PHARMACOKINETICS AND TOLERABILITY OF

A HIGHER RIFAMPICIN DOSE VERSUS THE STANDARD DOSE

IN PULMONARY TUBERCULOSIS PATIENTS

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Running title: Higher versus standard dose of rifampicin

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ABSTRACT

Rifampicin is a key drug for tuberculosis treatment. Available data suggest that the currently applied 10 mg/kg dose of rifampicin may be too low, and that increasing the dose may shorten the treatment duration.

A double-blind randomized phase II clinical trial was performed to investigate the effect of a higher dose of rifampicin in terms of pharmacokinetics and tolerability. Fifty newly diagnosed adult Indonesian TB patients were randomized to receive a standard (450 mg; i.e. 10 mg/kg in Indonesian patients) or higher (600 mg) dose of rifampicin besides other TB drugs. A full pharmacokinetic curve for rifampicin, pyrazinamide and ethambutol was recorded after 6 weeks of daily TB treatment. Tolerability was assessed during the 6-month treatment period.

Geometric means of exposure to rifampicin (AUC$_{0-24}$) were increased by 65% (p<0.001) in the higher-dose group (79.7 mg.h/L) compared to the standard-dose group (48.5 mg.h/L). Maximum rifampicin concentrations ($C_{\text{max}}$) were 15.6 mg/L vs. 10.5 mg/L (49% increase, p<0.001). The percentage of patients with rifampicin $C_{\text{max}} \geq 8$ mg/L was 96% vs. 79%, p=0.094. The pharmacokinetics of pyrazinamide and ethambutol were similar in both groups. Mild (grade I or II) hepatotoxicity was more common in the higher-dose group (46 vs. 20%, p=0.054), but no patient developed severe hepatotoxicity.

Increasing the rifampicin dose is associated with a more than dose-proportional increase in the mean AUC$_{0-24}$ and $C_{\text{max}}$ of rifampicin without affecting the incidence of serious adverse effects. Follow-up studies are warranted to assess whether high dose rifampicin may enable shortening of TB treatment.
INTRODUCTION

Each year eight million persons develop active tuberculosis (TB) and 2 to 3 million people die from this infectious disease. The treatment of TB is complicated by the length and complexity of currently available drug regimens, which invites problems of nonadherence, inadequate response and resistance development. Therefore, a long-term goal for TB control has been to shorten the duration of treatment. This may possibly be achieved by increasing the dose of the pivotal TB drug rifampicin, considering that early bactericidal activity studies in TB patients [10] and recent work in the mouse model [6] suggest that the typical 10 mg/kg dose of rifampicin is rather low. A 50% increase in the rifampicin dose may reduce the duration of treatment by one-third [6]. Apart from these studies, we and others have found low two-hour (peak) plasma concentrations of rifampicin in TB patients treated with 10 mg/kg rifampicin daily [3,9,20], which also suggests that a higher dose of rifampicin merits further study.

So far, only few clinical data have been available with regard to the pharmacokinetics, tolerability and effectiveness of drug regimens based on a higher dose of rifampicin [8,17]. Hence we performed a pilot study in Indonesian patients, in which we compared a higher (600 mg) and the standard dose (450 mg or 10 mg/kg, considering the body weight of Indonesian people) of rifampicin. It appeared that 78% of patients in the higher-dose arm versus 48% of those in the standard-dose arm achieved a rifampicin peak (two-hour) plasma concentration above a reference value of 8 mg/L [19]. As the pilot study evaluated only a single time point in the rifampicin pharmacokinetic curve and was non-blinded, we decided to conduct a double-blind randomized clinical trial with intensive pharmacokinetic sampling to
compare the steady-state pharmacokinetics and tolerability of a higher and the standard dose of rifampicin.

MATERIALS AND METHODS

Subjects
The study was conducted in an urban outpatient tuberculosis clinic in Bandung, Indonesia. Study subjects were patients with newly diagnosed, untreated pulmonary tuberculosis. The diagnosis of pulmonary tuberculosis was based on clinical symptoms and chest X-ray examination, confirmed by microscopic detection of acid-fast bacilli. Patients were excluded if they were below 18 years of age, had a body weight less than 33 kg, were pregnant or lactating, had a history of liver or kidney disease or any other disease that might affect the pharmacokinetics of TB drugs, or if they had been treated for tuberculosis previously. HIV status was assessed anonymously at the end of the study. Informed consent was obtained from all subjects and the study was approved by the Independent Ethics Committee, Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia and by the Ethical Review Board Region Arnhem/Nijmegen, The Netherlands.

