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Isoniazid, Rifampin, and Pyrazinamide Plasma Concentrations in Relation to Treatment Response in Indonesian Pulmonary Tuberculosis Patients

Erlina Burhan,[a] Carolien Ruesen,[b] Rovina Ruslami,[c] Arum Ginanjar,[a] Hadiarto Mangunnegoro,[a] Purwantyastuti Ascobat,[d] Rogier Donders,[e] Reinout van Crevel,[b] Rob Aarnoutse[f]

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia[a]; Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands[b]; Department of Pharmacology and Therapy, Medical Faculty, University of Padjadjaran, Bandung, Indonesia[c]; Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia[d]; Department of Epidemiology, Biostatistics and HTA, Radboud University Medical Centre, Nijmegen, The Netherlands[e]; Department of Pharmacy, Radboud University Medical Centre, Nijmegen, The Netherlands[f]

Numerous studies have reported low concentrations of antituberculosis drugs in tuberculosis (TB) patients, but few studies have examined whether low drug concentrations affect TB treatment response. We examined steady-state plasma concentrations of isoniazid, rifampin, and pyrazinamide at 2 h after the administration of drugs ($C_{2\,h}$) among 181 patients with pulmonary tuberculosis in Indonesia and related these to bacteriological response during treatment. $C_{2\,h}$ values below reference values for either isoniazid, rifampin, or pyrazinamide were found in 91% of patients; 60% had at least two low $C_{2\,h}$ concentrations. The isoniazid $C_{2\,h}$ was noticeably lower in fast versus slow acetylators (0.9 mg/liter versus 2.2 mg/liter, $P < 0.001$). At the end of treatment, 82% of the patients were cured, whereas 30 patients (17%) had dropped out during the study, and 2 patients (1%) failed treatment. No association was found between $C_{2\,h}$ concentrations and sputum culture results at 8 weeks of treatment. *Post hoc* analysis showed that patients with low pyrazinamide $C_{2\,h}$ ($P = 0.01$) and patients with large extensive lung lesions ($P = 0.01$) were at risk of at least one positive culture at week 4, 8, or 24/32. Antituberculosis drug concentrations were often low, but treatment response was nevertheless good. No association was found between drug concentrations and 8 weeks culture conversion, but low pyrazinamide drug concentrations may be associated with a less favorable bacteriological response. The use of higher doses of pyrazinamide may warrant further investigation.

Generally, first-line treatment of drug-susceptible tuberculosis (TB) is highly effective. However, a number of patients do not respond adequately to treatment, develop drug resistance or experience a relapse of TB after completion of treatment. Inadequate exposure to anti-TB drugs may constitute one of the factors underlying suboptimal treatment response (1, 2). Among adults, low plasma concentrations of anti-TB drugs have been found in patients with HIV infection, gastrointestinal tract disorders, high body weight, male gender, or diabetes mellitus (DM) (3–11) and in fast acetylators for isoniazid (12). Low plasma concentrations can also result from interindividual variability in drug absorption, metabolism, or excretion (3, 13). Some studies have reported associations between low concentrations of anti-TB drugs and poor treatment response (1–3, 14), although this was not found in other studies (7, 15). In a recent study performed in a preclinical model, pharmacokinetic variability appeared to be more important in the emergence of multidrug-resistant tuberculosis (MDR-TB) than nonadherence (16). Furthermore, a systematic review showed that pharmacokinetic variability to isoniazid in multi-drug TB regimens is significantly associated with therapy failure and acquired drug resistance (17).

Nevertheless, the number of studies examining the relation between plasma concentrations of anti-TB drugs and treatment response remains limited and the majority of them investigated the relation between plasma concentrations of separate anti-TB drugs and treatment response. Therefore, in the present study, we assessed the relation between plasma concentrations of isoniazid, rifampin, and pyrazinamide, studied separately and in combination, in relation to treatment response in patients with pulmonary TB. The study was a practical field pharmacokinetic study conducted under routine conditions in a hospital setting in Indonesia, which has the fourth highest case-load of TB worldwide, a high MDR-TB burden, and cure rates between 72% (retreatment) and 91% (new smear-positive cases) (18).

