Clinical significance of 2 h plasma concentrations of first-line anti-tuberculosis drugs: a prospective observational study

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Objectives: To study 2 h plasma concentrations of the first-line tuberculosis drugs isoniazid, rifampicin, ethambutol and pyrazinamide in a cohort of patients with tuberculosis in Denmark and to determine the relationship between the concentrations and the clinical outcome.

Methods: After 6–207 days of treatment (median 34 days) 2 h blood samples were collected from 32 patients with active tuberculosis and from three patients receiving prophylactic treatment. Plasma concentrations were determined using LC-MS/MS. Normal ranges were obtained from the literature. Clinical charts were reviewed for baseline characteristics and clinical status at 2, 4 and 6 months after the initiation of treatment. At a 1 year follow-up, therapy failure was defined as death or a relapse of tuberculosis.

Results: Plasma concentrations below the normal ranges were frequently observed: isoniazid in 71%, rifampicin in 58%, ethambutol in 46%, pyrazinamide in 10% and both isoniazid and rifampicin in 45% of the patients. The plasma concentrations of isoniazid correlated inversely with the C-reactive protein level at the time of sampling (P = 0.001). During 1 year of follow-up, therapy failure occurred in five patients. Therapy failure occurred more frequently when the concentrations of isoniazid and rifampicin were both below the normal ranges (P = 0.013) and even more frequently when they were below the median 2 h drug concentrations obtained in the study (P = 0.005).

Conclusions: At 2 h, plasma concentrations of isoniazid and rifampicin below the normal ranges were frequently observed. The inverse correlation between the plasma concentrations of isoniazid and C-reactive protein indicate a suboptimal treatment effect at standard dosing regimes. Dichotomization based on median 2 h drug concentrations was more predictive of outcome than dichotomization based on normal ranges.

Keywords: anti-tuberculosis drugs, tuberculosis, therapeutic drug monitoring

Introduction

Worldwide, acquired drug resistance, microbiological failure and relapse of tuberculosis (TB) are matters of great concern. Even in Denmark, a country with a low burden of TB, where the diagnostics and treatment for TB are free of charge, a favourable treatment outcome is achieved in only ~85% of patients with pulmonary TB.1 The emergence of multidrug-resistant and extensively drug-resistant TB strains emphasizes the evolving threat of microbial resistance to the first-line TB drugs isoniazid, rifampicin, ethambutol and pyrazinamide, and the need to safeguard these drugs.

Insufficient drug concentrations of first-line TB drugs may be an important cause of acquired drug resistance, microbiological failure and the relapse of TB. However, clinically derived therapeutic ranges of first-line TB drugs remain to be established. Drug concentrations in blood collected 2 h after taking medication are often used as the estimated peak maximum plasma concentration (C_{max}) of first-line TB drugs and reference ranges representing the normal C_{max} concentrations that can be expected in healthy adults after the ingestion of just a single daily standard dose have been published.2

Two-hour plasma concentrations of one or more of the first-line TB drugs below these normal ranges have frequently been
reported in TB patients in both developed and developing countries. 3 17 Most studies investigating the association between first-line TB drug plasma concentrations and treatment response support a reduced treatment effect of low plasma concentrations. 3,8 14 However, some of these studies are limited by the absence of an adequate control group. 3,8,9,11 To date, no prospective studies including a population from an industrialized country have investigated the clinical significance of the plasma concentrations of first-line TB drugs below the normal ranges. 2

In this prospective study we investigated the prevalence of, possible risk factors for and clinical consequences of 2 h plasma concentrations of first-line TB drugs below the normal ranges in a sample of 32 closely supervised, adult patients with either pulmonary or extrapulmonary TB in Denmark.

Patients and methods

Study subjects

This study was evaluated by the local ethics committee and was assessed to be a quality assurance study. The study was approved by the Danish Data Protection Agency and conducted in compliance with the Declaration of Helsinki. All patients gave consent to blood sampling.

