A Pharmacokinetic Study of Rifabutin and its Interaction with Antiretrovirals in African Patients with TB-HIV Co-infection

by

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Declaration

I declare that the work presented in this thesis is my own except for all laboratory assays, which were done at the Division of Clinical Pharmacology, University of Cape Town and the Department of Molecular and Clinical Pharmacology, University of Liverpool. This thesis has not been submitted previously to another university.

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Suhashni Naiker

Dedication

I dedicate this thesis to my two children, Kirthi and Avantika Lee and my husband Amroshan. I would not have accomplished this without your love, patience and understanding.

Presentations at Conferences

1. S Naiker, C Conolly, L Wiesner, DC Phillips, AD Harries, C Lienhardt, H McIlleron and AS Pym. “Pharmacokinetic Evaluation of Different Rifabutin Dosing Strategies in African Tuberculosis Patients on Lopinavir/ritonavir-based Antiretroviral Therapy”. International Conference on Retroviruses and Opportunistic Infection (CROI), February 2011, Boston USA

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Ideally I should be thanking God first for giving me the strength and ability to pursue and complete a study of this magnitude. However, Hindu scriptures say that one should revere one's parents first, teachers second and then God. My parents have always supported and encouraged my studies and together with my mother-in-law have helped and supported me immensely throughout this study, for which I am indeed very grateful.

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During the course of this study I have learned about TB, HIV and pharmacokinetics, but most importantly I have learned the important role God plays in our lives. I have seen the hand of God many times during this study and I thank Her from the bottom of my heart. On that note I would also like to thank Guru Leeandran Narainsamy for his spiritual guidance and teaching, which have assisted me in achieving the goals I set for myself.

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Abstract

The management of HIV-associated tuberculosis (TB) is complicated by the pharmacokinetic interactions between rifampicin (RMP) and co-administered protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors. Rifabutin (RBT) is an alternative rifamycin, preferred in patients requiring PIs. Recent studies suggest the current recommended dose of RBT in combination with boosted lopinavir (LPV/r) is suboptimal and there are insufficient pharmacokinetic data evaluating the interaction between RBT co-administered with efavirenz (EFV) and nevirapine (NVP). Pharmacogenomic studies have shown that RMP concentrations are lower in patients from sub-Saharan Africa with polymorphisms of the SLCO1B1gene but there is currently no data on the pharmacogenetic determinants of RBT exposure.

The pharmacokinetics of RBT were evaluated at two different doses in HIV co-infected patients before and after the introduction of LPV/r, EFV and NVP-based antiretroviral therapy (ART). After six weeks of standard TB therapy, RBT 300 mg daily was started for four weeks. Thereafter patients were randomized to receive either RBT 150 mg daily or RBT 150 mg three times a week (TPW) with LPV/r, RBT 300mg or 450mg with NVP or RBT- 450mg or 600mg with efavirenz. After four weeks on the first RBT dose, patients switched to the alternate dose and continued until the end of TB treatment. Serial RBT and 25-O-desacetylrifabutin (dRBT) concentrations were measured during a dose interval before patients switched RBT doses.

The median AUC_{0-24} and C_{max}, of RBT in patients taking 150mg RBT TPW was significantly reduced when compared to the other treatment arms. 86% of patients whilst on this intermittent RBT arm had an $AUC_{0-24} < 4.5$ µg.h/mL, level that has been associated with acquired rifamycin resistance. Rifabutin exposure was maintained within the range of AUCs that have been shown to prevent acquired rifamycin resistance (ARR) with 150mg daily dosing in combination with LPV/r. In addition, the combination of RBT with NVP 300mg resulted in significantly increased exposure of RBT, with significantly higher exposure observed with 600mg RBT. However, the combination of RBT 450mg with EFV resulted in RBT exposure lower than 300mg RBT given alone in the same patients, whereas RBT 600mg plus NVP results in bioavailability of RBT equivalent to 300mg given alone.

Rifabutin was well tolerated at all doses. Only three grade 4 laboratory toxicities, elevated transaminases, neutropenia, and uveitis, possibly related to RBT were reported in patients taking NVP. SLCO1B1 rs4149032 C>T polymorphism occurs frequently in African patients in Durban and may be associated with low RBT bioavailability. These findings support recommendations for the higher dose of RBT in combination with LPV and EFV but not with NVP.