Study design
This was a double-blind, randomized, two-arm, phase II clinical trial. Eligible patients were randomized to a standard dose (450 mg, corresponding to 10 mg/kg in Indonesian people) or a higher dose (600 mg) of rifampicin besides other TB drugs. In accordance with the Indonesian National TB Program, TB treatment consisted of daily isoniazide 300 mg, rifampicin (higher or standard dose), pyrazinamide 1500...
mg, and ethambutol 750 mg for two months, followed by isoniazid 600 mg and rifampicin (higher- or standard-dose) three times weekly for four months [5]. All patients received TB drugs from the same manufacturer, formulated in separate tablets. Bioequivalence of the rifampicin tablets and an international reference standard has been established before [4].

Double blinding for the dose of rifampicin was accomplished by inserting rifampicin tablets of 450 mg and 600 mg into blank capsule shells with the same colour and size. Five capsules were sealed into an aluminium blister. The encapsulation and sealing of tablets was performed by the manufacturer of the drugs. Information about the randomization of the participants was not available to anyone involved in the study until it was completed.

A full pharmacokinetic curve was recorded at steady state after six weeks of treatment. Patients were followed every other week during the intensive phase and once-monthly thereafter for evaluation of possible adverse events as well as microbiological tests (sputum microscopy and culture).

Based on available data from the previous pilot study [19], it was estimated that at least 24 participants were required in each arm to be able to demonstrate a change (two-sided test) of at least 40% in the peak plasma concentration of rifampicin at a 5% significance level and with 80% statistical power. The study was not powered to detect differences in bacteriological outcome.

**Pharmacokinetic assessment**

Patients refrained from the intake of any food or any drugs (other than the study medication) from 11 p.m. on the day preceding the pharmacokinetic assessment until four hours after the intake of study medication. They took all antituberculosis
drugs with 230 ml of still water. Serial venous blood samples were collected just prior to, and 1, 1.5, 2, 2.5, 3, 4, 6 and 12 hours after witnessed drug intake. Plasma was immediately separated and frozen at -20°C, transferred to -80°C within 72 h and transported on dry ice to the Netherlands for bioanalysis.

**Bioanalysis**

The plasma concentrations of rifampicin, desacetylrifampicin, pyrazinamide and ethambutol were assessed with validated high performance liquid chromatographic (HPLC) methods. Concentrations of isoniazid were not assessed, as undue degradation of this unstable drug was anticipated during storage and transport.

Rifampicin and desacetylrifampicin were measured with protein precipitation followed by HPLC with ultraviolet (UV) detection. Hundred microliters of acetonitrile and 10 µL ascorbic acid solution (20 mg/ml) were added to 200 µL of plasma sample. The mixture was vortexed for 20 sec and centrifuged for five minutes. Then 200 µL of 10 mM potassium dihydrogen phosphate buffer was added and the mixture was vortexed and centrifuged again. One hundred microliters of the clear supernatant was injected in the HPLC system, which consisted of an Omnispher 5 C_{18} column (250 by 4.6 mm, Varian, Middelburg, The Netherlands) protected with a Chromguard RP guard column (10 by 3 mm, Varian, Middelburg, the Netherlands). The mobile phase consisted of 10 mM potassium dihydrogen phosphate (pH 4.5) and acetonitrile (62:38 % v/v). The flow rate was set at 1 ml/min and the wavelength for UV detection was 334 nm. Accuracy for standard concentrations was between 99.8% and 100.4% for rifampicin and between 102.4 and 103.9% for desacetylrifampicin, dependent on the concentration level. The intra- and inter-assay
coefficients of variation were less than 4% over the ranges of 0.28-30 mg/L and 0.15-3 mg/L for rifampicin and desacetylrifampicin, respectively. Lower limits of quantitation were 0.28 and 0.15 mg/L respectively. Rifampicin containing plasma samples were stable (< 5% loss) for at least 16 months at -20°C and -80°C.

