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Pharmacokinetics of thrice-weekly rifampicin, isoniazid and pyrazinamide in adult tuberculosis patients in India

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SUMMARY

OBJECTIVE: To study the pharmacokinetics of rifampicin (RMP), isoniazid (INH) and pyrazinamide (PZA) in adult tuberculosis (TB) patients and examine factors that influence drug pharmacokinetics.

METHODS: Adult TB patients ($n = 101$) receiving thrice-weekly anti-tuberculosis treatment in the Revised National TB Control Programme (RNTCP) were studied. The study was conducted at steady state after directly observed drug administration. RMP, INH and PZA concentrations were estimated using high-performance liquid chromatography and *NAT2* genotyping by real-time polymerase chain reaction.

RESULTS: RMP peak concentration (C_{max}) was subtherapeutic (<8 μg/ml) in 88% of the patients. The C_{max} of RMP, INH and PZA at 2 h was observed in respectively 83.2%, 97.0% and 92.1% of the patients. The C_{max} and area under the curve from 0 to 8 h ($AUC_{0–8}$) of PZA was lower in TB patients with diabetes mellitus than in non-diabetics. Significant associations were observed between the C_{max} and the $AUC_{0–8}$ of RMP, INH and PZA with drug doses; RMP with category of treatment; INH with smoking, body mass index and *N*-acetyl transferase 2 genotype; and PZA with sex and smoking.

CONCLUSIONS: Several risk factors for drug concentration variations were identified. Two-hour post-dosing drug concentrations mimicked C_{max}. A high proportion of TB patients had RMP C_{max} below the expected range, which is a matter of concern.

KEY WORDS: pharmacokinetics; anti-tuberculosis drugs; intermittent anti-tuberculosis treatment; Revised National Tuberculosis Control Programme

TUBERCULOSIS (TB) is a readily curable disease when adequate anti-tuberculosis treatment is properly administered. Using the DOTS strategy, many countries, including India, have successfully increased their TB cure and completion rates. The majority of persons with uncomplicated TB are treated with a 6-month intermittent regimen under India's Revised National TB Control Programme (RNTCP). However, treatment failures, relapses and the development of multidrug-resistant strains of *Mycobacterium tuberculosis* occur and continue to threaten TB control programmes.[1,2]

Although favourable treatment outcomes are achievable in a high proportion of patients, low drug levels may be critical where there is variable drug quality, different disease presentations, malnutrition, human immunodeficiency virus (HIV) co-infection, severe illness and other comorbidities. Anti-tuberculosis drug pharmacokinetics (PK) is known to be influenced by the patient's age, sex, ethnicity, gastro-intestinal infections and disorders and drug–drug interactions.[3] Potential sequelae of inadequate anti-tuberculosis drug concentrations include prolonged infectiousness, increased risk of relapse and death and development of drug-resistant *M. tuberculosis*.[4,5] There is a paucity of information available on the possible mechanisms to explain treatment failure, relapses and acquired drug resistance in the DOTS setting.

While several potential determinants of variability in drug concentration are recognised,[6–9] they are poorly characterised in populations of TB patients. Limited evidence suggests that anti-tuberculosis drug concentrations in patients might in some circumstances be related to alcohol use,[7] under-nutrition,[8] sex[6,9,10] and drug formulation.[6,7,9,11]

The aim of the present study was to determine the PK of rifampicin (RMP), isoniazid (INH) and pyrazinamide (PZA) in adult TB patients treated with thrice-weekly regimens in Chennai, India.

METHODS

Patients

A prospective PK study was conducted among 101 adult patients with both pulmonary and extra-

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pulmonary forms of TB. The study protocol was approved by the Institutional Ethics Committee of the National Institute for Research in Tuberculosis (NIRT), Chennai, India. All participants provided written consent before inclusion. Patients were diagnosed and treated for TB at RNTCP centres under the Chennai Corporation, India. All consecutive patients receiving anti-tuberculosis treatment at two treatment centres from April to June 2014 were included in the study. Patients who were too sick or moribund were excluded. Patients received RNTCP Category I (2 months of RMP, INH, PZA and ethambutol [EMB], followed by 4 months of RMP and INH) or Category II (2 months of RMP, INH, PZA, EMB and streptomycin [SM], 1 month of RMP, INH, PZA and EMB and 5 months of RMP, INH and EMB). The regimens were thrice-weekly for the entire duration of treatment and administered under directly observed treatment (DOT). The drug doses were as follows: RMP 450 mg (600 mg for those with body weight $\geqslant$60 kg), INH 600 mg, PZA 1500 mg, EMB 1200 mg and SM 0.75 g.