List of Abbreviations

AE adverse event

ALT alanine aminotransferase

ARR acquired rifamycin resistance

ART antiretroviral therapy

ARV antiretroviral

AST aspartate aminotransferase

ATV atazinavir

AUC area under the plasma concentration time curve

AUC_{0-24} / MIC ratio of 24-hour area under the curve to MIC

BMI body mass index

BSV between subject variability

C_0 pre-dose concentration

C_{12} / C_{24} trough concentration

CAMELIA Cambodian Early versus Late Introduction of Antiretrovirals

CAR constitutive androstane receptor

CDC Centers for Disease Control and Prevention

CFR case fatality rates

CL/F apparent oral clearance

C_{max} maximum concentration

C_{max}/ MIC ratio of the peak concentration to minimum inhibitory concentration

COMESEM Madrid South-Eastern Metropolitan Crown

CTX cotrimoxazole

CV coefficient of variation

CYP cytochrome

d-RBT 25-O-desacetylrifabutin

ddI didanosine

DOH department of health

DOT directly observed therapy

DST drug sensitivity test

EBA early bactericidal activity

EFV efavirenz

EMB; E ethambutol

FBC full blood count

HAART highly active antiretroviral therapy

HPLC high pressure liquid chromatography

IDV indinavir

IFN interferon

INH; H isoniazid

IRIS immune reconstitution inflammatory syndrome

ka first order absorption rate constant

KZN Kwa-Zulu Natal

LC/MS/MS liquid chromatography mass spectrometry

LFT liver function test

LLQ lower limit of quantification

LPV/r boosted lopinavir / lopinavir/ritonavir

M.tuberculosis Mycobacterium tuberculosis

MAC *Mycobacterium avium* complex

MBC minimal bactericidal concentration

MDR TB muti drug resistant tuberculosis

MIC minimum inhibitory concentration

NFV nelfinavir

NRTI nucleoside reverse-transcriptase inhibitors

NVP nevirapine

OATP organic anion transporting peptide

PAS; P P-aminosalicylic acid

PCR polymerase chain reaction

PD pharmacodynamic

PGP P-glycoprotein

PI protease inhibitor

PK pharmacokinetic

PXR pregnane X receptor

PZA; Z pyrazinamide

QC quality control

RBT rifabutin

RCTs randomized control trials

RMP; R rifampicin

rtPCR real time polymerase chain reaction

RTV; r ritonavir

RXR retinoid X receptor

SAE serious adverse event

SAPIT Starting Antiretroviral Therapy at Three Points in Tuberculosis

SCC short-course chemotherapy

SM ; S streptomycin

SNPs single nucleotide polymorphisms

SQV saquinavir

TB Tuberculosis

T_{max} time at which maximum concentration attained

TPW three time per week

UV ultra violet

WHO World Health Organization

ZDV zidovudine

List of Figures

Figure 5. 3. Median (IQR) concentration-time profile of lopinavir when lopinavir/ritonavir was administered as part of antiretroviral treatment with rifabutin 150 mg daily (blue) and 150mg thrice weekly (red) and isoniazid

List of Tables

Table of Contents

Chapter 1. Introduction & Literature Review

Section 1 Introduction

1.1. Tuberculosis – a recurring epidemic

The presence of tuberculosis (TB) in human remains from pharaonic Egypt indicates that it has plagued mankind since an early stage of human history. A large expansion of TB occurred in the early 1600s during the industrial revolution in Europe, as a result of over-crowding and unhygienic living conditions, and was subsequently spread to North America by European migration (Bates and Stead 1993). For decades preceding the discovery of antibiotics, TB was treated by lung collapse and thoracoplasty, confinement in a sanatorium, bed rest and exposure to fresh air (Ducati et al 2006) resulting in high case fatality rates (CFRs). The probability of death in patients diagnosed with pulmonary TB between 1925 and 1934 in Denmark was reported to be 17-29% 1 year after diagnosis, 32-43% 3 years after diagnosis and 42-55% 5 years after diagnosis (Buhl and Ngboe 1925). An observational study of sputum-positive TB patients diagnosed between 1928 and 1938 by (Thompson et al 1943) reported that the probability of dying in the first year after diagnosis was 40%.