Total plasma concentrations of pyrazinamide were measured by solid phase extraction followed by HPLC-UV. Briefly, Waters Oasis HLB 1 cc 30 mg extraction cartridges were washed sequentially with methanol and HPLC-grade water. A half ml of plasma sample and 0.5 ml of water were drawn slowly onto the column and allowed to stand. The column was washed with water and elution was performed with methanol. The eluate was dried and after addition of 10 mM phosphate buffer pH 6.0/methanol (95/5% v/v), vortexing and centrifugation, the clear supernatant was injected into the HPLC apparatus. Chromatographic analysis was performed on an Atlantis dC\textsubscript{18} column (150 by 4.6 mm, Waters). The mobile phase consisted of 10 mM phosphate buffer pH 6.0 / acetonitrile (99/1% v/v) (fluid A) and acetonitril (fluid B), in which the percentage of acetonitril (fluid B) was changed linearly as follows: 0 min, 0%; 11.5 min, 0 %; 12 min, 45 %; 17 min, 45%; 17.5 min, 0% and 25 min, 0%. The flow rate was set at 1.3 ml/min and the wavelength for UV detection was 266 nm. The accuracy for standard concentrations was between 96.0% and 109.2%, intra- and inter-assay coefficients of variation were less than 10% over the range of 0.1 to 66.8 mg/L and the lower limit of quantitation was 0.1 mg/L. Plasma samples with pyrazinamide were stable (< 5% loss) for at least 3 months at -20°C and -80°C.

Ethambutol was quantitated with liquid-liquid extraction, followed by derivatization and HPLC-UV. Plasma samples (100 µL) were spiked with internal standard (N,N-
diisopropylethylendiamine 99%), alcalized and extracted with chloroform. The chloroform layer was poured into other tubes containing derivatization reagens (0.01% phenylethylthyocyanate), and evaporated to dryness. Samples were resolved in acetonitrile/0.05 M phosphate buffer (40:60 v/v). After vortexing and centrifugation the supernatant was injected in the HPLC apparatus. Chromatographic analysis was performed on an Omnispher 5 C\textsubscript{18} column (100 by 4.6 mm, Varian, Middelburg, the Netherlands). The mobile phase was 0.05 M phosphate buffer (pH 5.6) – acetonitril gradient in which the percentage of phosphate buffer was changed linearly as follows: 0 min, 65%; 21 min, 46 %; 22 min, 65 %; 25 min, 65%. Flow rate was set at 1.5 ml/min and the wavelength for UV detection was 215 nm. Accuracy was between 101% and 105%, intra- and inter-assay coefficients of variation were less than 4% over the range of 0.05 to 10 mg/L, and the lower limit of quantitation was 0.05 mg/L. Plasma samples with ethambutol were stable (< 5% loss) for at least 8 months at -20°C and -80°C.

**Pharmacokinetic analysis**

A noncompartmental analysis with WinNonLin version 4.1 (Pharsight Corp., Mountain View, California) was performed to compute the pharmacokinetic parameters of rifampicin, desacetyl rifampicin, pyrazinamide and ethambutol. The maximum concentration of drug in plasma was defined as $C_{\text{max}}$ and the time to this maximum concentration as $T_{\text{max}}$. $C_{\text{max}}$ and $T_{\text{max}}$ were determined directly from the plasma concentration-time data. The value of the slope ($-\beta/2.303$, where $\beta$ is the first-order elimination rate constant) was calculated by least-squares linear regression analysis. If the concentration at 12 h post dose ($C_{12}$) was quantifiable, the concentration at 24 h ($C_{24}$) was estimated using the equation $C_{24} = C_{12} \cdot e^{-\beta(t24-t12)}$. 
The area under the concentration-time curve from 0 to 24 h post dose (AUC$_{0-24}$) was assessed using the linear-log trapezoidal rule from 0 up to the last concentration. Half life ($t_{1/2}$) was calculated from the expression $0.693/\beta$. Apparent clearance (CL/F, where F is bioavailability) was calculated by dividing dose by AUC$_{0-24}$, and apparent volume of distribution (V/F) was obtained by dividing CL/F by $\beta$.

The relative exposure of metabolite desacetylrifampicin to rifampicin was expressed as the ratio of the metabolite and the parent drug.

**Tolerability**

Safety and tolerability were assessed by questioning patients actively (before the study and in weeks 2, 4, 6 and 8 in the intensive phase and weeks 12, 16, 20 and 26 in the continuation phase, in total 8 times after inclusion), guided by a list of nine possible adverse events that could occur during treatment with TB drugs. Patients were questioned by a field investigator, who was a medical doctor at every time.

