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Randomised Pharmacokinetic Trial of Rifabutin with Lopinavir/Ritonavir-Antiretroviral Therapy in Patients with HIV-Associated Tuberculosis in Vietnam

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Abstract

Background: Rifampicin and protease inhibitors are difficult to use concomitantly in patients with HIV-associated tuberculosis because of drug-drug interactions. Rifabutin has been proposed as an alternative rifamycin, but there is concern that the current recommended dose is suboptimal. The principal aim of this study was to compare bioavailability of two doses of rifabutin (150 mg three times per week and 150 mg daily) in patients with HIV-associated tuberculosis who initiated lopinavir/ritonavir-based antiretroviral therapy in Vietnam. Concentrations of lopinavir/ritonavir were also measured.

Methods: This was a randomized, open-label, multi-dose, two-arm, cross-over trial, conducted in Vietnamese adults with HIV-associated tuberculosis in Ho Chi Minh City (Clinical trial registry number NCT00651066). Rifabutin pharmacokinetics were evaluated before and after the introduction of lopinavir/ritonavir -based antiretroviral therapy using patient randomization lists. Serial rifabutin and 25-O-desacetyl rifabutin concentrations were measured during a dose interval after 2 weeks of rifabutin 300 mg daily, after 3 weeks of rifabutin 150 mg daily with lopinavir/ritonavir and after 3 weeks of rifabutin 150 mg three times per week with lopinavir/ritonavir.

Results: Sixteen and seventeen patients were respectively randomized to the two arms, and pharmacokinetic analysis carried out in 12 and 13 respectively. Rifabutin 150 mg daily with lopinavir/ritonavir was associated with a 32% mean increase in rifabutin average steady state concentration compared with rifabutin 300 mg alone. In contrast, the rifabutin average steady state concentration decreased by 44% when rifabutin was given at 150 mg three times per week with lopinavir/ritonavir. With both dosing regimens, 2 – 5 fold increases of the 25-O-desacetyl- rifabutin metabolite were observed when rifabutin was given with lopinavir/ritonavir compared with rifabutin alone. The different doses of rifabutin had no significant effect on lopinavir/ritonavir plasma concentrations.

Conclusions: Based on these findings, rifabutin 150 mg daily may be preferred when co-administered with lopinavir/ritonavir in patients with HIV-associated tuberculosis.

Trial Registration: ClinicalTrials.gov NCT00651066
Introduction

In 2011, there were an estimated 34 million adults and children living globally with HIV/AIDS and an estimated 8.7 million new cases of tuberculosis: 1.1 million persons had HIV-associated tuberculosis and 430,000 persons with HIV-associated tuberculosis died [1,2].

Since 2003, there has been a remarkable scale up of antiretroviral therapy with 8 million people estimated to be on therapy by the end of 2011 [1]. The most recent data show that 97% of adults and children on antiretroviral therapy are taking a first-line regimen, in general consisting of two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor [3]. The remainder is on a second-line regimen, usually consisting of a nucleoside reverse transcriptase inhibitor backbone and a protease inhibitor. The low number of patients on second-line treatment reflects the poor availability of viral load monitoring during antiretroviral therapy in resource-limited countries, and thus a limited ability to correctly diagnose treatment failure and switch patients accordingly to more effective therapy. With the development of point-of-care tests for viral load under the World Health Organization (WHO) new Treatment 2.0 initiative [4], and recommendations from the WHO that 12-monthly viral load monitoring should become the norm for monitoring antiretroviral therapy [5], it is likely that increasing numbers of patients will be identified with treatment failure and will need switching to a second-line regimen with a protease inhibitor. While this is a welcome move, this change will have implications for the care and treatment of patients with HIV-associated tuberculosis.

Observational studies have clearly shown that antiretroviral therapy improves the prognosis of patients with HIV-associated tuberculosis [6], and clinical trials have also established the importance of early initiation of antiretroviral therapy in reducing early mortality [7,8,9]. While first-line antiretroviral therapy using efavirenz is safe and effective when combined with rifampicin-based anti-tuberculosis treatment [10], there are challenges when it comes to using second-line regimens. The combination of rifampicin and protease inhibitors is problematic because rifampicin significantly reduces the bioavailability of all known protease inhibitors by 75% to 95% by induction of cytochrome 3A4 (CYP3A4) enzymes [11]. Attempts to overcome this adverse drug-drug interaction by either increasing the dose of the protease inhibitor or altering the dose of rifampicin have been thwarted by hepatotoxicity and other problems with tolerance [12], and such approaches are anyway incompatible with large-scale and decentralised public sector roll-out of ART.