The subsequent development of modern TB therapy during the 1950s and 1960s and the use of treatment regimens incorporating combinations of these drugs resulted in cure rates of over 90% (Fox et al 1999). However, an upsurge of the global incidence of TB began in the 1980s despite the availability of highly efficacious treatment and is

attributed to various factors including HIV. Globally, in 2010 there were an estimated 8.8 million people infected with TB of which 1.1 million (13%) were also infected with HIV. The majority (82%) of TB-HIV co-infected people reside in sub-Saharan Africa (Figure 1.1). Tuberculosis was the cause of approximately 1.4 million deaths globally in 2010, 0.35 million of which were also HIV co-infected. South Africa has the largest population of HIV infected people in the world, estimated at 5.9 million in 2009 with Kwa-Zulu Natal (KZN) being the most affected province in the country. Consequently South Africa has the highest incidence of TB-HIV co-infected patients among countries in the African Region (World Health Organization 2011b).

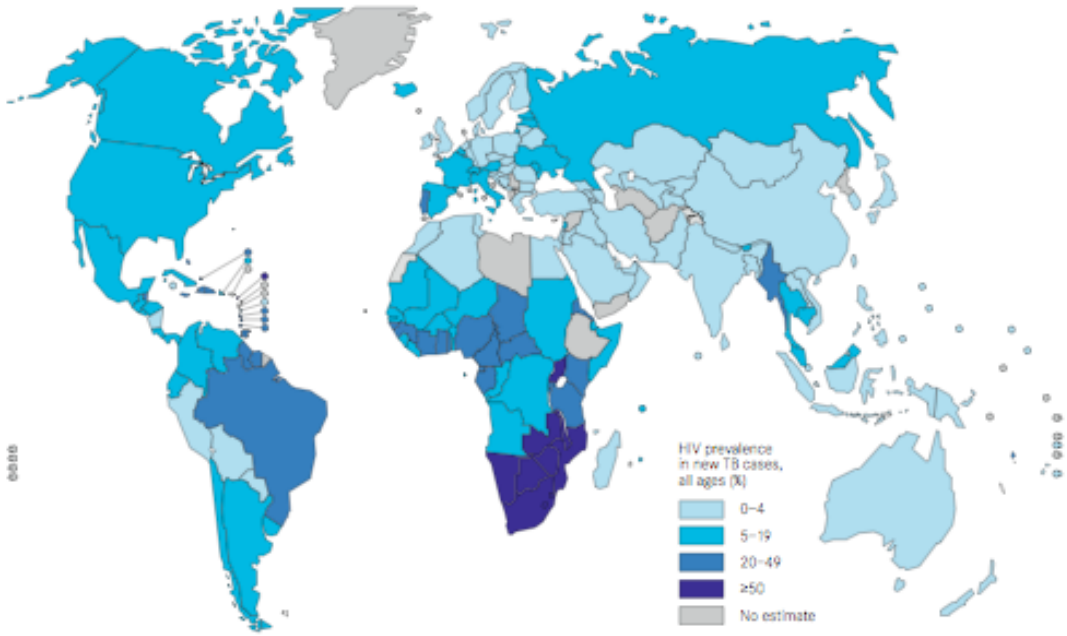

Figure 1.1. Estimated HIV prevalence in new TB cases, 2010 (World Health Organization 2011b).

1.2. Development of modern tuberculosis chemotherapy

Although Dr. Robert Koch discovered that infection with the bacillus, *Mycobacterium tuberculosis,* caused TB as early as 1882 (Koch 1882), effective treatment of TB began only after the discovery of streptomycin (SM; S) in 1943 (Waksman 1943). In fact, the very first clinical trial of TB therapy conducted in 1946 compared the efficacy of bed rest to the combination of bed rest and treatment with SM. Treatment with SM proved to be superior to bed rest alone thus SM was the first drug to be used for the treatment of TB (BMRC 1948). However, treatment with SM alone was associated with acquired resistance and the use of drug combinations was proposed when more anti-TB drugs were introduced, namely P-aminosalicylic acid (PAS; P) in 1948 and isoniazid (INH; H) in 1952. Subsequent clinical trials incorporating SM, INH and PAS, demonstrated almost complete suppression of acquired resistance by the addition of INH (Medical Research Council 1955). Thus a triple combination anti-TB regimen consisting of INH, SM and PAS for 2 or 3 months followed by INH and PAS for the remaining months of at least 12 months of treatment was developed and was used as standard chemotherapy for the treatment of TB for 20 years (International Union against Tuberculosis 1964).

