Plasma Levels of Rifampin Correlate with the Tuberculosis Drug Activity Assay

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ABSTRACT The plasma tuberculosis drug activity (TDA) assay may be an alternative tool for therapeutic drug monitoring in resource-limited settings. In tuberculosis (TB) patients (n = 30), TDA and plasma levels of first-line drugs were analyzed 2 h post-dose, 2 weeks after treatment initiation. Patients with plasma levels of rifampin lower than 8 mg/liter had a significantly lower median TDA (1.40 versus 1.68, P = 0.0013). TDA may be used to identify TB patients with suboptimal rifampin levels during TB treatment.

KEYWORDS pharmacokinetics, Mycobacterium tuberculosis, rifampin, isoniazid


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after an overnight fast at week two, 2 h after the observed intake of the drugs, the time at which peak drug concentrations for RIF and INH are expected. The definitions of lower-than-recommended drug concentration levels for RIF (<8 mg/liter) and INH (<3 mg/liter) were based on clinical practice and previous studies (3, 11), although these cutoffs are poorly validated against clinical outcomes (7). The determinations of plasma concentrations of INH were performed using high-pressure liquid chromatography (Agilent 1100 HPLC-system; Agilent, Santa Clara, CA, USA) and those of RIF by LC-MS (Agilent, Santa Clara, CA, USA) at Karolinska University Laboratory. TDA was determined as previously described (9). Briefly, *M. tuberculosis* H37Rv (ATCC 27294) equivalent to a 0.5 McFarland standard was diluted 1:10, washed and resuspended in 300 μl phosphate-buffered saline, and incubated for 72 h at 37°C with 300 μl patient plasma or 300 μl antibiotic-free human control plasma in duplicates. After washing, the suspensions were incubated in a Bactec MGIT 960 instrument until the time to detection (TTD). TDA was calculated as the ratio of the TTD for H37Rv in the MGIT tube with patient plasma in relation to that in the tube with control plasma. In each experiment, control plasma samples (n = 4 to 8) and plasma with 8 mg/liter RIF were analyzed to evaluate the assay precision (coefficient of variation [CV]). The data are presented as medians and interquartile ranges (IQRs). Spearman’s rank correlation, Mann-Whitney U, and Fisher’s tests were used for comparisons, and a P value of <0.05 was considered statistically significant.

The median age of the patients was 32 years (IQR, 26 to 44 years), 60% (18/30) were female patients, and 60% (18/30) were from sub-Saharan Africa. No patient had an HIV infection, and 80% (24/30) of the study participants had PTB. There were no differences in RIF doses (median, 9.4 mg/kg; IQR, 8.2 to 10.1 mg/kg) between patients with plasma RIF concentrations higher than or lower than 8 mg/liter (P = 0.82). The correlation between TDA and RIF levels at 2 h after drug intake was significant (r = 0.54, P = 0.002) (Fig. 1), and TDA levels of <1.5 correlated with lower RIF levels (P < 0.001) (Fig. 2). The median TDA was significantly lower in TB patients with RIF levels <8 mg/liter than in
patients with RIF levels >8 mg/liter (1.40 [1.30 to 1.48] versus 1.68 [1.60 to 1.82], respectively, \( P = 0.0013 \)) (Fig. 3). In patients with INH levels of less than or greater than 3 mg/liter, the median TDA values were similar (1.64 [1.44 to 1.69] versus 1.52 [1.35 to 1.71], respectively, \( P = 0.64 \)), which was also the case for pyrazinamide (PZA) and ethambutol (EMB) (data not shown). The median TTDs for control plasma (\( n = 30 \)) and the positive control (\( n = 14 \)) were 239.5 h (172 to 264 h) and 412.5 h (323 to 446 h), respectively. The CV for intraassay variability was 9.6% for the negative controls (\( n = 8 \)), whereas the CVs for interassay variability were 23.0% for the negative (\( n = 30 \)) and 18.2% for the positive (\( n = 14 \)) controls.

We observed an association between TDA and levels of RIF, whereas the effects of PZA and EMB were negligible, similar to a previous study (9). The correlation between exact levels of TDA and RIF was significant but with a rather low correlation coefficient. However, we found a highly significant relation between clinically used cutoff levels for low plasma RIF concentrations (<8 mg/liter) and low TDA levels. Although additional clinical studies are needed to confirm the suggested cutoff for TDA, our data indicate significantly lower plasma drug levels of RIF at TDAs of <1.5, in accordance with a previous study, where an association with a lower degree of sputum smear conversion was observed (9). A strength of the current study is the precision analysis of the TDA assay, as high intra- and interassay variabilities may potentially limit useful applications. Even if the TDA assay variability rarely affects clinical decisions regarding dose adjustments, we recommend that until a refined TDA method is developed focusing on the initial bacterial inoculum, samples taken before dose adjustment should be reanalyzed together with the follow-up sample. It is also worth considering an additional TDA sample 6 h after drug intake to identify patients with delayed peak RIF levels (12). A further optimization of the TDA assay could include the \( M. \) tuberculosis isolate of each patient to get a more precise measurement of the ratio between the peak drug levels and the individual MICs (9); but, method variability and potential delays would then need to be thoroughly evaluated.

![FIG 2 Comparison of plasma rifampin levels and TDA. Horizontal bold lines indicate medians. Patients with TDA of <1.5 exhibited significantly lower rifampin levels (\( P < 0.001 \), Mann-Whitney U test).](image-url)
In conclusion, patients with plasma levels of rifampin lower than 8 mg/liter had low TDA values. Our data support the use of TDA as an indicator for low rifampin exposure in settings where LC-MS methods are not available but TB culture facilities are. Further studies are needed to establish TDA as a therapeutic management tool in TB treatment.

ACKNOWLEDGMENTS

This work was funded by the Research Council of Southeast Sweden (FORSS), the Marianne and Marcus Wallenberg Foundation, the Swedish Heart and Lung Foundation, ALF grants from the Region of Östergötland, Sweden, and the Swedish Research Council.

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. We declare no conflict of interest.

REFERENCES