Rifabutin is an attractive alternative to rifampicin as it is a less potent inducer of CYP3A4 [13], and the drug can safely be combined with ritonavir-boosted protease inhibitors without protease inhibitor dose adjustment. Rifabutin is recommended at a standard dose of 300 mg daily for the prophylaxis and treatment of Mycobacterium avium complex and for the treatment of drug-susceptible tuberculosis. Plasma concentrations of rifabutin are increased in the presence of protease inhibitors [11], and therefore dose adjustments are recommended. Guidelines from the Centers for Disease Control (CDC, Atlanta, USA) recommended in 1998 that the dose of rifabutin be reduced from 300 mg to 150 mg in the presence of a protease inhibitor [14], and the guidelines further recommended in 2004 that the dose be reduced to 150 mg three times a week (TPW) when used in combination with lopinavir/ritonavir (LPV/r) [15]. However, two recent reports have suggested that rifabutin given at a dose of 150 mg TPW in combination with LPV/r in patients with HIV-positive tuberculosis may result in inadequate rifamycin levels [16,17]. Case reports of tuberculosis relapse in patients administered rifabutin 150 mg TPW with LPV/r [18] and further data showing that low rifamycin concentrations are associated with acquired rifamycin resistance in patients taking intermittent doses of rifabutin [19] add to concerns that rifabutin given intermittently with protease inhibitor-based antiretroviral therapy is sub-optimal.

The present study was therefore undertaken with the primary objective of comparing the pharmacokinetic parameters of two doses of rifabutin (150 mg TPW and 150 mg daily) in patients with HIV-associated tuberculosis in Vietnam who initiated antiretroviral therapy with LPV/r. Secondary objectives were to investigate (i) the pharmacokinetics of LPV/r in combination with RBT, and (ii) the safety and toxicity of rifabutin in combination with antiretroviral therapy during the initial phase of anti-TB treatment.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Ethics Statement

The study was approved by the Institutional Review Board and Ethical Review committee at Pham Ngoc Thach Hospital, the Health Department of Ho Chi Minh City and the Ministry of Health, Vietnam, as well as the Union Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France.

Study design

This study was a randomized, open-label, multi-dose, two-arm, cross-over trial, conducted in Vietnamese patients with HIV-associated tuberculosis - Clinical trial registry number: NCT00651066.

Study setting

The study was carried out in Pham Ngoc Thach Hospital, Ho Chi Minh, Vietnam, a tertiary care facility that has 800 beds and cares for TB patients, about 10% of whom have associated HIV-infection. In Vietnam, patients with suspected tuberculosis are investigated according to National Tuberculosis Guidelines [20] which are based on smear microscopy for acid-fast bacilli and

Competing Interests: The authors declare that the French National Institute for Health and Medical Research-French National Agency for Research on AIDS and Viral Hepatitis (Institut national de la sante et de la recherche medicale-Agence Nationale de Recherche sur le Sida) was the Sponsor and Funder of the study. Fondation Total also provided a grant to support the study. The authors declare that they received a donation of rifabutin from Laboratoires SERB and a donation of rifabutin and 25-O-desacetyl rifabutin reference powder from Pfizer (SA) which was used for the preparation of standard curves and quality control of assays. The French National Institute for Health and Medical Research-French National Agency for Research on AIDS and Viral Hepatitis (INSERM-ANRS), Fondation Total, Laboratoires SERB and Pfizer (SA) had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Furthermore, receipt of the donations from Laboratoires SERB and Pfizer (SA) does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.

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chest radiography for those with pulmonary disease. Anti-
tuberculosis treatment is given for 6 months and consists of a 2-
months initial phase of rifampicin, isoniazid, pyrazinamide and
ethambutol given as fixed dose combination tablets under direct
observation, followed by 4-months continuation phase with rifampicin
and isoniazid as fixed dose combination tablets. HIV
testing is done at the time of tuberculosis registration [20], and
HIV-positive patients are assessed with a CD4 lymphocyte count
and started as soon as possible on a standard first-line antiretro-
viral therapy regimen - usually consisting of stavudine or
zidovudine – lamivudine – efavirenz as a standard fixed dose
combination.

