การศึกษาทางด้านเภสัชจลนศาสตร์ ประสิทธิผล ความปลอดภัย และความทนทานต่อยาโลพินาเวียร์/ยาริโทนาเวียร์ขนาดมาตรฐานร่วมกับยาไรฟาบูตินขนาด 150 มิลลิกรัมต่อวัน (ขนาดมาตรฐาน) หรือขนาด 300 มิลลิกรัม 3 ครั้งต่อสัปดาห์ (ขนาดต่ำ) ในผู้ป่วยติดเชื้อเอชไอวีที่เป็นวัณโรค

นายคริส ฟูจิตนิรันดร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2559

A study of the pharmacokinetics and safety of rifabutin 150 mg once daily versus rifabutin 300 mg thrice weekly with Lopinavir/ritonavir based HAART in HIV/TB co-infected patients

Mr. Chris Fujitnirun

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Medicine

Department of Medicine

Faculty of Medicine

Chulalongkorn University

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Thesis Title	A study of the pharmacokinetics and safety of rifabutin 150 mg once daily versus rifabutin 300 mg thrice weekly with Lopinavir/ritonavir based HAART in HIV/TB co-infected patients
By	Mr. Chris Fujitnirun
Field of Study	Medicine
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คริส ฟูจิตนิรันดร์ : การศึกษาทางด้านเภสัชจลนศาสตร์ ประสิทธิผล ความปลอดภัย และความทนทานต่อยาโลพินาเวียร์/ยาริโทนาเวียร์ขนาดมาตรฐานร่วมกับยาไรฟาบูตินขนาด 150 มิลลิกรัมต่อวัน (ขนาดมาตรฐาน) หรือขนาด 300 มิลลิกรัม 3 ครั้งต่อสัปดาห์ (ขนาดต่ำ) ในผู้ป่วยติดเชื้อเอชไอวีที่เป็นวัณโรค (A study of the pharmacokinetics and safety of rifabutin 150 mg once daily versus rifabutin 300 mg thrice weekly with Lopinavir/ritonavir based HAART in HIV/TB co-infected patients) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. นพ.โอภาส พุทธเจริญ, 52 หน้า.

บทนำ: ยาไรแฟมปิซินสามารถกระตุ้นเอนไซม์ไซโตโครม P450 ได้ซึ่งทำให้ระดับยาโลพินาเวียร์ลดลงอย่างมากเมื่อให้ยาคู่กันจึงมีคำแนะนำให้ใช้ยาไรฟาบูตินแทนไรแฟมปิซินเมื่อต้องใช้คู่กับยาโลพินาเวียร์ อย่างไรก็ตามขนาดยาไรฟาบูตินที่แนะนำอาจไม่เพียงพอ จุดประสงค์หลักของการศึกษานี้คือเพื่อเปรียบเทียบค่า เภสัชจลนศาสตร์ของยาไรฟาบูตินขนาด 150 มก. วันละครั้งกับ 300 มก. สามครั้งต่อสัปดาห์ เมื่อให้คู่กับยาโลพินาเวียร์/ริโทนาเวียร์ขนาด 400/100 มก. วันละสองครั้ง ในผู้ป่วยติดเชื้อ HIV ร่วมกับวัณโรค

วิธีการวิจัย: การศึกษานี้เป็นการศึกษาแบบสุ่มซึ่งไม่ปกปิดทั้งผู้วิจัยและผู้ร่วมการศึกษาโดยแบ่งผู้ป่วยเป็น 2 กลุ่ม เป็นการศึกษาทางเภสัชจลนศาสตร์ และประสิทธิภาพของยาที่ 48 สัปดาห์หลังการรักษา ในผู้ป่วยชาวไทยที่ติดเชื้อ HIV ร่วมกับวัณโรค การศึกษานี้จะวัดค่าเภสัชจลนศาสตร์ของยาไรฟาบูตินก่อนและหลังให้ยาไรฟาบูตินร่วมกับยาโลพินาเวียร์/ริโทนาเวียร์ ระหว่าง 2 ถึง 8 สัปดาห์ โดยวิธี high performance liquid chromatography (HPLC)

ผลการศึกษา: ผู้ร่วมการศึกษาทั้งหมด 21 รายโดย 10 รายได้รับยาไรฟาบูตินขนาด 150 มก. วันละครั้งและ 11 รายได้รับยาไรฟาบูตินขนาด 300 มก. สามครั้งต่อสัปดาห์ ค่าชีวปริมาณออกฤทธิ์ (AUC) ของยาไรฟาบูติน ขนาด 150 มก. วันละครั้งร่วมกับยาโลพินาเวียร์/ริโทนาเวียร์สูงกว่าเมื่อให้ยาไรฟาบูตินตัวเดียวร้อยละ 41.9 แต่ผู้ป่วยที่ได้ยาไรฟาบูตินขนาด 300 มก. สามครั้งต่อสัปดาห์ร่วมกับยาโลพินาเวียร์/ริโทนาเวียร์นั้นมี AUC สูงกว่าเมื่อให้ยาไรฟาบูตินตัวเดียวร้อยละ 145.2 หลังจากให้ยาไรฟาบูตินร่วมกับยาโลพินาเวียร์/ริโทนาเวียร์นาน 2-8 สัปดาห์พบว่าค่าเฉลี่ยเลขคณิต (สัมประสิทธิ์ความผันแปร) ของค่าความเข้มข้นสูงสุด (Cmax) ของยาไรฟาบูตินทั้งสองขนาดมีค่าใกล้เคียงกัน [0.65 (36%) เทียบกับ 0.82 (30%) mg/L] ค่าเฉลี่ยเลขคณิต (สัมประสิทธิ์ความผันแปร) ของค่าชีวปริมาณออกฤทธิ์ (AUC) ของยาไรฟาบูตินขนาด 300 มก. สามครั้งต่อสัปดาห์สูงกว่าขนาด 150 มก. วันละครั้งถึงร้อยละ 72.7 [15.5 (43%) เทียบกับ 8.97 (37%) mg.h/L] ค่าทางเภสัชจลนศาสตร์ของยาโลพินาเวียร์/ริโทนาเวียร์นั้นอยู่ในระดับรักษา (therapeutic level) โดยระดับยาก่อนให้ยาครั้งถัดไป [trough concentration (C0)], ระดับยาสูงสุด [peak concentrations (Cmax)], ระดับยาต่ำสุด [minimum concentrations (Cmin)] และระดับยาเฉลี่ย [average concentrations (Cave)] ของผู้ป่วยทั้งสองกลุ่มนั้นใกล้เคียงกัน ค่าเฉลี่ยของ C0 ของยาโลพินาเวียร์ในผู้ป่วยที่ได้ยาไรฟาบูติน 150 มก. วันละครั้งและ 300 มก.สามครั้งต่อสัปดาห์เป็น 8.709 เทียบกับ 10.473 µg/mL, ค่าเฉลี่ย Cmax เป็น 13.455 เทียบกับ 14.027µg/mL, ค่าเฉลี่ย Cmin เป็น 5.287 เทียบกับ 4.155 µg/mL และค่าเฉลี่ย Cave เป็น 9.695 เทียบกับ10.252 µg/mL พบการอักเสบของยูเวีย (Uveitis) ในผู้ป่วย 2 รายโดยทั้งคู่ได้รับไรฟาบูติน 300 มก. สามครั้งต่อสัปดาห์

สรุปผล: การศึกษานี้เสนอว่ายาไรฟาบูตินขนาด 150 มก. วันละครั้งควรเป็นขนาดที่แนะนำให้ใช้ในผู้ป่วยชาวไทยเมื่อต้องใช้ยาชนิดนี้ร่วมกับยาโลพินาเวียร์/ริโทนาเวียร์ นอกจากนี้ยังพบว่ายาโลพินาเวียร์/ริโทนาเวียร์ขนาด400/100 มก. วันละสองครั้งสามารถให้ระดับยาในเลือดที่เพียงพอเมื่อให้ร่วมกับยาไรฟาบูตินทั้งสองขนาด

คำสำคัญ: ไรฟาบูติน, โลพินาเวียร์/ริโทนาเวียร์, เภสัชจลนศาสตร์

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559

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KEYWORDS: RIFABUTIN / LOPINAVIR / RITONAVIR / PHARMACOKINETICS

CHRIS FUJITNIRUN: A study of the pharmacokinetics and safety of rifabutin 150 mg once daily versus rifabutin 300 mg thrice weekly with Lopinavir/ritonavir based HAART in HIV/TB co-infected patients. ADVISOR: ASST. PROF. OPASS PUTCHAREON, M.D., 52 pp.

Introduction: Rifampicin is a potent cytochrome P450 inducer that can markedly reduce serum lopinavir level. Rifabutin based anti TB is an alternative when boosted protease inhibitors are in need, however recommended doses of rifabutin may be subtherapeutic. The primary aim of this study was to compare pharmacokinetics parameters of rifabutin 150 mg once daily, versus rifabutin 300 mg thrice weekly in combination with LPV/r 400/100 mg based HAART in HIV/TB infected patients in Thailand.

Method: This was a randomized, open-label, 2- arm, intensive pharmacokinetic study, as well as a 48 week efficacy study, conducted in Thai HIV and TB coinfected patients. Rifabutin pharmacokinetics were evaluated before and between week 2 to week 8 after coadministration of lopinavir/ritonavir. We used a high performance liquid chromatography (HPLC) technique to determine rifabutin and lopinavir/ritonavir concentrations.

Results: Twenty one patients were enrolled in the study. Ten patients were randomized to rifabutin 150 mg daily and eleven patients received rifabutin 300 mg thrice weekly. AUC of rifabutin 150 mg once daily combined with lopinavir/ritonavir is moderately higher than rifabutin alone for 41.9%. By contrast, AUC in patients with rifabutin 300 mg thrice weekly combined with lopinavir/ritonavir is markedly higher than rifabutin alone for 145.2%. After week 2 to week 8 of rifabutin and lopinavir/ritonavir concurrent administration, pharmacokinetic parameters of rifabutin included peak concentrations (Cmax), and area under the curve (AUC) were studied. Geometric mean Cmax (CV) of rifabutin 150 mg daily and 300 mg thrice weekly were similar [0.65 (36%) vs. 0.82 (30%) mg/L]. Geometric mean AUC (CV) of rifabutin 300 mg thrice weekly was higher than 150 mg daily for 72.7% [15.5 (43%) vs 8.97 (37%) mg.h/L]. Pharmacokinetic parameters of lopinavir/ritonavir are in therapeutic level [trough concentration (C0), peak concentrations (Cmax), minimum concentrations (Cmin) and average concentrations (Cave)] and were similar in both arms [mean C0 of rifabutin 150 mg daily and of rifabutin 300 mg three times weekly were 8.709 vs. 10.473 µg/mL, mean Cmax was 13.455 vs. 14.027µg/mL, mean Cmin was 5.287 vs. 4.155 µg/mL and mean Cave was 9.695 vs.10.252]. Uveitis which isassociated to rifabutin developed in two patients who received rifabutin 300 mg three times weekly.

Conclusion: Our study suggests that rifabutin 150 mg daily should be recommended in Thai patient who concurrently use lopinavir/ritonavir because of optimal pharmacokinetic parameters and clinical safety. Moreover, this study shows that lopinavir/ritonavir 400/100 mg BID can give adequate lopinavir levels in HIV and TB coinfected patients who were treated with rifabutin both 150 mg daily and 300 mg thrice weekly.

Rifabutin, lopinavir/ritonavir, pharmacokinetics

Department: Medicine
Field of Study: Medicine
Academic Year: 2016

Student's Signature .....

