Low plasma concentrations of rifampicin in tuberculosis patients in Indonesia

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SUMMARY

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SETTING: Although rifampicin is a key drug in tuberculosis treatment, little is known about its quality and bioavailability in countries endemic for tuberculosis. High drug levels may lead to increased toxicity, while low drug levels may predispose to treatment failure and relapse.

OBJECTIVE: To investigate possible variations in the bioavailability of plasma rifampicin in tuberculosis patients in Indonesia.

DESIGN: Plasma concentrations of rifampicin and the rifampicin content of drug formulations in use were measured among 62 non-selected tuberculosis patients in Jakarta, Indonesia.

RESULTS: Plasma concentrations of rifampicin were generally low: 70% of patients had 2-hour plasma concentrations (C max) below 4 mg/L. No toxic plasma concentrations of rifampicin (>20 mg/L) were found. The strongest predictive factor for the magnitude of rifampicin concentrations was the drug manufacturer. The rifampicin content of the different drug preparations used was normal (90.5–103.6% of the reference standard). No association was found between low plasma rifampicin concentrations and delayed sputum conversion or treatment failure.

CONCLUSION: The unexpectedly low plasma concentrations of rifampicin in this setting are most likely due to reduced bioavailability of local drug preparations, as the rifampicin content of the drug preparations was found to be normal. The clinical significance of these findings remains to be determined.

KEY WORDS: tuberculosis treatment; rifampicin; clinical pharmacology; biological bioavailability; Indonesia

METHODS

Patients

Between September and December 2000, 62 consecutive patients with microbiologically proven pulmonary tuberculosis were investigated in an out-patient tuberculosis clinic in Jakarta, Indonesia. In accordance with the Indonesian national guidelines, treatment consisted of isoniazid (INH) 300 mg, rifampicin (RMP) 450 mg, pyrazinamide 1500 mg and ethambutol 750 mg daily for 2 months, followed by INH 600 mg and RMP 450 mg three times weekly for 4 months. No fixed-dose combinations were used, and according to local guidelines treatment was not adjusted for body weight. For every patient, a single box with all of the medications for 6 months was kept at the clinic throughout the period of treatment. All

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patients visited the clinic at weekly intervals, when drug intake was observed by health personnel and medication was provided for the remaining days of the week. Patients received all drugs free of charge. The outcome of treatment was monitored using standard criteria. This study was conducted with informed consent from all patients and with permission from the University of Indonesia.

**Microbiology**

At least two sputum samples were collected for microscopy and culture before treatment and after 2 and 6 months. Culture of *Mycobacterium tuberculosis* was performed in 3% Ogawa medium. Drug susceptibility testing of isolates was done using serial dilutions of anti-tuberculosis drugs on Middlebrook’s medium. Minimal inhibitory drug concentrations (MIC) to define antimicrobial resistance were 0.2 mg/L for INH, 1 mg/L for RMP and 5 mg/L for ethambutol and streptomycin.

**Measurement of rifampicin in plasma samples and drug preparations**

Patients were evaluated after 4 and 8 weeks of treatment, when stable drug levels were expected. Apart from analgesics and antitussives, no other co-medication was allowed. Patients were asked not to have breakfast on the morning of blood sampling. Witnessed intake of all four anti-tuberculosis drugs took place in the clinic between 8:00 and 10:00 am, at least 24 hours after the previous drug intake. Two hours after drug intake, corresponding with the estimated time to maximum plasma concentrations of rifampicin, 10 mL of venous blood was collected. Following immediate centrifugation, plasma was separated and frozen at −20°C in polypropylene tubes containing 20 mg/mL ascorbic acid. All samples were stored at −80°C within 4 hours. Measurement of rifampicin in plasma samples was done by high performance liquid chromatography. For measurement of rifampicin in drug formulations, two blisters, each containing medication for a single day of intensive treatment, were collected from every individual patient. The manufacturer, batch number and expiration date of medication were recorded. The medications were kept in closed plastic bags at room temperature and protected from sunlight until analysis. For chromatographic measurement of rifampicin content, single tablets were dissolved by addition of MeOH, followed by ultrasonic centrifugation and homogenisation. Internationally recognised drug preparations (Rifadin®, Aventis) were used as controls.

**Data analysis**

In patients with plasma concentrations of rifampicin available at weeks 4 and 8, the mean value was used for further analysis unless one value was below 1 mg/L and less than 25% of the second value, in which case the lower value was excluded. Two-hour plasma concentrations (Cmax) of rifampicin >20 mg/L were considered toxic, values between 8 and 20 mg/L therapeutic, values between 4 and 8 mg/L low, and <4 mg/L subtherapeutic (very low). Plasma concentrations of rifampicin were correlated with sex, body weight, presence or absence of diarrhoea, drug manufacturer and human immunodeficiency virus (HIV) infection. Collected drug preparations of rifampicin were defined as substandard if they contained less than 85% or more than 115% of the reference standard. The possible significance of low plasma concentrations was investigated by calculating the ratio of Cmax/MIC for rifampicin, and by comparing the clinical and bacteriological outcome in patients with rifampicin concentrations below and above 4 mg/L.