During the study period from 1 January 2009 to 31 March 2011 two groups of patients receiving first-line TB drugs were eligible for inclusion. Group A comprised patients from inpatient and outpatient clinics in the eastern part of Denmark undergoing therapeutic drug monitoring (TDM) performed for clinical reasons. Group B comprised consecutive inpatients and outpatients identified at the Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet and Copenhagen University Hospital Hvidovre for whom plasma concentrations were determined solely for the purpose of the present study. Patients suspected of non-adherence or in whom blood sampling did not fulfil the requirements were excluded from the study.

Patients eligible for inclusion in the study were ≥ 18 years of age and were either receiving prophylactic isoniazid treatment or standard TB therapy. Because of the expected steady-state in the pharmacokinetics of rifampicin, patients eligible for inclusion had received first-line drugs for at least 6 days before blood sampling. Only patients treated for active TB caused by fully susceptible strains were included when investigating the clinical significance of the plasma concentrations of the first-line TB drugs.

Patients were treated with the Danish national guidelines. Standard TB treatment is divided into a 2 month initial ‘intensive’ phase consisting of 5 mg/kg of isoniazid to a maximum of 300 mg once daily, 10 mg/kg of rifampicin to a maximum of 600 mg once daily, 20 mg/kg of ethambutol to a maximum of 1200 mg once daily and 30 mg/kg of pyrazinamide to a maximum of 2000 mg once daily, followed by a second 4 month ‘continuation’ phase consisting of 5 mg/kg of isoniazid to a maximum of 300 mg once daily and 10 mg/kg of rifampicin to a maximum of 600 mg once daily. Both single drug products and fixed-dose combination products were administered.

Data collection and covariates

The clinical charts were reviewed for baseline characteristics and clinical and paraclinical status 2, 4 and 6 months after the initiation of treatment. The concentration of the acute-phase protein C-reactive protein (CRP) was recorded as a biomarker of disease severity and treatment response. In addition, patient factors previously reported to influence the plasma concentrations of first-line TB drugs [age, gender, anemia, hyperalbuminemia, comorbidity (HIV infection, diabetes mellitus), drug dose/kg of body weight, drug formulation, earlier TB infection, being homeless, excessive alcohol use and hospital status (inpatient or outpatient)] were recorded.

A 1 year follow-up was carried out reviewing the clinical files at the hospitals and the laboratory records at the International Reference Laboratory of Mycobacteriology at Statens Serum Institute, Copenhagen, Denmark. Therapy failure was defined as death due to any cause or a relapse of TB within 1 year after the end of treatment. A successful outcome was defined as cure (spontaneous culture negative in the last month and on ≥ 1 previous occasion) or completed (completed treatment but does not meet the criteria of cure or failure).

TDM

Sample collection

Patients were given (inpatients) or instructed to take (outpatients) their daily dose of TB medication as usual in order to obtain representative 2 h plasma concentrations. Venous blood was collected 2 h after ingestion of the medication, and within a further 1 h the plasma was separated and frozen. The plasma was stored at −80 °C until analysis. The drug concentrations from blood collected 2 h after medication were used as the estimated Cmax. Low Cmax values were defined using normal ranges from the literature: isoniazid < 3 mg/L, rifampicin < 8 mg/L, ethambutol < 2 mg/L and pyrazinamide < 20 mg/L. 7 The results of the plasma concentration analysis were reported to the treating physician, generally within 3 weeks after sampling.

Drug concentration analysis

The total plasma concentrations of all four drugs were quantified using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method developed in collaboration with Department of Biochemistry, Immunology and Genetics, Statens Serum Institut, Copenhagen, DK. The method was validated over the concentration range 0.5 – 10 mg/L for isoniazid, 0.75 – 30 mg/L for rifampicin, 0.25 – 10 mg/L for ethambutol and 4 – 80 mg/L for pyrazinamide. The lower limit of quantification was 0.5 mg/L, 0.75 mg/L, 0.25 mg/L and 4.0 mg/L, respectively. The precision estimated by the coefficient of variation was < 15% and the accuracy estimated by the relative errors was within ±15% for all four drugs. Plasma concentrations below the lower limit of quantification were assumed to be zero.