Advisor's Signature .....

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Chapter 1

INTRODUCTION

Background

Fixed dose combination of stavudine, lamivudine and nevirapine has been widely used in Thai HIV infected since June 2002. The prevalence of NNRTI resistant HIV has increased since 2005. Tuberculosis can develop following NNRTI-based regimen failure or after introduction of a new salvage regimen with a boosted PI (immune recovery syndrome). Although, efavirenz based HAART is preferred in TB/HIV with rifampicin containing antituberculosis, efavirenz could not be used in case of NNRTI failure, intolerance or toxicity. Lopinavir/ritonavir (LPV/r) is widely used for NNRTI failure in most resource limited settings. Rifampicin, most important drug of effective antituberculosis therapy, is a potent induction of hepatic cytochrome P450 3A (CYP3A) and the drug transporter P-glycoprotein resulting markedly lowers plasma concentrations of most HIV protease inhibitors. Thus, this drug is contraindicated with most PIs. Rifabutin is recommended to replace rifampicin when use concomitant with boosted PI (4, 5). However LPV/r can increase plasma level of rifabutin and cause toxicity (bone marrow suppression, arthalgia, uveitis). Thus current guidelines recommend that rifabutin needs dose adjustment when use with boosted PI (decrease form 300 mg once daily to 150 mg once daily or 300 mg three times a week). This recommendation based on limited data from other countries. Prior to implementation of rifabutin to the TB/HIV program of Thailand, it is extremely important to determine the appropriate dosing regimens of rifabutin concomitant used with LPV/r in HIV/TB co-infected Thai patients.

Purpose and Benefit

This study was undertaken with the main purpose to compare pharmacokinetics parameters of rifabutin 150 mg once daily versus rifabutin 300 mg thrice weekly in combination with an LPV/r 400/100 mg based HAART in HIV/TB infected patients in

Thailand. The others purposes are to assess the safety of the both doses and describe the pharmacokinetics of LPV/r 400/100 mg BID when concomitant use with rifabutin 150 mg once daily or rifabutin 300 mg thrice weekly and to assess the TB and HIV outcomes when using rifabutin with an LPV/r based HAART

Research Questions

A. Primary research question: Are there the difference of pharmacokinetics parameters between rifabutin 150 mg once daily and rifabutin 300 mg thrice weekly in combination with LPV/r 400/100mg based HAART in HIV/TB infected patients.
   - The main pharmacokinetic parameters are both Cmax and AUC which were reported that are correlated with rifabutin efficacy (1-3).

B. Secondary research questions: Are there the difference of safety, pharmacokinetics of LPV/r, TB and HIV outcomes between rifabutin 150 mg once daily and rifabutin 300 mg thrice weekly in combination with LPV/r 400/100mg based HAART in HIV/TB infected patients

Conceptual Framework

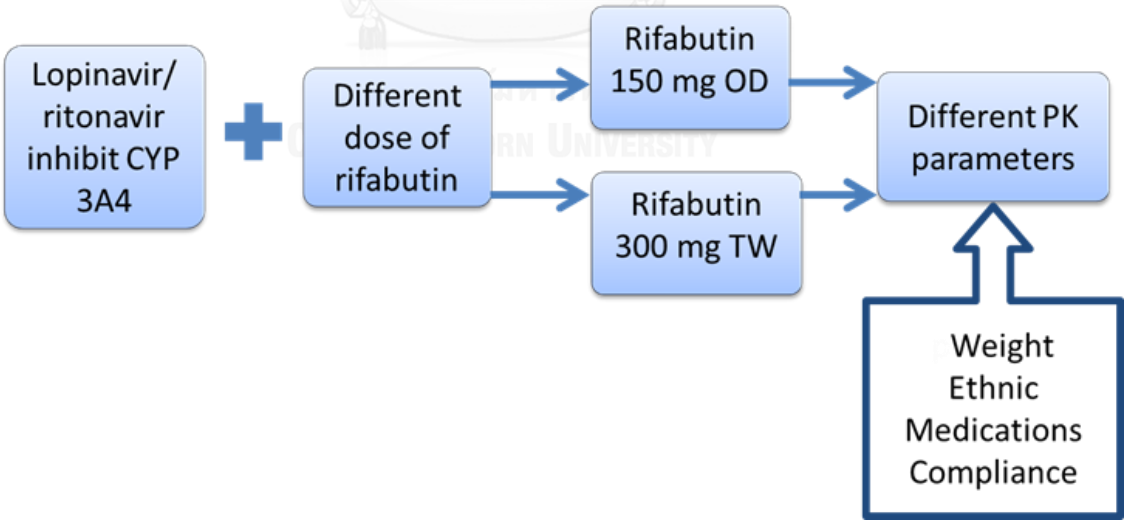

Figure 1. conceptual frame work. Difference in weight, ethic, other medications and compliance can cause difference in pharmacokinetic parameters from previous studies (4-6)

Operational Definition

Diagnosis of HIV infection

It was made by positive of fourth generation enzyme-linked immunosorbent assay (ELISA) for anti HIV test.

Diagnosis of tuberculosis

The diagnostic criteria for TB in this study is: At least one acid fast staining smear positive, positive TB culture or positive nucleic amplification test for TB in specimen, with a typical syndrome and/or a chest radiography consistent with TB.

TB outcome (cure, relapse, or treatment failure : resist ant TB)

1. Cure refers to smear negative at the end of TB treatment

2. Relapse refers to the circumstance in which a patient becomes and remains culture negative while receiving therapy but, at some point after completion of therapy, either becomes culture positive again or has clinical or radiographic deterioration that is consistent with active tuberculosis.

3. Treatment failure is defined as continued or recurrently positive cultures during the course of antituberculosis therapy. Patients whose sputum cultures remain positive after 4 months of treatment should be deemed treatment failures.

Area under the curve (AUC)

Area under the curve is the area under drug concentration-time curve that represent total drug exposure over time.

Cmax is maximum concentration of drugs

Cmin is minimum concentration of drugs

Research Design

This study is a randomized, open-label, 2- arm, intensive pharmacokinetic study.

Study design

After the HIV/TB coinfected patients were enrolled to the study they will receive rifabutin 300 mg once daily for at least 2 weeks prior baseline visit.

At baseline visit (Day 0), patients will be randomized to receive rifabutin 150 mg once daily or rifabutin 300 mg three times a week. All patients will get LPV/r 400/100 mg twice daily and at least 2 NRTIs. The timing of the initiation of the cART will depend on the clinical judgement of the treating physician. Pharmacokinetic of rifabutin will be performed at baseline. Pharmacokinetics of rifabutin at baseline should be skipped if the patients have taken antiretroviral drugs prior enrollment to this visit.The randomized dose of rifabutin and all ARV will be started at the next following day (Day1), exceptional for patients who could not perform PK of rifabutin at baseline, they can start randomized dose of rifabutin at baseline visit.

The intensive pharmacokinetic (PK) study of LPV/r and rifabutin will be performed again after at least 2 weeks have elapsed since the patient has been receiving assigned rifabutin anti-TB therapy in combination with LPV/r + NRTIs twice daily (between week 2 to week 8).

Chapter 2

LITERATURE REVIEW

Coinfection with tuberculosis and human immunodeficiency virus (HIV) is common in Thailand (7). Despite the availability of effective therapy for both diseases, simultaneous treatment is one of the most complex scenarios because of drug interactions, high pill burden, paradoxical reactions and overlapping toxicities (8, 9). Rifampicin (RIF) is a cornerstone of effective antituberculosis therapy but it is a potent inducer of hepatic cytochrome P450 3A (CYP3A) and the drug transporter P-glycoprotein (10), resulting in markedly lower plasma concentrations of most HIV protease inhibitors by more than 80% (11, 12). Thus, this drug is contraindicated with most PIs (13).

Rifabutin induces CYP3A activity less than rifampicin and may therefore be a better choice for coadministration with antiretrovirals including PI. The guidelines recommend a rifabutin based anti TB when boosted PI is in need (13). However, rifabutin has more toxicity (bone marrow suppression, arthalgia, uveitis) and under dosing of rifabutin can result in selection rifamycin resistant *M. tuberculosis*, therefore, dose adjustment of ART regimens and rifabutin is needed when used together.

Lopinavir/ritonavir (LPV/r) is widely used for NNRTI failure in most resource limited settings. LPV/r is inhibitors of CYP3A4, thus significantly reducing the clearance of rifabutin (6) resulting in a higher dose of rifabutin (Rifabutin AUC increased by 303%; 25-O-des-acetyl rifabutin AUC increased by 47.5 fold in healthy volunteer). This interaction can result in increased risk of rifabutin toxicity (14).

Current guidelines (13) suggest that when using LPV/r containing HAART, the dose of rifabutin should be reduced from 300 mg daily to 300 mg every other day or three thrice weekly or 150 mg daily and therapeutic drug monitoring should be

considered to ensure that rifabutin Cmax is in the range of 0.45-0.9 mg/L which is the adequate level from previous studies (15)

Some studies investigate a lower dose of rifabutin (150 mg thrice weekly). Khachi et al (16) reported that a rifabutin 150 mg thrice weekly dose resulted in subtherapeutic rifabutin concentration in HIV/TB coinfected patients who were treated with LPV/r containing HAART. Furthermore, Khachi et al also found that 2 cases with low rifabutin concentrations had clinical deterioration of tuberculosis. Jenny-Avital ER et al (17) also reported that 3 cases of active HIV/TB acquired rifampin resistance following alternate-day rifabutin and boosted PI therapy. The pharmacokinetic study of Boulanger C et al (18) on higher dose of rifabutin 300 mg thrice weekly showed that increasing of rifabutin from 150 mg thrice weekly to 300 mg thrice weekly can increase both Cmax and AUC for 60 and 55% respectively. This study also showed that rifabutin 300 mg thrice weekly can give acceptable rifabutin Cmax. However population of this study is only 10 patients and 8 of them are African American, and 2 were white.

Study of Lan NT, et al (5) that was conducted in Vietnam in 2014 investigated pharmacokinetic parameters of rifabutin 150 mg thrice weekly and 150 mg daily. This study found that concurrent use of rifabutin 150 mg daily with lopinavir/ritonavir was associated with increase in mean rifabutin concentration for 32% compared with rifabutin 300 mg alone. The mean rifabutin Cmax is adequate (0.67 mg/L) with dose of 150 mg daily. In contrast, the average rifabutin level decreased by 44% when rifabutin 150 mg thrice weekly was used with lopinavir/ritonavir. These findings imply that rifabutin 150 mg daily is an adequate dose.

However study of Naiker S, et al (4) that was conducted in South Africa in 2009 to 2014 showed different results. That study investigated pharmacokinetic parameters of rifabutin 150 mg thrice weekly and 150 mg daily. The pharmacokinetic analysis showed that rifabutin 150 mg daily give quite low median Cmax of 0.31 mg/L (IQR = 0.26-0.38 mg/L) which is lower than adequate therapeutic level. Moreover with this dose 29% of patients have too low rifabutin AUC (< 4.5 mg.h/L) which increase risk of emergence of resistance to rifabutin (4).

In summary, the results from these trials are different and indicate that adequate dose of rifabutin concurrent use with lopinavir/ritonavir is still controversial. Moreover data of rifabutin 300 mg thrice weekly are limited and there is no data of rifabutin pharmacokinetics when it is concurrent used with lopinavir/ritonavir in Thai patients.

