INT J TUBERC LUNG DIS 17(6):800–806

http://dx.doi.org/10.5588/ijtld.12.0628

Age, nutritional status and INH acetylator status affect pharmacokinetics of anti-tuberculosis drugs in children

G. Ramachandran,* A. K. Hemanth Kumar,* P. K. Bhavani,* N. Poorana Gangadevi,* L. Sekar,* D. Vijayasekaran,† V. V. Banu Rekha,* S. Ramesh Kumar,* N. Ravichandran,‡ G. Mathevan,§ S. Swaminathan*

*National Institute for Research in Tuberculosis, Indian Council of Medical Research, Chetpet, Chennai, †Institute of Child Health, Chennai, ‡Government Hospital of Thoracic Medicine, Chennai, §Government Rajaji Hospital, Madurai, India

SUMMARY

SETTING: The currently recommended dosages of rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA) and ethambutol in children are extrapolated from adult pharmacokinetic studies, and have not been adequately evaluated in children.

OBJECTIVE: To describe the pharmacokinetics of RMP, INH and PZA given thrice weekly in children with tuberculosis (TB), and to relate pharmacokinetics to treatment outcomes.

METHODS: Eighty-four human immunodeficiency virus negative children with TB aged 1–12 years in Chennai and Madurai, India, were recruited. Phenotypic INH acetylator status was determined. Nutritional status was assessed using *Z* scores. During the intensive phase of anti-tuberculosis treatment, a complete pharmacokinetic study was performed after directly observed administration of drugs. At 2 and 6 months, drug levels were measured 2 h post-dose. Drug concentrations were measured using high performance liquid chromatography and pharmacokinetic variables were calculated. Multivariable regression analysis was performed to explore factors impacting drug levels and treatment outcomes.

RESULTS AND CONCLUSIONS: Children aged <3 years had significantly lower RMP, INH and PZA concentrations than older children, and 90% of all children had sub-therapeutic RMP C_{max} (<8 μg/ml). Age, nutritional status and INH acetylator status influenced drug levels. Peak RMP and INH concentrations were important determinants of treatment outcome. Recommendations for anti-tuberculosis treatment in children should take these factors into consideration.

KEY WORDS: tuberculosis; children; pharmacokinetics; rifampicin; intermittent, therapeutic drug monitoring

TUBERCULOSIS (TB) in children is an important but neglected public health problem, with a global estimate of about 0.5 million new cases and about 65 000 deaths per year.[1] While outcomes are good in most children, younger children face morbidity, and there are increasing concerns about rising drug resistance rates.[2]

In India's Revised National TB Control Programme (RNTCP), anti-tuberculosis treatment is given thrice weekly and drug dosages are based on body weight.[3] Recent reports suggest that the currently recommended dosages of rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB) are inadequate in children.[4–10] Young children experience significant changes in the relative sizes of body compartments and their pharmacokinetic profile.[11,12] Genetic polymorphisms are reported to influence RMP (*SLCO1B1*) and INH (*NAT2*) pharmacokinetics.[13–17]

For anti-tuberculosis treatment to be efficacious, adequate plasma drug levels should be achieved; therapeutic ranges have previously been determined in adult pharmacokinetic and pharmacodynamic studies.[18] We evaluated the pharmacokinetics of RMP, INH and PZA in non-human immunodeficiency virus (HIV) infected children with TB who were receiving anti-tuberculosis treatment according to the RNTCP guidelines in India, and related the drug pharmacokinetic parameters to treatment outcomes.

METHODS

Patients

HIV-negative children aged 1–12 years attending the out-patient TB treatment centres at the Institute of Child Health, Chennai, the Government Hospital of Thoracic Medicine, Chennai, and the Government Rajaji Hospital, Madurai, were recruited. A diagnosis of TB (pulmonary and extra-pulmonary) was made using standard RNTCP definitions, and patients had received anti-tuberculosis treatment according

Correspondence to: Soumya Swaminathan, National Institute for Research in Tuberculosis, Mayor VR Ramanathan Road, Chetpet, Chennai 600 031, India. Tel: (+91) 44 2836 9600. Fax: (+91) 44 2836 2528. e-mail: doctorsoumya@yahoo.com; soumyas@trcchennai.in

Article submitted 13 August 2012. Final version accepted 9 January 2013.

to RNTCP guidelines for at least 2 weeks (six doses). Individual drugs were made available in blister packs for the entire 6 months as a 'patient-wise box' in four different weight bands.

Parents/guardians gave informed written consent, and children aged >7 years gave assent. The study was approved by the Institutional Ethics Committees of all the study sites.

Clinical assessment

Clinical examination and documentation of baseline demographic data were performed for all the eligible children. Clinical history was collected from the children's RNTCP records. Adherence to anti-tuberculosis treatment was assessed by questioning the parent/guardian and reviewing the treatment card. Anthropometric measurements (body weight, height, head circumference and mid-arm circumference) were taken on the day of the pharmacokinetic study. Clinical management of patients was performed by site physicians.

Determination of phenotypic isoniazid acetylator status

Two ml of saliva was collected at 5 h after administration of INH syrup at a dose of 2.5 mg/kg body weight. Salivary INH was determined using high-performance liquid chromatography (HPLC) and an INH concentration of ≤0.3 μg/ml was indicative of rapid acetylator status.[19,20]

Pharmacokinetic study

The pharmacokinetic study was conducted in the in-patient wards in the hospitals. On the day of the study, serial blood samples (2 ml) at pre-dosing, 2, 4, 6 and 8 h were collected. The drugs were administered by the clinic nurses under direct observation. Children were asked to swallow the tablets with water. For children who could not swallow, the tablets were powdered, taken in a small quantity of water and the entire contents were given to the children. Plasma RMP and INH were measured at 2 h post-dose at 2 and 6 months.