Patient recruitment

Study patients were adults aged 18 – 65 years, HIV-positive,
with a CD4 count less than or equal to 250 cells/μL and with
newly diagnosed tuberculosis. Eligibility requirements included:
provision of written informed consent; having a firm home address
that was readily accessible; if female, having a negative pregnancy
test on day of enrolment; having a diagnosis of pulmonary
tuberculosis confirmed by smear microscopy, culture or a chest
radiograph compatible with active tuberculosis and associated
with a typical clinical history and two negative sputum smears; no
previous history of antiretroviral therapy; weight ≥40 kg; a
Karnofsky score Q ≥80%; no grade 3 or 4 clinical or laboratory
findings according to Division of AIDS tables [21]. Patients with
the following conditions were excluded from the trial: a previous
episode of tuberculosis within the last 12 months, a history of prior
Treatment under study

The detailed planned trial timeline describing the intended
allocation of treatments in the two arms of the trial in relation to
the initial and continuation phases of anti-tuberculosis treatment
and randomization is shown in Figure 1. As no wash out period
was possible, all pharmacokinetic parameters were estimated at
steady state, at least 2 weeks after initiation of rifabutin treatment
or with the new dosing regimens. At enrolment into the trial,
patients were started on rifabutin 300 mg once a day (OD), in
combination with standard doses of isoniazid, pyrazinamide and
ethambutol. After two weeks (representing the first 2 weeks of the
initial phase of treatment) the first pharmacokinetic study (PK1)
was done. Patients were continued on the same anti-tuberculosis
treatment and at two weeks from the start of anti-tuberculosis
treatment were started on antiretroviral therapy with stavudine-
lamivudine-lopinavir/ritonavir (d4T/3TC/LPV/r – standard
doses of stavudine 30 mg/lamivudine 150 mg/lopinavir/ritonavir
400 mg/100 mg – taken twice daily) and randomized to one of
two arms: Arm A = Rifabutin 150 mg TPW or Arm B =
Rifabutin 150 mg OD. After a further three weeks, the second PK
(PK2) study was done and the treatments crossed-over: patients on
the “A” dose of Rifabutin were switched to the “B” dose and vice
versa. Patients remained on these doses along with isoniazid,
pyrazinamide and ethambutol and antiretroviral therapy for a
further three weeks and the third PK study (PK3) done. After PK3,
the patients stopped rifabutin and started the continuation phase
of anti-tuberculosis treatment with rifampicin and isoniazid under
the care of the National Tuberculosis Program. They were also
referred to the National AIDS Program to be treated according
to standard care with stavudine/lamivudine/efavirenz. Patients were
followed up to the end of anti-tuberculosis treatment for another
16 weeks. Physical examinations and laboratory investigations
were done at every PK study.

Laboratoires SERB supplied rifabutin 150 mg capsules for oral
administration (Ansaïpine 150 mg, Pfizer) and the new film-
coated tablet formulation of LPV/r, Aluvia® was purchased from
Abbott Laboratories (USA).

Sample size

Based on the area under the curve (AUC0–24) for rifabutin
determined in previous studies [19], it was estimated that a sample
size of 12 participants had a power of 80% to detect a 20% relative
change between the geometric means of the AUC0–24 for the
participants taking rifabutin without antiretroviral therapy and the
AUC0–24 for the participants taking rifabutin when combined with
antiretroviral therapy. To provide a target of 12 evaluable patients
in each arm, because patients with low CD4 cell counts recruited
into the study might experience high mortality and morbidity
resulting in a high attrition rate, it was decided that 32 patients
should be enrolled (16 in each arm).

Pharmacokinetic (PK) sampling and drug analysis

All patients were admitted to the Clinical Trial Unit facility the
night before each PK study and were fasted from midnight. On
the morning of the PK sampling day, serial blood samples were
obtained. The first blood sample (0 h) was drawn prior to
administration of study drugs and a standard hospital breakfast
was served exactly two hours (2 h) after drug ingestion.
Subsequent bloods were drawn at 2, 3, 4, 5, 6, 8, 12, 24 and
48 h (in the case of intermittent RBT dosing) after drug ingestion.
The samples were placed on ice immediately and centrifuged at
3000 rpm at 4°C for 10 minutes within 30 minutes of collection.
Separated plasma was transferred to polypropylene tubes and
stored immediately at -70°C until analysis. The drug assays for
RBT and its metabolite (25-O-desacytrolfabin) as well as
lopinavir and ritonavir are described in the following section [22].