Clinical Pharmacology of Rifabutin (19)

The chemical name for rifabutin is 1',4-didehydro-1-deoxy-1,4-dihydro-5'-(2-methylpropyl)-1-oxorifamycin XIV. Molecular formula of rifabutin is $C_{46}H_{62}N_4O_{11}$ with a molecular weight of 847.02.

Absorption

With a single oral dose of 300 mg to 9 healthy adult volunteers, rifabutin was readily absorbed from the alimentary tract and give mean (±SD) peak plasma levels (Cmax) of 375 (±267) ng/mL (with the range of 141 to 1033 ng/mL) attained in 3.3 (±0.9) hours (Tmax is in the range of 2 to 4 hours).

The bioavailability of capsule rifabutin, relative to an oral solution, was 85% in 12 healthy adult volunteers. High-fat meals can slow the rate of absorption but cannot influence the extent of total absorption. The post-Cmax plasma concentrations of rifabutin declined in biphasic manner. Pharmacokinetic dose-proportionality was in the range of 300 to 600 mg (based on pharmacokinetic study in nine healthy adult volunteers) and in the range of 300 to 900 mg (based on study in human immunodeficiency virus (HIV)-positive patients)

Distribution

Rifabutin is a lipophilic drug and demonstrates a high propensity of distribution and intracellular uptake. With intravenous dosing, at the steady-state distribution volume is estimated to be 9.3 ± 1.5 L/kg in 5 HIV-positive patients. Markedly higher intracellular levels than in plasma have been found in both rat and man. With oral dose, the lung-to-plasma concentration ratio (at 12 hours) was approximately 6.5 in four surgical patients. At the steady-state mean rifabutin trough levels ranged from 50 to 65 ng/mL in HIV infected patients and in healthy adult volunteers. About 85% of rifabutin is bound to plasma proteins over a concentration range of 0.05 to 1 μg/mL. Renal or hepatic

dysfunction does not influence protein binding. A mean terminal half-life of rifabutin is 45 (±17) hours (range: 16 to 69 hours) so rifabutin was slowly eliminated from plasma. Although the levels of rifabutin after multiple doses decrease by 38%, its terminal half-life unchanges.

Metabolism

The most predominant metabolites of rifabutin that have been identified included 25-O-desacetyl and 31-hydroxy rifabutin and show an area under the curve ratio of 0.10 and 0.07, respectively. The 25-O-desacetyl rifabutin has an activity equal to rifabutin and contributes up to 10% of total antimicrobial activity.

Excretion

A study with ^{14}C-labeled rifabutin showed that oral dose of rifabutin was excreted in urine for 53% and 30% of the dose was excreted in feces. Mean systemic clearance (CLs/F) after a single oral dose of rifabutin was 0.69 (±0.32) L/hr/kg with the range of 0.46 to 1.34 L/hr/kg. Each biliary and renal clearance of unchanged drug contribute approximately 5% of systemic clearance.

CHAPTER 3

RESEARCH METHODOLOGY

Study Ethics Approval

The study protocol and all relevant documents were submitted to the local ethical committees for each site.

Informed Consent

All patients will be asked to give written informed consent prior to enrolment in the study.

Study Site

This study was carried out in 2 sites which were King Chulalongkorn Memorial Hospital/The HIV Netherlands Australia Thailand Research Collaboration site, Bangkok, Thailand and Bamrasnaradura Infectious Diseases Institute, Nontaburi, Thailand

Study population and sample size

In this study, Thai ART-naïve or treatment failure (PI naïve) HIV/TB co-infected patients receiving (or planned to receive) rifampicin containing anti-TB therapy. This study is a pilot study. Generally there is no sample size calculation in pharmacokinetic study. The estimated sample size of 12 patients per arm are enough to show statistical significant at 80% power when compare the result to historical study in Thai population and previous pharmacokinetic study of rifabutin (5).

Patients were recruited to this study between January 2016 and March 2017

Randomization

On the day of enrolment, patients were randomized to receive one of two treatment arms. Randomization lists were produced prior to the start of the study with a 1:1 ratio by mixed size blocks.

Study Inclusion and Exclusion Criteria

Thai active HIV/TB co-infected patients receiving rifampicin / rifabutin containing anti-TB therapy.

Inclusion criteria

1. Confirmed HIV positive after voluntary counseling and testing
2. Aged ≥18-65 years of age
3. ARV-naïve or PI-naïve (NNRTI intolerance/failure) or PI experience (TB developed during on salvage regimen) without prior PI mutation (Viral load less than 50 copies/mm^3 while they have taken PI regimen)
4. Any CD4 cell count
5. Alanine aminotransferase (ALT) <5 times upper limit of normal (ULN)
6. Serum creatinine <1.4 mg/dl
7. Hemaglobin >7 mg/L
8. TB is diagnosed and planned to receive stable doses of rifampicin or rifabutin containing anti-TB therapy for at least another 4 week period after initiation of ART
9. No other active OI (CDC class C event), except oral candidiasis or disseminated MAC
10. Body weight >40kg
11. Able to provide written informed consent

Exclusion criteria

1. Current use of steroid (except short course steroid for IRIS) and other immunosuppressive agents.
2. Current use of any prohibited medications related to drug pharmacokinetics.
3. Patients with current alcohol or illicit substance use that in the opinion of the site Principal Investigator would conflict with any aspect of the conduct of the trial.
4. Unlikely to be able to remain in follow-up for the protocol defined period.
5. Patients with proven or suspected acute hepatitis. Patients with chronic viral hepatitis are eligible provided ALT, aspartate aminotransferase (AST) < 5 x ULN.
6. Karnofsky performance score <30%
7. TB meningitis and bone/joints (due to longer period of anti TB drug)
8. Pregnancy
9. Patient chooses to use efavirenz, not LPV/r. However, in ART naïve, EFV is allowed after intensive PK of LPV/r and rifabutin at week2-8.

Antiretroviral study regimen

- Lopinavir/ritonavir 400/100 mg BID (LPV/r 200/50 2 tablet every 12 hours) will be used.
- The NRTI is based on physician direction ie. TDF+3TC or AZT +3TC (FTC) for ARV naive or TDF+3TC (FTC) or AZT +TDF in resistance patients
- 3TC 150 mg oral every 12 hour or 300 mg once a day
- AZT 200 mg (or 300 if BW > 60 kg) oral every 12 hour
- TDF 300 mg once daily or 300 mg alternate day if GFR < 60 cc/min
- FTC 200 mg once daily

Administration of anti TB drugs

Rifabutin in this study is donated from Lupin Ltd. B/4 Laxmi Towers, Bundra Kurla Complex, Bandra (E), Mumbai400 051, India

All TB drug will be followed standard short course regimen and upon physician's decision. TB regimen will consist of isoniazid (INH) 300 mg (3 tablets) once daily, rifampicin (RIF) 450-600 mg (1 capsule) once daily, ethambutol (EMB) 800 mg (2 tablets) once daily and pyrazinamide (PZA) 1500-2000 mg (3-4 tablets) once daily for 2 months and then isoniazid (INH) 300 mg once daily plus rifampicin 450-600 mg once daily for another 4-7 months. Totally 9 months course would be preferred.

All TB drugs will be administrated orally 30 minutes before or after lunch. After screening period, rifampicin will be switched to rifabutin 300 mg (+ others TB drug such as INH, EMB, and PZA if patient get TB drug for less than 2 months or INH if patient get TB drug for >2 months) 2 weeks. Rifabutin plasma levels will be checked at baseline (T=0, 1,2, 4, 6, 8, 12 and 24). Baseline visit and the beginning of rifabutin intake will be at least 2 weeks apart. At Baseline visit, patients will be randomized to receive rifabutin 150 mg (1 capsule) once daily or 300 mg 3 times a week (such as 2 capsules on Monday, Wednesday, and Friday) with LPV/r 400/100 mg 2 times a day and 2 NRTIs.

Study Procedure

HIV-infected patients diagnosed with TB infection and already receiving or deemed to require therapy with rifampicin-containing anti-TB therapy will be asked to give their informed consent to participate in the study. The standard process of informed consent will be followed. At identification patients may already have commenced rifampicin-containing anti-TB therapy, or be planning to commence.

In any event, if patients are deemed eligible for study (i.e. confirm to all study inclusion/exclusion criteria) then they will receive rifabutin 300 mg once daily for at least 2 weeks prior baseline visit.

At baseline visit (Day0), patients will be randomized to receive rifabutin 150 mg once daily or rifabutin 300 mg three times a week. All patients will get LPV/r 400/100 mg twice daily and at least 2 NRTIs that conform to current Thai guidelines. The timing of the initiation of the cART will depend on the clinical judgement of the treating physician, and may be at any time during the course of anti-TB therapy. However, if cART is not initiated until after anti-TB therapy is completed, the patient will not be eligible for study. Pharmacokinetic of rifabutin at T=0, 1, 2, 4, 6, 8, 10,12 and 24 hour will be performed at baseline. If the patients have taken antiretroviral drugs prior enrollment to this visit, pharmacokinetics of rifabutin at baseline should be skipped. The randomized dose of rifabutin and all ARV will be started at the next following day (Day1), exceptional for patients who could not perform PK of rifabutin at baseline, they can start randomized dose of rifabutin at baseline visit.

The intensive pharmacokinetic (PK) study of LPV/r and rifabutin will be performed again after at least 2 weeks have elapsed since the patient has been receiving assigned rifabutin anti-TB therapy in combination with LPV/r + NRTIs twice daily (between week 2 to week 8).

On the day of the intensive pharmacokinetic study (between week 2-week4) antiretroviral drugs and TB drugs will be ingested at the time of arrival at the research facility in the morning. At the start of the PK study the patients will ingest LPV/r 400/100mg (2 tablets) twice daily with rifabutin 150 mg (1 capsule) once daily or rifabutin 150 mg (2 capsules) 3 times a week plus other anti TB drugs that patient will be taken at that period. The PK of rifabutin 150 mg (2 capsules) 3 times a week i.e. Monday, Wednesday, or Friday and then PK will be performed on Monday, Wednesday, or Friday. The time point will be at T=0, 1, 2, 4, 6, 8, 10, and 12 hours post dose (and additional 24 hours post dose or 48 hours for patient who received rifabutin 300 mg thrice weekly). Standardized breakfast will be offered. Other prescription medication(s) will be ingested during the course of the day according to the patient's usual routine. All medications taken on the study day will be recorded. All regular study medications taken by the patient on a regular basis for the past month will also be recorded.

At designated time intervals associated with the ingestion of LPV/r, blood samples will be drawn. A volume of 5 ml of venous blood will be drawn just before ingestion of the study drugs (Time 0) and then at 1, 2, 4, 6, 8, 10, 12 hours post drug ingestion. Blood samples will be collected in heparinized tubes and plasma will be isolated within 6 hours after centrifugation at 4000 g for 10 minutes. Samples will be appropriately stored at HIV-NAT laboratory until analysis. Timeline of the study was showed in figure 2.

