Importance of Therapeutic Drug Monitoring of Rifampicin

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Abstract
Therapeutic Drug Monitoring (TDM) is a routinely practised clinical laboratory technique which aids the clinicians with a clear clinical judgement of the drug therapy and optimize the doses if necessary. Rifampicin is the most important and potent component of first line therapy of tuberculosis (TB). Several factors like age, weight, gender, doses and formulations, gastro-intestinal disorders, ethnicity etc alter the absorption and bioavailability of rifampicin thus altering the drug levels. Low plasma levels of rifampicin may play a plausible role in slow response to therapy, treatment failure or relapse or acquired drug resistance. TB Patients with further complicated conditions like diabetes or HIV are at an increased risk for poor drug absorption and drug-drug interactions. A standard treatment regimen may be inadequate for some cases as the clinical status of patients vary from case to case. TDM can be used as a clinical tool for identifying patients at high risk of treatment failure, delayed response, drug-drug interactions and help optimization of therapy. In the past two decades numerous reports of TDM of anti-tuberculosis drugs have been reported wherein low rifampicin levels have been a major concern. Rifampicin exhibit concentration dependent killing of mycobacteria. A 2 hour post-dose sample approximates the peak plasma rifampicin concentration (Cmax) and is recommended for TDM of rifampicin. An additional 6 hour sample may be collected to distinguish between delayed absorption and malabsorption. Combined with clinical and bacteriological data, TDM can help clinicians treat slow response / complicated TB patients.

Introduction
Tuberculosis (TB) is one of the oldest known infectious disease and is a leading cause of mortality in developing countries. India has a high prevalence of TB accommodating 11% of the total TB cases worldwide. Treatment for TB requires good patient adherence to combination chemotherapy for an extended period. Despite directly observed therapy (DOT) in TB programs, treatment failure, relapse, acquired drug resistance, multi-drug resistance, drug toxicities and extended treatment duration remain as ongoing complications. The bioavailability, pharmacokinetics and plasma levels of the orally administered anti-TB drugs vary differently and are influenced by patient age, sex, gastro-intestinal disorders, drug formulations or drug interactions. In such cases performing therapeutic drug monitoring (TDM) may benefit TB patients.

Rifampicin: Kinetics and Mechanisms of Action
Rifampicin is a critical and potent component of first-line TB therapy having unique properties of a rapid onset action once in contact with M. tuberculosis. It is absorbed from the gastro-intestinal (GI) tract and the rate of absorption is most variable among all TB drugs. Following an oral dose, the peak levels (time to attain maximum concentration – tmax) are attained within 2 hours with a Cmax ranging between 4 – 32 mg/l as widely reported in literature. Rifampicin is better absorbed in an acidic condition than in neutral or alkaline conditions. The peak levels (Cmax) and Tmax is delayed in presence of high-fat meals, so the drug should be given empty stomach whenever possible. Its absorption is fairly reduced in fixed dose combinations with isoniazid and pyrazinamide. A 2 hour drug level is usually preferred to estimate peak rifampicin levels. If delayed absorption (Late peak levels) or malabsorption (low levels at all-time points) is suspected, an additional 6 hour sample may be collected to distinguish between delayed absorption and malabsorption.
Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase. Rifampicin binds to the RNA polymerase at a site adjacent to the RNA polymerase active center and blocks RNA synthesis by physically blocking the formation of the phosphodiester bond in the RNA backbone, preventing extension of RNA products beyond a length of 2-3 nucleotides (“steric-occlusion” mechanism). Resistance to rifampicin arises from mutations that alter residues of the rifampicin binding site on RNA polymerase, resulting in decreased affinity for rifampicin. Resistant mutations map to the rpoB gene, encoding RNA polymerase beta subunit.