Serum glutamine pyruvate transferase (SGPT) was measured to evaluate for hepatotoxicity in weeks 2, 4, 6 and 8 during the intensive phase, i.e. 4 times during the study. All possible adverse events were categorized according to an adverse event grading system [12]. For elevations of SGPT, grade 1 was 1.25 - 2.5 times upper limit of normal (ULN); grade 2 was 2.6 - 5 times ULN; grade 3 was 5 - 20 times ULN and grade 4 was $\geq$ 20 times ULN. Patients were withdrawn if they experienced grade 3 or 4 hepatotoxicity. After the reversal of hepatotoxicity, treatment was gradually resumed.
**Bacteriological examinations and treatment outcome**

Microscopic examination of Ziehl-Neelsen stained sputum slides was done for acid-fast bacilli (AFB) and *M. tuberculosis* culture was performed on Ogawa 3%. Drug susceptibility testing for rifampicin, isoniazid, ethambutol and streptomycin was performed on cultured isolates, using an absolute concentration method with supranational control.

After 6 months of TB treatment a patient was cured (referring to an initially smear-positive patient who was smear-negative in the last month of treatment and on at least one previous occasion), failed treatment (i.e. a smear-positive patient who remained smear-positive at month 5 or later during treatment), completed treatment (a patient who completed treatment but did not meet the criteria for cure or failure because no sputum examination was possible during the last month of treatment, as the patient did not produce sputum), defaulted (treatment was interrupted for ≥ 2 consecutive months) or died (death from any cause during treatment) [21].

**Statistical analysis**

Pharmacokinetic parameters were log-transformed before statistical analysis. Differences in AUC_{0-24}, C_{max}, t_{1/2}, CL/F and V/F in the higher- versus standard-dose group were tested with the independent-samples t-test and a geometric mean ratio plus 95% confidence interval was calculated for every comparison. Values for T_{max} were not transformed and were compared using Wilcoxon rank sum test. Pearson Chi-square test was used to compare the proportions of patients who reached a reference peak plasma concentration of 8 mg/L for rifampicin [16], as well as the incidence of adverse events, as reported at least once in eight consecutive reporting times during the study.
Univariate analyses were performed in the higher-dose and standard-dose arms separately to assess the effects of gender, age, body weight and the occurrence of nausea or vomiting on the AUC$_{0-24}$ and C$_{\text{max}}$ of rifampicin, pyrazinamide and ethambutol. A multivariate linear regression analysis was performed to assess the variation in AUC$_{0-24}$ and C$_{\text{max}}$ attributable to the presence of those variables that emerged from the univariate analyses.

All statistical evaluations were performed with SPSS for Windows, version 12.0.1 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered statistically significant in all analyses.

RESULTS

Patients

Fifty patients were included in the study. They presented with a history of cough (100%), shortness of breath (70%), fever (76%), night sweats (62%) and weight loss (84%). All patients showed chest X-ray abnormalities and M. tuberculosis culture was positive in 92% of them. Fifty-two percent of the patients was male, median age was 28 years (range: 18 – 55 years), and mean body weight was 46.1 kg (range 35.6 – 71.2 kg). One patient was HIV-positive and type-2 diabetes was found in four patients (8%). One patient used glibenclamide as co-medication, a drug which is not known to affect the pharmacokinetics of TB drugs. Twenty-five patients were allocated to each of the two study arms. Both at baseline (data not shown) and at the time of the pharmacokinetic assessment (table 1), patient characteristics were similar in the two arms, except for rifampicin dose per kg.
Pharmacokinetic data were available from 47 patients (of whom 23 in the higher-dose arm), tolerability data were available from 49 patients (24 in the higher-dose arm) and 47 patients were available for an evaluation of treatment response (23 in the higher-dose arm).

**Pharmacokinetics of TB drugs**

All pharmacokinetic assessments occurred as planned without any events (e.g. vomiting) that may affect the pharmacokinetic profiles that were recorded. Marked inter-individual variability in $AUC_{0-24}$ and $C_{\text{max}}$ values for rifampicin was observed both in the higher-dose and standard-dose arm (table 2).

Exposure to rifampicin ($AUC_{0-24}$) was 65% (or 1.65 fold) higher in the higher-dose group, which reflects a more than dose-proportional increase of the exposure upon increasing the dose (table 2 and figure 1). Likewise, rifampicin $C_{\text{max}}$ was significantly higher in the higher-dose group (table 2). The percent of patients who reached a reference value of at least 8 mg/L [16] was 96% in the higher-dose group and 79% in the standard-dose group ($p=0.094$). At two hours post dose, 87% of patients in the higher-dose group and 58% in the standard-dose group had a concentration of at least 8 mg/L ($p=0.01$).