Statistical analysis

Regression analysis was applied to identify factors influencing the 2 h plasma concentrations. To obtain an approximate normal distribution of the plasma concentrations of isoniazid, rifampicin, ethambutol and pyrazinamide, log transformation was applied. Back-transformed mean values are reported as geometric means. For each drug, the occurrence of therapy failure was investigated by dividing the patients into two groups according to the lower normal 2 h plasma concentration obtained from the literature. Since the observed plasma concentrations were not evenly distributed above and below these cut-offs, patients were also dichotomized according to the median 2 h plasma concentration of each drug obtained in the study. To further adjust for disease severity, the effects of CRP level at baseline and of plasma concentrations of each drug were analysed using logistic regression. For group comparison the Student’s t-test was applied for normally distributed variables, and the Mann–Whitney test for non-normally distributed variables. Categorical variables were analysed using Fisher’s exact test (two-sided).

P < 0.05 was considered statistically significant. Due to the exploratory nature of the present analysis Bonferroni correction was not applied.

Results

During the study period 2 h blood samples from a total of 39 patients were collected. Of these, four patients were excluded
from the study, one because of non-adherence, two because of delayed blood sampling and one because of delayed separation and freezing of the plasma. A total of 35 patients were therefore included in the study: 10 patients in Group A and 25 patients in Group B. As there were no significant differences between the two groups with regard to the plasma concentrations of any of the four first-line drugs or the occurrence of therapy failure, no distinction between the two groups is made in the analysis. The duration of receiving treatment at the time of sampling ranged between 6 and 207 days. At the time of sampling four patients had progressed from the intensive phase to the continuation phase of treatment.

Active TB (pulmonary and extrapulmonary) was diagnosed in 32 patients, two patients received prophylactic isoniazid treatment, and one patient stopped TB treatment after 10 days of therapy because of uncertainty related to the diagnosis of TB. Mycobacterium tuberculosis growth in culture was confirmed in 27 (84%) of the 32 patients with active TB. A diagnosis of extrapulmonary TB was based on clinical grounds in five patients (16%). The extrapulmonary manifestations included TB-related spondylitis and meningitis, and liver and gastrointestinal TB.

A 2 h plasma concentration of isoniazid (prophylactic isoniazid treatment only, n=2), was available from all 35 patients, a 2 h plasma concentration of rifampicin from 33 patients, one of pyrazinamide from 29 patients and one of ethambutol from 28 patients.

The distribution of 2 h plasma concentrations of each of the four first-line drugs is displayed in Figure 1. In two patients the plasma level of rifampicin was assumed to be zero since the measured plasma concentration was below the lower limit of quantification. The median plasma concentration of isoniazid was 2.1 mg/L (range 0.5–12.1 mg/L), of rifampicin 6.5 mg/L (range 0–31.0 mg/L), of ethambutol 2.2 mg/L (range 0.5–5.9 mg/L) and of pyrazinamide 31.3 mg/L (range 14.9–110.2 mg/L).

Of the 35 patients included, 86% (30/35) had plasma concentrations of at least one drug below the normal range: isoniazid in 71% (25/35), rifampicin in 58% (19/33), ethambutol in 46% (13/28) and pyrazinamide in 10% (3/29). A total of 45% (15/33) had plasma concentrations of both isoniazid and rifampicin below the normal ranges, and of these 47% (7/15) had ethambutol plasma concentrations and 20% (3/15) had pyrazinamide plasma concentrations below the normal ranges. The frequencies of concurrent low plasma drug concentrations using the normal ranges from the literature are shown in Table 1.