The half-life of rifampicin is 2–3 hours. Rifampicin induces its own hepatic metabolism and hence the Cmax and t1/2 of rifampicin decrease over the first two weeks of therapy. Therefore, patients on longer than 3 weeks of rifampicin levels tend to have lower rifampicin levels than their initial baseline values. Therapeutic drug monitoring (TDM) of plasma levels of rifampicin is performed since decades and a huge inter-individual variability is observed among several ethnic groups. Some patients are slow to respond to the standard treatment and TDM may help identifying this and provide the clinicians a justified reason to increase the respective doses or decrease / increase the treatment duration.

Performing TDM and its Interpretation

Therapeutic drug monitoring (TDM) involves a clinical laboratory measurement of a chemical parameter which with appropriate medical interpretation helps better patient management and influence drug dose prescribing procedures. The general approach for TDM in various patient populations is the same. In case of rifampicin, measurement of peak levels after the steady state attainment is recommended. Therapeutic peak plasma concentration for rifampicin has been established in literature and ranges from 8–24 mg/l. Drug concentration at the site of action cannot be routinely measured, but the desired or adverse effects may be correlated better with the plasma concentrations. In most cases, rifampicin attains its peak level after about 2 hours post dose ingestion and hence a blood collection at 2 hour post dose is usually recommended for TDM. Given the variability of oral absorption, a single time point may miss the actual peak concentration. Therefore, a second sample, typically 6-hours post-dose, will help provide to a better judgement on the rate and completeness of absorption. The normal pattern for serum concentrations is that the 2-hour value is substantially higher than the 6-hour. Malabsorption may be seen if the 2-hour and 6-hour values are roughly the same, while delayed absorption is seen if the 6-hour values are higher than the 2-hour drug levels. In these situations, it is possible that the actual peak occurred between the two blood collections. Cases of malabsorption, wherein the rifampicin levels are below the therapeutic range, warrant the need for dose increment and a follow-up drug level to judge the achievement of the targeted therapeutic value. Rifampicin exhibits concentration – dependent killing of mycobacteria and hence Cmax < 4 mg/l prompt the need for dose increment.

Detailed pharmacokinetic-pharmacodynamic (PK-PD) data from human studies are lacking for the TB drugs. Precise targets for peak serum concentrations (Cmax) relative to the minimal inhibitory concentration (MIC) [Cmax : MIC], or time above MIC, are scarce from human studies. While few studies have looked at Cmax as a marker for PK-PD target, others have considered the area under curve of rifampicin (AUC) i.e the complete concentration of rifampicin from the time of ingestion to the elimination or infinity (AUC 0–∞). Several studies also correlate AUC (at varied timepoints) and MIC of rifampicin suggesting as a better predictor of the PK-PD target of rifampicin in hollow fibre models. The AUC24/MIC ratio required for a 1-log10 CFU reduction in vivo and inside macrophages were 271 and 665, respectively, while that required in vitro was 30. Attainment of the PK-PD target may also reflect the therapeutic effect. In rare situations, with severely ill patients, higher concentrations may be warranted with proper clinical judgements.

Several assays are available for estimation of rifampicin drug levels however, sensitivity and specificity of assays play a vital role. In general, high-performance liquid chromatography (HPLC) and gas chromatography (GC) with the commonly used detection systems (ultraviolet or fluorescence detection for HPLC, mass spectrometry for HPLC or GC) are preferred. Several precautions like extensive interference checks, sample stability should be performed prior to validating the assays.