**Statistics**

Variables are represented as mean (standard deviation [SD]) when normally distributed and as median (range) in all other cases. Pearson χ²-test, Student’s t-test, Mann-Whitney test and univariate regression analysis were used as appropriate. Statistical analysis was performed using SPSS version 9.0 for Windows (SPSS Inc., Chicago, IL). All reported P values are two-sided, and the level of significance was set at P < 0.05.

**RESULTS**

**Plasma concentrations of rifampicin**

Tuberculosis patients included in this study were mostly young adults and male (Table). Moderate or severe malnutrition (body mass index <17 kg/m²) was present in 45%. From 62 patients, 97 samples were available for measurement of rifampicin. The mean time recorded between witnessed drug intake and blood sampling was 2 hours (±5 min). Values of two separate measurements were available from 35 patients. In these patients, the coefficient of variation (SD/n) of plasma rifampicin concentrations 4 and 8 weeks after the start of treatment was 36%. Toxic

**Table Patient characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male (%)</th>
<th>Male (years)*</th>
<th>Sputum microscopy</th>
<th>HIV-positive (%)</th>
<th>Body weight (kg)*</th>
<th>Medication (mg/kg body weight)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>34 (16–62)</td>
<td>34 (16–62)</td>
<td>18</td>
<td>1 (1.6%)</td>
<td>45 (29–63)</td>
<td>INH 7.31 (4.76–10.34)</td>
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<tr>
<td>Sputum microscopy</td>
<td></td>
<td></td>
<td>+</td>
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<td>Rifampicin 11.97 (7.14–15.52)</td>
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<td>++</td>
<td></td>
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<td>Pyrazinamide 36.58 (23.81–51.72)</td>
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<td></td>
<td></td>
<td></td>
<td>+ + +</td>
<td></td>
<td></td>
<td>Ethambutol 18.29 (11.90–25.86)</td>
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</tr>
<tr>
<td>Male sex</td>
<td>43 (69%)</td>
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<tr>
<td><strong>Body</strong></td>
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* Median (range).
HIV = human immunodeficiency virus; INH = isoniazid.
concentrations (>20 mg/L) of rifampicin were not detected in any of the 97 samples. Two-hour plasma concentrations of rifampicin were within the therapeutic range in two patients (3%), low (4–8 mg/L) in 17 patients (27%), and very low (<4 mg/L) in 43 patients (70%) (Figure). Three patients had undetectable rifampicin concentrations. Rifampicin concentrations were significantly lower in male patients than in female patients (median 2.67 vs. 4.62 mg/L; P = 0.04; Figure).

Fifty-three patients (85%) had been treated with drug formulations produced by manufacturer ‘A’, while nine patients (15%) had been treated with drugs from manufacturer ‘B’ (both are leading manufacturers in Indonesia). Plasma rifampicin concentrations were 2.35-fold higher (95% confidence interval 1.45–3.26; P = 0.001) in patients treated with drugs from manufacturer ‘B’ than in patients treated with drugs from manufacturer ‘A’. No significant correlation was found between body weight and plasma concentrations of rifampicin (R² = 0.06). Diarrhoea (n = 6) was not associated with lower plasma rifampicin concentrations. HIV infection was established in one patient whose 2-hour plasma rifampicin concentrations were <1 mg/L on two separate occasions.

Rifampicin content of drug formulations
Medication was available for analysis from 59 patients, 51 produced by manufacturer ‘A’, and eight by manufacturer ‘B’. None were fixed-dose combinations. Drug preparations from ‘A’ were from three different batches. No medication had overrun its expiry date at the time of treatment. The weight of the tablets was 625 ± 4.9 mg for ‘A’, and 901 ± 18.9 mg for ‘B’. The rifampicin content was 93.6 ± 1.3% of the standard reference for tablets from ‘A’, and 100.1 ± 2.4% for tablets from ‘B’. No single preparation had a content that was <85% or >115% of the reference standard (range 90.5–103.6%).