With regard to metabolite desacetylrifampicin, it appeared that absolute values for $AUC_{0-24}$ and $C_{\text{max}}$ were approximately two fold higher in the higher-dose group compared to the standard-dose group (table 2). In addition, desacetylrifampicin / rifampicin ratios for $AUC_{0-24}$ and $C_{\text{max}}$ were significantly higher in the higher-dose group.
The pharmacokinetics of pyrazinamide and ethambutol did not differ between the two study groups (table 3). Of note, there were strong correlations between \( AUC_{0-24} \) values of rifampicin, pyrazinamide and ethambutol, and the same applied to \( C_{\text{max}} \) values (data not shown).

**Tolerability and bacteriological examinations**

No significant differences were found between the high and standard dose group in the incidence of nausea (33% resp. 24%, \( p=0.47 \)), vomiting (21% resp. 12%, \( p=0.40 \)), abdominal pain (4% resp. 8%, \( p=0.58 \)), itching (50% resp. 64%, \( p=0.32 \)), arthralgia (21% resp. 28%, \( p=0.57 \)), hyperuricemia (21% resp. 20%, \( p=0.94 \)), dizziness (13% resp. 8%, \( p=0.60 \)), fever (13% resp. 4%, \( p=0.28 \)), paresthesia (8% resp. 8%, \( p=0.97 \)), and hepatotoxicity grade 3 (4% resp. 12%, \( p=0.32 \)).

Hepatotoxicity grade 1 or 2 was more common in the higher dose group (46% vs. 20%, \( p=0.054 \)), but none of the patients developed serious hepatotoxicity and no action had to be taken. The grade 3 hepatotoxicity which developed in four of 49 patients (8%, three from the standard-dose arm and one from the higher-dose arm) was reversible in all patients. The majority of adverse events (99%) occurred in the first weeks of the intensive phase. No ‘flu-like syndrome’ was reported during intermittent dosing of rifampicin in the continuation phase.

Among 47 patients available for an evaluation of treatment response, nobody died, 38 patients were cured (81%), four completed the treatment (9%), three (6%) showed bacteriological failure (one received rifampicin 600 mg) and two patients defaulted (4%, one from the higher-dose group). Drug susceptibility tests revealed
isoniazid monoresistance in two patients (one from the higher-dose group), rifampicin monoresistance in one (from the standard-dose group), and multidrug-resistant TB in one patient (from the standard-dose group). There was no significant difference in the cumulative culture conversion rate between the higher- and standard-dose group, but it should be noted that the study was not powered to detect a difference in this respect.

The low number of undesirable events precludes firm conclusions about relationships between pharmacokinetic data on the one hand and the occurrence of adverse effects or inadequate response on the other. Patients with rifampicin $C_{\text{max}}$ values above an upper reference value of 24 mg/L [16] did not report any serious adverse event and patients with grade 3 and 4 hepatotoxicity did not show unduly high exposure to rifampicin or pyrazinamide. Likewise, the few patients with bacteriological failure had rifampicin $C_{\text{max}}$ values within the reference range.

**Determinants of the pharmacokinetics of rifampicin, pyrazinamide and ethambutol**

In univariate analyses, both in the higher- and in the standard-dose group, gender and age did not show a significant relationship with the $\text{AUC}_{0-24}$ and $C_{\text{max}}$ of rifampicin, pyrazinamide and ethambutol. However, body weight correlated with the $\text{AUC}_{0-24}$ of all three drugs in both study groups; for rifampicin, the Pearson correlation coefficient was -0.371 ($p=0.081$) in the higher-dose group and -0.445 ($p=0.029$) in the standard-dose group. Patients in the separate study arms who had reported nausea or vomiting at least once did not have lower exposures to rifampicin, pyrazinamide or ethambutol compared to those patients who never
reported nausea or vomiting. For example, in the higher dose arm, the mean value for the AUC$_{0-24}$ of rifampicin was 80.6 h$^\ast$mg/L (geometric mean: 76.1 h$^\ast$mg/L) among patients who once experienced nausea or vomiting compared to 85.1 h$^\ast$mg/L (geometric mean 81.7 h$^\ast$ mg/L) who did not ($p=0.62$, independent-samples t-test on log-transformed data). Similarly, the occurrence of just vomiting was not associated with large or significant decreases in exposure to the TB drugs in each of the study arms. Rifampicin, pyrazinamide and ethambutol C$_{\text{max}}$ values for three patients with diabetes mellitus (one in the higher-dose group and two in the standard-dose group) and one HIV-infected patient were within reference ranges.