Short-course chemotherapy (SCC), was introduced in the early 1970s after the discovery of rifampicin (R; RMP). Following extensive *in vitro* studies in a murine model of TB which showed the unique sterilizing ability of RMP and PZA in combination with INH (Grosset 1978), a clinical trial in East Africa and Zambia investigated the efficacy

of 2 six-month regimens of daily chemotherapy, viz. SM, INH, RMP and SM, INH, PZA in the treatment of newly diagnosed extensive smear-positive pulmonary TB. In this study the relapse rate was reduced to 8% and 3% by the addition of PZA and RMP respectively (East African/ British Medical Research Councils 1973). Subsequent studies showed that adverse events were associated with SM but not with ethambutol (EMB; E) and chemotherapy with RMP and INH for 9 months supplemented in the first 2 months by EMB was recommended as the treatment of choice for pulmonary TB in Britain (British Thoracic and Tuberculosis Association 1976). Further studies showed that treatment could be reduced to 6 months by combining RMP and PZA (British Medical Research Council 1978).

The World Health Organization (WHO) currently recommends standard SCC for the treatment of all patients with active TB. Short course chemotherapy consists of 2 months of an intensive phase of treatment with RMP, INH, PZA and EMB followed by 4 months of a continuation phase of INH and RMP (World Health Organization Stop TB Department 2009). The aim of SCC is to shorten the treatment period by killing the bacterial population quickly and to prevent the emergence of drug resistance. The 4 drug combination in the intensive phase ensures early bacterial and sterilizing activity and the prevention of drug resistance while the 2 drugs in the continuation phase continue with sterilizing activity to prevent relapse (Mitchison 1979; Jindani et al 1980).

Various studies have shown the superiority of 6 months of RMP-based therapy for the treatment of TB in HIV-infected and HIV-negative patients. Studies by the British

Medical Research Council Tuberculosis Units in Africa, Singapore and India recorded 2-year relapse rates of 0%-3% in patients taking RMP for the entire treatment period of 6 months compared to 2-year relapse rates of 7%-18% in patients that took RMP for 2/6 months of therapy (Fox et al 1999). A subsequent study confirmed that RMP-based therapy for 6 months was superior to 8-month EMB-containing regiments in HIV-infected and HIV-negative patients. In this study, newly diagnosed, sputum smear-positive TB patients were randomized to receive either 2 RHZE daily followed by 6EH daily, or 2 RHZE three times per week (TPW) followed by 6EH daily, while a control arm received 2 RHZE daily followed by 4RH daily. Sputum negative cultures 12 months after the completion of therapy were highest for patients on the control regimen thus verifying that both 8-month EMB-based regimens were inferior to the 6-month RMP-based regimen (Jindani et al 2004). Similar observations were reported in a Ugandan study of 549 TB-HIV co-infected patients. After treatment with 2 RHZE, patients were randomized to receive either 4 months of RMP and INH, 6 months of RMP and INH or 6 months of EMB and INH TPW. After 2 months of treatment, more than 94% of patients had negative sputum mycobacterial cultures. However, during follow-up 26% of patients that took EMB-based therapy relapsed, compared to 7% and 13% of patients that took RMP-based therapy for 6 and 4 months respectively (Okwera et al 2006).

1.3. The Treatment of HIV-associated Tuberculosis

In 2003, the WHO declared TB a global public health emergency and since 2004 has embarked on a strategy known as collaborative TB/HIV activities to prevent, diagnose

and treat TB in persons living with HIV. One of the recommended interventions is the provision of antiretroviral therapy (ART) to all persons living with HIV (World Health Organization 2004) and the WHO has since begun up scaling access to ART in resource poor countries. By the end of 2010, 6.65 million adults and children received ART in low and middle-income countries (World Health Organization 2011a). In many resource-limited countries with a high burden of TB, a large proportion of TB-HIV co-infected patients present at TB clinics late and with advanced immunosuppression (Dean et al 2001; Manosuthi et al 2006) and thus require TB therapy and ART urgently.

Antiretroviral therapy plays a key role in the prevention of HIV-associated TB. In patients taking ART, the risk of developing TB is reduced by 70 - 90% (Badri et al 2002; Lawn et al 2005; Lawn et al 2009; Lawn et al 2011). However, the burden of TB among patients taking ART is still high (and above the background rates in HIV-negative patients) in resource-limited settings, particularly in patients with more advanced immunodeficiency and incomplete or delayed immune restoration (Lawn et al 2005; Lawn et al 2006; Houlihan et al 2010; van Rie et al 2011; Gupta et al 2012). Bonnet et al (2006) investigated the incidence of TB in 5 countries with a high TB burden and reported that the incidence of TB among HIV-infected patients with CD4 counts < 200 cells/μL ranged from 4.8/100 person-years in Cameroon to 17.7/100 person years in Kenya. In a more recent cross-sectional survey of 24 ART treatment programs in Africa, Asia and Latin America, the rate of incident TB in the first year of ART was reported to be 8.2 cases per 100 person-years (Fenner et al 2011). In

patients on ART, TB may occur as unmasking of sub-clinical disease (TB present but not diagnosed at ART initiation), activation of latent TB or new infections.