Conducting the study

The PK study was conducted during the first month of anti-tuberculosis treatment after patients had received a minimum of 2 weeks of treatment. All anti-tuberculosis drugs were administered under fasting conditions, and drug administration was observed by an investigator. Blood samples were obtained before and at 2, 4, 6 and 8 h after drug ingestion. Samples were centrifuged immediately and plasma samples were stored at –20°C. Ascorbic acid was added to plasma to prevent oxidation of RMP. A small portion of the blood was used for DNA extraction, *NAT2* genotyping and clinical biochemistry testing.

Plasma drug estimations

Estimation of RMP, INH and PZA was undertaken within a week of blood collection according to previously validated and published methods.[12,13] The methods were validated over the concentration range of 0.25–10.0 μg/ml for RMP and INH and 1.25–50.0 μg/ml for PZA. The per cent recoveries were respectively 95%, 102% and 99% for RMP, INH and PZA. Within- and between-day variabilities of precision were less than 10%.

Calculation of pharmacokinetic parameters

Non-compartmental analysis with WinNonlin version 6.4 (Certara, Princeton, NJ, USA) was used to compute the peak drug concentration (C_{max}), the time to C_{max} (T_{max}) and the area under the curve for 0–8 h ($AUC_{0–8}$), the AUC for 0 to infinity ($AUC_{0–\infty}$) and the half-life ($t_{1/2}$).

Genotyping of N-acetyl transferase 2

Genotyping of the *N*-acetyl transferase 2(*NAT2*) gene was performed in a subgroup of 88 patients. Genomic DNA was extracted using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) and quantitated on Thermo Fischer's NanoDrop™ 2000 spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). Six single nucleotide polymorphisms (*rs*1041983, *rs*1801280, *rs*1799929, *rs*1799930, *rs*1208 and *rs*1799931) in the *NAT2* gene were analysed using Taqman SNP genotyping assays (Applied Biosystems 7500 Real-Time PCR System and Sequence Detection Software v1.3.1; Applied Biosystems, Waltham, MA, USA). The genotypes were predicted using NAT2PRED.[14]

Covariates

The patient factors taken for analysis included age, sex, body mass index (BMI), type of TB, category of anti-tuberculosis treatment, smoking, alcoholism, diabetes mellitus (DM), HIV infection, dose per kg body weight and INH acetylator status. Patients with a known history of DM, irrespective of blood glucose on the study day, and those with random blood glucose >200 μg/ml were considered as having DM in this study.

Statistical evaluation

Data were analysed using SPSS version 20.0 (Statistical Package for the Social Sciences, IBM Corp, Armonk, NY, USA). The target concentration range of C_{max} was taken as 8–24 μg/ml for RMP, 3–6 μg/ml for INH and 20–50 μg/ml for PZA.[15] Values were expressed as percentage, median and interquartile range (IQR). The Shapiro-Wilks test showed that PK data were not normally distributed. The Mann-Whitney *U*-test was used to compare the baseline characteristics of the two groups. Proportions between groups were compared using the *Z* proportion test. The squared ranks test was used to assess equality of variance across the different time points of drug levels. Multivariate regression analysis by stepwise method was used to identify factors that influenced drug concentrations. $P < 0.05$ was considered statistically significant.

Sample size

The sample size was calculated based on observations from a study undertaken at the NIRT in adult TB patients given intermittent anti-tuberculosis treatment under clinical trial conditions, in which the mean RMP concentration was 4.6 μg/ml (standard deviation 3.3). Assuming a variation of 1 μg/ml in RMP under field conditions, the sample size was calculated as 93 at 95% confidence level and 90% power.

Table 1 Patient details

Factors	*n* (%)
Age, years, median [IQR]*	34.0 [23.5–45.0]
Sex	
Female	35 (34.7)
Male	66 (65.3)
BMI, kg/m^2, median [IQR]*	18.6 [16.5–20.6]
Type of TB	
EPTB	29 (28.7)
PTB	72 (71.3)
Category of anti-tuberculosis treatment	
Category I	92 (91.1)
Category II	9 (8.9)
Dose, mg/kg body weight, median [IQR]*	
RMP	9.6 [8.7–10.7]
INH	12.8 [11.0–14.3]
PZA	31.9 [27.5–35.7]
HIV status	
No	99 (98.0)
Yes	2 (2.0)
Diabetes	
No	78 (77.2)
Yes	23 (22.8)
NAT2 genotype ($n = 84$)*	
Slow	54 (64.3)
Intermediate	26 (31.0)
Rapid	4 (4.8)
Smoking status	
No	83 (82.2)
Yes	18 (17.8)
Alcoholism	
No	78 (77.2)
Yes	23 (22.8)
Glucose, mg/dl, median [IQR]*	92.0 [82.5–121.0]
AST, U/l, median [IQR]*	19.0 [16.0–23.0]
ALT, U/l, median [IQR]*	13.0 [11.0–17.5]

* $n = 101$.
IQR = interquartile range; BMI = body mass index; TB = tuberculosis; EPTB = extra-pulmonary TB; PTB = pulmonary TB; RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; HIV = human immunodeficiency virus; NAT = *N*-acetyl transferase; AST = aspartate transaminase; ALT = alanine transaminase.