In a simple linear regression analysis the plasma concentrations of rifampicin decreased with increasing age (P=0.006, $R^2=0.23$) and were also significantly lower among patients with a low haemoglobin level at baseline compared with patients with a normal haemoglobin at baseline (geometric mean 5.2 versus 11.9 mg/L, $P=0.004$). Neither anaemia nor age was associated with lower drug concentrations of isoniazid, ethambutol or pyrazinamide. In addition, no associations were found between the 2 h plasma concentrations of isoniazid, rifampicin, ethambutol or pyrazinamide and the following covariate factors: gender, hypoalbuminaemia, dose (mg/kg), drug formulation, hospital status or number of days on treatment at the time of sampling.

As three patients with active TB transferred out and the original isolate from one patient was found to be resistant to isoniazid, follow-up was possible in only 28 patients 1 year after treatment was completed. Therapy failure occurred in five patients. Three patients died during treatment, and two patients experienced a relapse of TB within 1 year after the end of therapy.

![Figure 1](image_url). Distributions of plasma concentrations of first-line TB drugs. The upper and lower normal range for each drug are shown by vertical lines.
The demographic characteristics of the patients experiencing therapy failure and those with a successful outcome are presented in Table 2. At baseline, patients with subsequent therapy failure more often had an excessive alcohol use and a higher CRP level compared with those with a successful outcome. Patients with an excessive alcohol use tended to have a higher CRP at baseline but the difference was not significant (median CRP 87 versus 42 mg/L, \(P = 0.089\)). The median number of days on treatment at the time of sampling was 34 days for patients experiencing therapy failure versus 61 days for patients with a successful outcome (\(P = 0.928\)).

Compared with the group with a successful outcome, the patients with therapy failure attained significantly lower plasma concentrations of isoniazid despite the fact that they received a higher dosage of isoniazid per kg of body weight (Table 3). The difference in dosage per kg of body weight was primarily due to the lower body weight (median 51 versus 62.4 kg, \(P = 0.074\)) among the patients experiencing therapy failure. Patients experiencing therapy failure also received higher dosages of pyrazinamide per kg of body weight.

When dividing the patients into two groups based on the normal values suggested in the literature, it was observed that...
Discussion

In this study we describe the distribution of 2 h plasma concentrations of first-line TB drugs in a population of 32 closely supervised adult patients with TB in Denmark. It is one of relatively few studies to report on the clinical significance of the 2 h plasma concentrations of first-line drugs in TB patients receiving standard therapy. The main findings are: (i) 2 h plasma concentrations of isoniazid, rifampicin and ethambutol below the lower normal values are frequently observed; (ii) according to regression analysis, the CRP level at the time of sampling correlates inversely with the 2 h plasma concentration of isoniazid; and (iii) therapy failure occurs more frequently in patients with lower plasma concentrations of isoniazid and/or rifampicin. These findings confirm a high frequency of 2 h plasma concentrations below lower normal values and indicate an association between low drug concentrations 2 h after ingestion and a poor treatment outcome.

In the present study, 86% of the included patients had plasma concentrations of at least one drug below the normal range. Several previous studies have reported similar high frequencies of the plasma concentrations of TB drugs being below the normal ranges.3 – 11,15 – 17

Variations in the plasma concentrations of TB drugs have been associated with gender,4 drug formulation6,8,18 and drug dose (mg/kg).6 We did not observe such associations in our population apart from the plasma concentrations of rifampicin being lower with increasing age and with low baseline haemoglobin concentrations. The negative correlation with age is surprising as a reduced activity of the metabolic and excretory pathways could be expected with ageing. However, reduced absorption with age may explain the lower bioavailability. With regard to haemoglobin concentrations anaemia may be considered to be a marker of disease severity. Thus, anaemia may indicate severe illness resulting in decreased absorption and, as a result, lower rifampicin plasma concentrations.