For precise interpretation of the results it is important to record the time of sample collection, the time of the last dose, the dosage regimen and the indication for drug monitoring. Drug concentrations need to be interpreted in the context of the individual patient without rigid adherence to a target range. Before making dose adjustments, it is important to consider if the sample was taken at the correct time with respect to the last dose, if a steady state has been reached and whether the patient has adhered to their treatment.
The pharmacokinetics of rifampicin varies in different ethnic groups especially in countries or territories with low incidence of TB like Canada, United States of America etc as compared to countries with high incidences like India. A recent study by Prahl et al reported the clinical significance of 2 hour plasma levels of first line drugs. In this prospective observational study of 32 adults, about 56% of the population had rifampicin levels below the therapeutic range. The authors measured the drug levels of all four first line drugs (rifampicin, isoniazid, ethambutol and pyrazinamide) wherein 86% patients had atleast one drug below the normal range. A median plasma rifampicin level of only 6.5mg/L was observed in this study group. A similar study reported by Fahimi et al in 60 TB patients reported a median peak Cmax of 4.0 mg/L and that 92% of the patients were in the sub-therapeutic range for rifampicin and 81% of the study group had either of the first line anti-TB drugs below the normal ranges. The authors reported auto-induction of rifampicin as the plausible reason of low drug levels in the study group. In another study, 68% of the study group had low rifampicin levels among a total of 52 patients enrolled in the study. About 90% of the study group had either of the first line drugs in the lower range. A follow-up drug level in patients with a low baseline level and dose increase reflected an increase in the drug levels. However, the sample size of the group was small to represent and explain the pharmacokinetics of these drugs. Table 1 summarizes the various TDM studies performed worldwide with respect to rifampicin.

While several studies are reported for low rifampicin levels in various ethnic groups, a few have focussed on understanding the pharmacokinetics of these drugs. The ratio of Cmax : MIC of rifampicin is evaluated in a study reported by Van Crevel et al wherein an estimated ratio of 30 was targeted with a Cmax of > 8 mg/l and MIC 0.25 of rifampicin [16]. In the same study more than 50% of the study group had Cmax : MIC < 10. The PK-PD targets have not yet been established in human while several studies report varied target points like 271 in vitro, 665 in macrophages etc. using the AUC:MIC ratio. The differences in the target points are dependent on the MIC of rifampicin considered.

Till date no direct association of a particular factor for low rifampicin levels have been established however, differences in patient characteristics, dosing formulations, methods of pharmacokinetic evaluation, ethnicity, auto-induction of rifampicin etc have been suggested.

Table 1: Rifampicin TDM studies worldwide

<table>
<thead>
<tr>
<th>Country</th>
<th>Author, Year</th>
<th>Sample size</th>
<th>% with sub-therapeutic levels</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>Kayhan et al, 2011</td>
<td>49</td>
<td>75.50%</td>
<td>Diabetes an independent risk factor; Dose adjustments necessary to attain therapeutic level</td>
</tr>
<tr>
<td>Turkey</td>
<td>Babalik et al, 2013</td>
<td>21</td>
<td>81%</td>
<td>Acetylator status strongly influenced absorption of drugs</td>
</tr>
<tr>
<td>Canada</td>
<td>Van Tongeren et al, 2013</td>
<td>52</td>
<td>8.40%</td>
<td>Low levels responsible for high rate of treatment failure and relapse</td>
</tr>
<tr>
<td>Iran</td>
<td>Fahimi F et al, 2013</td>
<td>60</td>
<td>92.50%</td>
<td>Auto induction of rifampicin metabolism</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Tostmann et al, 2013</td>
<td>20</td>
<td>35%</td>
<td>Food interaction; low drugs levels correlated with treatment failure.</td>
</tr>
<tr>
<td>Korea</td>
<td>Um S.W. et al, 2004</td>
<td>41</td>
<td>NA</td>
<td>Low rifampicin levels tend to increase risk of drug resistance</td>
</tr>
<tr>
<td>India</td>
<td>Gurumurthy et al, 2004</td>
<td>69</td>
<td>23.50%</td>
<td>Malabsorption of rifampicin is common in HIV infected patients</td>
</tr>
<tr>
<td>India</td>
<td>Prakash J et al, 2007</td>
<td>41</td>
<td>NA</td>
<td>Rifampicin levels are increased in presence of PGP/CYP3A4 blocker; PGP/CYP3A4 plays an important role in rifampicin absorption</td>
</tr>
<tr>
<td>India</td>
<td>Arya A et al, 2015 (children only)</td>
<td>20</td>
<td>100%</td>
<td>Patients were receiving &lt;10 mg/kg dose and auto-induction could be reasons for low drug levels</td>
</tr>
<tr>
<td>America</td>
<td>Mehta J et al, 2003</td>
<td>124</td>
<td>4.80%</td>
<td>Only 6 patients were Slow responders in view of co conditions. Follow-up analysis on increasing drug dose showed increase in rifampicin level</td>
</tr>
<tr>
<td>India</td>
<td>Present authors #</td>
<td>100</td>
<td>59%</td>
<td>Majority patients were in sub-therapeutic range. 50% of the population were partial responders to the therapy.</td>
</tr>
</tbody>
</table>

