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Short Paper

Pharmacokinetics of rifampicin, isoniazid & pyrazinamide during daily & intermittent dosing: A preliminary study

Geetha Ramachandran[1], A.K. Hemanth Kumar[2], T. Kannan[3], Balamugesh Thangakunam[4], Deepa Shankar[4] & Devasahayam J. Christopher[4]

Departments of [1]Biochemistry & Clinical Pharmacology, [2]Clinical Pharmacology, [3]Statistics (Epidemiology Unit), ICMR-National Institute for Research in Tuberculosis, Chennai & [4]Department of Pulmonary Medicine, Christian Medical College, Vellore, Tamil Nadu, India

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***Background & objectives*: The National Tuberculosis (TB) Control Programme has transitioned from thrice-weekly to daily drug treatment regimens in India. This preliminary study was conceived to compare the pharmacokinetics of rifampicin (RMP), isoniazid (INH) and pyrazinamide (PZA) in TB patients being treated with daily and thrice weekly anti-TB treatment (ATT).**

***Methods*: This prospective observational study was undertaken in 49 newly diagnosed adult TB patients receiving either daily ATT (n=22) or thrice-weekly ATT (n=27). Plasma RMP, INH and PZA were estimated by high-performance liquid chromatography.**

***Results*: The peak concentration (C_{max}) of RMP was significantly higher (RMP: 8.5 µg/ml *vs.* 5.5 µg/ml; *P*=0.003) and C_{max} of INH was significantly lower (INH: 4.8 µg/ml *vs.* 10.9 µg/ml; *P*<0.001) in case of daily dosing compared to thrice-weekly ATT. C_{max} of drugs and doses was significantly correlated. A higher proportion of patients had subtherapeutic RMP C_{max} (8.0 µg/ml) during thrice-weekly compared to daily ATT (78% *vs.* 36%; *P*=0.004). Multiple linear regression analysis showed that C_{max} of RMP was significantly influenced by the dosing rhythm, pulmonary TB and C_{max} of INH and PZA by the mg/kg doses.**

***Interpretation & conclusions*: RMP concentrations were higher and INH concentrations were lower during daily ATT, suggesting that INH doses may need to be increased in case of a daily regimen. Larger studies are, however, required using higher INH doses when monitoring for adverse drug reactions and treatment outcomes.**

Key words Anti-tuberculosis drugs - daily regimen - drug concentration - intermittent regimen - pharmacokinetics - tuberculosis

Tuberculosis (TB) is a curable disease in the vast majority of patients, when adequate anti-TB treatment (ATT) is administered. The success of the current World Health Organization (WHO) recommended regimen, which results in a cure rate of over 95 per cent in drug-sensitive cases, is due to its ability to achieve early bacterial killing activity, sterilization of dividing bacilli and action against non-replicating mycobacterial persisters[1].

In spite of having established standard short-course chemotherapy regimens containing rifampicin (RMP), isoniazid (INH) and pyrazinamide (PZA), the occurrence of treatment failures, relapses and acquired drug resistance remains unexplained[2]. Treatment outcomes are driven by multiple factors, such as plasma drug concentrations, bacillary load, bacterial strain, virulence, minimal inhibitory concentration in relation to drug concentrations, drug concentrations at the site of lesion, duration of infection, extent of disease, immune status and nutritional status of the subject. Subtherapeutic drug concentrations have also been reported as a risk factor for unfavourable treatment outcome[1-7]. A study from Indonesia showed that most patients had good treatment outcome, in spite of having low RMP, INH and PZA concentrations[6]. Low peak concentrations of RMP were found to influence treatment outcome and/or acquired RMP resistance[1,2]. A retrospective cohort study from Virginia reported that most patients who responded slowly to treatment had two-hour RMP and INH concentrations below the expected range[7]. It has been reported that longer time to culture conversion and treatment failures were more frequent among those having drug concentrations below the expected range[4,5]. A prospective study undertaken in adult TB patients treated with thrice-weekly regimens in the Revised National Tuberculosis Control Programme (RNTCP) at Chennai in South India under programmatic settings identified low RMP concentration as one of the risk factors for poor outcome[3]. A recent systematic review and meta-analysis of first-line anti-TB drug concentrations and treatment outcome showed that the prevalence of low drug concentrations was high for all the drugs, but low PZA concentration increased the risk of poor outcome at the end of treatment and low RMP slightly increased this risk[8].