Drug analyses for rifabutin, 25-O-desacytrolfabin,
lamivudine-lopinavir/ritonavir. Rifabutin and 25-O-desacytrolfabin
were analyzed simultaneously with a validated HPLC assay.
Rifabutin and 25-O-desacytrolfabin standards were kindly
provided by Pfizer. In brief, after addition of medazepam as
internal standard both chemicals were extracted from 0.2 mL of
plasma with a hexane/dichloromethane solution (6/4 v/v). After
vortex and centrifugation, the organic phase was evaporated to
dryness. Dry residue was reconstituted with 100 μL of mobile
phase constituted of [Phosphate mono potassic dihydrogen
solution 0.05 M, pH = 3.85]/acetonitrile: 600/400 (v/v). 50 μL
is injected onto the Eclipse XDB RP-C18, 150×4, 6 mm, 5 μm –
Agilent column. The spectrophotometer for UV detection was set

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at 272 nm. Lower limits of detection were 12.5 ng/mL and 6.25 ng/mL for rifabutin and desacetyl rifabutin respectively. Linearity of standard curves was demonstrated up to 500 ng/mL and 250 ng/mL for rifabutin and desacetyl rifabutin respectively. Variability of day to day quality controls inserted in each analytical run was lower than 9% for median and high concentrations and lower than 15% for low concentrations. The accuracies (as % of nominal value) for rifabutin and 25-O-desacyl rifabutin were between 97% and 106% at low, medium and high QC levels during inter-run validation.

Plasma lopinavir and ritonavir concentrations were quantified by a validated reverse phase HPLC method as described elsewhere [22] with slight modifications. The limit of quantification was 50 ng/mL for lopinavir and ritonavir. Linearity of standard curves was demonstrated up to 10000 ng/mL and 5000 ng/mL for lopinavir and ritonavir respectively. Variability of day to day quality controls inserted in each analytical run was lower than 6% for median and high concentrations and lower than 9% for low concentration. The accuracies (as % of nominal value) for lopinavir and 25-O-desacyl rifabutin were between 98% and 110% at low, medium and high QC levels during inter-run validation. Asqualab quality controls (France) were inserted in each lopinavir and ritonavir analytical runs.

Pharmacokinetic analysis

The main pharmacokinetic measures for rifabutin, 25-O-desacyl rifabutin and lopinavir and ritonavir were derived by non-compartmental analysis using WinNonLin software (Pharsight, USA). The peak concentration (C_{\text{max}}), and time to C_{\text{max}} (T_{\text{max}}) were obtained directly from the concentration-time profiles. Drug concentrations at the end of a dosing interval were reported as C_{\text{min}} and pre-dose concentrations on the day of pharmacokinetic evaluation reported as C_{0}. The steady-state AUC (AUC_{\text{ss}}) during a dosing interval \( t \) 24 hours or 48 hours for rifabutin and 12 hours for lopinavir/ritonavir were calculated for each drug by the linear up/log down trapezoidal method. As an index of exposure during a dosing interval, the average concentration at steady state (Cave) was calculated for rifabutin and its metabolite as Cave = AUC_{\text{ss}}/t where \( t \) is the dosing interval. The metabolite ratio was calculated as the ratio of metabolite to parent drug AUCs.

Analysis and statistics

The steady state pharmacokinetics of rifabutin and 25-O-desacyl rifabutin were determined at each of the three pharmacokinetic evaluations and the pharmacokinetics of lopinavir were determined after the second and third pharmacokinetic evaluations. In order to identify an effect of sequence randomization on the pharmacokinetic measures, a linear mixed effects regression model using baseline dose considered as reference (rifabutin 300 mg daily) as a covariate was applied. As no sequence or day effect was found, the drug groups were pooled and dose levels were compared. Rifabutin parameters for assessing the interaction when combined with LPV/r were C_{\text{max}}, C_{0}, and Cave. These parameters were logarithmically (log) transformed and a linear mixed model fit was used which included treatment, period and sequence as fixed effects and the patient as a random effect. Ninety percent confidence intervals (90% CIs) for the difference in mean log-transformed (log) PK parameters for a particular rifabutin combination therapy (150 mg OD or 150 mg TPW) compared to rifabutin monotherapy (300 mg OD) were calculated. These differences in mean log PK parameters and 90% CIs were back transformed and presented in their original units as geometric means and 90% CIs. The geometric mean ratio presented in Table 1 can be interpreted as a relative change (either fold or percentage) in geometric mean PK parameters for a particular combination therapy compared to rifabutin monotherapy. The rifabutin regimen combined with LPV/r was deemed equivalent to rifabutin alone when the 90% CI for the ratio fell within the

Figure 1. Timeline of the pharmacokinetic trial of rifabutin with antiretroviral treatment in HIV-infected patients with tuberculosis in Vietnam. PK = pharmacokinetic analyses; TPW= three times per week; OD = once per Day; d4T = stavudine; 3TC = lamivudine; LPV/r = lopinavir/ritonavir; TB = tuberculosis; SCC = short course chemotherapy; RH= rifampicin and isoniazid; ART = antiretroviral therapy; EFV = efavirenz