Drug estimations

Plasma INH, PZA and RMP concentrations were determined by HPLC using validated methods.[20,21] The between- and within-run variations for all the drugs were below 10%. The lower limits of quantification (LLOQ) and lower limits of detection for RMP, INH and PZA were respectively 0.25 μg/ml, 0.25 μg/ml and 1.25 μg/ml and 0.1 μg/ml, 0.1 μg/ml and 0.5 μg/ml. Respectively 19%, 23% and 15% of samples were below the LLOQ for RMP, INH and PZA. The actual value was taken for analysis.

Calculation of pharmacokinetic variables

Peak concentration (C_{max}) and time to attain C_{max} (T_{max}) were determined by visual inspection of data. The linear trapezoidal rule was used to compute exposure or area under the time concentration curve ($AUC_{0–8}$).

Assessment of nutritional status

The *Z* scores for weight and height were computed based on the child's age and sex using the EPI-NUT component of the Epi-Info 2002 software package, version 3.4.3 (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA) based on reference median values of the National Center for Health Statistics, CDC.[22]

Treatment and follow-up

Children continued anti-tuberculosis treatment per programme guidelines. Treatment outcomes were noted; cured/treatment completion was considered as favourable outcome, while failure, death and default were taken as unfavourable outcomes.

Sample size

Based on the pharmocokinetic data from the study by Schaaf et al. among children with TB,[8] and using a precision of 20% and $\alpha = 5\%$, the sample size was estimated at 70. Considering a loss of 15% during follow-up, the required sample size was 81 children.

Statistical analysis

Data analysis was performed using SPSS, version 14.0 (Statistical Product and Service Solutions, Chicago, IL, USA). Shapiro-Wilks test showed that the pharmocokinetic data were not normally distributed. The Mann-Whitney *U* test was used to compare two groups, while the Kruskal-Wallis test with Bonferroni correction was used for multiple group comparisons. The analysis was stratified by age groups: 1–3, 3.1–6, 6.1–9 and 9.1–12 years. Sub-therapeutic C_{max} values (RMP <8 μg/ml, INH <3 μg/ml, PZA <35 μg/ml) were defined as per the National Jewish Medical Research Centre study.[23] The χ^2 test was used to test the association between the proportion of children with sub-therapeutic C_{max} of drugs and age (<3 years vs. >3 years). Univariate and multivariable regression analysis by stepwise method were performed to identify factors that influenced treatment outcome. Multiple regression analysis by backward elimination method was used to determine factors that influenced the C_{max} and $AUC_{0–8}$ of RMP, INH and PZA. $P < 0.05$ was considered statistically significant.

RESULTS

Eighty-four children were included in the study; their demographic and clinical characteristics are shown in Table 1. Extra-pulmonary sites included lymph node TB, TB of the abdomen, spinal TB and brain tuberculoma. Respectively 22, 31 and 16 children had stunting, underweight and wasting (*Z* scores < −2

Table 1 Demographic and clinical features of study participants ($N = 84$)

Details	Age 1–3 years ($n = 17$) *n* (%) or median [IQR]	Age 3.1–6 years ($n = 22$) *n* (%) or median [IQR]	Age 6.1–9 years ($n = 23$) *n* (%) or median [IQR]	Age 9.1–12 years ($n = 23$) *n* (%) or median [IQR]	Total ($N = 84$) *n* (%) or median [IQR]
Males	8 (50)	10 (45)	11 (48)	11 (48)	40 (48)
Body weight, kg	12 [10 to 12]	15 [13 to 17]	21 [17 to 22]	26 [23 to 30]	18 [13 to 23]
Rapid acetylators	7 (44)	3 (14)	8 (35)	9 (39)	27 (32)
Nutritional status					
Height for age *Z*-score	−1.4 [−2.5 to 0.3]	−1.2 [–2.0 to −0.5]	−0.6 [−1.6 to −0.3]	−1.3 [−2.1 to −0.96]	−1.2 [−2.1 to −0.3]
Weight for age *Z*-score	−2.0 [−2.6 to 0.3]	−1.8 [–2.1 to −0.6]	−1.4 [−2.3 to −0.8]	−1.9 [−2.5 to −1.1]	−1.8 [−2.4 to −1.1]
Weight for height *Z*-score	−1.4 [−1.6 to −0.003]	−1.2 [−1.8 to −0.4]	−1.7 [−2.9 to −0.7]	−0.2 [−1.8 to 0.3]	−1.2 [−1.9 to −0.3]
Mid-arm circumference, cm	14 [13 to 14]	15 [14 to 16]	15 [14 to 16]	16 [15 to 19]	15 [14 to 16]
Head circumference, cm	47 [42 to 48]	49 [47 to 49]	50 [50 to 51]	51 [49 to 53]	49 [48 to 51]
Serum albumin, g/dl	3.8 [3.6 to 4.2]	4.2 [3.6 to 4.4]	4.1 [3.9 to 4.4]	4.1 [3.5 to 4.5]	4.1 [3.6 to 4.4]
Drug dose, mg/kg					
RMP	9.4 [6.9 to 12.5]	10.1 [8.6 to 11.4]	10 [8.8 to 10.7]	9.1 [8.2 to 10.0]	9.9 [8.3 to 11.2]
INH	9.4 [6.9 to 12.5]	10.1 [8.6 to 11.4]	10 [8.8 to 10.7]	9.1 [8.2 to 10.0]	9.9 [8.3 to 11.2]
PZA	31.0 [23.1 to 40.7]	33.6 [28.5 to 39.3]	33.3 [29.4 to 35.7]	30.4 [27.4 to 33.3]	32.9 [27.8 to 37.2]
Duration of anti-tuberculosis treatment, months	0.5 [0.5 to 1]	0.75 [0.5 to 1]	0.5 [0.5 to 1]	1 [0.5 to 1]	1 [0.5 to 1]
Regimen					
Category I	10 (63)	12 (55)	11 (48)	15 (65)	48 (57)
Category II	0	1 (5)	0	2 (9)	3 (4)
Category III	6 (37)	9 (40)	12 (52)	6 (26)	33 (39)
Type of TB					
Pulmonary	7 (44)	6 (27)	3 (13)	3 (13)	19 (23)
Extra-pulmonary	9 (56)	15 (68)	19 (83)	20 (87)	63 (75)
Both	0	1 (5)	1 (4)	0	2 (2)