The finding of an association between low plasma concentrations of isoniazid and rifampicin and therapy failure is in line with previous retrospective10,13 and prospective10,13 studies linking below-normal range values with an impaired treatment response or outcome. However, other studies have not found an association between plasma concentrations and treatment response.16,17

The finding of lower isoniazid plasma concentrations among patients experiencing treatment failure in spite of a lower body weight and consequently a higher isoniazid dose (mg/kg) contradicts the earlier finding that isoniazid clearance is positively correlated with weight.19

There could be several reasons why not all studies have been able to find an association between drug plasma concentrations and treatment response. The normal ranges often used to define low plasma concentrations have not been validated with regard

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**Table 3. Drug doses and 2 h plasma concentrations by treatment outcome status**

<table>
<thead>
<tr>
<th>Drug dose (mg/kg)</th>
<th>Therapy failure (n = 5)</th>
<th>Successful (n = 23)</th>
<th>P value for differencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoniazid (95% CI)</td>
<td>5.8 (5.0–6.6)</td>
<td>4.9 (4.6–5.2)</td>
<td>0.027</td>
</tr>
<tr>
<td>rifampicin (95% CI)</td>
<td>10.4 (8.5–12.2)</td>
<td>9.6 (9.0–10.3)</td>
<td>0.328</td>
</tr>
<tr>
<td>ethambutol (95% CI)</td>
<td>19.9 (16.5–23.3)</td>
<td>17.6 (16.3–18.8)</td>
<td>0.112</td>
</tr>
<tr>
<td>pyrazinamide (95% CI)</td>
<td>32.0 (25.5–38.4)</td>
<td>26.5 (24.4–28.5)</td>
<td>0.031</td>
</tr>
<tr>
<td>Plasma concentration (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>isoniazid (95% CI)</td>
<td>1.0 (0.4–2.2)</td>
<td>2.6 (2.0–3.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>rifampicin (95% CI)</td>
<td>4.0 (2.2–7.6)</td>
<td>7.3 (5.1–10.5)</td>
<td>0.129</td>
</tr>
<tr>
<td>ethambutol (95% CI)</td>
<td>2.5 (0.7–9.2)</td>
<td>2.0 (1.4–2.8)</td>
<td>0.551</td>
</tr>
<tr>
<td>pyrazinamide (95% CI)</td>
<td>26.4 (14.0–50.0)</td>
<td>35.2 (28.8–43.1)</td>
<td>0.232</td>
</tr>
</tbody>
</table>

aStudent’s t-test.
bGeometric mean.
to clinical outcome, and the plasma concentrations required for effective therapy are not known.2 When using the lower-normal concentrations to evaluate the impact of low plasma concentrations on treatment efficacy we might therefore draw the wrong conclusions. We analysed data both after dichotomizing the patients according to the normal concentrations from the literature, and after dividing the patients into two groups according to the median 2 h plasma concentrations in the present study. When analysing the data this way we found that therapy failure was observed more frequently in patients with below-median values of isoniazid, of rifampicin and of both drugs. Using the normal ranges to dichotomize the patients, therapy failure was only observed more frequently in patients with both rifampicin and isoniazid below the suggested ‘benchmark values’. It is possible that the currently used normal values are not predictive of treatment efficacy and might better be regarded as desirable rather than necessary. We therefore need to review the normal ranges and define new ranges for TDM.