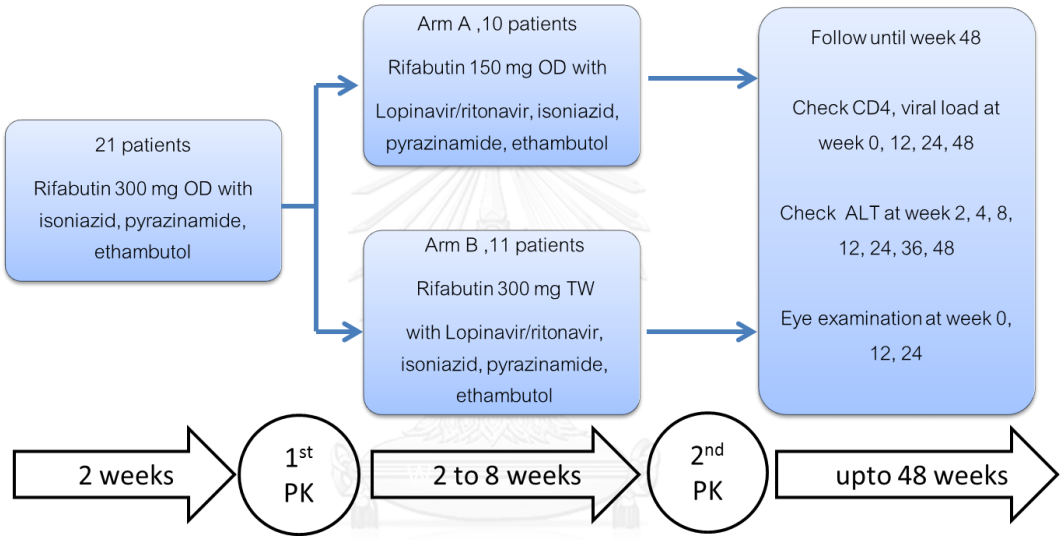

Figure 2. Timeline of the study; PK: pharmacokinetic, OD: once daily, TW: thrice weekly

Pharmacokinetic of rifabutin

All patients enrolled will be treated daily with INH (5mg/kg) and rifabutin (150 mg once daily or 300 mg three times a week) (plus Ethambutol and pyrazinamide during first 2 moths of TB course). The timing for administrating anti-tuberculosis drugs will be fixed in the morning, together with cART. Before and after administration of these drugs, the blood specimens will be collected at the time of 0, 1, 2, 4, 6, 8, 10, 12 and 24 hours post dose for baseline visit (prior taking LPV/r). Pharmacokinetic of rifabutin will be performed again at time of LPV/r pharmacokinetic study (T 0, 1, 2, 4, 6, 8, 10, 12 and 24 hours post dose or 48 hours post dose for Rifabutin 300 mg thrice weekly). Rifabutin will

be measured in patient's plasma employing high performance liquid chromatography (HPLC). Two millilitres of plasma sample will be required for testing. The separated plasma samples should be kept in shield at –20˚C until experiment.

Again, all TB drug will be followed standard short course regimen (6-9 months, 9 months course would be preferred)

Eyes Examination

Eyes examination will be performed while the patients are receiving rifabutin at baseline, week12, 24(optional if patient still used rifabutin) and at time of event.
This examination will focus on whether patient has rifabutin related uveitis.

Blood Sample Volume on the day of pharmacokinetic study of rifabutin at baseline

Pharmacokinetic sample of rifabutin: at baseline 9 samples x 2 mL per sample = 18 mL

Liver function tests: 1 sample x 5 mL = 5 mL.

HIV RNA: 1 sample x 7 mL = 7 mL

CD4/CBC 1 sample X 5 mL = 5 mL

Lipid profile, fasting blood sugar, creatinine 1 sample x 7 mL=7mL

Peripheral blood mononuclear cell (PBMC) 1 sample x 16 mL = 16 mL

Total volume of blood drawn on pharmacokinetic study day = 58ml

Blood Sample Volume on the day of pharmacokinetic study (LPV/r and rifabutin)

Pharmacokinetic sample of LPV/r: 8 samples x 5 mL per sample = 40 mL

Pharmacokinetic sample of rifabutin: 9 samples x 2 mL per sample = 18 mL

For patient who received rifabutin 300 mg thrice weekly; Pharmacokinetic sample of rifabutin: 10 samples x 2 mL per sample = 20 mL

Liver function tests, creatinine: 1 sample x 5 mL = 5 mL

HIV RNA: 1 sample x 7mL = 7 mL

Total volume of blood drawn on pharmacokinetic study day = 70 mL and 72 mL for rifabutin 300 mg thrice weekly arm

Bioanalysis of LPV RTV and rifabutin

Plasma concentrations of lopinavir and ritonavir, rifabutin and 25-0-desacetyl-rifabutin (dAc-rifabutin) are measured using sensitive and validated reversed phase high-performance liquid chromatographic assays. Plasma concentrations of lopinavir and ritonavir are analyzed at the HIV-NAT/Chula PK-laboratory facility. Rifabutin and 25-0-desacetyl-rifabutin concentrations are analyzed at Department of Pharmacy and Radboud Institute for Health Sciences (RIHS), Radboud university medical center, Nijmegen, Netherlands.

Pharmacokinetic analysis

Plasma concentration (C) versus time (t) data of LPV/r and rifabutin were analysed by non-compartmental methods. The highest observed concentration was defined as Cmax, with the corresponding sampling time as tmax. The terminal log-linear period (log C versus t) was defined by visual inspection ($n \geq 4$). The absolute value of the slope (β/ln10) was calculated using least-squares analysis. The elimination half-life was calculated using the equation $t1/2 = \ln 2/\beta$. The concentration 12-hours post drug ingestion was defined as Cmin. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal rule from 0 to 12 h (AUC[0-12h]). The apparent clearance (Cl/F) was calculated by dividing the dose by the AUC[0-12h]. The apparent volume of distribution (V/F) was calculated by dividing Cl/F by β.

Statistical Analysis

Descriptive statistics for the lopinavir/ritonavir and rifabutin pharmacokinetic parameters were made as geometric mean (coefficient of variation, CV), mean (standard

deviation, SD), median (interquartile range, IQR) and percentage. The data was analysed by Statistical Package for Social Sciences (SPSS) Version 22 for Windows.

CHAPTER 4

RESULTS

Baseline characteristics of HIV and TB coinfected patients in this study

Twenty one patients were evaluated for eligibility for this study. Ten patients were randomized to arm A (Rifabutin 150 mg daily) and eleven patients were in arm B (Rifabutin 300 mg thrice weekly). All of these patients underwent pharmacokinetic analysis of lopinavir/ritonavir and rifabutin.

Table 1 shows demographic data and baseline clinical characteristic of the 21 patients. Fifteen patients were diagnosed with pulmonary tuberculosis. Two patients suffered from disseminated tuberculosis. The other three patients had tuberculous colitis, tuberculous lymphadenitis and tuberculous pleuritis.

Pharmacokinetics of rifabutin and 25-O-desacetylrifabutin (coadministered with lopinavir/ritonavir)

The geometric mean minimum, peak, area under the curve and median time to reach peak concentration (Cmin , Cmax , AUC and Tmax) of rifabutin and 25-O-desacetylrifabutin in arm A and B were shown in table 2. All of plasma 25-O-desacetylrifabutin concentrations were lower than rifabutin concentrations in both arms. Peak concentration (Cmax) and minimum concentration (Cmin) in both arms are quite similar but area under the curve (AUC) of rifabutin is higher in the patients who recieved 300 mg 3 thrice weekly concurrent with lopinavir/ritonavir. AUC of rifabutin and 25-O-desacetylrifabutin in arm B were higher than in arm A for 72.7% and 57.8% respectively. Compare to rifabutin 300 mg once daily alone, combination of lopinavir/ritonavir and rifabutin 150 mg once daily give quite similar rifabutin Cmax for 0.66 and 0.65 mg/L respectively. Rifabutin AUC of rifabutin 150 mg once daily combined with

lopinavir/ritonavir is moderately higher than rifabutin alone for 41.9%. By contrast, combination of lopinavir/ritonavir and rifabutin 300 mg thrice weekly gives slightly higher Cmax than rifabutin alone for 24%. However the rifabutin AUC in patients with rifabutin 300 mg thrice weekly combined with lopinavir/ritonavir is markedly higher than rifabutin alone for 145.2%. Plasma concentration vs. time of rifabutin and 25-O-desacetyl rifabutin in relation to whether administered alone (300 mg) or combined with lopinavir/ritonavir are showed in figures 3 and 4.

Table 1. Baseline characteristics of HIV and TB coinfected patients in this study

	Total N=21	Arm A N=10	Arm B N=11
Mean age (years)	34.7	35.09	34.22
Sex; N (%)			
Male	18(90)	10 (90.9)	8(88.9)
Female	2(10)	1 (9.1)	1(11.1)
Ethnic; N(%)			
Thai	19(95)	11(100)	8(88.9)
Weight (kg)	57.2	57.0	57.5
CD4			
Absolute CD4 (cells/μL)	126.2	125.1	127.6
%CD4	9.4	9.4	9.3
Sites of TB infection			
● Pulmonary	16	8	8
● Disseminated	2	1	1
● Colitis	1		1
● Lymphadenitis	1		1
● Pleura	1	1	

Rifabutin pharmacokinetic parameters in combination with lopinavir/ritonavir of all patients are showed in table 3. In the arm A (rifabutin 150 mg once daily) range of rifabutin Cmax is 0.339 to 1.13 mg/L there is only one patient who has Cmax lower than therapeutic level (0.45-0.9 mg/L). Range of rifabutin AUC is 4.8 to 15.6 mg.h/L, there is no patient who has AUC less than 4.5 mg.h/L. In the arm B (rifabutin 300 mg thrice weekly) range of rifabutin Cmax is 0.54 to 1.23 mg/L, none of them has rifabutin Cmax less than 0.45 mg/L. Range of AUC is 7.3 to 31.7 mg.h/L, no patient who has AUC less than 4.5 mg.h/L.

Table 2. Pharmacokinetic parameters of rifabutin and 25-O-desacetylrifabutin. LPV/r: lopinavir/ritonavir, OD: once daily, TW: thrice weekly, Cmax: maximum concentration, Tmax: time to maximum concentration, AUC: area under the curve.

	Rifabutin			25-0-desacetyl-rifabutin		
	Alone	With LPV/r		Alone	With LPV/r	
	300 mg OD	150 mg OD	300 mg TW	300 mg OD	150 mg OD	300 mg TW
C_{max} (mg/L)	0.66 (42%)	0.65 (36%)	0.82 (30%)	0.06 (49%)	0.33 (25%)	0.3 (39%)
T_{max} (h)	2 (1-4.0)	3 (1-8)	2 (2-6)	2.03 (1-6)	4.02 (2-10)	4 (4-12)
AUC (mg.h/L)	6.32 (35%)	8.97 (37%)	15.5 (43%)	0.58 (64%)	6.15 (27%)	9.71 (45%)

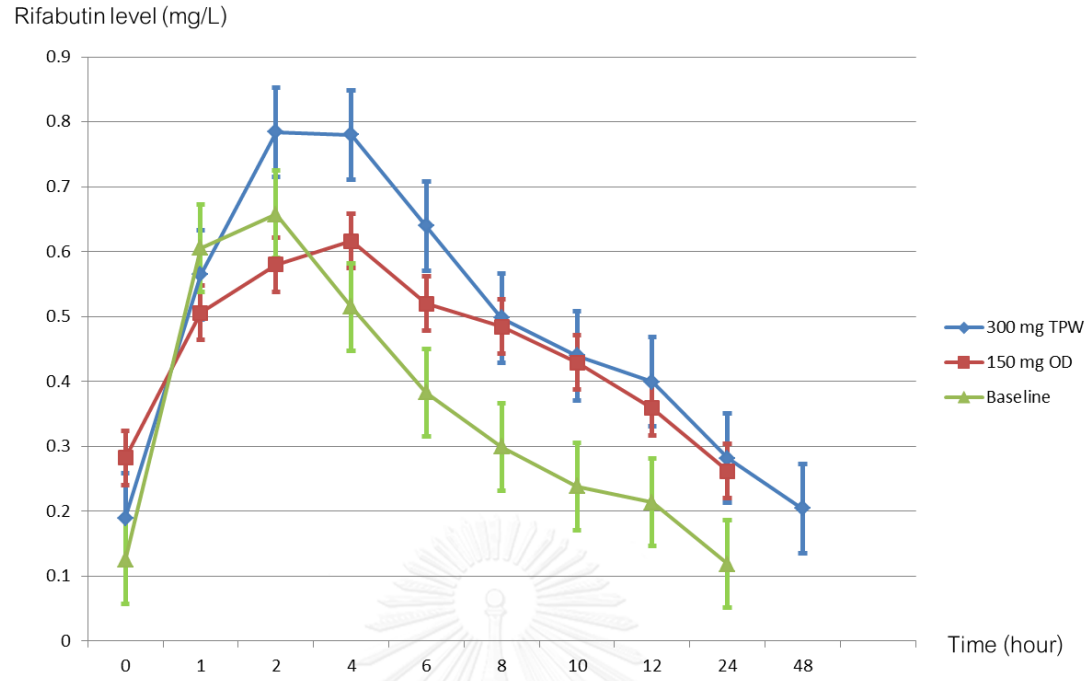

Figure 3. Plasma concentration of rifabutin vs time at baseline (rifabutin 300 mg alone) or with lopinavir/ritonavir