Multivariate analysis in all study patients revealed that both the dose of rifampicin and body weight were independent predictors of the AUC$_{0-24}$ of rifampicin, according to the formula \( \ln \text{AUC}_{0-24} \text{ (in h.mg/L)} = 3.288 + [0.003 \times \text{rifampicin dose (mg)}] - [0.017 \times \text{body weight (kg)}] \).

**DISCUSSION**

This phase II clinical study shows that an increase in the rifampicin dose from 10 to 13 mg/kg daily results in a more than dose-proportional increase in the mean AUC$_{0-24}$ (65% increase) and mean C$_{\text{max}}$ (49 % increase) of rifampicin without a significant increase in the incidence of serious adverse events. An increase in the AUC$_{0-24}$ or C$_{\text{max}}$ of rifampicin predicts an increase in effectivity, as rifampicin exhibits exposure-[6] or concentration-dependent [15] activity against *M. tuberculosis*. Therefore increasing the dose of rifampicin appears to be effective (from a pharmacokinetic point of view) and feasible. This calls for follow-up phase II studies that evaluate an
even higher (15 or 20 mg/kg) dose of rifampicin. In-vitro and murine data [6] and data in man [7] indicate that TB treatment duration could possibly be shortened to 4 months by using higher doses of rifampicin. This should eventually be tested in larger numbers of patients, within the context of a phase III trial.

The main problem of currently available TB treatment is its length and complexity. Strategies to shorten treatment include further optimization of the dosing of available TB drugs and the evaluation of new antituberculosis drugs (of which the quinolone moxifloxacin seems most promising [2]), or a combination of these [18]. Based on available data [6,10], increasing the dose of rifampicin seemed a promising means to optimize the response to this drug. This intervention is particularly attractive when it is acknowledged that rifampicin is widely available at low costs, and that the properties of this drug are well-known to physicians all over the world. If increasing the dose of rifampicin proves worthwhile, this intervention could be implemented broadly and quickly to the benefit of many patients.

Only few clinical studies have addressed the concept of high-dose rifampicin in TB treatment so far [17]. In one study a short regimen that incorporated a high dose of rifampicin (1200 mg daily or every other day) yielded very high sputum culture negativity by two months [7]. On the other hand, another study demonstrated no difference in effectivity between patients who used 600 (10 mg/kg) or 750 mg rifampicin daily. In the latter study, 750 mg of rifampicin was well-tolerated [8]. Past attempts to use large intermittent doses of rifampicin were met with a high incidence of a ‘flu-like syndrome’, but this was ascribed to the intermittency of dosing rather than the height of the dose [1, 17].
In the current study, a moderate (one-third) increase in the dose of rifampicin was evaluated. We chose to be cautious, considering the paucity of clinical data regarding high-dose rifampicin in general, and taking into account that there are only scarce pharmacokinetic and tolerability data for even a standard dose of rifampicin in Asian populations. A high dose of rifampicin (20 mg/kg) is used in the treatment of brucellosis [17], but it should be considered that tolerability data for high dose rifampicin in brucellosis can not be directly extrapolated to TB. In the treatment of brucellosis, high dose rifampicin is combined with just one other drug (doxycyclin) instead of several toxic TB drugs, and treatment of this infection takes only 45 days.

The moderate increase in rifampicin dose, as applied in this study, resulted in a relatively strong, more than dose-proportional increase in rifampicin plasma concentrations, which is consistent with the non-linear pharmacokinetics of rifampicin [1,11,14]. Increasing the dose of rifampicin also caused a more than proportional (around 2-fold) increase in the AUC\textsubscript{0-24} of active metabolite desacetylrifampicin and an increase in the desacetylrifampicin/rifampicin ratio, which is in agreement with previous data [11]. Considering the relatively small contribution of the active metabolite to the exposure and effectivity of rifampicin, these findings do not seem to be clinically relevant. Importantly, the increase in rifampicin dose did not affect the mean AUC\textsubscript{0-24} and C\textsubscript{max} of pyrazinamide and ethambutol, despite the observed correlations between the pharmacokinetics of rifampicin on the one hand and those of pyrazinamide and ethambutol on the other.