RESULTS

Patient details are shown in Table 1. HIV co-infected patients constituted 2% of the study cohort, while 22.8% had DM. The majority of the patients had newly diagnosed TB. INH acetylator genotype was classified as slow, intermediate or rapid. Respectively 54, 26 and 4 patients belonged to the slow, intermediate and rapid genotypes. The genotype distribution followed Hardy-Weinberg Equilibrium. Slow acetylators for the *NAT2* gene constituted 64.3% of the cohort.

PK parameters of RMP, INH and PZA are shown in Table 2. The number of patients with RMP C_{max} < 8.0 μg/ml was 89 (88.1%). The corresponding numbers for INH (<3.0 μg/ml) and PZA (<20.0 μg/ml) C_{max} were respectively 1 (1%) and 0. The proportion of patients with measured C_{max} of RMP, INH and PZA at 2 h were respectively 83.2%, 97.0% and 92.1%.

Drug C_{max} and $AUC_{0–8}$ of the different groups of patients are shown in Table 3. The C_{max} and $AUC_{0–8}$ of RMP and PZA were significantly higher in female than in male patients. Patients aged ⩾60 years had higher RMP C_{max} and $AUC_{0–8}$ than those aged <60; the difference was significant for $AUC_{0–8}$. Patients with BMI < 18.5 kg/m^2 had significantly higher INH and PZA C_{max} and $AUC_{0–8}$. Smokers had lower PZA C_{max} and $AUC_{0–8}$ than non-smokers. Although INH and PZA C_{max} and $AUC_{0–8}$ were lower in those with DM and TB than in those with only TB, the difference was statistically significant for PZA only. Differences in INH C_{max} and $AUC_{0–8}$ among slow, intermediate and rapid genotypes were statistically significant. Significant differences were not observed between patients who consumed alcohol and those who did not and between those who received Category I and those on Category II treatment.

The median plasma RMP, INH and PZA concentrations at different time points are shown in the Figure (A–C). Variations in INH ($\chi^2 = 355.19$, $P < 0.0001$) and PZA concentrations ($\chi^2 = 316.14$, $P < 0.0001$) were higher than for RMP ($\chi^2 = 137.10$, $P < 0.0001$).

Using C_{max} as a dependant variable, the multiple linear regression model described respectively 8%,

Table 2 Pharmacokinetic parameters of RMP, INH and PZA ($n = 101$)

Parameters	RMP median [IQR]	INH median [IQR]	PZA median [IQR]
C_{max}, μg/ml	5.0 [3.8–6.9]	11.3 [8.2–13.2]	40.2 [34.2–43.7]
T_{max}, h	2 [2–2]	2 [2–2]	2 [2–2]
$AUC_{0–8}$, μg/ml.h	27.9 [20.1–33.9]	41.1 [33.0–59.9]	228.0 [194.5–252.0]
$AUC_{0–\infty}$, μg/ml.h	45.0 [29.8–68.0]	53.4 [36.9–81.8]	544.8 [407.6–712.3]
$t_{1/2}$, h	4.7 [3.5–7.4]	3.1 [1.9–3.8]	8.6 [7.2–11.4]
Sub-therapeutic C_{max}*			
<	89 (88.1)	1 (1.0)	0
⩾	12 (11.9)	100 (99.0)	101 (100)

* Cut-off (<8.0 μg/ml for RMP, <3.0 μg/ml for INH and <20.0 μg/ml for PZA).
RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; IQR = interquartile range; C_{max} = peak concentration; T_{max} = time at which peak concentration was attained; AUC = area under the time concentration curve; $t_{1/2}$ = half-life.