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these patients, and 11 who had smear-negative pulmonary tuberculosis. In 18 of who completed PK analyses, all had a Karnosky score of 90. and the 25 who completed the PK analyses. Of the 25 patients and in one of these patients resistance to rifampicin and isoniazid Rifabutin and 25-O-desacetylrifabutin pharmacokinetics Patient demographic and clinical characteristics

Results

Patient flow chart

Thirty nine patients were assessed for eligibility for the trial, with Figure 2 showing the numbers randomized, allocated to interventions in Arm A and B, followed-up and subsequently having blood measurements for pharmacokinetic analysis. Altogether 33 patients were randomized. One patient in Arm B did not receive the allocated intervention due to early consent withdrawal, leaving 16 to receive the allocated intervention in each arm. In Arm A, four patients discontinued the intervention – one due to consent withdrawal and three due to serious adverse events (one with cryptococcal meningitis and two with hepatitis in the first two months of treatment. In Arm B, three patients discontinued the intervention – one due to impossible venous puncture as a result of being a previous intravenous drug user, one due to severe anaemia and one starting antiretroviral therapy in another setting before the first PK analysis. Thus, 25 patients underwent the three pharmacokinetic visits (12 in Arm A and 13 in Arm B). One patient in Arm B was lost-to-follow-up after the pharmacokinetic analysis, leaving 24 to complete anti-tuberculosis treatment.

Rifabutin and 25-O-desacetylrifabutin pharmacokinetics

Plots of mean concentrations of rifabutin and its desacetyl metabolite against time are shown in Figure 3. Plasma concentrations of 25-O-desacetylrifabutin were always lower than those of rifabutin concentrations at whatever dose of rifabutin used. Concentrations of rifabutin and 25-O-desacetylrifabutin were higher when rifabutin was combined with LPV/r compared with when it was administered alone, and higher concentrations were observed with the 150 mg OD dose compared with the 150 mg TPW dose. Pharmacokinetic parameters of rifabutin and 25-O desacetylrifabutin are compared in Table 3. Morning pre-dose trough (C0) concentrations were higher when rifabutin was administered OD with LPV/r compared with TPW. The peak concentrations (Cmax) and the area under the curve (AUCt) were similar whatever the dosing regimen, although slightly higher levels were observed with rifabutin 150 mg OD. Overall, a large inter-individual variability in pharmacokinetic parameters of rifabutin was observed. Individual metabolic ratios (25-O-desacetylrifabutin/rifabutin) showed a similar pattern with higher ratios observed when rifabutin was combined with LPV/r (medians 0.57 and 0.64 for 150 mg OD and 150 mg TPW respectively compared with when rifabutin was used alone with a median of 0.13) The geometric mean ratios of rifabutin and 25-O-desacetylrifabutin are shown in Table 1. When rifabutin 150 mg OD was combined with lopinavir/ritonavir, Cmax was only slightly lower than when rifabutin was administered alone, and a 2 to 3-fold increase in trough concentrations was observed. With the TPW dosing, a 35% decrease in Cmax was observed although pre-dose concentrations were close to meeting equivalence with rifabutin monotherapy. Assuming that the average concentration at steady state (Cave) represents plasma exposure, the two tested rifabutin dosing regimens combined with lopinavir/ritonavir failed to show bioequivalence. Only rifabutin at 150 mg OD with LPV/r led to a significantly 32% higher rifabutin Cave compared with when it was administered alone. Rifabutin Cave reached after the TPW regimen was lower compared with rifabutin alone. A large increase in 25-O-desacetyl rifabutin concentrations was observed when rifabutin was co-administered with lopinavir/ritonavir. Cave was increased by a factor of two to five with the OD and TPW dosing respectively.

Lopinavir and ritonavir pharmacokinetics

The median trough and peak concentrations (C0 and Cmax) of lopinavir and ritonavir with rifabutin 150 mg OD and 150 mg TPW are shown in Table 4. There was again wide inter-individual variation in individual trough concentrations, which were similar across rifabutin dose regimens. The study design did not allow comparison of lopinavir and ritonavir concentrations when combined with and without rifabutin.

Adverse events

The 33 enrolled patients had a total of 124 adverse events (all grades together). Eighty percent of the adverse events were low grade (grades 1 and 2). Hepatic events with raised levels of liver enzymes were the commonest adverse events with 56 events occurring in 25 patients. Of these, seven were grade 3 or 4. Of these hepatic events, 33 occurred in the first 2 months and 23 after rifabutin was stopped; their average duration was more than 66 days. There was one case of IRIS (immune reconstitution inflammatory syndrome) grade 3 and no uveitis. There were 4 cases of neutropenia but only one that was grade 3 and none that was grade 4. Serious adverse events are shown in Table 5.

