Systemic exposure to rifampicin in patients with tuberculosis and advanced HIV disease during highly active antiretroviral therapy in Burkina Faso

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Objectives: Low plasma concentrations of rifampicin, an essential antituberculosis drug, have been reported particularly among HIV co-infected persons. In a prospective, longitudinal study we measured rifampicin systemic exposure at different timepoints during highly active antiretroviral therapy (HAART).

Patients and methods: From May 2006 to April 2007, 16 tuberculosis (TB)/HIV co-infected patients were enrolled in Ouagadougou, Burkina Faso. All patients received fixed dose combinations of rifampicin, isoniazid, pyrazinamide and ethambutol under direct observation and HAART, consisting of a fixed dose combination of stavudine, lamivudine and nevirapine. Rifampicin concentrations during the dosing interval were determined by HPLC at three different timepoints: (i) after 2 weeks of TB therapy and before starting HIV therapy (T0); (ii) after 4 weeks of combined therapy (T1); and (iii) after 10 weeks of combined therapy (T2).

Results: The median values of the area under the curve (AUC0–24) of rifampicin increased by 39% at T1 (15.69 μg·h/mL; P=0.01) and by 83% at T2 (20.65 μg·h/mL; P=0.001) compared with T0 (11.28 μg·h/mL). Similar variations were observed for the median Cmax at T0 (2.24 μg/mL) compared with T2 (2.83 μg/mL; P=0.003). However, none of the subjects had Cmax levels >8 μg/mL at either T0 or T2.

Conclusions: Rifampicin systemic exposure increased during combined TB and HIV therapy, possibly due to increased drug absorption or decreased oral clearance, but remained invariably low in this population. Studies to define the Cmax rifampicin concentrations, which are associated with a significantly increased risk of treatment failure, are urgently warranted.

Keywords: TB, pharmacokinetics, HAART

Introduction

Rifampicin is the backbone of the standard first-line antituberculosis regimen and allows for the shortest possible duration of therapy (6 months) in HIV-negative and HIV-positive patients.1 Although favourable treatment outcomes are achievable in approximately 95% of patients with drug-susceptible pulmonary tuberculosis who receive 6 month rifampicin-based regimens,2 there is evidence that lower plasma concentrations of antituberculosis drugs are associated with clinical failure and acquired drug resistance.3,4

Several reports have suggested that rifampicin systemic exposure is reduced in HIV-infected patients,5–7 but none has described if and how the drug pharmacokinetic profile changes during antituberculosis treatment.

We report the variations in rifampicin plasma concentrations at different timepoints during combined tuberculosis (TB) and HIV treatment in a group of TB/HIV co-infected patients in Ouagadougou, Burkina Faso.

Patients and methods

Study design and population

From May 2006 to April 2007 we conducted a prospective cohort study comparing the pharmacokinetic profile of rifampicin at different timepoints during TB treatment, in combination with a standard nevirapine-
based highly active antiretroviral therapy (HAART) regimen. Nevirapine plasma concentrations in the same study population had been previously reported.  

HIV-1-infected subjects ≥18 years were enrolled if diagnosed with TB, had a CD4+ T cell count <100 cells/mm³, had not previously received any antiretroviral therapy, and were able and available to provide written informed consent. Diagnosis of TB was performed according to international standards for low resource settings. Participants were enrolled at four TB clinics in Ouagadougou from May 2006 to May 2007. Patients were hospitalized at the Centre d’Accueil Notre Dame de Fatima in Ouagadougou to perform blood collection for the pharmacokinetic study and whenever required by their clinical conditions.

**Treatment and follow-up procedures**

Patients were given standard daily antituberculosis therapy with a fixed dose combination of rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months, followed by 4 months of rifampicin and isoniazid. The antituberculosis drugs were provided by the Global Drug Facility (GDF). Rifampicin was given in tablets of 300, 450, or 600 mg on the basis of the patient's weight, in order to approximate a dose of 10 mg/kg/day. Treatment was administered in the morning on an empty stomach under direct supervision by the health staff at the health facility for the intensive phase and during pharmacokinetic sessions, and unsupervised thereafter, with weekly replenishment of the drugs. Antiretroviral therapy was started 2 weeks after initiation of antituberculosis therapy. It consisted of a fixed combination of stavudine (30 mg), lamivudine, and nevirapine at a standard dose.