Table 3 C_{max} and AUC_{0-8} of drugs among different patient groups

		RMP (μg/ml)*		INH (μg/ml)*		PZA (μg/ml)*	
Variables	*n*	C_{max} μg/ml	AUC_{0-8} μg/ml.h	C_{max} μg/ml	AUC_{0-8} μg/ml.h	C_{max} μg/ml	AUC_{0-8} μg/ml.h
Sex							
Female	35	5.8 (4.4–7.4)	31.0 (25.0–38.0)	12.1 (9.5–14.3)	45.0 (35.0–64.0)	42.8 (38.5–49.8)	242.0 (227.0–274.0)
Male	66	4.8 (3.6–6.5)	26.0 (19.0–31.0)	10.8 (7.9–13.2)	41.0 (32.0–58.0)	36.9 (31.5–41.7)	217.0 (181.0–241.0)
P value		0.018	<0.001	0.088	0.148	<0.001	0.001
Age, years							
<60	96	4.9 (3.7–6.9)	26.9 (20.0–33.5)	11.4 (8.3–13.2)	42.9 (33.4–61.1)	40.1 (34.1–43.8)	229.0 (194.0–252.3)
⩾60	5	6.8 (5.2–7.2)	35.4 (30.3–39.5)	11.1 (4.7–13.2)	38.1 (16.1–39.3)	41.1 (32.3–43.8)	223.7 (185.7–233.8)
P value		0.159	0.044	0.476	0.072	0.969	0.453
BMI, kg/m^2							
<18.5	50	5.5 (3.6–7.3)	29.4 (20.0–36.2)	12.0 (9.2–13.7)	48.2 (36.3–62.4)	42.1 (37.7–46.9)	240.6 (227.1–272.3)
⩾18.5	51	4.8 (4.0–6.8)	26.7 (21.8–32.1)	10.3 (7.6–12.1)	38.5 (27.2–53.9)	35.3 (30.8–41.3)	205.9 (171.0–232.5)
P value		0.233	0.294	0.021	0.02	0	0
Smoking status							
No	83	5.0 (4.0–6.7)	28.0 (20.5–34.1)	11.6 (8.5–13.5)	41.6 (34.1–61.1)	40.9 (34.7–45.0)	229.7 (203.9–258.1)
Yes	18	5.0 (3.1–7.0)	24.7 (18.2–33.9)	9.1 (7.6–11.8)	37.8 (27.5–53.9)	35.4 (30.7–40.2)	199.6 (169.8–236.2)
P value		0.635	0.446	0.053	0.205	0.01	0.029
Alcoholic							
No	78	5.0 (4.2–6.7)	28.0 (21.4–34.1)	11.4 (8.5–13.5)	41.2 (34.2–61.1)	40.5 (34.7–45.1)	228.0 (200.1–258.7)
Yes	23	4.8 (3.3–7.0)	24.7 (18.6–33.9)	10.4 (7.7–13.1)	38.5 (27.9–59.6)	37.8 (31.5–41.9)	230.9 (174.9–240.6)
P value		0.636	0.485	0.397	0.489	0.1	0.226
Diabetic							
No	78	4.9 (3.7–6.6)	27.9 (20.0–32.7)	11.5 (8.5–13.5)	42.9 (34.2–61.1)	40.5 (34.6–45.0)	232.3 (206.0–256.1)
Yes	23	5.7 (4.2–7.1)	27.6 (22.0–37.3)	10.7 (6.5–12.4)	37.8 (22.9–53.9)	36.4(28.8–41.6)	192.6 (158.4–230.4)
P value		0.235	0.35	0.174	0.083	0.007	<0.001
Treatment category							
I	92	4.9 (3.7–6.8)	27.1 (20.0–33.5)	11.4 (8.3–13.4)	42.5 (33.0–61.0)	39.5 (33.9–43.8)	227.9 (192.4–252.2)
II	9	6.6 (4.2–7.5)	32.5 (25.3–39.6)	11.1 (7.4–12.4)	38.1 (30.3–47.3)	41.3 (34.5–43.9)	236.8 (211.1–244.6)
P value		0.311	0.13	0.651	0.371	0.501	0.46
Disease type							
EPTB	29	4.3 (3.6–5.8)	23.5 (18.4–32.4)	9.8 (7.6–12.1)	39.5 (27.7–51.8)	36.4 (33.0–42.1)	212.6 (190.0–241.8)
PTB	72	5.2 (4.2–7.0)	28.5 (21.9–35.7)	11.6 (8.5–13.6)	43.5 (33.5–61.2)	40.7 (35.4–44.7)	230.4 (203.5–255.6)
P value		0.042	0.053	0.104	0.215	0.088	0.186
NAT2 genotype							
Slow	54			11.9 (8.5–13.6)	52.6 (37.8–62.4)		
Intermediate	26			10.0 (7.6–11.7)	34.3 (26.9–40.4)		
Rapid	4			6.9 (4.3–10.7)	30.5 (15.6–40.9)		
P value				0.021	<0.001		

C_{max} = peak concentration; AUC = area under the time concentration curve; RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; BMI = body mass index; EPTB = extra-pulmonary tuberculosis; PTB = pulmonary TB; NAT2 = *N*-acetyl transferase 2.