IQR = interquartile range; RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; TB = tuberculosis.

standard deviation). The drug doses received by these children did not significantly differ from those for normally nourished children. The C_{max} (7.0 ± 2.9 μg/ml vs. 4.6 ± 2.2 μg/ml, $P < 0.001$) and $AUC_{0–8}$ (28.8 ± 12.0 μg/ml.h vs. 15.6 ± 7.9 μg/ml.h, $P < 0.001$) were significantly higher in slow acetylators of INH.

The C_{max} and $AUC_{0–8}$ of all of the drugs were significantly lower in children aged 1–3 years than in the other age groups ($P < 0.01$); the drug pharmacokinetics were similar in the three older age groups (Table 2). The distribution of the C_{max} of RMP, INH and PZA among the different age groups is shown in Figure 1. Of the 84 children, 76 (90%) had subtherapeutic RMP C_{max} (<8 μg/ml) across all age groups (all children aged <3 years had subtherapeutic RMP C_{max}). In the case of INH and PZA, respectively 10/84 (12%) and 31/84 (37%) had subtherapeutic C_{max} (INH <3 μg/ml, PZA <35 μg/ml). A significantly higher number of children aged <3 years had subtherapeutic concentrations than those aged >3 years (INH 6/10 vs. 10/74, $P = 0.003$; PZA 14/31 vs. 2/53, $P < 0.001$). The distribution of slow and rapid acetylators of INH and proportions of stunted/underweight/wasted children in the 1–3 and 3.1–12 years age groups were not significantly different. The mean doses (mg/kg) of RMP and INH in these two age groups were respectively 9.7 mg and 9.6 mg, while the corresponding values for PZA were 31.5 mg and 32.7 mg; however, these differences were not statistically significant.

The C_{max} and $AUC_{0–8}$ of RMP, INH and PZA were lower in children with stunting and underweight compared to normal children; the differences were statistically significant for RMP, INH and PZA for stunting and RMP and PZA for underweight ($P < 0.05$). No significant difference in the C_{max} and $AUC_{0–8}$

Table 2 Peak concentration and exposure of RMP, INH and PZA among different age groups

Drug	Pharmakinetic variable	Age 1–3 years ($n = 17$) median [IQR]	Age 3.1–6 years ($n = 22$) median [IQR]	Age 6.1–9 years ($n = 22$) median [IQR]	Age 9.1–12 years ($n = 23$) median [IQR]
RMP	C_{max}, μg/ml	3.1 [2.4 to 4.0]*	5.5 [4.4 to 6.6]	7.0 [4.2 to 7.7]	5.9 [4.1 to 7.1]
	$AUC_{0–8}$, μg/ml.h	15.0 [9.6 to 19.2]*	25.9 [18.4 to 32.5]	30.0 [19.5 to 37.1]	31.8 [18.8 to 38.1]
INH	C_{max}, μg/ml	3.3 [2.4 to 4.6]*	6.1 [3.1 to 8.4]	6.3 [4.4 to 9.0]	7.2 [5.6 to 8.5]
	$AUC_{0–8}$, μg/ml.h	14.9 [7.2 to 19.5]	26.7 [12.5 to 40.7]	21.9 [15.0 to 28.8]	28.7 [18.6 to 40.6]
PZA	C_{max}, μg/ml	30.4 [26.2 to 33.4]*	38.5 [31.4 to 44.2]	40.9 [38.3 to 47.4]	38.0 [30.1 to 45.1]
	$AUC_{0–8}$, μg/ml.h	175.9 [131.5 to 193.9]*	226.5 [132.2 to 265.3]	234.2 [207.4 to 269.7]	221.8 [194.7 to 256.6]

*Significant compared to other age groups based on the Kruskall-Wallis test followed by Bonferroni multiple comparison test, at a significance level of $P < 0.01$.
RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; C_{max} = peak concentration; $AUC_{0–8}$ = area under the time concentration curve.