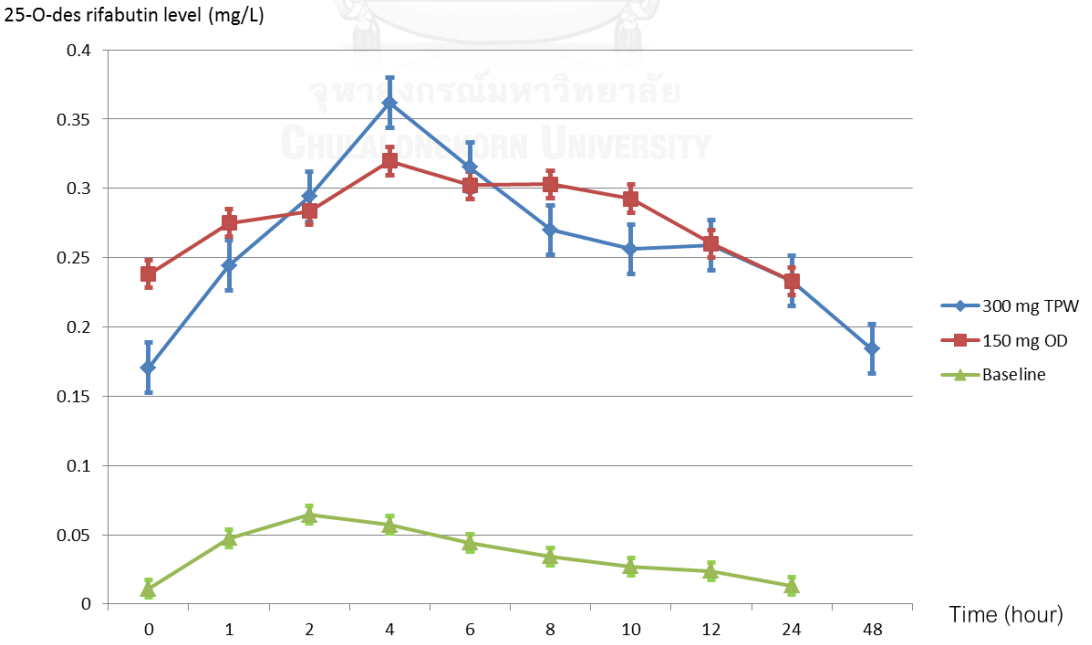

Figure 4. Plasma concentration of 25-O-desrifabutin vs. time at baseline (rifabutin 300 mg alone) or with lopinavir/ritonavir

Pharmacokinetic of lopinavir/ritonavir

The mean trough, peak and average concentrations (C0, Cmax and Cave) of lopinavir/ritonavir in arm A and B were shown in table 3. The results show variation of lopinavir/ritonavir concentrations among HIV and TB coinfected patients. However the mean lopinavir/ritonavir concentrations were similar between the two arms.

Table 3. Pharmacokinetics of lopinavir and ritonavir. OD: once daily, TW: thrice weekly, C_{max}: maximum concentration, C_{min}: minimum concentration,C_{ave}: average concentration

	Lopinavir (µg/mL) (range)		Ritonavir (µg/mL) (range)	
	150 mg OD	300 mg TW	150 mg OD	300 mg TW
C_{max}	13.455 (8.84- 16.54)	14.027 (10.6- 21.95)	0.946 (0.71- 1.38)	0.786 (0.54- 1.14)
C_{min}	5.287 (2.93- 7.92)	4.155 (1.33- 5.91)	0.223 (0.15- 0.31)	0.178 (0.07- 0.26)
C_{ave}	9.695 (5.75- 11.51)	10.252 (8.06- 15.24)	0.545 (0.39- 0.82)	0.468 (0.34- 0.66)

Adverse event

The 21 HIV and TB coinfected patients, there are 32 adverse events. These occurred in 12 patients. Fifty percent of the events were moderate to severe. Overall 4 percent of adverse events were considered probable or certainly rifabutin related. Uveitis which is the well-known and serious side effect of rifabutin developed in two patients. Interestingly, both of them were treated with rifabutin 300 mg 3 times/week. Eighty-four percent of the events recovered or resolved included uveitis. Moderate and severe adverse events in both arms were shown in table 4.

Table 4. Adverse events

9 adverse events in 7 patients in arm A (150 mg OD)	Hyperpigmented skin
	Hypovolemic hypotension
	Lopinavir intolerance
	Appendiceal abscess
	Rifabutin overdose
	Thrombocytopenia
	Rash
	Ocular tuberculosis
	Hepatitis
	Latent syphilis
5 adverse events in 5 patients in arm B (300 mg TW)	Uveitis
	Endophthalmitis
	Drug reaction
	Diarrhea
	Anemia
	Depression
	Gastritis

Tuberculosis and HIV outcome

Among HIV and TB coinfected patients in this study, all of them are cured of tuberculosis (sputum AFB negative at the end of treatment). About HIV outcome, 9 of 10 patients of the arm A have viral load less than 50 copies/mL and 8 of 11 patients of the arm B have viral load less than 50 copies/mL. Summary of characteristics, pharmacokinetic parameters and outcomes of HIV and TB are showed in table 5 and 6

Patient Number	Age (year)	sex	Weight (kg)	CD4 Cells/μL	CD4%	TB site	Tmax (hour)	Cmax (mg/L)	AUC (mg.h/L)	VL at 48 weeks (copies/mL)	Outcome of TB
1	21	male	71.6	444	22	Pulmonary	8	0.664	8.714941134	<50	Cure
2	39	male	78.9	374	23	Pleura	2	0.722	8.930216233	<50	Cure
3	19	male	47.9	4	1	Pulmonary	1	0.577	7.458452867	<50	Cure
4	46	male	50.6	31	5	Pulmonary	4.033333	1	15.64601504	<50	Cure
5	44	female	40.5	172	17	Pulmonary	4	1.13	15.5677539	<50	Cure
6	35	male	65.5	418	22	Pulmonary	4	0.667	10.94386102	<50	Cure
7	28	male	62.1	48	3	Disseminated	4.033333	0.339	4.782514985	<50	Cure
8	22	male	63.1	390	22	Pulmonary	2	0.456	7.583928604	37561	Cure
9	34	male	54.5	43	18	pulmonary	1	0.754	8.215232058	<50	Cure
10	55	female	72.8	110	7	Pulmonary	2	0.569	7.277807015	<50	Cure

Table 5. Characteristics, pharmacokinetic parameters and outcomes of HIV and TB of the patients in arm A (rifabutin 150 mg daily), TB; tuberculosis, Tmax;time to maximal concentration, Cmax; maximum concentration, AUC; area under the curve, VL; viral load

Patient Number	Age (year)	sex	Weight (kg)	CD4 Cells/μL	CD4%	TB site	Tmax (hour)	Cmax (mg/L)	AUC (mg.h/L)	VL at 48 weeks (copies/mL)	Outcome of TB
11	38	male	48	41	4	Pulmonary	2	1.23	31.74369956	<50	cure
12	60	male	48	77	5	Pulmonary	2	0.926	18.16060254	<50	cure
13	48	male	68.9	55	14	Pulmonary	6	0.561	13.01857247	<50	cure
14	58	male	70.2	8	2	Pulmonary	2	0.818	15.41892534	<50	cure
15	30	male	43.1	91	11	Pulmonary	4	1.19	20.20455439	148	cure
16	29	male	54.4	252	13	LN	6	0.768	11.76969861	<50	cure
17	31	male	53	70	17	Pulmonary	2	0.911	16.8766815	921871	cure
18	20	male	56.8	493	11	Colitis	2	0.547	7.319354783	<50	cure
19	33	male	51	92	7	Disseminated	4	0.594	10.59681274	64	cure
20	32	male	52.3	159	8	Pulmonary	4	0.729	13.89673822	<50	cure
21	44	male	67.6	302	11	Pulmonary	2	1.14	24.8809324	<50	cure

Table 6. Characteristics, pharmacokinetic parameters and outcomes of HIV and TB of the patients in arm B (rifabutin 300 mg thrice weekly) TB; tuberculosis, Tmax;time to maximal concentration, Cmax; maximum concentration, AUC; area under the curve, VL; viral load

CHAPTER 5

DISCUSSION AND CONCLUSION

Pharmacokinetics of rifabutin

This study designed to investigate the adequate dose of rifabutin in the treatment of patients with HIV associated tuberculosis who receiving lopinavir/ritonavir. We compared pharmacokinetics parameters of rifabutin 150 mg daily versus rifabutin 300 mg thrice weekly in combination with LPV/r 400/100 mg twice daily based HAART. The major findings of our study are peak (Cmax) and minimum concentration (Cmin) of rifabutin were quite similar in both of 150 mg daily and 300 mg three times/week. However area under the curve (AUC) of rifabutin in the patients who received rifabutin 300 mg thrice weekly are 72.7% higher than 150 mg daily.

From previous pharmacokinetic studies, recommended target Cmax of rifabutin is 0.45-0.9 mg/L (15). Contrast to the previous study of Khachi et al (16) and that use rifabutin 150 mg three times/week with lopinavir/ritonavir 400/100 mg BID and showed subtherapeutic rifabutin level, our study shows that both doses of rifabutin,150 mg once daily and 300 mg three times/week, can give adequate Cmax of 0.69 and 0.86 mg/L respectively. However in our study, there is one patient in arm A (150 mg daily) who had subtherapeutic rifabutin level of 0.34 mg/L, fortunately he had a good clinical response to anti tuberculosis therapy. These findings were similar to previous study of Lan et al (5) that compared pharmacokinetic parameters of rifabutin 150 mg daily and 150 mg three times/week concurrent with lopinavir/ritonavir which was conducted in Vietnam. In that study there was a patient who received rifabutin 150 mg daily had rifabutin Cmax of 0.246 mg/L but median Cmax is quite similar to the mean Cmax of our study (0.67 mg/L). Our finding is different to study of Naiker et al (4) which investigate

pharmacokinetic parameters of rifabutin 150 mg three times/week and 150 mg daily concurrent with lopinavir/ritonavir and conducted in South Africa. The study showed that rifabutin 150 mg daily gave lower median Cmax of 0.31 mg/L (IQR = 0.26-0.38 mg/L).