26.5% and 36.2% of the variability associated with RMP, INH and PZA (see Appendix Table A.1).* A reduction of 4.14 μg/ml in PZA C_{max} was observed in male patients. Patients who smoked had a reduction of respectively 1.87 and 4.13 μg/ml in INH and PZA C_{max}. An increment of 1 mg/kg dose caused RMP, INH and PZA C_{max} to increase by respectively 0.38, 1.09 and 0.75 μg/ml. Rapid acetylators of INH had a 3.52 μg/ml reduction in INH C_{max}.

Using AUC_{0-8} as the dependent variable, sex (INH and PZA), smoking (PZA), type of TB (RMP), mg/kg dose (INH and PZA), category of anti-tuberculosis treatment (RMP) and slow *NAT2* genotype (INH) were significant (Appendix Table A.2).

Although factors such as sex (RMP and INH), DM (PZA), BMI (PZA) and type of TB (RMP) were significant in univariate analysis, they were non-significant when adjusted for other factors in the multiple linear regression analysis.

DISCUSSION

In this study, we examined RMP, INH and PZA PK in a cohort of TB patients and identified patient- and treatment-related factors that influenced drug C_{max}. The high proportion of patients (88%) with low RMP C_{max} is a matter of concern. It has been reported that higher doses are associated with improved early bactericidal activity and better treatment response.[16–18] A study in patients with pulmonary TB in Virginia, USA, showed that most patients with slow response to treatment had RMP and INH concentrations below the expected range.[19]A study from Botswana in a predominantly HIV-infected cohort of adults with TB showed that the C_{max} of first-line anti-tuberculosis drugs was frequently be-

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000009/art000 .....

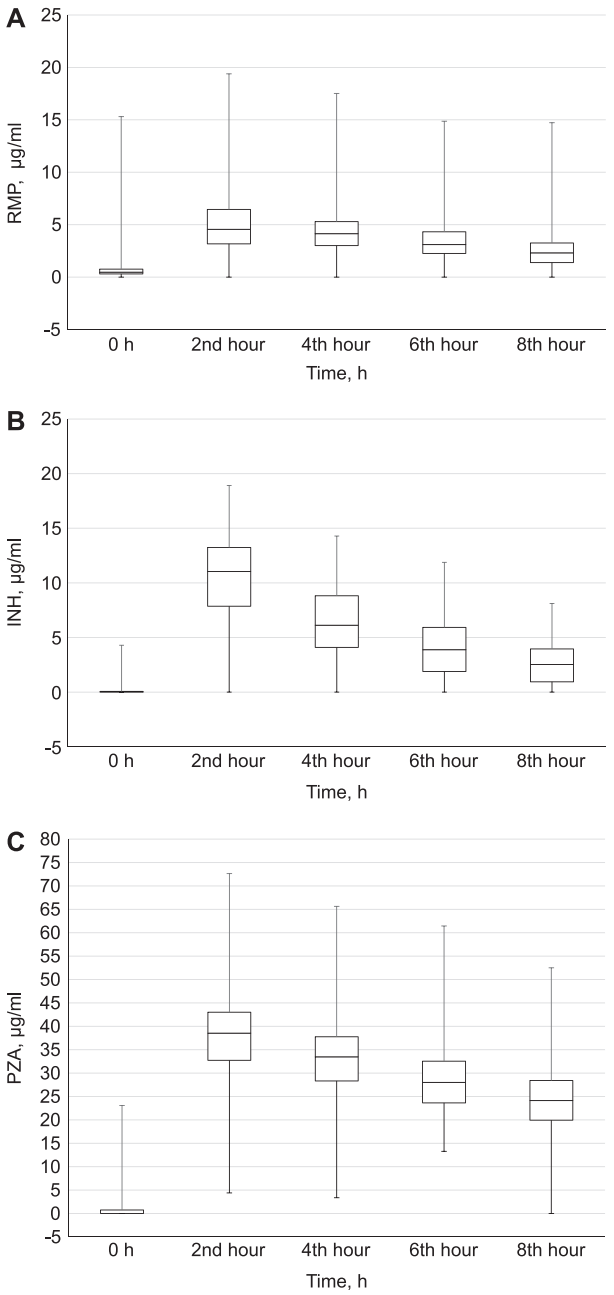

Figure Median concentrations of **A)** RMP, **B)** INH, and **C)** PZA at each sampling time ($n = 101$). The error bars denote the ranges of concentrations at each sampling time, and the boxes represent the 25% to 75% percentile ranges. RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide.

low the target range.[20] Low serum concentrations of anti-mycobacterial drugs were reported by Tappero et al. in a cohort of ambulatory patients with TB in Botswana.[21] Using a mouse model, it has been suggested that RMP activity is concentration-dependent and is related to the AUC/minimum inhibitory concentration (MIC) ratio.[22] Assuming the MIC of RMP to be 1 µg/ml for *M. tuberculosis*, we obtained a median $AUC_{0–8}$/MIC ratio of 27.9, which is several times lower than the estimated levels required for optimal efficacy.[21]

Our findings of low RMP C_{max} in the majority of TB patients are in agreement with several other studies.[6,8,9,21] This can be explained in part due to auto-induction of RMP metabolism, resulting in lower levels after repeated doses.[18,23] However, other factors, and particularly the drug dose, could be crucial.