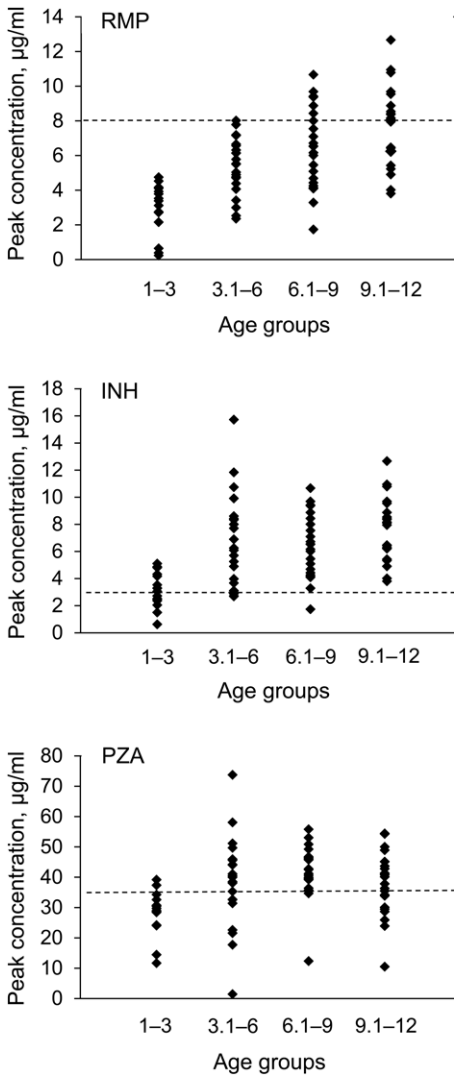

Figure 1 Distribution (dot-plot) of peak drug concentrations among the various age groups, showing lowest levels in the youngest age group. Dotted horizontal lines denote presumed lower limit of therapeutic margin (RMP 8 µg/ml, INH 3 µg/ml, PZA 35 µg/ml).[20] Peak concentration of drugs are in µg/ml. RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide.

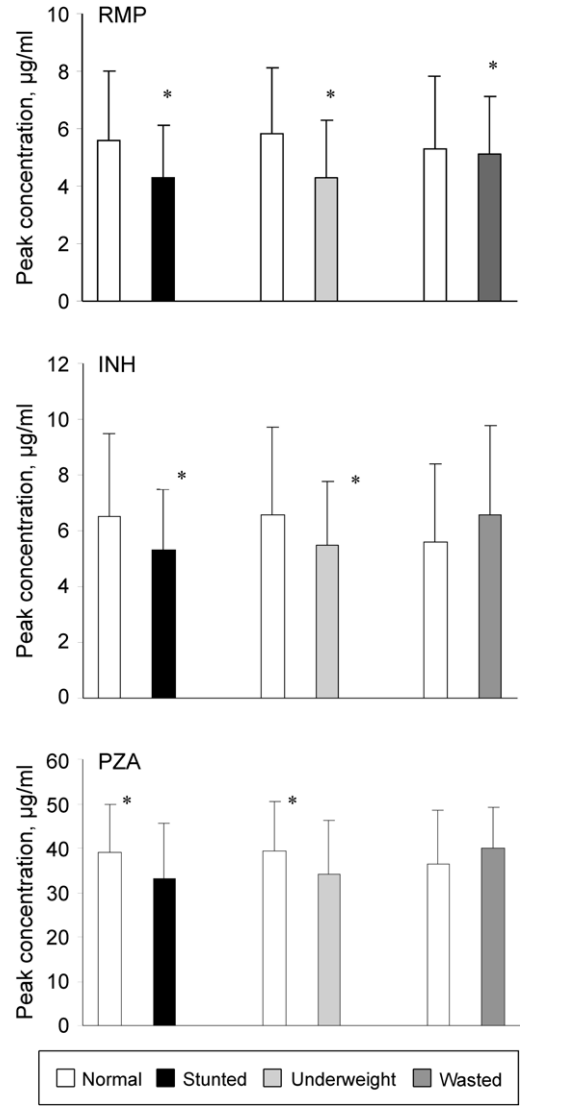

Figure 2 Stunted and underweight children have significantly lower peak levels of anti-tuberculosis drugs (values are mean; vertical bars denote standard deviation). *$P < 0.05$ vs. normal group. RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide.

of RMP, INH and PZA was observed in children with wasting compared to normal children (Figure 2).

Among factors such as age, body mass index (BMI), serum albumin, INH acetylator status, weight for age *Z*-score (WAZ), height for age *Z*-score (HAZ) and weight for height *Z*-score (WHZ), multiple regression analysis showed that age significantly influenced the C_{max} and $AUC_{0–8}$ of RMP, INH and PZA. In addition, WAZ (underweight) had a significant influence on the C_{max} and $AUC_{0–8}$ of RMP and acetylator status on the C_{max} and $AUC_{0–8}$ of INH ($P < 0.05$; Table 3).

TB treatment outcomes were available for 70 children; 14 had migrated and could not be followed up. Among the 70 children, 15 had unfavourable outcomes: death ($n = 1$), relapse ($n = 1$) failure ($n = 13$). The C_{max}, $AUC_{0–8}$, 2- and 6-month RMP and INH concentrations were significantly lower in children with unfavourable outcomes than in those with favourable outcomes (Table 4). There was a delay in the time to attain C_{max} of RMP among unfavourable responders compared to favourable responders ($P = 0.03$). The pharmacokinetics of PZA did not significantly differ between favourable and unfavourable responders. A favourable outcome was achieved in 75% of the children when RMP, INH and PZA C_{max} were within the therapeutic range. This dropped to 56% when all the drugs were below the therapeutic range; this difference was not significant, however. A higher proportion of children aged ⩾3 years had a favourable outcome compared to those aged <3 years (84% vs. 54%, $P = 0.016$). There was also a significantly higher proportion of slow INH acetylators among children with favourable outcome (89% vs. 60%, $P = 0.005$).