The National Tuberculosis Elimination Programme (NTEP) in India was providing intermittent ATT for several years. However, recently, the programme guidelines were revised, and ATT is now provided daily and drug dosages are based on body weight[9]. The transition from intermittent to daily treatment was based on the mounting evidence of increased rate of relapse with the former mode of treatment[10]. When the rhythm of ATT is altered from intermittency to daily therapy, the doses also get altered. This is likely to impact drug levels and eventually treatment outcome. The currently used dosages of anti-TB drugs are not based on careful pharmacokinetic (PK) studies.

Not many studies have compared the PK of anti-TB drugs during daily and intermittent ATT. So, the aim of this study was to determine the PK of RMP, INH and PZA and provide data on drug concentrations achieved during daily and thrice-weekly ATT regimens.

Material & Methods

A prospective observational PK study was undertaken in newly diagnosed adult patients with pulmonary/extrapulmonary TB receiving ATT at the department of Pulmonary Medicine and Directly Observed Treatment Short-Course Clinic, Christian Medical College (CMC), Vellore, southern India, between September 2015 to April 2018. The study was approved by the Institutes Ethics Committees of ICMR-National Institute for Research in Tuberculosis (NIRT), Chennai and CMC, Vellore. Patients who were on intermittent treatment according to the NTEP guidelines or daily ATT as per the WHO guidelines were eligible for recruitment. Patients were receiving treatment with NTEP category I (RMP, INH, PZA and ethambutol for two months, and followed up with RMP and INH for four months). Patients meeting the following study criteria were recruited: (*i*) aged 18 yr or above, (*ii*) >30 kg body weight, (*iii*) on ATT for minimum two weeks, (*iv*) not too sick or moribund, (*v*) HIV seronegative, (*vi*) no history of diabetes mellitus and HbA1c ≥7, (*vii*) no history of renal impairment and (*viii*) willing to participate and give written informed consent. Known or suspected multidrug-resistant TB patients were excluded. The study was adequately powered (>80%) to detect statistically significant differences between the two groups of patients.

Sample collection for pharmacokinetic (PK) studies: This PK study was conducted during the first month of ATT, after the participants had received a minimum of two weeks of treatment. The sample collection was carried out on the day of treatment for the patients who were being treated with thrice-weekly ATT. The anti-TB drugs were administered under fasting conditions and drugs administered under supervision. Blood samples (2 ml) were obtained before (0) and at two, four, six and eight hours after drug ingestion in both the groups. The samples were centrifuged immediately and plasma samples were stored at −20°C. Ascorbic acid was added to plasma to prevent oxidation of RMP. The plasma samples were transported to the ICMR-NIRT, Chennai, in dry ice.

Plasma drug estimations: Estimation of RMP, INH and PZA was undertaken at ICMR-NIRT, Chennai, within

a week of blood collection according to previously validated and published methods[11,12]. 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 95, 102 and 99 per cent, respectively, for RMP, INH and PZA. Within- and between-day variabilities of precisions were <10 per cent.

Calculation of pharmacokinetic (PK) parameters: Based on plasma drug concentrations obtained at different time points, certain PK variables were calculated by non-compartmental analysis using WinNonlin version 6.4 (Certara). Peak drug concentration (C_{max}), the time to attain C_{max} (T_{max}), the area under the curve (AUC_{0-8}), clearance (Cl) and half-life ($t_{1/2}$) were calculated.

Statistical evaluation: Stata V.15.0 (StataCorp, College Station, TX, USA) was used for data analysis. Shapiro-Wilk test was used for verification of data and checking of normality. There was non-normal distribution of PK observations. Categorical variables were presented as proportions and continuous variables were presented as median with interquartiles. Drug concentrations between the patient groups were compared using Mann-Whitney U test. Comparison of proportions was performed using z proportion test. The subtherapeutic cut-off concentrations were taken as <8 µg/ml for RMP, <3 µg/ml for INH and <20 µg/ml for PZA[13]. Spearman's rank correlation test was performed to test the correlations between C_{max}/AUC_{0-8} and drug doses.