Clinical and bacteriological significance of plasma rifampicin concentrations

During treatment, one patient died (1.6%) and six defaulted (9.7%). After 6 months of treatment, 50 patients were cured (80.6%), two (3.2%) showed bacteriological failure, and three (4.8%) were still on treatment because of delayed sputum conversion. The cure rate was higher in patients with rifampicin concentrations >4 mg/L than in patients with concentrations <4 mg/L (94.7% vs 74.4%), but this was mainly caused by a higher default rate in the latter group. Weight gain and resolution of symptoms were similar in both groups (data not shown). No jaundice or symptomatic hepatitis occurred during treatment. Three patients (4.8%) developed a mild elevation of plasma transaminases (twice the upper limit of normal); no patient had transaminases greater than three times the upper limit of normal. Plasma concentrations of rifampicin in these patients were respectively 3.31, 3.76 and 7.38 mg/L. After 4 weeks of treatment, concentrations of plasma rifampicin showed a weak correlation with plasma transaminases (R² = 0.10; P = 0.017).

The bacteriological response was similar in patients with therapeutic and subtherapeutic concentrations of rifampicin: after 2 months of treatment, sputum culture was positive for M. tuberculosis in respectively 25% and 20% of patients with plasma rifampicin concentrations <4 mg/L and >4 mg/L. MICs for rifampicin were available for 43 M. tuberculosis isolates. The MIC for rifampicin was >1 mg/L in two patients (resistant, 4.6%), between 0.5 and 1 mg/L in 20 patients (intermediate, 46.5%), and <0.5 mg/L in 21 patients (sensitive, 48.8%). The median ratio Cmax/MIC for rifampicin was 9.7 (range 0–48.8). In 64% of patients with rifampicin concentrations <4 mg/L, and in 16% of patients with concentrations >4 mg/L, the Cmax/MIC was <10 (P = 0.018).

DISCUSSION

In this Indonesian tuberculosis control clinic, 70% of patients had very low 2-hour rifampicin plasma concentrations (<4 mg/L), and no toxic concentrations of rifampicin were observed. The absence of more than two-fold elevated serum transaminases during treatment, which normally occurs in 10–20% of cases, is in line with low exposure to anti-tuberculosis medication in this population.

Our study is not the first to report low plasma concentrations of rifampicin in tuberculosis patients. Low or absent 2-hour plasma concentrations of rifampicin concentrations...
rifampicin and reduced total drug exposure have been reported in HIV-infected patients. In 22 non-HIV-infected tuberculosis patients selected due to a slow clinical response, treatment failure or relapse, 14 (64%) had plasma concentrations of rifampicin below the 2-hour target range of 8 mg/L. HIV infection (1.6% in this patient group), diabetes and gastric surgery, which may all result in delayed absorption of rifampicin, cannot explain our results. As rifampicin is rather unstable, patient studies are vulnerable to artefacts. However, we circumvented the breakdown of rifampicin by using a cold chain and stabilisation by ascorbic acid. The distribution of the rifampicin concentrations also argues against decay. To increase precision, the majority of the patients were evaluated twice on two separate days. Given the likelihood of biological variability, there was relatively little variation between the two measurements. Although drug dosages were not adjusted to body weight (in line with standard procedures in the National TB Programme in Indonesia), this did not account for the variations in rifampicin concentrations. In accordance with a previous study, rifampicin concentrations were significantly lower in male than in female patients.

Several recent reports have shown that the drug content of anti-tuberculosis drugs may be insufficient. Trade of ‘fake’ drugs is widespread in some parts of the world. Indonesia has had serious problems related to drug supply in recent years, and we and others have occasionally found degraded and expired medication in Indonesia. However, no reduced rifampicin content was found in the tablets that had been prescribed to the patients in this study. Apart from drug content, other factors such as particle size, excipients and manufacturing process may affect the bioavailability of rifampicin. For example, major problems have been encountered in the manufacture of combined formulations of rifampicin plus INH and pyrazinamide (not used in this study). In this study, a more than two-fold difference was found in plasma concentrations from patients treated with formulations from two different drug companies. The drug content of the respective formulations was only slightly different, which indicates that the problem must lie in reduced bioavailability. This finding re-emphasises the need for pharmacokinetic studies to ensure the bioavailability of rifampicin.

The clinical significance of finding low plasma concentrations of anti-tuberculosis drugs remains unclear because of the size of this study, which was only designed to test for bioavailability. To evaluate therapeutic efficacy would require a much larger study, since under supervised treatment the bacteriological failure rate is in the order of 1–3%. However, several case reports suggest that low drug levels are associated with a poor clinical response, treatment failure and acquisition of drug resistance.