Table 3 Multiple regression analysis showing factors significantly influencing peak concentration and exposure of RMP, INH and PZA

Factor*	β (non-standardised)	95%CI	*P* value
RMP			
C_{max}, μg/ml			
Age	0.417	0.25 to 0.59	<0.001
WAZ	0.555	0.08 to 1.03	<0.05
$AUC_{0–8}$, μg/ml.h			
Age	2.04	1.14 to 2.95	<0.001
WAZ	2.81	0.31 to 5.30	<0.05
INH			
C_{max}, μg/ml			
Acetylator status	2.719	1.45 to 3.99	<0.001
Age	0.403	0.19 to 0.62	<0.001
$AUC_{0–8}$, μg/ml.h			
Acetylator status	13.670	8.49 to 18.85	<0.001
Age	1.292	0.43 to 2.15	<0.01
PZA			
C_{max}, μg/ml			
Age	1.201	0.23 to 2.18	<0.05
$AUC_{0–8}$, μg/ml.h			
Age	7.456	1.97 to 12.94	<0.01

*Age, acetylator status (for INH), BMI, serum albumin, HAZ, WAZ and WHZ (all were significant at < 0.1 level on univariate analysis). Multiple regression analysis by stepwise method was used.
RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; CI = confidence interval; C_{max} = peak concentration; WAZ = weight-for-age *Z*-score denoting underweight; $AUC_{0–8}$ = area under the time concentration curve; BMI = body mass index; HAZ = height for age *Z*-score; WHZ = weight for height *Z*-score.

However, the proportion of children with stunting, underweight and wasting did not significantly differ between favourable and unfavourable responders (*P* values respectively 0.11, 0.063 and 0.073).

In univariate analysis, age, BMI, serum albumin, INH acetylator status, WAZ, HAZ, WHZ and C_{max} of RMP, INH and PZA, were tested for association with treatment outcome. Those variables with $P < 0.10$ (C_{max} of RMP and INH, WAZ, INH acetylator status and serum albumin) were fed into the multiple logistic regression model using the stepwise method. Among these, the C_{max} of RMP (adjusted odds ratio [aOR] 1.5, 95% confidence interval [CI] 1.1 to 2.1, $P = 0.014$) and INH acetylator status (aOR 4.2, 95%CI 1.1 to 15.4, $P = 0.033$) were found to significantly impact treatment outcome.

A significant (decreasing) trend ($P < 0.001$) in the 2-h concentration of RMP ($P < 0.001$) and INH ($P < 0.01$) was seen over time (baseline, 2 months and 6 months).

DISCUSSION

Our findings of low anti-tuberculosis drug levels in children receiving standard short-course chemotherapy according to RNTCP guidelines in India, and their impact on treatment outcome, highlight the importance of considering children's dosage requirements separately. Data on anti-tuberculosis drug pharmacokinetics in children globally are limited, and there are none in Indian children treated under the RNTCP with intermittent regimens.

Differences in absorption, distribution, metabolism and excretion due to growth and development in children are known to have a profound effect on serum drug concentrations.[24] In addition, drug levels are influenced by other factors such as sex, ethnicity, genetic and nutritional status, HIV infection, drug-drug interactions and drug-food interactions.[4,6,16,17,25–32]

Children aged <3 years had markedly lower drug levels, despite receiving similar drug doses on mg/kg basis to older children. This is probably due to faster elimination of drugs by infants and younger children due to the relatively greater mass of the liver in proportion to total body weight. Several studies have

Table 4 Comparison of drug levels between children with favourable and unfavourable TB treatment outcomes

	n	Unfavourable outcome median [IQR]	*n*	Favourable outcome median [IQR]	*P* value*
RMP					
C_{max}, μg/ml	15	3.4 [2.5 to 4.2]	55	5.9 [4.4 to 7.1]	0.002
$AUC_{0–8}$, μg/ml.h	15	14.0 [9.2 to 22.4]	55	29.4 [18.0 to 34.4]	0.003
T_{max}, h	15	4 [2 to 4]	55	2 [2 to 4]	0.007
Occasion 1 (2-h)	15	2.2 [0.6 to 3.9]	55	5.7 [3.4 to 7.1]	0.003
2 month (2-h)	14	3 [1.0 to 4.7]	48	4.3 [2.9 to 6.6]	0.024
6 month (2-h)	14	0.6 [0.2 to 1.9]	47	3.7 [2.1 to 4.8]	<0.001
INH					
C_{max}, μg/ml	15	4.3 [2.5 to 6.3]	55	6.3 [4.2 to 8.4]	0.031
$AUC_{0–8}$, μg/ml.h	15	15.7 [9.6 to 21.4]	55	25.4 [15.3 to 35.2]	0.017
T_{max}, h	15	2 [2 to 4]	54	2 [2 to 4]	NS
Occasion 1 (2-h)	15	3.8 [1.6 to 5.4]	55	6.2 [3.5 to 8.4]	0.010
2 month (2-h)	14	2.3 [1.0 to 3.8]	48	6.3 [4.2 to 8.7]	<0.001
6 month (2-h)	14	1.4 [0.6 to 2.1]	47	5.1 [2.6 to 6.4]	<0.001
PZA					
C_{max}, μg/ml	15	30.7 [25.9 to 42.5]	55	39.4 [32.6 to 44.1]	NS
$AUC_{0–8}$, μg/ml.h	15	190.4 [159.1 to 223.2]	55	221.8 [185.5 to 257.7]	NS
T_{max}, h	15	2 [2 to 4]	55	2 [2 to 4]	NS

*Mann-Whitney *U* test.
IQR = interquartile range; RMP = rifampicin; C_{max} = peak concentration; $AUC_{0–8}$ = area under the time concentration curve; T_{max} = time to attain peak concentration; INH = isoniazid; NS = non-significant at 5% level; PZA = pyrazinamide.