Response to treatment

Among the 24 patients who completed anti-tuberculosis treatment with all PK visits scheduled, 22 (92%) had negative cultures for Mycobacterium tuberculosis and 2 had positive cultures (one patient was sputum smear negative but had drug-resistant TB with resistance to isoniazid and rifampicin and one patient was sputum-smear positive for acid-fast bacilli with the culture.
indicating non-tuberculous mycobacteria). For the 24 study patients, the median (IQR) increase in CD4 cells/mm³ was 127 (64–170) – there were two patients who had a decrease from 229 to 188 and 223 to 219 cells/mm³. Plasma HIV-RNA was undetectable (<250 copies/mL) for 19 (79%) of the 24 study completers. Five patients had a detectable HIV-RNA without any resistance mutations at HIV genotyping.

Table 2. Base-line characteristics of HIV-infected tuberculosis patients in Vietnam.

<table>
<thead>
<tr>
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<th>Median (IQR) N = 33 enrolled patients</th>
<th>Median (IQR) N = 25 completing PK studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>32.7 (28.6 – 35.1)</td>
<td>32.7 (27.6 – 35.1)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>28 (85%)</td>
<td>21 (84%)</td>
</tr>
<tr>
<td>Weight in Kg</td>
<td>50.4 (45.5 – 54.50)</td>
<td>49 (44.50 – 53.50)</td>
</tr>
<tr>
<td>BMI (1)</td>
<td>18.6 (17.31 – 20.52)</td>
<td>18.0 (17.26 – 19.92)</td>
</tr>
<tr>
<td>CD4 Count cells/mm³</td>
<td>65 (23 – 135)</td>
<td>65 (26 – 126)</td>
</tr>
<tr>
<td>Plasma HIV-RNA logcopies/mL (2)</td>
<td>5.79 (5.26 – 6.22)</td>
<td>5.87 (5.32 – 6.18)</td>
</tr>
</tbody>
</table>

IQR = inter-quartile; PK = pharmacokinetic analyses; BMI = body mass index;
(1)16/33 or 13/25 patients were underweight (BMI<18.5) and 17/33 or 12/25 were normal (BMI>18.5–25.6)
(2)Measured at the second visit (Day 14) before antiretroviral therapy initiation
N = 30

doi:10.1371/journal.pone.0084866.t002

Discussion

This is one of the first studies to investigate whether doses of rifabutin at 150 mg once daily or 150 mg three times per week are suitable in combination with the tablet formulation of LPV/r in an antiretroviral therapy regimen in the treatment of patients with HIV-associated tuberculosis. The main findings were that peak concentrations (C_{max}) and the area under the curve (AUC_{t}) of the...
drugs were in the same range, regardless of the dose used. There was a significant and almost one third higher average concentration at steady state of rifabutin when used with LPV/r at a dose of 150 mg daily compared with 300 mg alone. The intermittent dosing of rifabutin co-administered with LPV/r led to a lower average concentration compared with 300 mg alone, although pre-dose concentrations remained in the same range. The different doses of rifabutin had no significant effect on the concentrations of lopinavir or ritonavir. Although there were a large number of recorded adverse effects, these were largely low grade and mainly related to an increase in serum liver enzyme levels. Of the 24 patients who completed the pharmacokinetic studies and who completed six months of anti-tuberculosis treatment, over 90% had negative Mycobacterium tuberculosis cultures, all but two patients had a measurable increase in CD4 cell counts and over 70% of patients had undetectable viral loads.

There have been previous studies assessing the pharmacokinetic interaction of rifabutin with ritonavir-boosted HIV protease inhibitors (fosamprenavir, darunavir, atazanavir and saquinavir [23,24,25,26]. All these studies were conducted in healthy volunteers with various rifabutin dosing regimens, 150 mg or 300 mg OD when rifabutin was administered alone and 150 mg once every other day, twice weekly or every 3 days when combined with a protease inhibitor. All these studies showed that when the rifabutin dose was reduced in the presence of a potent drug metabolizing enzyme inhibitor (namely a protease inhibitor) this led to unchanged or moderate increases in rifabutin concentrations and a large increase in rifabutin metabolite concentrations. Interestingly, the steady state concentrations seen with the daily dose of rifabutin in the absence of antiretroviral therapy were in the same range as or somewhat lower than those described in our Vietnamese population [25,26]. There have not been previous published studies assessing these drug-drug interactions when using the tablet formulation of LPV/r (Aluvia), which is now the most widely used protease inhibitor formulation in global HIV programs due to its heat stable properties [22].