A single and multivariable linear regression analysis was performed to determine factors, such as age, gender, treatment regimen, type of TB, body mass index (BMI) and drug dosage that influenced drug concentrations. Statistical significance was determined at $P<0.05$.

Results & Discussion

A total of 49 patients took part in the study; 22 and 27 patients, respectively, were receiving daily and thrice-weekly ATT. All patients were smear positive. Patients did not significantly differ in age and body weight. There were 15 (68%) and 16 (59%) males in the daily and thrice-weekly treatment arms, respectively. The mg/kg RMP and INH doses received (as per recommended dosing) by patients in the daily and thrice-weekly treatment arms differed significantly. Patients received significantly higher RMP dose and lower INH and PZA dose during daily ATT than during thrice-weekly ATT (RMP: 10.8 *vs*. 9.8 mg/kg; $P=0.008$, INH: 5.8 *vs*. 13.0 mg/kg; $P<0.001$ and PZA: 26.3 *vs*. 32.4 mg/kg; $P=0.002$).

Based on the drug plasma concentrations obtained at different time points, PK variables were calculated (Table). The C_{max} of RMP was significantly higher in patients who received daily ATT than those who received thrice-weekly ATT (8.5 µg/ml *vs*. 5.5 µg/ml; $P=0.003$). The C_{max} and AUC_{0-8} of INH were significantly lower and Cl significantly higher in patients who received daily ATT than those who received thrice-weekly ATT. There were significant differences in the PK of PZA between the two treatment groups.

Significant correlations were obtained between C_{max} and drug doses; the *r* and *P* values were as follows: RMP (0.38 and 0.007), INH (0.77 and <0.001) and PZA (0.58 and <0.001). The corresponding values for AUC_{0-8} and drug doses were as follows: RMP (0.38 and 0.006), INH (0.80 and <0.001) and PZA (0.58 and <0.001). The number (%) of patients with RMP C_{max}<8.0 µg/ml in the daily and thrice-weekly treatment groups was eight (36%) and 21 (78%), respectively ($P=0.004$).

A single and multiple linear regression analysis was carried out to identify factors such as age, gender, dosing rhythm, type of TB, BMI and drug doses (mg/kg) that influenced drug concentrations. After adjusting for factors, RMP C_{max} was significantly influenced by the dosing rhythm and type of TB; RMP C_{max} was likely to be 2.14 µg/ml lower during thrice-weekly than daily dosing. INH and PZA mg/kg doses significantly influenced C_{max} of these drugs. An increase in one unit of drug doses was likely to increase INH and PZA C_{max} by 1.34 µg/ml and 1.62 µg/ml, respectively.

Acquired drug resistance can emerge even when a robust TB control programme is in place. Hence, there is the need to ensure good compliance to treatment. PK assessment has been reported to have a role in the context of anti-TB treatment[14]. Hence, it is important to understand TB treatment from a PK perspective.

This prospective study provides PK data of key first-line anti-TB drugs, RMP, INH and PZA during daily and thrice-weekly dosing in adult TB patients. Currently, the NTEP is treating TB patients with daily ATT, the doses being body weight based[9]. This study was undertaken at a time when there was a transition from thrice-weekly to daily ATT in India, thus giving us an opportunity to compare the PK of RMP, INH and PZA during daily and intermittent dosing.