MATERIALS AND METHODS

Study design and recruitment of subjects. We conducted a prospective cohort study among TB patients who visited the Persahabatan Hospital, Jakarta, for treatment between March 2010 and March 2011. Patients were included if they had a positive acid-fast bacilli (AFB) smear, were starting treatment with anti-TB drugs according to the direct observed treatment short course (DOT) strategy, and gave informed consent. Patients who were pregnant, below 18 or above 65 years of age, with drug-resistant isolates, or with impaired liver or renal function were excluded.

Drug dosing was according to World Health Organization (WHO)-recommended weight bands. Study subjects received 150, 225, 300, or 375 mg of isoniazid, 300, 450, 600, or 750 mg of rifampin, and 550, 825, 1,100, or 1,375 mg of pyrazinamide according to their body weight (the body weight bands were 30 to 39 kg, 40 to 54 kg, 55 to 70 kg, and >70 kg). The

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Address correspondence to Carolien Ruesen, carolienruesen@gmail.com.
E.B. and C.R. share first authorship.

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anti-TB drugs were administered in fixed dose combinations from Kimia Farma that are approved by the Indonesian national program based on bio-equivalence studies. Anti-TB drugs were taken without food.

Basic demographic and clinical information was collected from all participants, including age, sex, body weight, and length (to calculate body mass index [BMI]), comorbidities (including HIV infection and DM), and concomitant drug use. HIV and DM status was checked through blood samples collected after 4 weeks. AFB and sputum culture were collected at baseline and at 4 weeks, 8 weeks, and 24 or 32 weeks (32 weeks for retreatment cases). Spoligotyping was performed at baseline. Drug susceptibility testing was performed on all isolates using the proportion method (19) in a WHO-accredited laboratory. Patients with resistant isolates were excluded from the study. Chest X-rays were made at baseline, and after 24 or 32 weeks and extensive lesions were categorized by size (area less than 5 vertebrae, 5 to 9 vertebrae, or >9 vertebrae).

Blood sampling, bioanalysis, and pharmacokinetic data analysis. Patients had refrained from food at least 8 h before drug intake at the pharmacokinetic sampling day. Plasma drug concentrations from blood collected 2 h after administration of drugs ($C_{2\,h}$) were used as the estimated peak plasma concentration (C_{max}). Drug sampling took place at 4 weeks after inclusion in the study, because of the expected steady state in the pharmacokinetics of the TB drugs at that time. In addition, 6 weeks after inclusion in the study, full pharmacokinetic curves were obtained in nine subjects. Serial venous blood samples were collected just before, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after observed TB drug intake. Plasma was separated and stored at −80°C immediately until transport on dry ice to the Netherlands for bioanalysis. Isoniazid and acetylisoniazid concentrations were measured with a validated method comprising liquid-liquid extraction, followed by ultraperformance liquid chromatography with UV detection. Accuracy was between 97.8 and 106.7% for isoniazid and between 98.0 and 108.9% for acetylisoniazid, dependent on the concentration level. The intra- and interassay coefficients of variation were <13.4 and <3.2% (dependent on the concentration) over the range of 0.05 to 15.1 mg/liter for isoniazid, and <4.2% and <5.7% over the range of 0.16 to 16.2 mg/liter for acetylisoniazid. Lower limits of quantification were 0.05 mg/liter for isoniazid and 0.16 mg/liter for acetylisoniazid. The concentrations of rifampin and pyrazinamide were assessed with validated high-performance liquid chromatographic assays by methods described previously (9). All analytical methods performed well at two concentration levels in the first round of a recently developed interlaboratory proficiency testing program for TB drugs (20). In patients with full pharmacokinetic curves, pharmacokinetic parameters were assessed using standard noncompartmental methods in WinNonLin Version 5.3 as described before (9).