The majority of patients achieved INH and PZA levels within or above the expected ranges. While this finding is similar to that reported by McIlleron et al.,[6] it contrasts with the findings from other studies.[20,21] These discrepancies may be due to differences in patient characteristics or dosing practices.

Female patients had higher RMP, INH and PZA C_{max} and AUC_{0-8} than their male counterparts. Although the mechanism of sex-related differences in drug PK has been poorly understood, our findings are consistent with other studies.[6,10] Our finding of patients with higher BMI having lower drug concentrations is not surprising, and was in line with estimates. Drugs are distributed over a larger volume in patients with higher BMI, who are therefore likely to have lower drug levels in the blood. The relationship between drug concentrations and mg/kg drug dose supports the widely followed strategy of using body weight to guide dosing practice. The association between smoking and PZA concentrations is unclear, although this was shown to be significant in both univariate and multivariate analysis.

Our finding of low C_{max} and INH C_{max} $AUC_{0–8}$ in slow genotype of the *NAT2* gene is not surprising, as INH concentrations are known to be driven by variations in the concentrations of the NAT2 enzyme, which is genetically controlled.

It has been reported that exposure to RMP is significantly reduced in patients with TB and DM.[24] This is probably the first study to report that patients with DM and TB had lower PZA C_{max} and $AUC_{0–8}$. Although a similar trend was observed in the case of INH (C_{max} 10.7 vs. 11.5 µg/ml, $P = 0.174$; $AUC_{0–8}$ 37.8 vs. 42.9 µg/ml.h, $P = 0.083$), the differences did not attain statistical significance. The reasons for the low INH and PZA concentrations in patients with DM and TB are unclear, although it could be due to malabsorption of drugs because of diabetic enteropathy or higher BMI among diabetic patients. In this study, we observed that patients with DM and TB had significantly higher BMI than those with TB alone (20.3 vs. 18.4 kg/m^2, $P = 0.021$). It would be interesting to evaluate whether PK drug interactions exist between INH/PZA and anti-diabetic medications, or whether the transport of INH and PZA in the body is glucose-mediated, leading to faster elimination of drugs from the blood.

It may not always be possible to conduct intensive or semi-intensive PK studies requiring collection of multiple blood samples in the clinical/field setting,

due to financial and logistical constraints. Studies are therefore typically limited to one or two time points. It has been suggested that the 2-h post-dose RMP, INH and PZA concentrations are usually most informative.[15] Our observation that the majority of patients had RMP, INH and PZA C_{max} at 2 h is in line with the report published by Peloquin.[15] Future studies planned in large populations can choose the 2-h post dosing time-point to examine RMP, INH and PZA concentrations. This is supported by the fact that there was no bias in recruiting patients in this study. All consecutive TB patients with pulmonary or extra-pulmonary TB, newly diagnosed or on retreatment, smear-positive or -negative, were recruited into this study. The PK study procedure was similar for all patients; timing of food intake on the study day was uniform.

In conclusion, several risk factors for variations in drug concentrations were identified. Plasma concentrations of RMP, INH and PZA at 2-h post dosing mimic C_{max}. A high proportion of TB patients in this cohort had RMP C_{max} below the expected range, which is a matter of concern. Measuring drug levels would help clinicians achieve better patient management. This could guide them when altering drug doses, and can be correlated with treatment outcome and adverse drug reactions. Sub-therapeutic drug levels are likely to pose a problem in a subset of patients. It is important to direct public health efforts towards this subset of patients who either suffer relapse, fail to convert sputum in a timely fashion or develop acquired drug resistance. Future research should be directed towards identifying these patients before treatment failure, as this would help prioritise public health resources.