There was no patient in arm B (300 mg three times/week) who had subtherapeutic rifabutin level, however there were 5 patients from 11 pateints who had Cmax of 0.926-1.23 mg/L which is greater than recommended target. The mean weight of patient with too high rifabutin Cmax is 51.94 kg (SD = 8.4) which is lower than the patient with normal Cmax (58.9 kg , SD = 8.4). Lower weight may be the reason why Cmax in this population is too high.

Because pharmacodynamic studies suggest that the mycobacterial killing activity of rifamycins is concentration dependent (1, 2), and some studies found that AUC is the better parameter (2, 3), our study show both Cmax and AUC of rifabutin. A previous study found that AUC of rifabutin below 4.5 mg.h/L is associated with higher risk of acquired rifamycin resistance (20). None of our patients in both arms had AUC below 4.5 mg.h/L. This finding is different to results from study of Naiker et al (4) which showed that 29% of patients with 150 mg daily had inadequate AUC (< 4.5 mg.h/L). However our findings are similar to Vietnam study that showed only one patient who received rifabutin 150 mg daily and did not have adequate AUC (> 4.5 mg.h/L). This difference may be due to ethnicity or difference in weight of patients in these studies. Median weight of Vietnamese patient was 49 kg but 59.9 kg in South African patients. However, in our study, median weight was 57.2 kg which is a bit lower than South African patients.

Greater AUC of rifabutin was found to have positive correlation with higher Cmax. Previous study showed that concurrent use of lopinavir/ritonavir and rifabutin 150 mg daily caused increasing in AUC of rifabutin for 203% (13). Our study showed that concurrent use of lopinavir/ritonavir and rifabutin 300 mg three times/week produced much greater AUC than 150 mg daily for 75.8% which is very high and may increase risk of side effects.

Pharmacokinetic of lopinavir/ritonavir

A study of Khachi et al (16) showed that coadministration of lopinavir/ritonavir and rifabutin can cause a subtherapeutic lopinavir level. In that study 2 patients who were treated with LPV/r (400/100 mg) BID and rifabutin 150 mg three times a week had lower lopinavir trough levels, 1.030 and 1.549 μg/mL, compared to standard lopinavir trough levels of 5.5 ± 4.0 μg/mL (21). However, the study did not show a clinical significance of a lower lopinavir level. In our study, both trough (C0) and peak (Cmax) lopinavir concentrations were in the range of standard levels that have been reported in the previous study (trough and peak level are 5.5 ± 4.0 and 9.6 ± 4.4 μg/mL respectively) (21) despite the higher rifabutin dose in our study. This phenomenon can be explained by the quite low body weight in our HIV and TB coinfected patients.

All pharmacokinetic parameters of lopinavir in our study were similar in both arms. This finding was comparable to previous studies (4, 5). These findings may reflect the lower potency of rifabutin in cytochrome P450 induction compared to rifampicin (22).

Adverse events

One of the most serious side effects of rifabutin is uveitis. It was reported as high as 6 to 9 percent in patient who was treated with rifabutin (23). In this study two patients had uveitis, both of them were in arm B (rifabutin 300 mg three times/week) one of them had both high Cmax and AUC of 1.19 mg/L and 20.2 mg.h/L respectively but another patient had normal Cmax and AUC. Data of rifabutin level and correlation to uveitis are limited but rifabutin-induced uveitis is usually associated with high dose of rifabutin (24) and commonly found in patient who concurrently use other drugs that can inhibit cytochrome P450 3A4 and increase serum rifabutin level (25). The patient with uveitis had clinical improvement after discontinuation of rifabutin. This is similar to other reports that showed good prognosis of rifabutin induced uveitis (26).

Our patients had other common side effects such as cytopenia, diarrhea, rash and hepatitis like in previous studies (23, 27). However, we also found hypovolemic hypotension, appendiceal abscess and endophthalmitis that may not be associated with

rifabutin. Because of the small sample size, this study had limited ability in determining which arm was more likely to cause adverse events.

Our pharmacokinetic study of rifabutin implies that rifabutin 150 mg daily and 300 mg three times/week produce adequate Cmax and AUC in Thai patient. However rifabutin 300 mg three times/week might associate with high Cmax and AUC which may be associated with adverse event especially uveitis.

Clinical outcomes

Our study shows that clinical outcomes of TB and HIV treatment are not different among the two groups of patients. With both doses (150 mg daily and 300 mg thrice weekly), there is no deterioration of tuberculosis, unlike the lower dose (150 mg thrice weekly) (16) and with both doses in Thai population there is no emergence of rifamycin resistance that was reported in previous study of rifabutin 150 mg daily (4). The HIV treatment outcome which is determined by viral load at the 48^{th} week are not different in the two groups which may be due to the same lopinavir/ritonavir pharmacokinetics.

Limitations of this study

First, this study is not a blinded study and cannot avoid some biases especially in the evaluation of side effect and some subjective data. However this limitation may not affect in the pharmacokinetic analysis. We use standard technique (HPLC) that was performed by the experts in pharmacokinetics to measure rifabutin and 25-o-desrifabutin level which is reliable. Second , because of the small sample size, this study cannot provide the best conclusion about efficacy and toxicity of the both doses of rifabutin therefore larger clinical trial is needed.

Conclusion

Our study suggests that rifabutin 150 mg daily should be considered in Thai patient who concurrently use lopinavir/ritonavir because of good pharmacokinetic parameters and safety. Moreover this study demonstrated that lopinavir/ritonavir 400/100 mg BID can give adequate lopinavir levels in HIV and TB coinfected patients who were treated with rifabutin both 150 mg daily and 300 mg thrice weekly.

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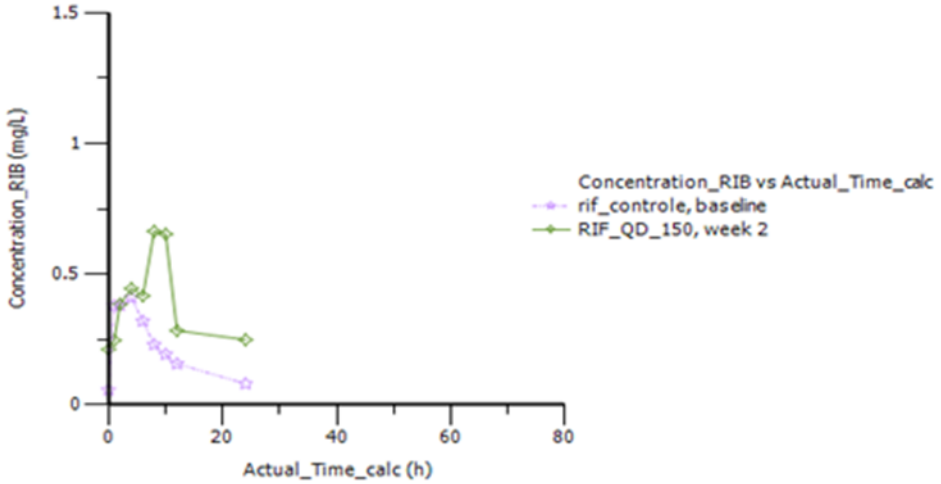
APPENDIX

Graph of rifabutin level vs. time in all 21 patients

Patient 1



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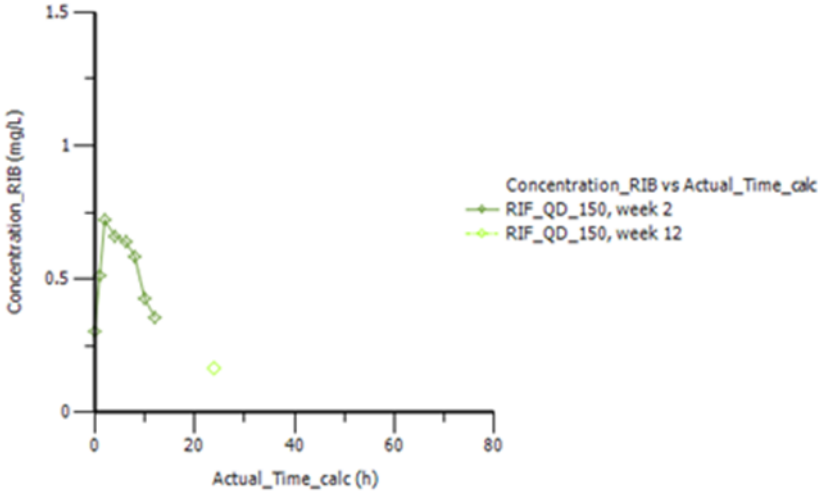

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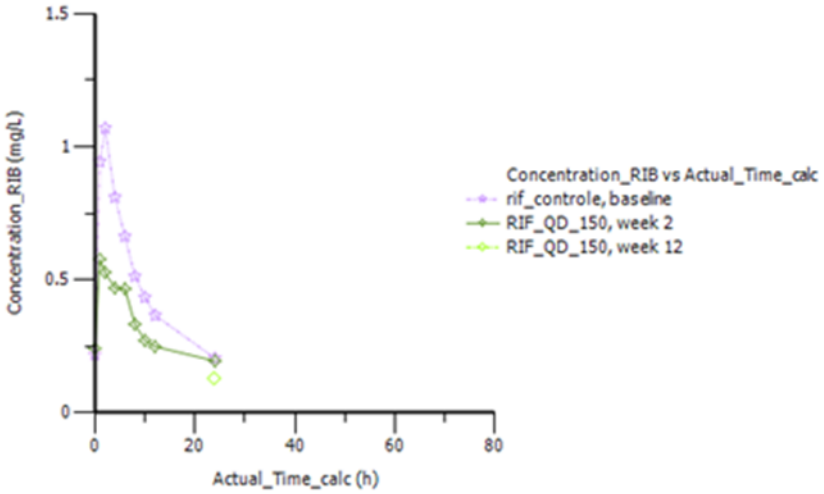

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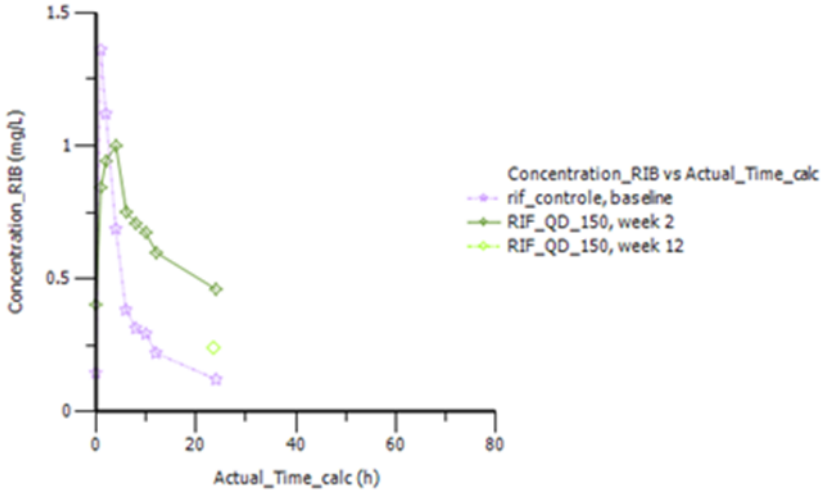