Acetylator status for isoniazid was determined phenotypically by calculating the metabolic ratio (MR) for isoniazid (acetylisoniazid $C_{2\,h}$/isoniazid $C_{2\,h}$), with fast acetylators having MRs ≥ 1.5 and slow acetylators having MRs < 1.5 (21).

Statistical analysis. Correlation analyses were performed for the patients with full pharmacokinetic curves to check whether $C_{2\,h}$ was a good representative of C_{max} or total exposure (AUC_{0-24}) for the three drugs. In addition, independent samples *t* tests and correlation analyses were carried out among all patients to identify determinants of pharmacokinetics of the three different antituberculosis drugs.

The relation between plasma concentrations of antituberculosis drugs and culture results at week 4 and 8 was explored among patients with a positive culture at week 0. Treatment response was defined as "poor" if patients had a positive culture at 8 weeks of treatment (22) (primary analysis), or if patients had any positive culture at 4, 8, or 24/32 weeks of treatment (*post hoc* analysis). Univariate and multivariate logistic regression analyses were used to detect determinants of poor treatment response. Determinants evaluated were demographic variables (i.e., gender, age, weight, and BMI), smoking, new case or retreatment case, Beijing genotype for *M. tuberculosis*, comorbidities, chest X-ray findings at baseline, sputum microscopy at baseline, and plasma concentrations of the three TB drugs. For the plasma concentrations of each of the three TB drugs, odds ratios (ORs) for a poor treatment response were assessed for an interquartile range increase in $C_{2\,h}$, i.e., an increase in $C_{2\,h}$ values from the 25th percentile to the 75th percentile of the observed $C_{2\,h}$ values (interquartile OR).

$C_{2\,h}$ values were ln transformed before statistical analysis. The multivariate models included $C_{2\,h}$ of the three drugs separately, any variable with $P < 0.1$ in univariate analysis, DM, HIV, prior TB treatment, sputum microscopy at baseline, and *M. tuberculosis* Beijing genotype. Concentrations of the different drugs were not analyzed in the same model as they were significantly correlated.

In addition to continuous measures for the $C_{2\,h}$ values of isoniazid, rifampin, and pyrazinamide, drug $C_{2\,h}$ values were also examined as dichotomized variables based on previously published ranges for C_{max} (13). These reference values represent the normal concentrations that can be expected in adults after the standard doses of anti-TB drugs. They are based on data that were compiled from all available sources (both healthy volunteers and TB patients) by, among others, Holdiness (23) and Peloquin (24). $C_{2\,h}$ values were defined as low or very low, respectively, if they were <8 or <4 mg/liter (rifampin), <3 or <1.5 mg/liter (isoniazid), and <35 or <20 mg/liter (pyrazinamide). ORs for a poor treatment response were assessed for the number of drugs (no drug, one drug, two drugs, all three drugs) with low (or very low) concentrations in patients. All statistical evaluations were performed with SPSS for Windows, version 16.0. *P* values of <0.05 were considered to be statistically significant in all analyses.

RESULTS

Patients. A total of 196 patients with pulmonary TB were recruited for the study, 15 of whom were excluded from further analysis because of missing pharmacokinetic results. The remaining 181 patients presented with a 2- to 3-week history of cough (99%), fever (67%), shortness of breath (57%), self-reported weight loss (81%), night sweats (64%), and/or chest pain (51%) (Table 1). The nine patients with a full pharmacokinetic curve included in the cohort showed comparable baseline demographic and clinical characteristics with the total cohort, although none of the nine patients were infected with a Beijing genotype strain, compared to 29% of the full cohort. In addition, the nine patients had a slightly higher body weight and younger age (data not shown). The number of fast acetylators was 5/8 (63%) among the nine patients, compared to 98/179 (55%) among the whole cohort, and this affects the average pharmacokinetics of isoniazid.