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Table A.1 Factors influencing peak concentrations of drugs

	RMP			INH			PZA		
Factors	Univariate (95%CI)	*P value*	Multivariate (95%CI)	Univariate (95%CI)	*P value*	Multivariate (95%CI)	Univariate (95%CI)	*P value*	Multivariate (95%CI)
Age	0.020 (−0.021 to 0.060)	0.340	—	0.001 (−0.051 to 0.052)	0.981	—	−0.063 (−0.199 to 0.073)	0.362	—
Sex	−0.958 (−2.075 to 0.159)	0.092	*	−1.255 (−2.667 to 0.157)	0.081	*	−6.555 (−10.124 to −2.986)	0.000	−4.137 (−7.439 to −0.834)
Smoking status	−0.521 (−1.926 to 0.884)	0.464	—	−1.553 (−3.309 to 0.203)	0.082	−1.869 (−3.471 to −0.267)	−5.490 (−10.088 to −0.893)	0.020	−4.126 (−8.195 to −0.058)
Alcoholism	−0.175 (−1.460 to 1.111)	0.788	—	−0.652 (−2.274 to 0.970)	0.427	—	−2.604 (−6.886 to 1.677)	0.230	—
Diabetes	0.429 (−0.854 to 1.712)	0.508	—	−1.278 (−2.885 to 0.329)	0.118	—	−5.618 (−9.783 to −1.453)	0.009	*
Treatment category	1.846 (−0.010 to 3.703)	0.051	1.866 (0.040 to 3.693)	−0.230 (−2.625 to 2.165)	0.849	—	2.180 (−4.153 to 8.514)	0.496	—
BMI	−0.066 (−0.221 to 0.089)	0.402	—	−0.197 (−0.390 to −0.004)	0.045	0.455 (0.057 to 0.852)	−0.997 (−1.480 to −0.515)	0.000	*
Type of TB	1.117 (−0.054 to 2.288)	0.061	*	1.112 (−0.380 to 2.604)	0.142	—	2.668 (−1.294 to 6.630)	0.185	—
Dose, mg/kg	0.374 (0.009 to 0.739)	0.045	0.378 (0.019 to 0.737)	0.475 (0.201 to 0.748)	0.001	1.094 (0.512 to 1.676)	0.785 (0.521 to 1.048)	0.000	0.747 (0.496 to 0.998)
NAT2 genotype									
Slow	—	—	—	1.285 (−0.059 to 2.629)	0.061	1.254 (0.009 to 2.499)	—	—	—
Intermediate	—	—	—	−1.009 (−2.812 to 0.158)	0.199	—	—	—	—
Rapid	—	—	—	−3.469 (−6.899 to −0.039)	0.047	−3.518 (−6.670 to −0.366)	—	—	—

* Non-significant at 5% level of significance onmultivariate linear regression using stepwise method.
RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; CI = confidence interval; BMI = body mass index; TB = tuberculosis; NAT = *N*-acetyl transferase.

Table A.2 Factors influencing area under time concentration curve of drugs

	RMP			INH			PZA		
Factors	Univariate (95%CI)	*P value*	Multivariate (95%CI)	Univariate (95%CI)	*P value*	Multivariate (95%CI)	Univariate (95%CI)	*P value*	Multivariate (95%CI)
Age	0.112 (−0.127 to 0.350)	0.355	—	−0.121 (−0.402 to 0.160)	0.396	—	−0.61 (−1.470 to 0.249)	0.162	—
Sex	−4.679 (−11.30 to 1.946)	0.164	—	−7.106 (−14.894 to 0.682)	0.073	−8.828 (−15.75 to −1.90)	−39.455 (−62.477 to −16.434)	0.001	−25.359 (−45.570 to −5.149)
Smoking	−3.631 (−11.83 to 4.575)	0.382	—	−5.518 (−15.045 to 4.008)	0.253	—	−32.487 (−61.256 to −3.717)	0.027	−26.015 (−50.376 to −1.654)
Alcoholism	−1.407 (−9.045 to 6.231)	0.715	—	−2.822 (−11.567 to 5.923)	0.523	—	−14.785 (−41.583 to 12.014)	0.276	—
Diabetes	1.39 (−6.248 to 9.028)	0.719	—	−8.194 (−16.799 to 0.410)	0.062	*	−37.516 (−63.388 to −11.644)	0.005	*
Treatment category	15.512 (4.280 to 26.744)	0.007	17.464 (6.396 to 28.533)	−5.648 (−19.164 to 7.868)	0.409	—	4.906 (−36.820 to 46.632)	0.816	—
BMI	−0.358 (−1.264 to 0.548)	0.435	—	−1.262 (−2.297 to −0.227)	0.017	*	−6.993 (−9.951 to −4.036)	0.000	*
Type of TB	6.753 (−0.17 to 13.676)	0.056	8.264 (1.566 to 14.962)	5.378 (−2.771 to 13.527)	0.193	—	16.032 (−9.056 to 41.119)	0.208	—
Dose, mg/kg	1.842 (−0.298 to 3.982)	0.091	*	2.743 (1.269 to 4.216)	0.000	2.546 (1.198 to 3.894)	5.323 (3.712 to 6.934)	0.000	5.186 (3.667 to 6.706)
NAT2 genotype									
Slow	—	—	—	12.695 (5.720 to 19.670)	0.000	14.342 (7.882 to 20.803)	—	—	—
Intermediate	—	—	—	−12.73 (−20.737 to −4.722)	0.002	*	—	—	—
Rapid	—	—	—	−16.447 (−34.920 to 2.025)	0.080	*	—	—	—