reported low RMP, INH and PZA concentrations in younger children.[6,7,10,32] Our findings of subtherapeutic RMP C_{max} in the majority of children across all age groups and that of INH and PZA in younger children are a matter of concern. Neither INH acetylator status nor nutritional status confounded this observation. Sustained suboptimal drug levels could predispose to development of drug resistance and poor treatment outcomes. This suggests the need to increase paediatric doses of RMP, INH and PZA; it would be preferable to calculate drug doses based on body surface area rather than body weight.[4,7]

Drug disposition and nutritional status have close interaction. The pathophysiological changes associated with malnutrition can alter pharmacokinetic processes, drug responses and toxicity.[33] Our finding of lower drug levels in stunted and underweight children than in normally nourished children is similar to that reported by Graham et al., who showed PZA, but not EMB levels, to be lower in malnourished children.[10] The observation of age and malnutrition being significant predictors of anti-tuberculosis drug levels has been reported previously in children on daily treatment.[6,8,10] These variables therefore appear to be important, regardless of the rhythm of administration of drugs.

Most previous pharmacokinetic studies did not correlate drug concentrations with treatment outcomes; hence, the significance of subtherapeutic plasma concentrations in relation to therapeutic efficacy was unclear. This study demonstrated that unfavourable responders had lower C_{max} and exposure of RMP and INH at different time points of treatment than favourable responders. Although RMP concentrations were low in most children, adequate INH and PZA concentrations could have helped in achieving satisfactory treatment outcomes. There were significantly higher proportions of children aged <3 years and rapid acetylators among unfavourable responders. Using Monte Carlo simulations, Jeena et al. concluded that individualising INH doses should be considered based on disease process, age and acetylator status in children.[31] Slow and rapid acetylators have been shown to differ in the speed of sputum smear and culture conversion.[34]

The cut-off values used to define subtherapeutic drug levels were those validated by the National Jewish Medical Research Centre.[23] While these values are commonly used, there is no evidence that they are optimal in terms of efficacy.

In 2010, the World Health Organization (WHO) issued revised anti-tuberculosis drug dosage recommendations for children, with daily doses being increased from 10 to 15 mg/kg for RMP, from 5 to 10 mg/kg for INH and from 25 to 35 mg/kg for PZA. Thee et al. compared the pharmacokinetics of RMP, INH and PZA between previous and revised WHO doses in children aged <2 years, and provided supportive evidence for the implementation of the revised WHO guidelines in younger children, showing that higher doses produced therapeutic drug levels.[18] However, treatment outcomes and occurrence of toxicity were not noted in this study. A recent study from Venezuela has provided supportive evidence for the implementation of the revised WHO paediatric TB drug dose recommendations.[35] This study also observed that younger children had significantly lower drug levels than older children, a finding similar to the present study.

In the RNTCP, new paediatric patients diagnosed with TB are initiated on treatment, with doses of 10 mg/kg for INH and RMP, 30–35 mg/kg for PZA and 30 mg/kg for EMB given thrice weekly. Children are divided into four weight bands: 6–10 kg, 11–17 kg, 18–25 kg and 26–30 kg. Patients receive the patient-wise box appropriate for their body weight; the same doses are given to all children within a particular weight band. Apart from overall underdosing, drug levels are likely to vary between children at the extremities of each weight band. Concerns have been raised about the current dosing strategy used in the RNTCP.[36] While daily regimens are used globally to treat TB in adults and children, the Indian Government's programme uses an intermittent regimen. Comparison of the data in this study with those from our adult studies showed that peak concentrations and AUCs of INH and RMP were lower in children.[37]

This is the first study to report on the pharmacokinetics of RMP, INH and PZA in children with TB treated with intermittent regimens, and to relate drug levels to treatment outcomes. This study has demonstrated that age (<3 years), acetylator status (rapid) and nutritional status (stunting and underweight) could influence drug pharmacokinetics, which could in turn influence treatment outcome. Drug doses may have to be increased, particularly for the youngest and lightest children. Future recommendations for anti-tuberculosis treatment in children should consider age, acetylator status and nutritional status to achieve optimal treatment outcomes.

Acknowledgements

The authors thank all the children for participation in the study, S Kalpana for assistance in patient recruitment at the Institute of Child Health, V Sudha for drug estimations, S Venkatesh for counselling and follow-up of patients and the clinic nurses at the National Institute for Research in Tuberculosis for blood collection. The study was funded by the Paediatric HIV Task Force of the Indian Council of Medical Research, New Delhi, India.

Conflict of interest: none declared.

References

1 World Health Organization. Global tuberculosis control: epidemiology, strategy, financing. WHO report 2009. WHO/HTM/TB/2009.411 Geneva, Switzerland: WHO, 2009. http://www.who.int/tb/publications/global_report/2009/en/index.html Accessed February 2013.

2 Swaminathan S, Banu Rekha V V. Pediatric tuberculosis: global overview and challenges. Clin Infect Dis 2010; 50: S184–S194.