Concomitant treatment with trimethoprim/sulfamethoxazole and supplementation with group B vitamin were provided to all patients. At enrolment, all patients underwent physical examination including medical history and laboratory tests. The presence of diarrhoea (three or more unformed stools per day) was recorded. Height (cm) and weight (kg) were measured, and the body mass index (BMI) was calculated (kg/m²) at baseline and during the follow-up.

The CD4+ T lymphocytes count and the HIV viral load in serum (HIV-RNA) were measured on the day of HAART initiation, 4 weeks later and then every 24 weeks until 72 weeks. Patients were followed-up for 72 weeks.

**Pharmacokinetic assessments**

Three full pharmacokinetic curves of rifampicin were measured: at T0, 2 weeks after starting TB therapy and before starting HIV drugs; at T1, after 4 weeks of combined TB and HIV therapy; and at T2, after 10 weeks of combined therapy. Serial venous blood samples were collected in heparinized hard plastic tubes just prior to and 1, 2, 4, 6, 8 and 12 h after observed dosing, and centrifuged at 2000 rpm for 10 min. Plasma samples were stored protected from light and frozen at −70°C immediately after being separated, in order to prevent degradation and oxidation of the drug. Shipment to the laboratory for analysis was carried out using dry ice. Rifampicin concentrations in plasma were measured at the Laboratory of Clinical Pharmacokinetics, San Matteo Hospital, by a validated reverse-phase HPLC method with ultraviolet detection, as previously described.  

The standard curves for rifampicin were linear within the range of 50–10 000 ng/mL in plasma, with a limit of quantification of 50 ng/mL and a limit of detection of 30 ng/mL. Within- and between-day imprecision were <10% for all the quality control samples.

**Statistical analysis**

Rifampicin main pharmacokinetic parameters and BMI at the different timepoints were compared using the Wilcoxon signed rank test for paired samples. SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The chosen level of significance was 5% and the P values reported were two-tailed.

**Ethical considerations**

The study was approved by the national Ethics Committee of Burkina Faso (reference number 2006-010, 12 April 2006) and conducted in accordance with the Declaration of Helsinki.

**Results**

The demographic and laboratory data of 16 patients enrolled in the study are shown in Table 1. None of the patients had a diagnosis of diabetes mellitus, and no patients reported diarrhoea at the time of the pharmacokinetic determination. Two persons died during TB treatment, and 14 successfully completed it. All 14 subjects who completed follow-up had sustained virological response to HIV treatment, and the median CD4+ T cell count after 24 weeks of HAART had risen from 73.5 to 194 (range 87–399). The median BMI and the median haemoglobin levels increased at the end of TB treatment from 17.0 to 18.3 kg/m² and from 8.8 to 12.6 g/dL, respectively. Rifampicin plasma concentration–time curves were drawn for 16 patients at T0 and T1, and for 14 patients at T2. We made a comparison between groups for paired pharmacokinetic data from the 14 subjects with all determinations (Table 2). AUCₐ₀–₄ values at T0 were significantly lower than at T1 (median value 11.28 µg·h/mL versus 15.69 µg·h/mL; P=0.01) and T2 (20.65 µg·h/mL; P=0.001), with an increase of 39% and 83%, respectively (Figure 1). Cₘₐₓ values were significantly lower at T0 (median value 2.24 µg/mL) compared with T2 (2.83 µg/mL; P=0.003), while the difference was not significant between T0 and T1. All patients had very low Cₘₐₓ values at all timepoints, and no patient reached the suggested threshold of 8 µg/mL at T0 and T2; 2 of 16 (12.5%) did reach it at T1 (9.7 and 9.0 µg/mL). The CL/F values steadily decreased over time and were significantly lower than at T1.