A different version of the current study was carried out in South Africa from 2008 to 2010, in which the start of antiretroviral therapy was delayed.

### Table 3. Pharmacokinetic parameters of rifabutin and 25-O desacetyl rifabutin.

<table>
<thead>
<tr>
<th></th>
<th>Rifabutin</th>
<th>25-O desacetyl rifabutin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>with lopinavir/ritonavir</td>
</tr>
<tr>
<td></td>
<td>300 mg OD</td>
<td>150 mg OD</td>
</tr>
<tr>
<td>Cmax ng/mL</td>
<td>792 (344 – 1105)</td>
<td>671 (246 – 1146)</td>
</tr>
<tr>
<td>Tmax h</td>
<td>3 (2 – 4)</td>
<td>3 (2 – 5)</td>
</tr>
<tr>
<td>C0 ng/mL</td>
<td>74 (13 – 161)</td>
<td>180 (121 – 310)</td>
</tr>
<tr>
<td>Cmin ng/mL n = 25</td>
<td>79 (13 – 170)</td>
<td>169 (71 – 320)</td>
</tr>
<tr>
<td>Cmin ng/mL n = 15</td>
<td>61 (13 – 118)</td>
<td>161 (71 – 289)</td>
</tr>
<tr>
<td>AUC Cmax h/mL</td>
<td>5640 (2715–8876)</td>
<td>7292 (3524–12514)</td>
</tr>
<tr>
<td>Cave ng/mL</td>
<td>235 (113–370)</td>
<td>304 (147–521)</td>
</tr>
</tbody>
</table>

Data are presented as medians with the range in parenthesis

OD – once daily; TPW - three times per week; Cmax - peak concentration; Tmax - time to reach peak concentration; C0 - concentration at time 0; Cmin - concentration at the end of a dosing interval (24 h or 48 h); NA non available, Cmin 48 h post dosing non available in 10 patients; AUC Cmax – area under the curve during a dosing interval, t is 24 h for OD dosing and 48 h for TPW. Cave – average concentration (AUC / t).

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doi:10.1371/journal.pone.0084866.g003
therapy was at 10 weeks after the start of anti-tuberculosis treatment when the patient was in the continuation phase on rifabutin and isoniazid [27]. In the South African study, it was found that the peak concentrations of rifabutin were significantly reduced in patients taking rifabutin three times a week, and, furthermore, over 85% of patients on the intermittent dose had areas under the curve less than 4.5 mg.h/mL, levels which have previously been associated with acquired rifamycin resistance. Interestingly, rifabutin concentrations were higher in our Vietnamese population, and only one patient had a Cmax less than 0.3 μg/mL on the 150 mg TPW regimen. The AUCt of rifabutin during the dosing interval were higher than those measured in the South African population (median levels for the 300 mg OD dose were 5640 ng.h/mL in Vietnam compared with 3053 ng.h/mL in South Africa). These differences may be due to ethnic differences or other differences in the two populations – for example, the median body mass index was 18 in Vietnam and 23 in South Africa. In both Vietnam and South Africa, LPV/r led to a significant increase in rifabutin concentrations with the 150 mg OD regimen and a decrease in rifabutin concentrations with the 150 mg TPW regimen. As a consequence of higher rifabutin concentrations in Vietnam, only one patient had an AUCtless than 4.5 μg.h/mL on the 150 mg OD regimen compared with four on the 150 mg TPW and six with the 300 mg OD regimen.

Although the study was not designed to compare lopinavir and ritonavir concentrations on and off anti-tuberculosis treatment, trough lopinavir concentrations were higher than those observed in previous studies [22]. There have been reports for example of increased lopinavir concentrations on rifabutin which have decreased once rifabutin was discontinued [28]. Importantly in our study, the findings showed that lopinavir/ritonavir concentrations were not reduced during rifabutin therapy.

It was initially planned that the same study design run in South Africa would be implemented in Vietnam. However, for various reasons implementation of the Vietnam study was delayed, and by the time patients were being recruited, the WHO had released their 2010 Guidelines for ART, recommending that antiretroviral therapy should start between 2 – 8 weeks after the start of anti-tuberculosis treatment [29]. Investigators in the Vietnam study felt that the Vietnam study protocol starting antiretroviral therapy at 10 weeks was in conflict with recommended international best practice [30]. The trial was stopped and an amended study protocol with patients starting antiretroviral therapy two weeks after start of anti-tuberculosis treatment as presented in this paper was developed and implemented instead.