NA: Data unavailable; #: Laboratory data manuscript under publication

clinical judgment. TDM provides objective laboratory data that is incorporated into the decision but is not the sole basis for change in treatment strategy.

TDM Studies Worldwide

Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top 5 causes of death for women aged 15 to 44 years. The incidence of TB is more common in developing countries with India bearing the second largest burden of TB. Several studies in varied ethnic groups have reported low plasma rifampicin levels and hence the role of TDM of rifampicin in clinical practise. The pharmacokinetics of rifampicin varies in different ethnic groups especially in countries or territories with low incidence of TB like Canada, United States of America etc as compared to countries with
Diabetes was an independent risk factor (p=0.03) for low rifampicin levels in this study. A follow-up after increase in dose improved the drug levels as well as the clinical outcome.6,37 In a similar study reported by Babalik et al, the diabetics had half the rifampicin levels than the non-diabetics. After 30 days of therapy, all the diabetic patients had rifampicin levels below the reference range.4 These studies suggest diabetics need close monitoring in terms of drug levels and clinical outcome.

**Patients co-infected with HIV:**
HIV-positive patients are more likely to progress to active disease if exposed to or previously infected with TB.32 Patients with HIV infection have an increased risk of other concurrent opportunistic infections and due to large intake of heavy dose drugs, they are at an increased risk of malabsorption or drug – drug interactions.32-34,38-40

In an Indian study reported by Gurumurthy et al, a decreased bioavailability of rifampicin was seen in HIV infected TB patients than the non-HIV TB patients.32 Similar trends were reported in other ethnic groups as well. Both diseases require long durations of treatment thus demanding correct dosing and appropriate care taken in the treatment regimen. Also, when one considers the cost of the HIV drugs, TDM appears to be a relatively inexpensive way to verify the correct doses.

**Patients with Renal Failure or Hepatic Dysfunction:**
Chronic renal failure compromises the immune system. Patients receiving dialysis are far more likely to go on to have active TB disease than those with normal renal function, whether they are latently infected or newly exposed to TB.13,15,41 Rifampicin is cleared via the hepatic route. Toxic drug levels or one of the major side-effects of rifampicin include hepatotoxicity. Patients with either hepatic or renal dysfunction often experience nausea and vomiting. This can produce the dual problems of malabsorption and reduced drug clearance. Therefore, it is reasonable to check serum concentrations in patients with significant renal or hepatic dysfunction, so that each patient gets the correct, individualised dose.15

**Conclusion**
Thus to conclude, TDM may not be mandatory for all patients however, slow responders, patients with further complicated conditions warrant the need of TDM. The alternate to TDM is to watch, wait and hope for the best. Serum concentrations is just one of the several factors needed to be considered for clinical judgement and it is important to treat the individual patient and not the laboratory value. The clinical condition of the patient, the extent of disease, the susceptibility of the organisms once it becomes available, and the rapidity of the clinical and bacteriological response are also important parameters needing consideration. Drug concentrations may be used as surrogates for drug effects so therapeutic drug monitoring may assist with dose individualisation. It can also be used to detect toxicity, so therapeutic drug monitoring can optimise patient management and improve clinical outcomes.

**Disclosures**
All authors declare that they have no conflict of interest.

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17. Mehta J. Utility of rifampicin blood levels in the treatment and follow-up of active pulmonary tuberculosis in patients who were slow to respond to routine directly observed therapy. Chest 2001; 120:1520.