**Table 1. Demographic, clinical and laboratory characteristics of 16 patients with TB and HIV co-infection at baseline**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender, n (%)</td>
<td>9 (56.2)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>38 (27–47)</td>
</tr>
<tr>
<td>BMI (kg/m²), median (range)</td>
<td>17.0 (13–23)</td>
</tr>
<tr>
<td>WHO stage III, n (%)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td>WHO stage IV, n (%)</td>
<td>5 (31.3)</td>
</tr>
<tr>
<td>Pulmonary TB (PTB), n (%)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td>extra-pulmonary TB (EPTB) (pleural), n (%)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>PTB+EPTB, n (%)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>HIV-1 RNA (copies/mL), median (range)</td>
<td>202 643 (65 631–500 000)</td>
</tr>
<tr>
<td>CD4+ T lymphocytes (cells/mm³), median (range)</td>
<td>73.5 (5–99)</td>
</tr>
<tr>
<td>HBsAg+, n (%)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Haemoglobin level (g/dL), median (range)</td>
<td>8.8 (6.1–14.1)</td>
</tr>
</tbody>
</table>

HBsAg, hepatitis B surface antigen.

*a*n=15.
Discussion

We report very low median rifampicin C\textsubscript{max} and AUC\textsubscript{0–24} after doses of 10 mg/kg/day among HIV-infected persons with TB in Burkina Faso. We also describe for the first time a significant increase in both median rifampicin C\textsubscript{max} and AUC between the intensive and continuation phase of TB therapy.

The reasons for the significant increase in rifampicin C\textsubscript{max} and AUC during TB and HIV therapy are unknown. Rifampicin is known to induce its own clearance during the first week of treatment,\textsuperscript{12} but there is no evidence that this phenomenon is reversible during prolonged rifampicin intake. The most likely explanations for the 83% increase in systemic exposure to rifampicin over time may be either the improvement in the general health conditions of the patients (witnessed by the BMI rise) with increased drug absorption (increasing F) or changes in hepatic cytochrome P450 activity, which may account for the gradual decrease in oral clearance (CL/F decreased by 41%). Nevirapine and its 12-hydroxynevirapine metabolite themselves may cause liver toxicity leading to P450 inhibition, according to studies in vitro\textsuperscript{13} and in animal models.\textsuperscript{14}

Rifampicin levels were particularly low during the initial phase of tuberculosis therapy, exactly at the time when adequate drug concentrations would be mostly needed. Since microbial killing, resistance suppression and post-antibiotic effect of rifampicin are concentration dependent, the crucial pharmacodynamic parameters are C\textsubscript{max} and AUC.\textsuperscript{15,16} The C\textsubscript{max} value normally considered for therapeutic drug monitoring of rifampicin is ≥ 8 mg/mL, according to studies conducted worldwide in the 1950s\textsuperscript{17} and subsequently validated in the 1990s.\textsuperscript{17} Despite the significant increase observed during treatment, in our patients the median C\textsubscript{max} of rifampicin remained below the expected reference value of 8 mg/mL in virtually all subjects.

The causes of low serum concentrations of rifampicin may be various: poor quality of TB drugs (drug quality was ensured by the GDF in our study), HIV infection (although some studies did not observe differences between matched HIV-infected and uninfected TB patients),\textsuperscript{6,18–20} and wasting or malnutrition with associated hypoalbuminaemia, small-bowel oedema, villous atrophy and bacterial overgrowth leading to malabsorption.\textsuperscript{21}

The reduction of rifampicin plasma levels is a reason for concern due to the potential impact on treatment outcome and the development of rifamycin resistance.\textsuperscript{13,22} We did not evaluate the association between rifampicin C\textsubscript{max} and clinical outcome because of our limited sample size, but the two patients who died had AUC and C\textsubscript{max} rifampicin values above the median value at both T0 and T1.

In summary, we have shown that rifampicin C\textsubscript{max} and AUC\textsubscript{0–24} significantly increase during TB treatment, but they remain much below the target concentrations in a typical population of severely malnourished HIV-TB co-infected patients in Africa.

Acknowledgements

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Transparency declarations
None to declare.

References