic RM, Jonker DM, Kerbusch T, Karlsson MO. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J Pharmacokinet Pharmacodyn 2007; 34: 711–26.

26 Anderson BJ, Holford NHG. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol 2008; 48: 303–32.

27 Jonsson EN, Karlsson MO. Automated covariate model building within NONMEM. Pharm Res 1998; 15: 1463–8.

28 Savic RM, Karlsson MO. Importance of shrinkage in empirical Bayes estimates for diagnostics: problems and solutions. AAPS J 2009; 11: 558–69.

29 Guiastrennec B, Ramachandran G, Karlsson MO, Kumar AKH, Bhavani PK, Gangadevi NP, *et al*. Suboptimal antituberculosis drug concentrations and outcomes in small and HIV-coinfected children in India: recommendations for dose modifications. Clin Pharmacol Ther 2018; 104: 733–41.

30 Hemanth Kumar AK, Kannan T, Chandrasekaran V, Sudha V, Vijayakumar A, Ramesh K, *et al*. Pharmacokinetics of thrice-weekly rifampicin, isoniazid and pyrazinamide in adult tuberculosis patients in India. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis 2016; 20: 1236–41.

31 Pande JN, Pande A, Singh SPN. Acetylator status, drug metabolism and disease. Natl Med J India 2003; 16: 24–6.

32 Chirehwa MT, Rustomjee R, Mthiyane T, Onyebujoh P, Smith P, McIlleron H, *et al*. Model-based evaluation of higher doses of rifampin using a semimechanistic model incorporating autoinduction and saturation of hepatic extraction. Antimicrob Agents Chemother 2016; 60: 487–94.

33 Bekker A, Schaaf HS, Seifart HI, Draper HR, Werely CJ, Cotton MF, *et al*. Pharmacokinetics of isoniazid in low-birth-weight and premature infants. Antimicrob Agents Chemother 2014; 58: 2229–34.

34 Gumbo T, Louie A, Deziel MR, Liu W, Parsons LM, Salfinger M, *et al*. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob Agents Chemother 2007; 51: 3781–8.

35 Chan CY, Au-Yeang C, Yew WW, Hui M, Cheng AFB. Postantibiotic effects of antituberculosis agents alone and in combination. Antimicrob Agents Chemother 2001; 45: 3631–4.

36 Boeree MJ, Heinrich N, Aarnoutse R, Diacon AH, Dawson R, Rehal S, *et al*. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. Lancet Infect Dis 2017; 17: 39–49.

37 Boeree MJ, Diacon AH, Dawson R, Narunsky K, du Bois J, Venter A, *et al*. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med 2015; 191: 1058–65.

38 van Ingen J, Aarnoutse RE, Donald PR, Diacon AH, Dawson R, Plemper van Balen G, *et al*. Why do we use 600 mg of rifampicin in tuberculosis treatment? Clin Infect Dis 2011; 52: e194–9.

39 Frydenberg AR, Graham SM. Toxicity of first-line drugs for treatment of tuberculosis in children: review. Trop Med Int Health 2009; 14: 1329–37.

40 Peloquin C. What is the 'right' dose of rifampin? [editorial]. Int J Tuberc Lung Dis 2003; 7: 3–5.

41 Magis-Escurra C, Anthony RM, van der Zanden AGM, van Soolingen D, Alffenaar J-WC. Pound foolish and penny wise—when will dosing of rifampicin be optimised? Lancet Respir Med 2018; 6: e11–2.