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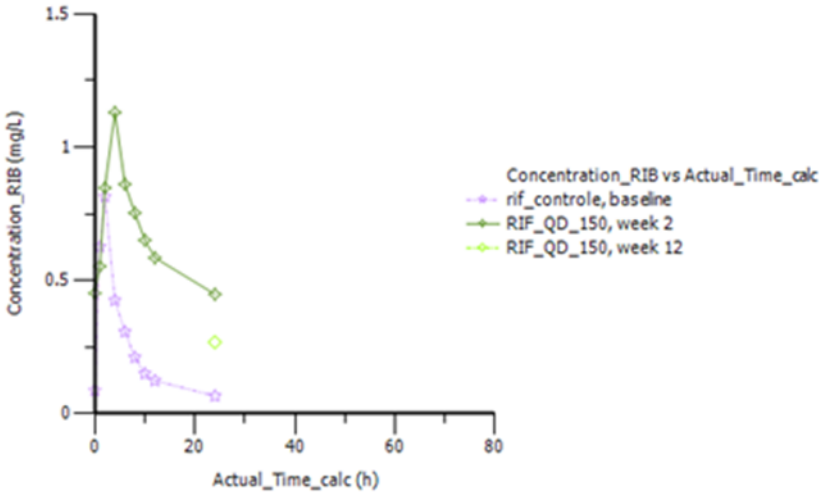

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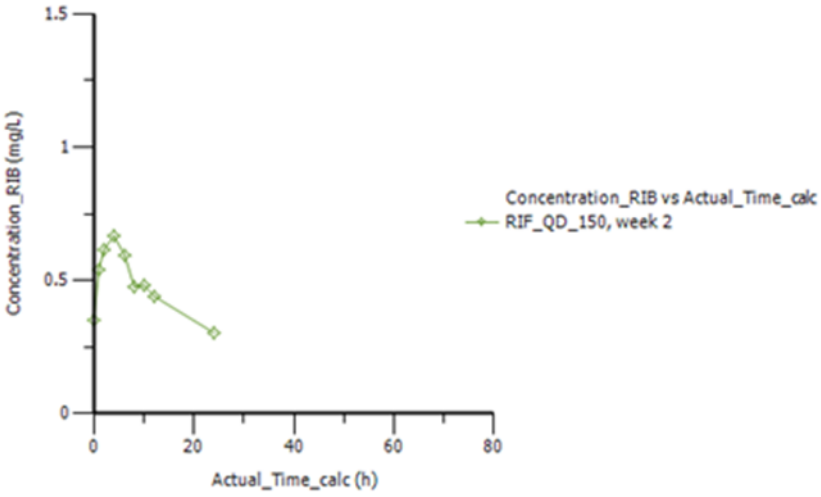

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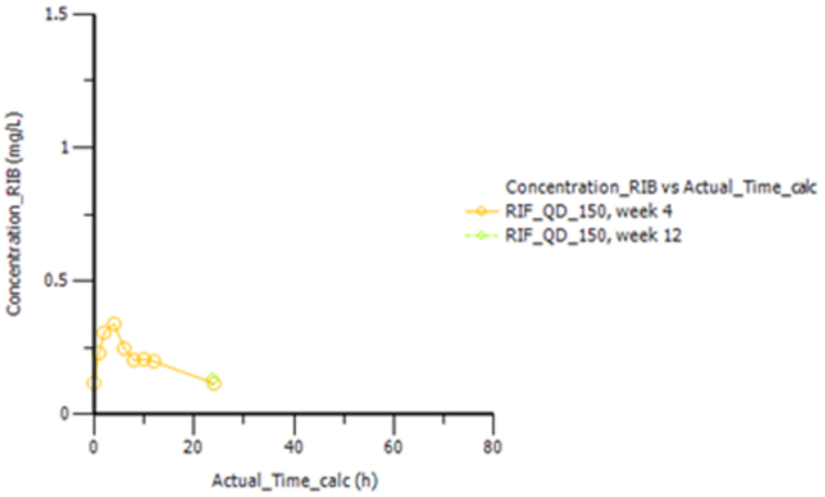

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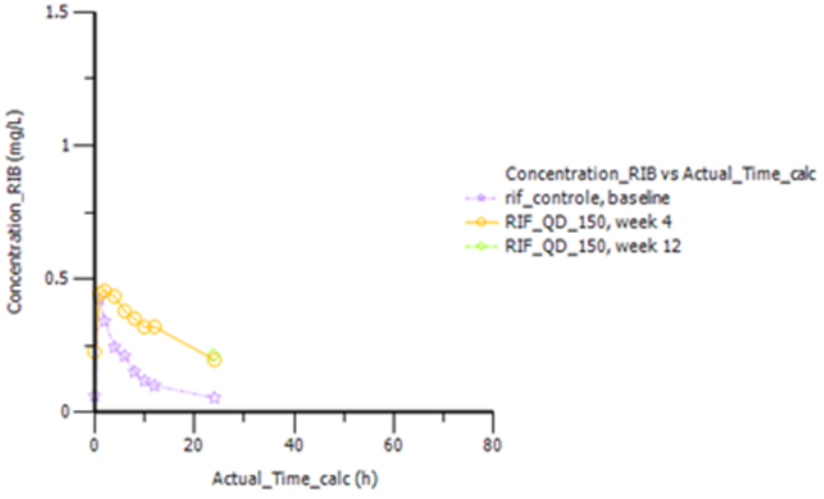

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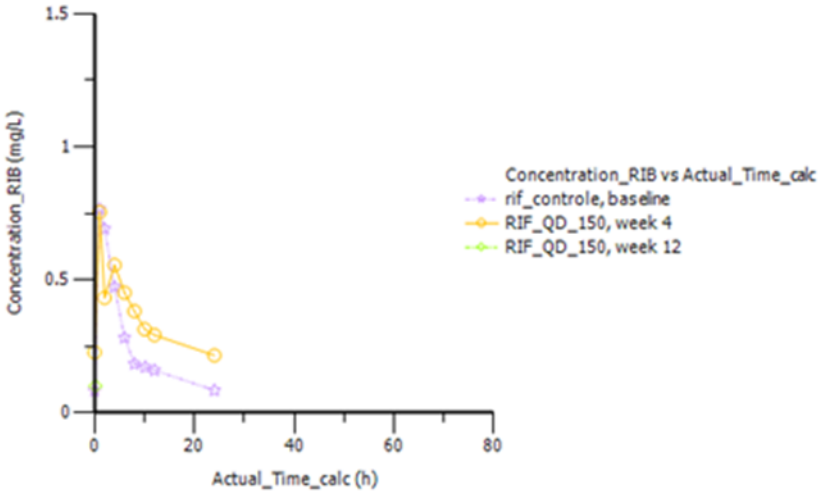

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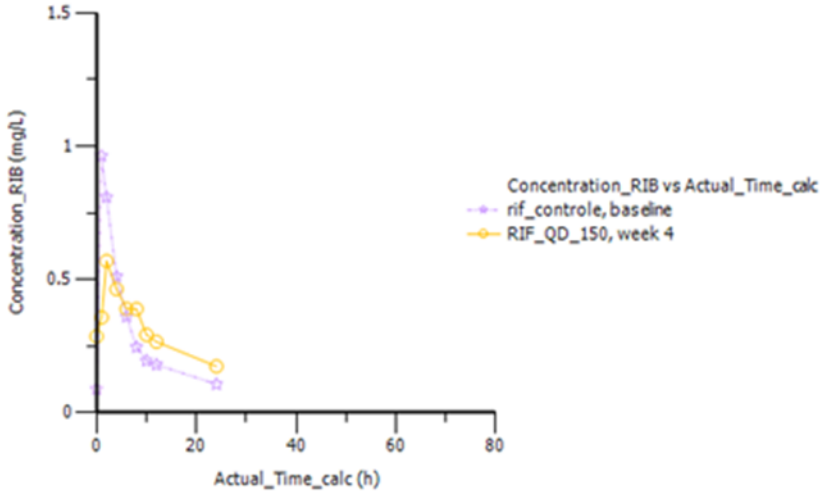

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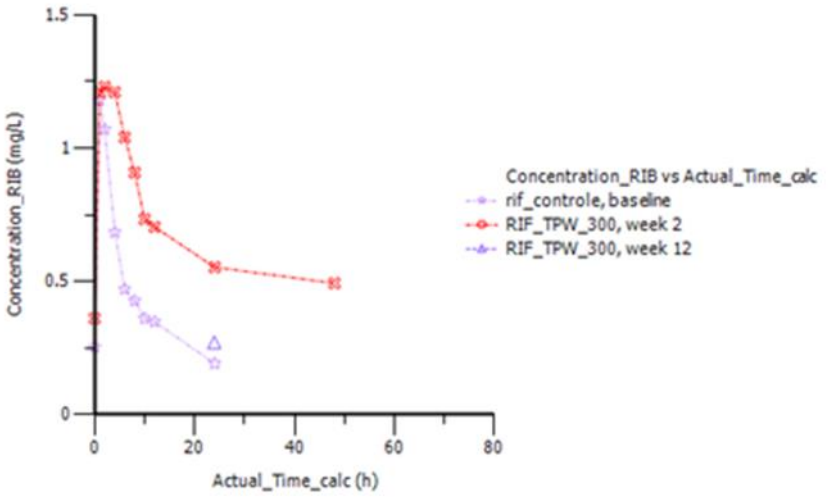

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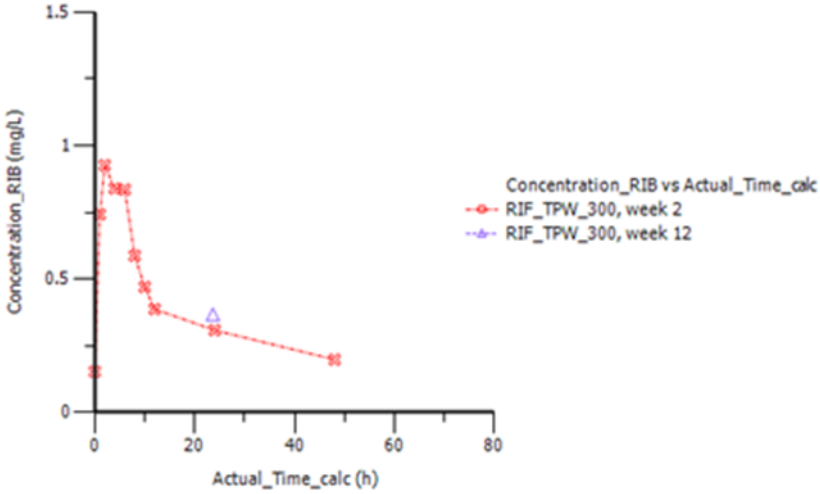
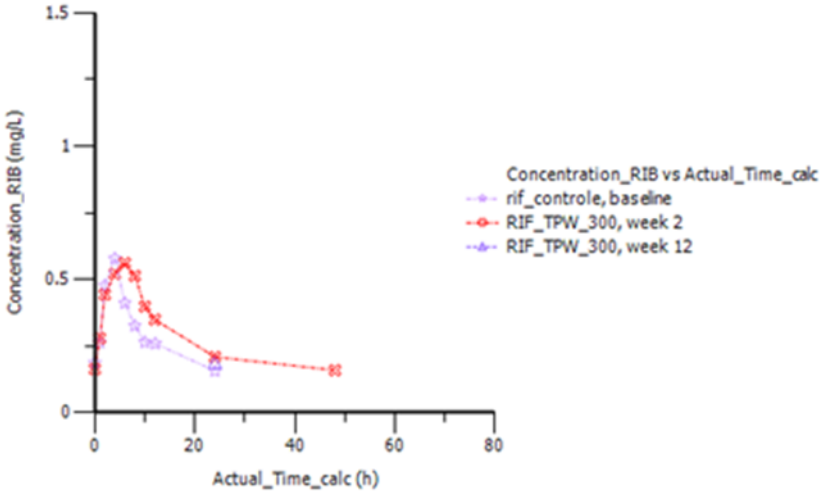