Pharmacokinetics of isoniazid, rifampin, and pyrazinamide. In the nine patients with full pharmacokinetic curves, the $C_{2\,h}$ values correlated well with the C_{max}s for isoniazid (Spearman's *r*, 0.850; $P = 0.004$) and pyrazinamide (Spearman's *r*, 0.817; $P = 0.007$) but not for rifampin (Spearman's *r*, 0.550; $P = 0.1$). However, the rifampin $C_{2\,h}$ and rifampin AUC_{0-24} were highly correlated (Spearman's *r*, 0.950; $P < 0.001$) and similar correlations were found for the $C_{2\,h}$ and AUC_{0-24} of isoniazid (Spearman's *r*, 0.967; $P < 0.001$) and pyrazinamide (Spearman's *r*, 0.700; $P = 0.04$).

Geometric mean (GM) $C_{2\,h}$ values of isoniazid and rifampin were below 3 and 8 mg/liter respectively, whereas the average pyrazinamide $C_{2\,h}$ values were in the reference range (Table 2). Marked interindividual variability was observed in the $C_{2\,h}$ plasma concentrations of the TB drugs, and low $C_{2\,h}$ values were commonly observed for each of the three separate TB drugs as can be seen in Table 2. In addition, 91% of the patients had at least one low drug level among the three drugs and 56% of the patients had at least one "very low" drug level. Furthermore, 60 and 11% of the

TABLE 1 Demographic, clinical, and laboratory characteristics of the study population

Characteristic[a]	Percentage or median (IQR)[b] ($n = 181$)
Median age in yrs	35 (25–46)
Male subjects	62.4
Diabetes mellitus	24.3
Median wt (kg)	50 (44–55)
DM	55 (47–58)
Non-DM	48 (44–53)
Median BMI	18 (17–20)
DM	21 (18–22)
Non-DM	18 (17–19)
HIV positive	1.1
Tobacco smoker	2.8
Prior TB treatment	8.8
Use of other medication	18.2
Extrapulmonary TB	4.4
Chest X-ray abnormalities	
Old lesion, calcification, fibrosis	8.9
Active lesion, infiltrates, cavity	91.1
Sputum microscopy at baseline	
≥10 AFB per 100 fields	4.4
1 to 10 AFB per field	31.5
>10 AFB per field	64.1
Positive *M. tuberculosis* sputum culture at baseline	92.3
M. tuberculosis Beijing genotype	29.0
Median metabolic ratio	1.9 (0.4–4.0)
Median dose (mg/kg)	
Isoniazid	4.9 (4.5–5.2)
Rifampin	9.8 (9.0–10.4)
Pyrazinamide	17.9 (16.5–19.1)

[a] BMI, body mass index (calculated as the weight in kilograms divided by the square of the height in meters); DM, diabetes mellitus; TB, tuberculosis; AFB, acid-fast bacilli; metabolic ratio, calculated as acetylisoniazid $C_{2\,h}$ divided by isoniazid $C_{2\,h}$; IQR, interquartile range. Data are missing for diabetes mellitus ($n = 1$), *M. tuberculosis* Beijing genotype ($n = 50$), and chest X-ray abnormalities ($n = 57$).
[b] Data are presented as percentage or median (interquartile range).

patients had at least two low or very low drug levels, respectively. A total of 98 patients (55%) and 81 patients (45%) were classified as fast acetylators and slow acetylators, respectively. Fast acetylators had noticeably lower isoniazid plasma $C_{2\,h}$ than slow acetylators (GM, 0.9 mg/liter; interquartile range [IQR], 0.7 to 1.4 mg/liter versus 2.2 mg/liter; IQR, 1.5 to 3.1 mg/liter; $P < 0.001$).

Correlation analyses demonstrated that the $C_{2\,h}$ values of the three different drugs were significantly correlated. A higher $C_{2\,h}$ of rifampin correlated with a higher $C_{2\,h}$ level of isoniazid (Pearson r, 0.21, $P = 0.004$), and similar correlations were found for rifampin and pyrazinamide ($r = 0.453$, $P < 0.001$) and isoniazid and pyrazinamide ($r = 0.424$, $P < 0.001$). In addition, independent sample t tests showed that patients with DM had lower isoniazid concentrations (GM, 1.1 versus 1.5 mg/liter, $P = 0.02$). Other variables, including age, body weight, BMI, and gender, did not affect the $C_{2\,h}$ plasma concentrations of the TB drugs.