The toxicity of rifampicin is known to be related to dose and administration interval [1]. In this study, the incidence of adverse events was relatively high, both in the
higher- and standard-dose group. This high incidence may be attributable in part to the active and frequent questioning of patients that we applied to guarantee their safety. The large majority of adverse events were mild in severity. The incidence of grade 1-2 hepatotoxicity was higher in the higher-dose group, but this was transient and did not cause treatment interruption or alteration. More importantly, this study did not show evidence for an increase in the incidence of serious hepatotoxicity related to a higher dose of rifampicin, which is in agreement with previous data [8]. Nausea and vomiting occurred some 10% more often in the higher dose arm, which reflects a (non-significant) difference of only 2-3 patients in this small phase II study. These adverse events should be monitored carefully in follow-up studies, as they may possibly affect adherence and the absorption of rifampicin, which may offset the advantages of high dose rifampicin. In the current study, nausea or vomiting were not associated with exposure to the TB drugs. This may not be surprising, considering that no vomiting occurred during the pharmacokinetic assessments.

A limitation of this study, inherent to phase II studies in general, is the relatively small number of participants. This means that only large differences in the incidence of adverse events became statistically significant. Furthermore, the number of participants, in combination with a relatively short (6-month) follow-up, did not allow for a valid comparison of bacteriological response between the two treatment arms. As another limitation, it should be considered that all participants were Indonesian. Although rifampicin doses were based on body weight, it can not be excluded that the pharmacokinetics and/or tolerability of high-dose rifampicin may be different in people with another race or genetic background. As a third limitation, the effect of
high-dose rifampicin on the pharmacokinetics of isoniazid was not assessed in this study.

In conclusion, this study shows that an increase in the rifampicin dose from 10 to 13 mg/kg daily causes a more than dose-proportional increase in the mean AUC$_{0-24}$ and C$_{\text{max}}$ of rifampicin without causing an increase in severe adverse events to the TB treatment. Therefore increasing the dose of rifampicin is feasible and effective from a pharmacokinetic point of view. Follow-up phase II studies should be performed to evaluate an even higher dose of rifampicin. These studies should be carefully monitored, considering the non-linear pharmacokinetics of rifampicin. Eventually, high dose rifampicin is to be tested in larger phase III trials. For certain risk groups, e.g. patients with drug resistance or patients with HIV infection [20] or diabetes mellitus [13], a higher dose of rifampicin may be useful as part of the current 6-month TB treatment regimen. In the absence of such risk factors, a higher dose of rifampicin (possibly in conjunction with new TB drugs) may enable shortening of TB treatment.

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Potential conflicts of interest. All authors: no conflicts.
REFERENCES


Table 1. Patient characteristics at the time of the pharmacokinetic assessment

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<tr>
<td>Mean (s.d.)</td>
<td>47.3 (6.9)</td>
<td>48.4 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (s.d.)</td>
<td>18.4 (2.6)</td>
<td>18.8 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1/23 (4%)</td>
<td>2/24 (8%)</td>
<td></td>
</tr>
<tr>
<td>HIV status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>0</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>Rifampicin dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (s.d.)</td>
<td>12.9 (1.7)</td>
<td>9.5 (1.4)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Steady-state pharmacokinetics of rifampicin and desacetylrifampicin after daily administration of a high (600 mg, 13 mg/kg) or standard (450 mg, 10 mg/kg) dose of rifampicin (geometric mean plus range, unless stated otherwise)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rifampicin 600 mg (n=23)</th>
<th>Rifampicin 450 mg (n=24)</th>
<th>Ratio 600mg/450mg (geometric mean + 95 CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rifampicin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (mg h/L)</td>
<td>79.7 (38.7-138.1)</td>
<td>48.5 (26.7-72.8)</td>
<td>1.65 [1.38 – 1.96]</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>15.6 (5.1-26.6)</td>
<td>10.5 (6.2-16.6)</td>
<td>1.49 [1.22 – 1.81]</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; &gt; 8 mg/L (%)</td>
<td>22/23 (96%)</td>
<td>19/24 (79%)</td>
<td></td>
<td>0.090&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h; median, range)</td>
<td>1 (1 – 6)</td>
<td>2 (1 – 4)</td>
<td></td>
<td>0.428&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2.2 (1.3-6.3)</td>
<td>1.9 (1.5-5.2)</td>
<td>1.13 [0.95 – 1.35]</td>
<td>0.176&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>7.5 (4.3-15.5)</td>
<td>9.2 (6.1-16.8)</td>
<td>0.82 [0.69 – 0.97]</td>
<td>0.021&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>23.3 (12.3-96.1)</td>
<td>25.3 (12.9-55.7)</td>
<td>0.92 [0.72 – 1.18]</td>
<td>0.502&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Desacetylrifampicin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (mg h/L)</td>
<td>13.2 (7.2-25.6)</td>
<td>6.4 (2.3-13.3)</td>
<td>2.07 [1.61 – 2.65]</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (mg/L)</td>
<td>2.2 (0.6-3.9)</td>
<td>1.2 (0.5-2.6)</td>
<td>1.83 [1.40 – 2.41]</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h; median, range)</td>
<td>4 (1.5 – 6)</td>
<td>4 (1.5 – 4)</td>
<td></td>
<td>0.586&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2.5 (1.7-9.9)</td>
<td>3.2 (2.0-18.9)</td>
<td>0.80 [0.63 – 1.03]</td>
<td>0.085&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Ratio desacetylrifampicin/rifampicin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;</td>
<td>0.17 (0.10-0.25)</td>
<td>0.13 (0.08-0.20)</td>
<td>1.26 [1.09 – 1.45]</td>
<td>0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.14 (0.07-0.22)</td>
<td>0.11 (0.05-0.19)</td>
<td>1.23 [1.03 – 1.48]</td>
<td>0.024&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>AUC<sub>0-24</sub>: area under the concentration-time curve from 0 to 24 h post-dose, C<sub>max</sub>: maximum concentration, T<sub>max</sub>: time to maximum concentration, CL/F: total clearance, V/F: apparent volume of distribution, F: bio-availability, CI: confidence interval