3 Government of India. TB India 2011. Revised National TB

Control Programme annual status report. New Delhi, India: Central TB Division, Government of India, 2011.
4 Schaaf H S, Parkin D P, Seifart H I, et al. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. Arch Dis Child 2005; 90: 614–618.
5 Thee S, Detjen A, Wahn U, Magdorf K. Isoniazid pharmacokinetic studies of the 1960s: considering a higher isoniazid dose in childhood tuberculosis. Scand J Infect Dis 2010; 42: 294–298.
6 Mcllleron H, Willemse M, Werely C J, et al. Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines. Clin Infect Dis 2009; 48: 1547–1553.
7 Thee S, Detjen A, Wahn U, Magdorf K. Rifampicin serum levels in childhood tuberculosis. Int J Tuberc Lung Dis 2009; 13: 1106–1111.
8 Schaaf H S, Willemse M, Cilliers K, et al. Rifampin pharmacokinetics in children, with and without human immunodeficiency virus infection, hospitalised for the management of severe forms of tuberculosis. BMC Med 2009; 7: 19–29.
9 Donald P R, Maher D, Maritz J S, Qazi S. Ethambutol dosage for the treatment of children: literature review and recommendations. Int J Tuberc Lung Dis 2006; 10: 1318–1330.
10 Graham S M, Bell D J, Nyirongo S, Hartkoon R, Ward S A, Molyneux E M. Low levels of pyrazinamide and ethambutol in children with tuberculosis and impact of age, nutritional status and human immunodeficiency virus infection. Antimicrob Agents Chemother 2006; 50: 407–413.
11 Bartelink I H, Rademaker C M, Schobben A F, van den anker J N. Guidelines on paediatric dosing on the basis of developmental physiology and pharmacokinetic considerations. Clin Pharmacokinet 2006; 45: 1077–1097.
12 Kearn G L, Abdel-Rahman S M, Alander S W, Blowey D L, Leeder J S, Kauffman R E. Developmental pharmacology—drug disposition, action, and therapy infants and children. N Engl J Med 2003; 349: 1157–1167.
13 Kinzig-schippers M, Tomalik-Scharte D, Jetter A, et al. Should we use *N*-acetyltransferase type 2 genotyping to personalize isoniazid doses? Antimicrob Agents Chemother 2005; 49: 1733–1738.
14 Parkin D P, Vandenplas S, Botha F J, et al. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. Am J Respir Crit Care Med 1997; 155: 1717–1722.
15 Zhu R, Kiser J J, Seifart H I, et al. The pharmacogenetics of NAT 2 enzyme maturation in perinatally HIV exposed infants receiving isoniazid. J Clin Pharmacol 2012; 52: 511–519.
16 Weiner M, Peloquin C, Burman W, et al. Effects of tuberculosis, race and human gene SLCO1B1 polymorphisms on rifampin concentrations. Antimicrob Agents Chemother 2010; 54: 4192–4200.
17 Chigutsa E, Visser M E, Swart E C, et al. The *SLCO1B1* rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin concentrations: dosing implications. Antimicrob Agents Chemother 2011; 55: 4122–4127.
18 Thee S, Seddon J N, Donald P R, et al. Pharmacokinetics of isoniazid, rifampin and pyrazinamide in children younger than two years of age with tuberculosis: evidence for implementation of revised world health organization recommendations. Antimicrob Agents Chemother 2011; 55: 5560–5567.
19 Sarma G R, Kailasam S, Datta M, Loganathan G K, Rahman F, Narayana A S L. Classification of children as slow or rapid acetylators based on concentrations of isoniazid in saliva following oral administration of body weight and surface area—related dosages of the drug. Indian Pediatr 1990; 27: 134–142.
20 Hemanth Kumar A K, Sudha V, Ramachandran G. Simple and rapid liquid chromatography method for simultaneous determination of isoniazid and pyrazinamide in plasma. SAARC J Tuberc Lung Dis HIV/AIDS 2012; 9: 13–18.
21 Hemanth Kumar A K, Chandra I, Ramachandran G, Chelvi K S, Victor L, Gurumurthy P. A validated high performance liquid chromatography method for the determination of rifampicin and desacetyl rifampicin in plasma and urine. Indian J Pharmacol 2004; 36: 231–233.
22 World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. Technical Report series no. 854. Geneva, Switzerland: WHO, 1995. http://www.who.int/childgrowth/publications/physical_status/en/index.html Accessed February 2013.
23 Peloquin C A. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs 2002; 62: 2169–2183.
24 King J R, Kimberlin D W, Aldrovandi G M, Acosta E P. Antiretroviral pharmacokinetics in the pediatric population—a review. Clin Pharmacokinet 2002; 41: 1115–1133.
25 Cranswick N, Mulholland K. Isoniazid treatment of children: can genetics help guide treatment? Arch Dis Child 2005; 90: 551–553.
26 Peloquin C A, Namdar R, Singleton M D, Nix D E. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest 1999; 155: 12–18.
27 Acocella G, Buniva G, Flauto U, Nicolis F B. Absorption and elimination of the antibiotic rifampicin in newborns and children. In: Proceedings of the Sixth International Congress of Chemotherapy, 1969. Volume 2. Tokyo, Japan: University of Tokyo Press, 1970: pp 755–760.
28 Mcllleron H, Rustomjee R, Vahedi M, 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 2012; 5665: 3232–3238.
29 Hall R G, Meek C, Swancutt M G T, Lee R. A surprising effect of weight on ethambutol pharmacokinetics. In: Abstract Book of the 3rd International Workshop on Clinical Pharmacology of Tuberculosis Drugs, 11 September 2010, Boston, MA, USA [Abstract O_12]. http://regist2.virology-education.com/abstractbook/3rd%20Int%20Workshop%20on%20TB-PK_abstractbook.pdf Accessed April 2013.
30 Lin M-Y, Lin S-J, Chan L-C, Lu Y-C. Impact of food and antacids on the pharmacokinetics of anti-tuberculosis drugs: systematic review and meta-analysis. Int J Tuberc Lung Dis 2010; 14: 806–818.
31 Jeena P M, Bishai W R, Pasipanodaya J G, Gumbo T. In silico children and the glass mouse model: clinical trial simulations to identify and individualise optimal isoniazid doses in children with tuberculosis. Antimicrob Agents Chemother 2011; 55: 539–545.
32 Thee S, Detjen A, Wahn U, Magdorf K. Pyrazinamide serum levels in childhood tuberculosis. Int J Tuberc Lung Dis 2008; 12: 1099–1101.
33 Krishnaswamy K. Drug metabolism and pharmacokinetics in malnourished children. Clin Pharmacokinet 1989; 17 (Suppl 1): S68–S88.
34 Selkon J B, Fox W, Gangadharam P R J, Ramachandran K, Ramakrishnan C V, Velu S. Rate of inactivation of isoniazid in South Indian patients with pulmonary tuberculosis–2. Clinical implications in the treatment of pulmonary tuberculosis with isoniazid either alone or in combination with PAS. Bull World Health Organ 1961; 25: 779–792.
35 Verhagen L M, Lopez D, Hermans P W, et al. Pharmacokinetics of anti-tuberculosis drugs in Venezuelan children younger than 16 years of age: supportive evidence for the implementation of revised WHO dosing recommendations. Trop Med Int Health 2012 [epub ahead of print 24 Oct].
36 Lodha R, Ramesh Menon P, Kabra S K. Concerns on the dosing of anti-tubercular drugs for children in RNTCP. Ind Pediatr 2008; 45: 852–854.
37 Gurumurthy P, Ramachandran G, Hemanth Kumar A K, et al. Decreased bioavailability of rifampin and other anti-TB drugs in patients with advanced HIV disease. Antimicrob Agents Chemother 2004; 48: 4473–4475.