Although we were only able to study the effect of rifabutin with LPV/r in the intensive phase of anti-tuberculosis treatment, we continued with the cross-over design to ensure that if there was any sequence effect of the different rifabutin doses on pharmacokinetic measures this would be identified. In the event, no sequence or day effect was found, and the drug groups could therefore be pooled and dose levels compared. There is still controversy over whether Cmax or AUCt is the best pharmacodynamic measure for rifamycins in general. Some studies on

Table 4. Lopinavir and ritonavir pharmacokinetic parameters.

<table>
<thead>
<tr>
<th></th>
<th>Lopinavir</th>
<th>Ritonavir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBT OD</td>
<td>RBT TPW</td>
</tr>
<tr>
<td></td>
<td>All patients</td>
<td>RBT OD</td>
</tr>
<tr>
<td></td>
<td>All patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RBT OD</td>
</tr>
<tr>
<td>Cmax – ng/ml</td>
<td>15439 (7540–34490)</td>
<td>18154 (7803–39550)</td>
</tr>
<tr>
<td>C0 – ng/mL</td>
<td>9155 (399–27567)</td>
<td>8014 (50–31171)</td>
</tr>
</tbody>
</table>

Data are presented as medians with the range in parenthesis.

RBT = rifabutin; OD = once daily; TPW = three times per week; Cmax = peak concentration; C0 = trough concentration. All patients: data pooled whatever the RBT dosing 150 mg OD or 150 mg TPW.
doi:10.1371/journal.pone.0084866.t004

Table 5. Serious adverse events in patients who completed all pharmacokinetic assessments (N = 25) and in patients who did not complete the assessments (N = 8).

<table>
<thead>
<tr>
<th>9 serious adverse events seen in 7 patients who completed the study</th>
<th>5 serious adverse events in 5 patients who did not complete the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hernia of an intervertebral disc</td>
<td>• Acute hepatitis followed by death</td>
</tr>
<tr>
<td>• Severe anaemia</td>
<td>• Severe hepatitis and recovered</td>
</tr>
<tr>
<td>• Immune reconstitution inflammatory syndrome</td>
<td>• Polyrthritis</td>
</tr>
<tr>
<td>• Cholestatic hepatitis</td>
<td>• Cryptococcal meningitis</td>
</tr>
<tr>
<td>• MDR-TB causing bilateral lymphadenopathy</td>
<td>• Severe anaemia and respiratory failure followed by death</td>
</tr>
<tr>
<td>• Unidentified abdominal mass</td>
<td></td>
</tr>
<tr>
<td>• Polyarthralgia (2 occurrences)</td>
<td></td>
</tr>
<tr>
<td>• Pneumocystis carinii (jerovici) pneumonia</td>
<td></td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0084866.t005
guinea pigs have found Cmax to be the critical pharmacokinetic parameter [31,32], while other studies on mice and using hollow fibers have found that AUC was a superior parameter [33,34]. For these reasons, both parameters were measured and reported on in this study, showing that rifabutin and 25-O-desacylrifabutin levels were higher using the daily dose with LPV/r than without, and with the average concentration at a steady state being one third higher using 150 mg daily and 40% lower using 150 mg TPW compared with rifabutin alone. A limitation of this study is that we cannot provide answers about the toxicity or efficacy of single dose rifabutin, and a more formal clinical trial is warranted to determine whether daily rifabutin with an increase in rifabutin concentrations is associated with improved efficacy and acceptable adverse effects.

In conclusion, this study supports the use of rifabutin given at a dose of 150 mg once daily when combined with LPV/r based antiretroviral therapy, at least in patients with a low body mass index. It is not possible to generalize the results of this study to other ethnic groups outside of South-East Asia who may differ in their body mass index and in the way in which they metabolize drugs. The WHO Guidelines for the treatment of HIV-associated tuberculosis [10] recommend that treatment is given daily throughout the intensive and continuation phases of antituberculosis treatment. Giving rifabutin as a daily dose is in line with these recommendations. This would facilitate the important programmatic issue of combining rifabutin with other antituberculosis medications as a fixed-dose combination pill to be taken on a daily basis, a necessary measure if the results of this and other research are going to reach patients being managed routinely within general health service care.

Supporting Information

Checklist S1 CONSORT Checklist. (DOC)

Protocol S1 Trial protocol. (PDF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: NTNL D. Lagarde AP CL ADH. Performed the experiments: NTNL NTNT NB HD NJ TL TXL ND. Laureillard LB CC CG DC D. Lagarde. Wrote the paper: NTNL NTNT NB HD NJ TL TXL LB CC CG D. Lagarde AP CL ADH.

References