Determinants of treatment response. Of all 181 patients, 167 (92%) had a positive culture at week 0 and were included in further analysis. A total of 131 patients (78% of 167) had culture results for weeks 4, 8, and 24/32. Of those, 49 (37%) had at least one positive culture during treatment with the majority ($n = 38$) having a positive culture only at week 4. The proportion of patients with a positive culture decreased sharply during the course of treatment. Fifty-six (34%) of 167 patients had a positive culture at week 4, 11 of 155 (7%) at week 8, and 2 of 131 (2%) at weeks 24/32. After 24 or 32 weeks, the majority (149 patients or 82%) of the 181 patients were cured, while 30 patients (17%) dropped out during the study, and 2 patients (1%), both new smear-positive cases, failed treatment. Of those who were cured, 140 patients were new smear-positive cases and 9 were retreatment cases.

In primary univariate logistic regression analyses, plasma concentrations of the antituberculosis drugs were not significantly associated with culture results at week 8, and none of the associations appeared to reach statistical significance either (Table 3, lower part). For example, patients with a negative culture at week 8 ($n = 142$) had a GM isoniazid $C_{2\,h}$ value of 1.4 mg/liter, and those with a positive culture ($n = 11$) had a GM isoniazid $C_{2\,h}$ of 1.3 mg/liter (Table 3, lower part), already indicating the lack of

TABLE 2 Steady-state pharmacokinetics of isoniazid, rifampin, and pyrazinamide for the nine patients with a full pharmacokinetic curve, as well as for the total cohort

Parameter[a]	GM (range)[b]		
	Isoniazid	Rifampin	Pyrazinamide
Total cohort ($n = 181$)			
$C_{2\,h}$ GM in mg/liter (range)	1.4 (0.2–5.0)	6.5 (0.1–19.7)	36.1 (13.5–68.0)
$C_{2\,h}$ GM in mg/liter (IQR)	1.4 (0.9–2.2)	6.5 (5.0–11.7)	36.1 (32.2–41.4)
Proportions with low $C_{2\,h}$ levels	50% (<1.5 mg/liter)	17% (<4 mg/liter)	2% (<20 mg/liter)
	88% (<3 mg/liter)	49% (<8 mg/liter)	39% (<35 mg/liter)
Patients with a full curve ($n = 9$)			
AUC_{0-24} in h · mg/liter (range)	4.4 (1.3–15.0)	35.4 (26.8–49.9)	382.4 (270.4–595.9)
C_{max} in mg/liter (range)	1.9 (1.0–3.5)	9.0 (6.6–12.8)	42.3 (37.3–52.5)
Median T_{max} in h (range)	1.0 (1.0)	3.0 (3.0)	1.0 (1.0)
$C_{2\,h}$ GM in mg/liter (range)	0.9 (0.2–2.7)	6.6 (3.7–8.8)	36.2 (30.6–47.0)
$C_{2\,h}$ GM in mg/liter (IQR)	0.9 (0.4–2.0)	6.6 (5.3–8.6)	36.2 (31.0–39.3)
Half-life in h (range)	1.9 (0.9–4.0)	1.6 (1.4–1.8)	6.7 (5.2–9.6)

[a] IQR, interquartile range; C_{max}, maximum observed plasma concentration; T_{max}, time to C_{max}; AUC_{0-24}, area under the plasma concentration-time curve from time zero until 24 h after dose administration; $C_{2\,h}$, plasma concentration 2 h after dose administration.
[b] Data are presented as the geometric mean (GM; range, minimum to maximum) except where stated otherwise. $C_{2\,h}$ data were missing for isoniazid ($n = 2$), rifampin ($n = 4$), and pyrazinamide ($n = 2$).