Patient 13



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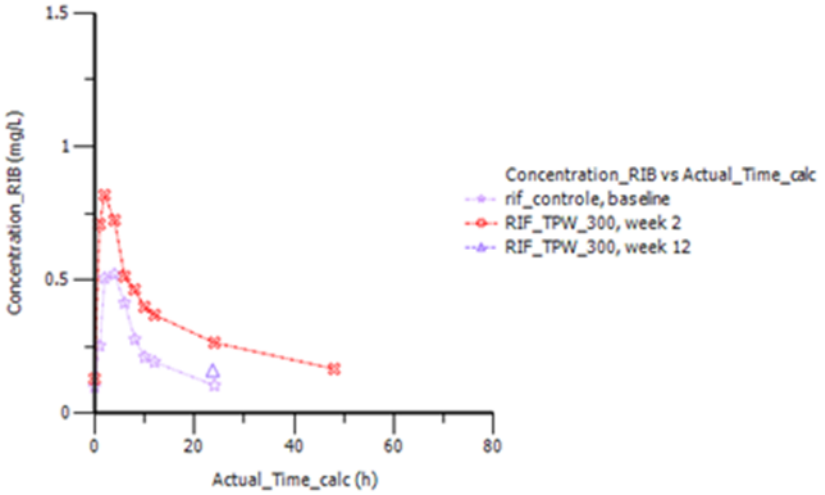

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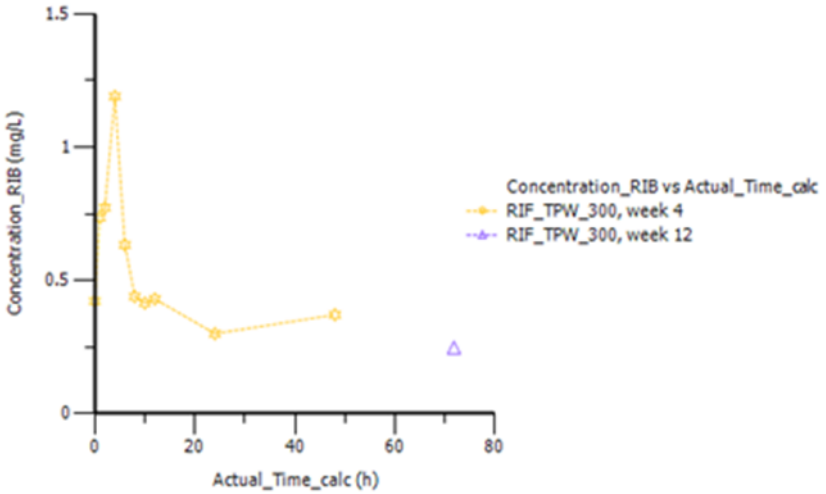

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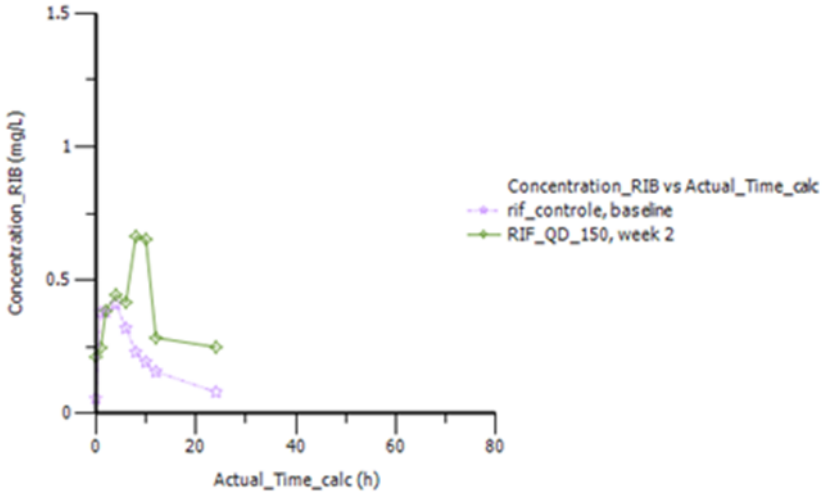
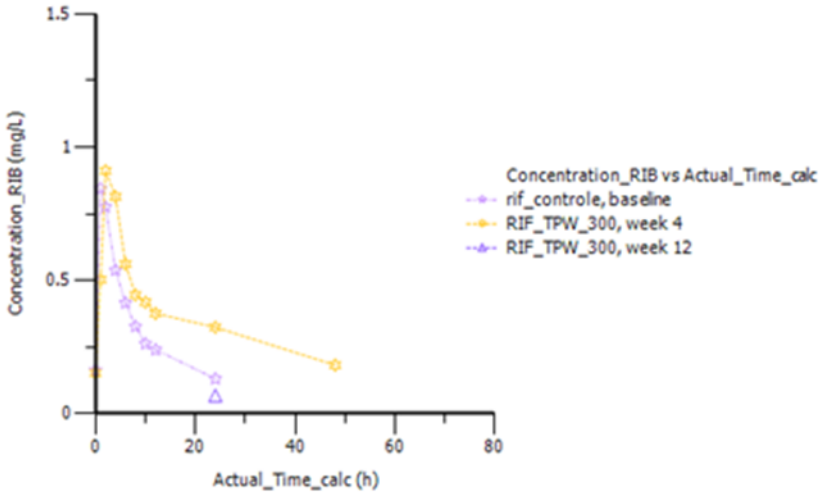


Patient 17



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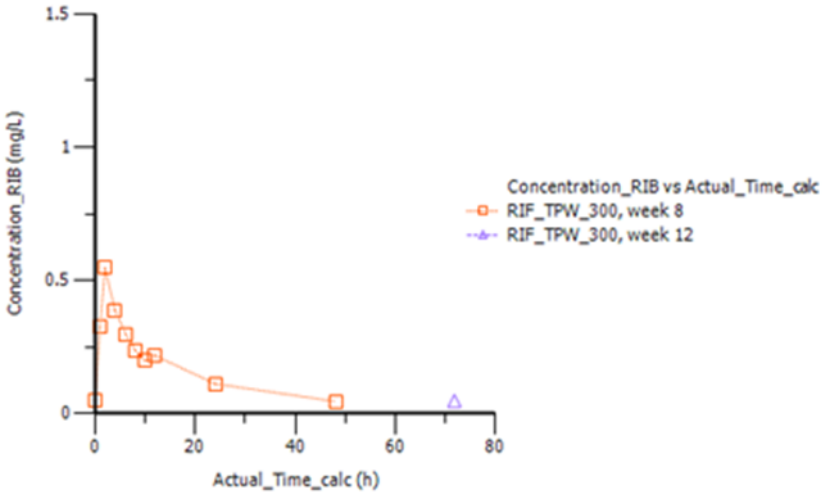

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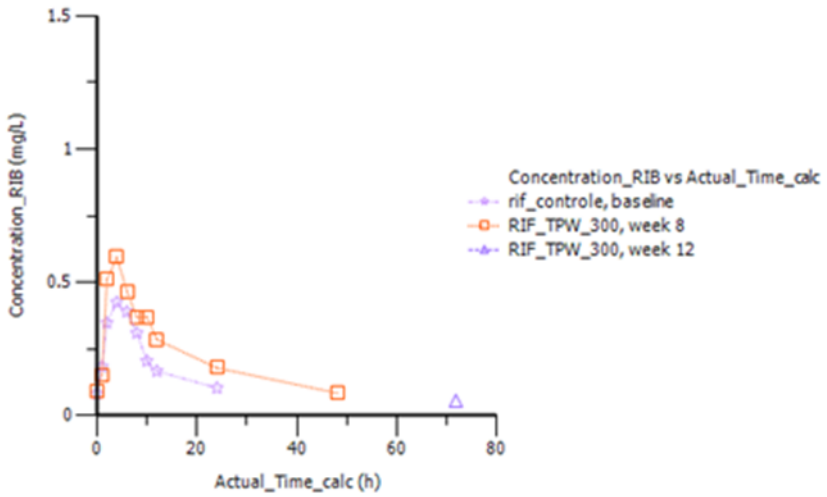

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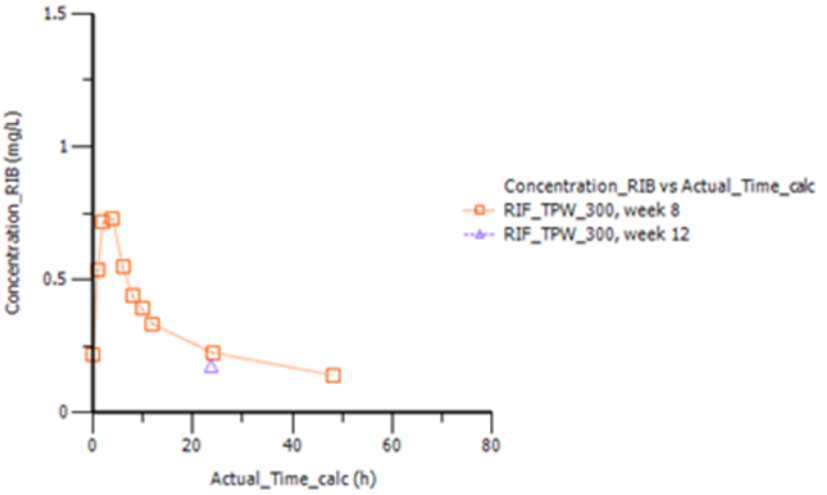
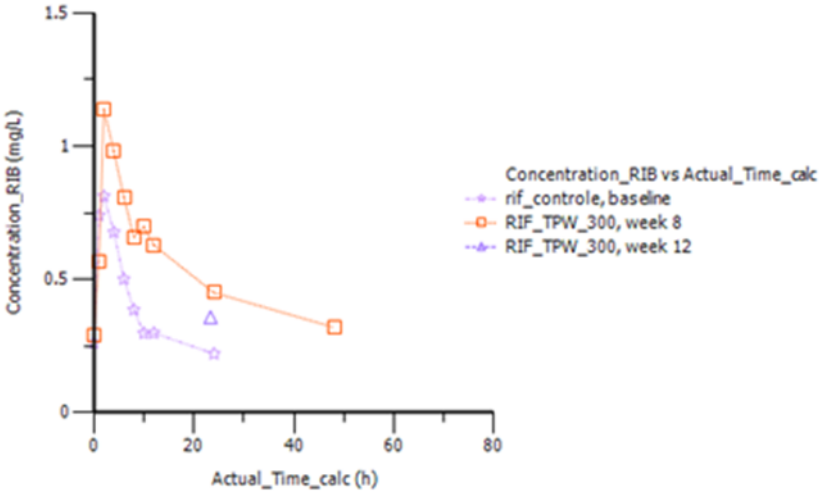


Patient 21



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Publications:

1. Fujitnirun C, Plongla R. Emerging drug-resistant organism around the world. In: Vanichanan J, Putcharoen O. Infectious Diseases Across the Regions. 1st ed. Bangkok: Trithep Book Process; 2017:352-428

2. Fujitnirun C, Jutivorakul K. Febrile neutropenia. In: Suwanpimolkul G, Jutivorakool K, Paitoonpong L, Putcharoen O, Suankratay C. Infectious Disease Emergencies. 1st ed. Bangkok: Trithep Book Process; 2016:251-301

3. Fujitnirun C, Suankratay C. Invasive Nontoxigenic Corynebacterium diphtheriae Infection: the 2 Case Reports. Poster session presented at: IDweek 2016, Annual Conference of the Infectious Diseases Society of America; 2016 October 26-30; New Orleans, LA, United States.