<sup>b</sup>Independent t-test on log transformed data

<sup>c</sup>Pearson Chi-square test

<sup>d</sup>Wilcoxon rank sum test
Table 3. Steady-state pharmacokinetics of pyrazinamide and ethambutol after daily administration of 1500 mg (30 mg/kg) and 750 mg (15 mg/kg) (geometric mean plus range, unless stated otherwise)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rifampicin 600 mg (n=23)</th>
<th>Rifampicin 450 mg (n=24)</th>
<th>Ratio 600mg/450mg (geometric mean ± 95 CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyrazinamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0-24}) (mg·h/L)</td>
<td>514.5 (266.4-775.3)</td>
<td>472.8 (258.9-705.1)</td>
<td>1.04 [0.93 – 1.27]</td>
<td>0.269(^b)</td>
</tr>
<tr>
<td>C(_{\text{max}}) (mg/L)</td>
<td>46.0 (23.3-71.8)</td>
<td>43.8 (19.2-62.1)</td>
<td>1.05 [0.91 – 1.21]</td>
<td>0.493(^b)</td>
</tr>
<tr>
<td>T(_{\text{max}}) (h; median, range)</td>
<td>2.5 (2-6)</td>
<td>2.75 (1-6)</td>
<td>0.897</td>
<td></td>
</tr>
<tr>
<td>t(_{1/2}) (h)</td>
<td>6.9 (4.1-11.7)</td>
<td>6.6 (3.9-12.7)</td>
<td>1.04 [0.89 – 1.22]</td>
<td>0.593(^b)</td>
</tr>
<tr>
<td><strong>Ethambutol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC(_{0-24}) (mg·h/L)</td>
<td>14.7 (10.0-23.4)</td>
<td>14.4 (9.6-22.5)</td>
<td>1.02 [0.88 – 1.17]</td>
<td>0.830(^b)</td>
</tr>
<tr>
<td>C(_{\text{max}}) (mg/L)</td>
<td>2.3 (1.2-5.6)</td>
<td>2.4 (1.1-4.9)</td>
<td>0.98 [0.77 – 1.24]</td>
<td>0.863(^b)</td>
</tr>
<tr>
<td>T(_{\text{max}}) (h; median, range)</td>
<td>2.5 (1.5-4)</td>
<td>2.5 (1.5-6)</td>
<td>0.397(^c)</td>
<td></td>
</tr>
<tr>
<td>t(_{1/2}) (h)</td>
<td>4.6 (1.8-6.8)</td>
<td>4.2 (2.6-5.5)</td>
<td>1.09 [0.95 – 1.24]</td>
<td>0.217(^b)</td>
</tr>
</tbody>
</table>

\(^a\) AUC\(_{0-24}\): area under the concentration-time curve from 0 to 24 h post-dose, C\(_{\text{max}}\): maximum concentration, T\(_{\text{max}}\): time to maximum concentration, CI: confidence interval

\(^b\) Independent t-test on log transformed data.

\(^c\) Wilcoxon rank sum test
Legend to figure 1:

FIGURE 1

Mean steady-state plasma concentration - time profiles of rifampicin in tuberculosis patients who received high dose (600 mg, 13 mg/kg, n=23) or standard dose (450 mg, 10 mg/kg, n=24) of rifampicin, with standard deviations.