TABLE 3 Relation between antituberculosis drug plasma concentrations and culture results at weeks 4 and 8[a]

Characteristic[b]	GM (IQR)		OR (95% CI)[c] for positive culture
	Positive culture	Negative culture	
Wk 4 (*n*)	56	108	
Isoniazid $C_{2\,h}$ (mg/liter)	1.3 (0.9–2.2)	1.5 (1.0–2.2)	0.76 (0.49–1.18)*
Rifampin $C_{2\,h}$ (mg/liter)	6.5 (5.1–10.8)	6.7 (4.8–11.8)	0.95 (0.66–1.36)*
Pyrazinamide $C_{2\,h}$ (mg/liter)	35.4 (31.8–40.9)	37.0 (33.2–41.5)	0.80 (0.55–1.15)*
Low plasma concentrations			
No drug	5 (36)	9 (64)	1.00
One drug	18 (35)	34 (65)	0.95 (0.28–3.27)
Two drugs	21 (37)	36 (63)	1.05 (0.31–3.55)
All three drugs	12 (29)	29 (71)	0.75 (0.21–2.69)
Very low plasma concentrations			
No drug	24 (33)	49 (67)	1.00
One drug	23 (30)	53 (70)	0.89 (0.44–1.77)
Two drugs	8 (57)	6 (43)	2.72 (0.85–8.73)
All three drugs	1 (100)	0 (0)	NA
Wk 8 (*n*)	11	142	
Isoniazid $C_{2\,h}$ (mg/liter)	1.3 (0.9–2.4)	1.4 (0.9–2.2)	0.88 (0.39–1.96)*
Rifampin $C_{2\,h}$ (mg/liter)	6.5 (6.1–12.1)	6.8 (5.0–11.7)	0.95 (0.47–1.90)*
Pyrazinamide $C_{2\,h}$ (mg/liter)	38.5 (34.0–44.6)	36.5 (32.4–41.0)	1.38 (0.65–2.93)*
Low plasma concentrations			
No drug	2 (15)	11 (85)	1.00
One drug	3 (6)	46 (94)	0.36 (0.05–2.41)
Two drugs	4 (7)	50 (93)	0.44 (0.07–2.71)
All three drugs	2 (5)	35 (95)	0.31 (0.04–2.50)
Very low plasma concentrations			
No drug	5 (7)	65 (93)	1.00
One drug	4 (6)	64 (94)	0.81 (0.21–3.16)
Two drugs	2 (14)	12 (86)	2.17 (0.38–12.49)
All three drugs	0 (0)	1 (100)	NA

[a] Data are presented as the GM (IQR) or number (percentage) unless stated otherwise in column 1. Pharmacokinetic parameters were log transformed. OR, odds ratio; CI, confidence interval; $C_{2\,h}$, plasma concentration 2 h after dose administration; NA, not assessed.

[b] For "low plasma concentrations," isoniazid $C_{2\,h}$ at <3 mg/liter, rifampin $C_{2\,h}$ at <8 mg/liter, and pyrazinamide $C_{2\,h}$ at <35 mg/liter were considered low. For "very low plasma concentrations," isoniazid $C_{2\,h}$ at <1.5 mg/liter, rifampin $C_{2\,h}$ at <4 mg/liter, and pyrazinamide $C_{2\,h}$ at <20 mg/liter were considered very low. "No drug" served as a reference category.

[c] *, That is, the OR for an interquartile range increase in $C_{2\,h}$, i.e., an increase in $C_{2\,h}$ values from the 25th percentile to the 75th percentile of observed $C_{2\,h}$ values (interquartile OR), based on continuous drug concentration data.

difference in exposure to isoniazid in these two groups. The interquartile OR for a positive culture at week 8 associated with an increase in isoniazid $C_{2\,h}$ from the 25th percentile (0.9 mg/liter, see Table 2) to the 75th percentile of isoniazid $C_{2\,h}$ values (2.2 mg/liter, Table 2) was not significantly lower than 1 (interquartile OR, 0.88; 95% confidence interval [CI] = 0.39 to 1.96). Similarly, plasma concentrations were not significantly related to culture results at week 4 (Table 3, upper part). The two patients with a positive culture at week 24/32 had higher $C_{2\,h}$ for the three drugs than the patients with a negative culture (data not shown). Oddly, four patients had a positive culture at week 8 or 24/32 after a negative culture at week 4 or 8.