Table. Pharmacokinetics of rifampicin, isoniazid and pyrazinamide during daily and thrice-weekly treatment

PK variables	RMP			INH			PZA		
	Daily	Thrice weekly	*P*	Daily	Thrice weekly	*P*	Daily	Thrice weekly	*P*
C_{max} (µg/ml)	8.5 (7.2-9.3)	5.5 (4.0-8.0)	0.003	4.8 (3.4-5.4)	10.9 (6.6-16.2)	<0.001	38.2 (26.9-45.6)	39.6 (28.8-49.8)	0.540
T_{max} (h)	2 (2-2)	2 (2-4)	0.042	2 (2-2)	2 (2-2)	0.832	2 (2-4)	2 (2-4)	0.960
AUC_{0-8} (µg/ml.h)	38.0 (29.7-47.8)	30.9 (18.9-37.9)	0.077	14.9 (10.6-26.6)	45.5 (31.2-67.0)	<0.001	223.8 (153.8-243.5)	235.7 (170.2-289.8)	0.309
Cl (ml/min)	11.1 (9.3-14)	10.3 (7.1-16.1)	0.732	18.7 (8.1-26.0)	9.9 (6.9-12.4)	0.050	2.9 (1.9-3.1)	2.3 (1.7-2.6)	0.141
$t_{1/2}$ (h)	2.6 (2.0-3.9)	4.1 (3.1-6.1)	0.016	2.1 (1.6-3.5)	3.1 (1.9-4.0)	0.115	8.8 (7.0-10.0)	10.7 (8.5-18.1)	0.046

Values were presented as median (IQR). IQR, interquartile range; PK, pharmacokinetics; RMP, rifampicin; INH, isoniazid; PZA, pyrazinamide; C_{max}, peak drug concentration; T_{max}, time to attain C_{max}; AUC, area under the curve; Cl, clearance; $t_{1/2}$, half-life

In the present study there seemed to exist a linear relationship between drug doses and plasma concentrations. The dose of RMP was higher during daily as compared to thrice-weekly ATT, which translated into a higher RMP C_{max} during the daily ATT compared to intermittent ATT. These findings suggest that daily RMP was likely to produce therapeutic drug concentrations that would improve outcome. Ongoing clinical trials evaluating the efficacy of RMP administered in much higher doses than presently used could throw light on this aspect.

Several studies have shown the association between low serum concentrations of anti-TB drugs and treatment failure/relapse/acquired drug resistance[1,2,15-18]. A study undertaken in TB patients in Chennai treated under the RNTCP programme setting showed that low RMP concentrations were one of the risk factors for poor treatment outcome[3]. A high proportion of patients (>90%) had sub-therapeutic RMP concentrations, which was likely due to inadequate dosing. In the present study, patients received higher mg/kg dose of RMP during the daily rather than the thrice-weekly ATT regimen, which led to elevated RMP C_{max} in the former compared to the latter group of patients.

The PK of INH seemed to fare better during thrice-weekly ATT, the drug dose, C_{max} and AUC being higher during thrice-weekly than during daily ATT. A higher proportion of patients had sub-therapeutic INH C_{max} (23%) during daily ATT than during thrice-weekly ATT. This is higher than a previous study undertaken in Chennai, in which 16 per cent of patients had sub-therapeutic two-hour INH during thrice-weekly ATT[3]. Of the five patients who had sub-therapeutic INH C_{max} during daily ATT, four had lower half-lives and higher clearance, suggesting that they could have been rapid acetylators of INH. Since there existed significant correlations between drug doses and C_{max}, increasing INH dose was likely to increase INH C_{max} as was shown in the multiple linear regression analysis. Based on these findings and from a PK standpoint, it can be suggested that the existing INH doses during daily ATT may have to be increased.

This study had some limitations. INH acetylator status, which is known to influence INH drug concentrations, was not determined in the study patients. Since this was a pure PK study, patients were not followed up for hepatotoxicity and other drug toxicities and treatment outcomes.

Overall, this study showed that the drug doses used in the NTEP for daily ATT produced plasma RMP

concentrations within the therapeutic range, but INH doses used during daily ATT resulted in suboptimal concentrations. However, these studies need to be carried out in larger number of patients in different settings, before policy decisions on dosing are made.

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***Conflicts of Interest*:** None.

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For correspondence: Dr Devasahayam J. Christopher, Department of Pulmonary Medicine, Christian Medical College, IDA Scudder Road, Vellore 632 004, Tamil Nadu, India
e-mail: djchris@cmcvellore.ac.in