In the present study the median plasma concentration of isoniazid of 2.1 mg/L and of rifampicin of 6.5 mg/L were used as peak concentration thresholds. Similar new threshold values have been suggested by other authors. For isoniazid Donald et al. estimated that a 2 h plasma concentration of 2.19 mg/L is associated with 90% of the maximal killing (EBA90) of metabolically active bacteria present in the sputum during the first 2 days of treatment.20 With regard to rifampicin, Pasipanodya et al. identified a peak concentration threshold of 6.6 mg/L to be predictive of 2 month sputum conversion in a study of 142 TB patients.13 The present study thus supports the suggestion that the clinically relevant thresholds are considerably lower than the lower normal values often used.4

The measured plasma concentrations were reported to the treating physician and doing so could potentially induce a change of therapy. However, this only occurred in a minority of cases and may in fact result in an underestimation of the effect of low plasma concentrations on treatment outcome. This study has several limitations. The sample size is small, and when including all four drugs in the analysis, the problem of multiple comparisons arises. Consequently, the findings may rather be considered suggestive of an association. However, the main findings of an association of plasma concentrations of isoniazid and rifampicin with outcome remained significant when applying a Bonferroni corrected significance level (P ≤ 0.013).

In addition, the timepoint in treatment when the sampling was executed was not standardized. Although a recent study has suggested that, due to autoinduction, rifampicin concentrations may not reach steady-state until 40 days of treatment,21 it is generally assumed that plasma concentrations of rifampicin do not change after the first week of treatment.24 The findings in the present study do not indicate a further time effect on plasma concentrations of rifampicin after 6 days of treatment.

In order to visualize the ‘true’ drug concentrations the patients were instructed to ingest their medication as usual, and venous blood samples were drawn 2 h later. Therefore the patients were not necessarily fasting and the 2 h drug plasma concentrations may be lower on that account.23–26 In addition, only one 2 h plasma concentration was measured instead of applying a more intensive multisample schedule that is more appropriate for evaluating pharmacokinetics and pharmacodynamics. In applying this method we risk underestimating the peak concentrations in patients who have either rapid or delayed absorption. On the other hand 2 h plasma concentrations of isoniazid, rifampicin, ethambutol and pyrazinamide are accepted as an estimated $C_{\text{max}}$ and the $C_{\text{max}}$ of rifampicin and isoniazid has been found to correlate with treatment outcome.27,28 Furthermore the 2 h plasma concentration correlates well with $C_{\text{max}}$ in earlier studies.2

In future studies a limited sampling strategy for TDM could be applied, with blood sampling at two or three timepoints after the ingestion of the medication in order to obtain a more representative value of $C_{\text{max}}$ without performing a full pharmacokinetic curve.

Of the patients included, 40% were outpatients on self-administered therapy. In these patients adherence to therapy could not be assessed. However, the finding of adequate pyrazinamide concentrations for those given pyrazinamide supports the fact that the patients were indeed adherent to the treatment, and we did not find any statistically significant difference between inpatients and outpatients with regard to the measured 2 h plasma concentrations of any of the four drugs. We therefore do not believe our results are weakened when including outpatients receiving self-administered therapy.

The multidrug TB treatment strategy currently employed worldwide to gain optimal treatment efficacy and avoid the development of acquired drug resistance is undeniably effective for the majority of patients. However, the interindividual pharmacokinetic variability of first-line TB drugs can result in a low exposure to one or more of the TB drugs, as frequently observed in our study population. This undermines the concept of the multidrug TB treatment strategy. Although no cases of acquired drug resistance were observed in the population studied, persistent suboptimal plasma concentrations of one or more of the TB drugs could predispose to development of drug resistance and a poor treatment outcome.12,14 With regard to our results on treatment outcome, it is important to keep in mind that the effectiveness of the first-line TB drugs should not be taken for granted. TDM is employed for a variety of drugs to optimize dosing that maximizes therapeutic benefit while minimizing toxicity. TDM is potentially useful for the treatment of active TB but is currently underused. Another important issue is the question of to what extent doses of the different anti-TB drugs can be increased without causing severe adverse events; there is very little information in the literature concerning the relationship between plasma concentrations and adverse reactions for the four antibiotics studied in this report. Such knowledge is crucial when recommending the implementation of therapeutic target ranges and the use of TDM in the treatment of TB.

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Transparency declarations
None to declare.

References