RÉSUMÉ

CONTEXTE : Les dosages actuellement recommandés pour la rifampicine (RMP), l'isoniazide (INH), le pyrazinamide (PZA) et l'éthambutol chez les enfants sont extrapolés à partir des études pharmacocinétiques chez l'adulte et n'ont pas été évalués de manière appropriée chez les enfants.

OBJECTIF : Décrire la pharmacocinétique de la RMP, de l'INH et du PZA administrés trois fois par semaine chez les enfants atteints de tuberculose (TB) et mettre la pharmacocinétique en relation avec les résultats du traitement.

MÉTHODES : On a recruté 84 enfants, âgés de 1 à 12 ans, séronégatifs pour le virus de l'immunodéficience humaine et atteints de TB à Chennai et Madurai, Inde. Le statut phénotypique d'acétylateur de l'INH a été déterminé. L'état nutritionnel a été évalué par des scores *Z*. Au cours de la phase intensive du traitement TB, on a mené une étude pharmacocinétique complète après administration directement observée des médicaments. On a mesuré les niveaux de médicaments 2 h après leur administration à 2 mois et à 6 mois. Les concentrations médicamenteuses ont été mesurées par chromatographie liquide à haute performance et l'on a calculé les variables pharmacocinétiques. On a mené une analyse de régression multivariée pour explorer les facteurs influençant les niveaux de médicaments et les résultats du traitement.

RÉSULTATS ET CONCLUSIONS : Les concentrations de RMP, INH et PZA sont significativement plus faibles chez les enfants âgés de <3 ans que chez les enfants plus âgés. De plus, la C_{max} est inférieure aux valeurs thérapeutiques pour la RMP chez 90% des enfants (<8 μg/ml). Les niveaux de médicament sont influencés par l'âge, le statut nutritionnel et le statut d'acétylateur de l'INH. Les pics de concentration de RMP et d'INH sont d'importants déterminants du résultat du traitement. Ces facteurs devraient être pris en considération pour les recommandations concernant le traitement antituberculeux chez les enfants.

RESUMEN

MARCO DE REFERENCIA: Las posologías de rifampicina (RMP), isoniazida (INH), pirazinamida (PZA) y etambutol que se recomiendan en la actualidad a los niños provienen de extrapolaciones de los estudios de farmacocinética realizados en adultos y no se han evaluado adecuadamente en la población infantil.

OBJETIVO: Describir la farmacocinética de la RMP, la INH y la PZA, cuando se administran tres veces por semana a los niños con tuberculosis (TB) y correlacionar la farmacocinética con los desenlaces terapéuticos.

MÉTODOS: Participaron en el estudio 84 niños de Chennai y Madurai, en la India, con TB y examen serológico negativo frente al virus de la inmunodeficiencia humana, cuya edad osciló entre 1 año y 12 años. Se determinó el fenotipo de acetilación de la INH. El estado nutricional se evaluó mediante las puntuaciones *Z*. Durante la fase intensiva del tratamiento antituberculoso se llevó a cabo un estudio farmacocinético completo, después de una observación directa de la administración de los medicamentos. A los 2 y los 6 meses de tratamiento se midieron las concentraciones de medicamentos 2 horas después de la administración mediante cromatografía líquida de alta eficiencia y se calcularon las variables farmacocinéticas. Un análisis de regresión multifactorial permitió analizar los factores que influían sobre las concentraciones de medicamentos y los desenlaces terapéuticos.

RESULTADOS Y CONCLUSIONES: Los niños <3 años de edad presentaron concentraciones de RMP, INH y PZA significativamente inferiores que los niños >3 años. Además, se observó en el 90% de todos los niños una concentración máxima de RMP inferior a la C_{max} (<8 μg/ml). La edad, el estado nutricional y el fenotipo de acetilación de la INH influyeron sobre las concentraciones máximas de RMP e INH y fueron factores determinantes importantes del desenlace terapéutico. Las recomendaciones de tratamiento antituberculoso en los niños deben tomar en consideración estos factores.