In view of the size of the study and the good treatment response in the cohort, culture conversion at 8 weeks seemed less useful as a measure of treatment response that would reveal possible determinants of response. In a *post hoc* analysis, treatment response was defined as "poor" if patients had at least one positive culture at either week 4, 8, or 24/32. This analysis also prevented inappropriate classification of patients who had inconsistent results of consecutive cultures ($n = 4$, see above). In univariate *post hoc* analyses, size of extensive lesions (OR for lesions >9 vertebrae versus lesions < 5 vertebrae, 3.94; 95% CI = 1.20 to 12.98), isoniazid $C_{2\,h}$ (interquartile OR, 0.58; 95% CI = 0.35 to 0.96) and pyrazinamide $C_{2\,h}$ (interquartile OR, 0.59; 95% CI = 0.37 to 0.93) were significantly associated with the occurrence of at least one positive culture at either week 4, 8, or 24/32. Also, patients in the poor response group had very low plasma concentrations for two or more drugs more frequently than the good response group (18% versus 6%, $P = 0.04$). $C_{2\,h}$ values of rifampin and the MR for isoniazid were not significantly related to treatment response. Age, DM, HIV, prior TB treatment, sputum microscopy, and *M. tuberculosis* Beijing genotype were also unrelated to response. In multivariate analysis, size of extensive lesions (OR for lesions > 9 vertebrae versus lesions < 5 vertebrae, 6.30; 95% CI = 1.25 to 31.87; $P = 0.01$) and pyrazinamide $C_{2\,h}$ (adjusted interquartile OR, 0.33; 95% CI = 0.13 to 0.80; $P = 0.01$) were the only independent predictors of treatment response.

DISCUSSION

This is one of relatively few studies that examined pharmacokinetics and pharmacodynamics in patients with plasma concentration samples of three antituberculosis drugs. In concordance with other studies (1, 3, 5, 13), we observed large interindividual variability in plasma concentrations of TB drugs. Many patients had

drug concentrations below reference values, despite administration of antituberculosis drug dosages through daily DOT. The frequently observed low isoniazid concentrations were probably the result of a majority of fast acetylators in our cohort, as expected in an Asian population (25, 26). In addition, in line with a recent publication, we found lower isoniazid concentrations in lower weight bands (27). Remarkably, the $C_{2\,h}$ values of the different drugs were significantly correlated to each other, indicating that patients with a low plasma concentration of one drug are also at risk of having low plasma concentrations of the other drugs.

Treatment response was generally good. After 8 weeks, only 7% of the patients had a positive culture, much less compared to another recent study in Indonesia, which found that 17 to 18% of patients had a positive culture at 2 months (28). Our primary data analysis showed no relationship between $C_{2\,h}$ of the three drugs and culture conversion at 8 weeks, the only currently accepted biomarker of sterilizing activity (22). Plasma drug concentrations were neither significantly associated with culture results at week 4. The *post hoc* data analysis suggests that only low plasma pyrazinamide concentrations may contribute to poor treatment response, regardless of prior TB treatment, DM, or HIV status, and sputum microscopy at baseline. It should be realized that this *post hoc* analysis was not planned in advance, it used a nonvalidated composite endpoint, and the findings are not supported by the week 4 or week 8 associations which comprise the bulk of the events in the composite endpoint. Nevertheless, the relevance of low pyrazinamide concentrations in this *post hoc* analysis would be consistent with findings from two recently published studies (3, 4). Therapeutic drug monitoring could identify patients who would benefit from pyrazinamide dose adjustments, but this is not feasible in developing countries at this time. Another possibility for achieving higher drug levels of pyrazinamide is to increase the standard doses of this drug. Recent studies in animal and *in vitro* models have revealed that the use of pyrazinamide doses higher than those currently used could increase the efficacy of pyrazinamide and lead to better outcomes (29, 30), but experience from the past indicated that higher doses of pyrazinamide are associated with more hepatotoxicity. Clinical studies that investigate the efficacy of these higher doses versus potential adverse events are warranted.