* Non-significant at 5% level of significance on multivariate linear regression using stepwise method.
RMP = rifampicin; INH = isoniazid; PZA = pyrazinamide; CI = confidence interval; BMI = body mass index; TB = tuberculosis; NAT = *N*-acetyl transferase.

RESUME

OBJECTIF : Etudier la pharmacocinétique de la rifampicine (RMP), de l'isoniazide (INH) et du pyrazinamide (PZA) chez les patients adultes avec la tuberculose (TB) et examiner les facteurs qui ont influencé la pharmacocinétique des médicaments.

MÉTHODE : Nous avons étudié des patients TB adultes ($n = 101$), recevant trois fois par semaine un traitement antituberculeux dans le programme national de lutte contre la TB révisé (RNTCP). L'étude a été réalisée à l'équilibre après administration sous observation directe des médicaments. Les concentrations de RMP, d'INH et de PZA ont été estimées par chromatographe liquide à haute performance et génotypage *NAT2* par réaction polymérase en chaîne en temps réel.

RÉSULTATS : Le pic de concentration de la RMP (C_{max}) a été inférieur au seuil thérapeutique (<8 μg/ml) chez 88% des patients. La C_{max} de la RMP, de l'INH et du PZA à 2 h a été observée chez 83,2%, 97% et 92,1% des patients, respectivement. La C_{max} et l'$AUC_{0–8}$ du PZA a été plus faible chez les patients TB atteints de diabète que les non diabétiques. Des associations significatives ont été observées entre la C_{max} et l'$AUC_{0–8}$ de la RMP, de l'INH et du PZA en fonction des dosages des médicaments, de la RMP en fonction de la catégorie de traitement, de l'INH avec le fait de fumer, l'indice de masse corporelle et le génotype *NAT2*, et enfin, du PZA avec le sexe et le fait de fumer.

CONCLUSION : Plusieurs facteurs de risque de variations des concentrations de médicaments ont été identifiés. Les concentrations obtenues 2 h après l'administration ont reproduit la C_{max}. Une proportion élevée de patients TB patients ont eu une C_{max} de RMP inférieure à la fourchette attendue, ce qui est une source de préoccupation.

RESUMEN

OBJETIVO: Analizar la farmacocinética de la rifampicina (RMP), la isoniazida (INH) y la pirazinamida (PZA) en los pacientes adultos con diagnóstico de tuberculosis (TB) y examinar los factores que la modifican.

METODOS: Se incluyeron en el estudio pacientes adultos con diagnóstico de TB ($n = 101$), que recibían tratamiento tres veces por semana en el marco del Programa Nacional Revisado contra la Tuberculosis. El análisis se llevó a cabo en situación de equilibrio, después de una administración observada de los medicamentos. Se determinaron las concentraciones de RMP, INH y PZA mediante cromatografía de líquidos de gran rendimiento y se practicó la genotipificación de *N*-acetyl transferasa 2 (*NAT2*) mediante la reacción encadena de la polimerasa en tiempo real.

RESULTADOS: La concentración máxima (C_{max}) de RMP fue subterapéutica (<8 μg/ml) en 88% de los pacientes. A las 2 h, se observó la C_{max} de RMP en 83,2% de los pacientes, la C_{max} de INH en 97,0% y la de PZA en 92,1% de los casos. La C_{max} y el área bajo la curva de 0 a 8 horas ($AUC_{0–8}$) de PZA fueron inferiores en los pacientes tuberculosos aquejados de diabetes que en los pacientes sin diabetes. La C_{max} y la $AUC_{0–8}$ exhibieron asociaciones significativas con las dosis de medicamentos para RMP, INH y PZA; con la categoría de tratamiento para RMP; con el tabaquismo, el índice de masa corporal y el genotipo del *NAT2* para INH; y con el sexo y el tabaquismo para PZA.

CONCLUSION: El estudio reveló diversos factores de riesgo de variación en las concentraciones de los medicamentos. Dos horas después de la administración, las concentraciones de medicamentos fueron equivalentes a la C_{max}. La alta proporción de pacientes con una C_{max} de RMP por debajo del intervalo previsto es fuente de preocupación.