It is clear from the literature that the microbicidal effect of rifampicin is concentration-dependent. The peak concentration after oral administration, which occurs around 2 hours after ingestion, should be between 8 and 20 mg/L. Rifampicin concentrations between 4 and 8 mg/L are in a grey zone, and concentrations below 4 mg/L are considered subtherapeutic. Such insights are derived from studies such as the US Public Health Service trial, in which a significantly higher percentage of bacteriological failures occurred in patients treated with 450 mg rifampicin than with 600 or 750 mg. Patients in that trial treated with <9 mg rifampicin per kg per day had a higher failure rate than those treated with >9 mg/kg. In another study, a dose reduction of rifampicin from 600 mg to 300 mg significantly decreased bactericidal activity. In line with a concentration-dependent effect, less frequent administration of rifampicin has no detrimental effects. Rifampicin requires a high ratio between maximum plasma concentrations in relation to MIC (high Cmax/MIC) for optimal activity. With a Cmax > 8 mg/L and a normal MIC of 0.25 mg/L, the estimated Cmax/MIC will be >32. In our study, Cmax/MIC was < 10 in 50% of cases.

In summary, very low 2-hour plasma rifampicin concentrations were found in the majority of a group of non-selected tuberculosis patients in Indonesia. The clinical significance of this finding is still unclear, but reduced bioavailability of rifampicin, and possibly other anti-tuberculosis drugs, may contribute to the low cure rates and frequent recurrence of tuberculosis in Indonesia. Additional investigations are needed to evaluate the bioavailability of anti-tuberculosis medication and the pharmacokinetic properties of patients in this setting. Bioavailability studies in healthy volunteers and comparative studies using locally produced medication and internationally recognised drug preparations are underway.

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RÉSUMÉ

CONTEXTE : La rifampicine est un médicament-clé dans le traitement de la tuberculose. Toutefois, on ne connaît que peu de choses au sujet de sa qualité et de sa biodisponibilité dans les pays où la tuberculose est endémique. Des taux élevés de médicament peuvent induire un accroissement de la toxicité alors que des taux faibles peuvent prédisposer à l’échec du traitement et à la rechute.

OBJECTIF : Investiguer les variations possibles de biodisponibilité de la rifampicine dans le plasma chez les patients tuberculeux en Indonésie.

SCHEMA : Les concentrations plasmatiques de la rifampicine et le contenu en rifampicine des formulations médicamenteuses utilisées ont été mesurées chez 62 patients tuberculeux non sélectionnés à Jakarta, Indonésie.

RÉSULTATS : Les concentrations plasmatiques de la rifampicine sont généralement faibles : chez 70% des patients, les concentrations plasmatiques après 2 heures (Cmax) sont inférieures à 4 mg/L. On n’a trouvé dans aucun cas des concentrations plasmatiques toxiques de rifampicine (>20 mg/L). Le facteur prédictif le plus pertinent de l’importance des concentrations de rifampicine est le fabricant du médicament. Le contenu en rifampicine des différentes préparations médicamenteuses utilisées est normal (de 90,5 à 103,6% du standard de référence). On n’a observé aucune association entre les faibles concentrations de rifampicine et les retards de la négativation des expectorations ou un échec du traitement.

CONCLUSION : Les faibles concentrations plasmatiques inattendues de rifampicine dans ce contexte sont le plus probablement dues à une diminution de la biodisponibilité des préparations locales du médicament, puisque le contenu des préparations de médicaments en rifampicine s’avère normal. L’importance clinique de ces observations reste à déterminer.
MARCO DE REFERENCIA: La rifampicina es un medicamento clave en el tratamiento de la tuberculosis, aunque se sabe poco acerca de su calidad y de su biodisponibilidad en los países donde la tuberculosis es endémica. Los niveles elevados de medicamentos pueden conducir a un aumento de la toxicidad, mientras que los niveles bajos pueden predisponer al fracaso del tratamiento y a la recaída.

OBJETIVO: Investigar la variaciones posibles de la biodisponibilidad de la rifampicina plasmática en los pacientes tuberculosos en Indonesia.

MÉTODO: Se midieron las concentraciones plasmáticas de rifampicina y el contenido en rifampicina de las formulaciones medicamentosas en uso, en 62 pacientes tuberculosos no seleccionados, en Jakarta, Indonesia.

RESULTADOS: Las concentraciones plasmáticas de rifampicina eran generalmente bajas: 70% de los pacientes tenían concentraciones plasmáticas después de 2 horas ($C_{\text{max}}$) inferiores a 4 mg/l. No se encontraron concentraciones tóxicas de rifampicina (>20 mg/l). El factor predictivo más poderoso para la magnitud de las concentraciones de rifampicina era el fabricante del medicamento. El contenido de rifampicina de las diferentes preparaciones medicamentosas utilizadas era normal (90,5-103,6% del estándar de referencia). No se encontró asociación entre una concentración plasmática baja de rifampicina y la demora de la negativización de las baciloscopias o el fracaso del tratamiento.

CONCLUSIÓN: Las inesperadas concentraciones plasmáticas bajas de rifampicina en este contexto son probablemente debidas a una biodisponibilidad reducida de las preparaciones locales, puesto que el contenido en rifampicina de las preparaciones medicamentosas era normal. Queda por determinar el significado clínico de estos hallazgos.