Given the available data on relationships between rifampin concentrations and response (31–33), the belief that the current rifampin dose is at the lower end of the dose-response curve (34), and the anticipated large interindividual variability in rifampin pharmacokinetics, we expected rifampin plasma concentrations to be related to treatment response as well. In addition, Pasipanodya showed that isoniazid acetylation-defined pharmacokinetic variability was associated with microbiological outcome (17). In the present study, these assumptions could not be confirmed. A first explanation could be that it is not the $C_{2\,h}$ or C_{max} of the TB drugs that is related to response, but the total drug exposure (AUC). However, according to Jayaram et al. both AUC and C_{max} show a good correlation with response, both for rifampin (32) and isoniazid (35). In addition, these parameters are often strongly related as demonstrated in this and other studies (6, 36, 37). As a second explanation, $C_{2\,h}$ was used as a surrogate of C_{max} instead of using a precisely estimated C_{max} derived from repeated sampling at different time points. Nevertheless, $C_{2\,h}$ correlated well with C_{max} and/or AUC in this and other studies (13). A third explanation could be that it is not the C_{max} or AUC that is related to response, but the C_{max}/MIC or AUC/MIC. However, the MIC for the various drugs was not determined. Fourth, response to rifampin may better correlate to protein-unbound (free) concentrations than to total (protein-unbound plus bound) concentrations (38). Fifth, concentrations would ideally be measured at the target locations, e.g., in the epithelial lining fluid instead of in plasma. Sixth, it should be acknowledged that many factors determine response in an individual patient apart from drug related factors, such as patient and bacterial factors. In this complex interplay with a limited number of participants we may not expect pharmacokinetic factors to be dominant. Finally, the statistical approach used to find associations between drug concentrations and response may be relevant. We used conventional, well-accepted logistic regression analyses resulting in ORs. A limitation of this method is that it does not account for the combined effects of the drugs in the regimen. We tried to solve this by evaluating the effect of the number of drugs with low plasma concentrations on treatment response, using cutoff values based on average (population) pharmacokinetic data.

Davies et al. argued that bacteriological biomarkers are likely to be the most directly relevant pharmacodynamic response during the treatment of TB (39). In a recent comprehensive evaluation of clinical trials a strong relation was shown between 2-month and 3-month culture result and long-term outcome (40). Culture conversion after 2 months of treatment is the only currently accepted biomarker of sterilizing activity (22), but due to the size of the study and the good response rate this measure seemed less useful to identify determinants of treatment response. It should be noted that our *post hoc* definition of treatment response—being "poor" if patients had at least one positive culture during treatment—is not well validated as a surrogate parameter of treatment response. Time to culture conversion might have been a better measure of treatment response, but this could not be analyzed since cultures were only sampled three times. Another limitation of this study is that we measured TB drug concentrations just once during treatment and that we only used a single sample at 2 h postdose to estimate C_{max}. Furthermore, *M. tuberculosis* Beijing genotype and chest X-ray abnormalities as possible determinants of response were not assessed in a subset of patients. The cutoff value of 1.5 regarding the MR for isoniazid, to differentiate between fast and slow metabolizers, was developed for plasma concentrations sampled at 3 h postdose, instead of 2 h. This could have led to a misclassification of patients.

In conclusion, we showed that low plasma concentrations of isoniazid, rifampin, and pyrazinamide occurred in many patients, even with DOT strategy. Most patients had a good treatment outcome despite these low drug concentrations. No association was shown between plasma concentrations and culture results at 4 and 8 weeks of treatment. *Post hoc* analysis showed that patients with low plasma pyrazinamide concentrations were at risk of at least one positive culture at weeks 4, 8, or 24/32. The same applied to patients with large lesions on chest X-ray. Dose adjustment or the use of higher standard doses of pyrazinamide might be beneficial for certain patients to improve treatment outcome.

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