The WHO promotes the use of SCC and directly observed therapy (DOT) for the treatment of TB in HIV-positive patients and in patients without HIV co-infection (World Health Organisation Stop TB Department 2009). Studies have shown that this approach is effective, including in patients with advanced immunosuppression (Small et al 1991; Schurmann et al 1993; Kassim et al 1995; Perriens et al 1995; Chaisson et al 1996; El-Sadar et al 2001). However, some African countries that have used "standard" regimens that do not contain RMP have reported high rates of relapse and death (Perriens et al 1991; Hawken et al 1993; Elliott et al 2009). A review of CFRs in sub-Saharan Africa by Mukadi et al (2001) reports that mortality was higher in HIV-infected TB patients compared to HIV-negative patients during the course of TB therapy and that countries that used non-RMP-based therapy or used RMP only in the intensive phase. A recent meta-analysis by Khan et al (2010) reported that patients treated with a rifamycin for only two months were more likely to experience relapse than those patients treated with 6 to 8 months of rifamycin based therapy.

The benefits of concurrent antiretroviral (ARV) and TB therapy compared to sequential therapy in the treatment of HIV-infected TB patients with advanced immunosuppression were shown recently by various large studies. In the Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPIT) study in South Africa, TB-HIV co-infected patients with CD4 cell counts < 500 cells/mm^3 were randomized to receive ART during TB therapy (integrated arm) or at the completion of TB therapy

(sequential arm). This trial showed that starting ART therapy during TB therapy increased the survival of TB patients co-infected with HIV (Abdool Karim et al 2010). Data from the Multicenter Cohort of Patients with HIV infection in the Madrid South-Eastern Metropolitan Crown (COMESEM) study showed that survival could be increased further if ART was started within 2 months of stating TB therapy (Velasco et al 2009). A subsequent study, the Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA) study investigated the timing of ART on the survival of ART-naive TB-HIV co-infected patients with CD4 cell counts < 200 cells/mm^3. . Enrolled patients on standard TB therapy were randomized to receive efavirenz (EFV)-based ART 2 weeks after starting TB therapy (earlier treatment) or 8 weeks after starting TB treatment (later treatment). Mortality was significantly lower in the earlier treatment group (18%) compared to the later treatment group (27%). Thus initiating ART within 2 weeks of starting TB therapy significantly increases the survival of TB-HIV co-infected patients with CD4 cell counts ≤ 200 cell/mm^3 (Blanc et al 2011). Another large open label multicenter randomized study compared immediate (within 2 weeks of TB treatment initiation) to early (8-12 weeks) ART among TB-HIV co-infected patients with $CD4^+$ cell counts < 250 cells/mm^3 and confirmed the results of Blanc and colleagues. In this study, in patients with $CD4^+$ counts < 50 cells/mm^3, AIDS defining illnesses and death were 42% lower in patients on immediate ART compared to early ART (Havlir et al 2011). The combined results from these studies have led to new treatment guidelines, which recommend that all TB patients receive ART soon after starting TB therapy (World Health Organization 2010 Revision).

1.4. Current challenges in the treatment of HIV-associated TB

1.4.1. Overlapping toxicities between anti-TB drugs and antiretrovirals

Adverse drug reactions to anti-TB therapy and ART are similar and common in TB-HIV co-infected patients. The majority of adverse events occur within the first two months of starting therapy. Hepatotoxicity is a common adverse event in patients taking rifamycins, INH and PZA and may be compounded in patients taking concomitant non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). The risk of hepatotoxicity is increased further in patients with concomitant hepatitis C virus infection (Sterling et al 2010). Dean et al (2001) reported peripheral neuropathy, rash and gastrointestinal upset as the most common adverse events in TB-HIV co-infected patients taking concomitant anti-TB therapy and highly active anti-retroviral therapy (HAART). Peripheral neuropathy, rash and gastrointestinal upsets are reported with TB and HAART, however they are more common in TB-HIV co-infected patients (40%) compared to non-infected patients (26%) (Breen et al 2006).

1.4.2. Immune reconstitution inflammatory syndrome (IRIS)

Despite the benefits of starting ART soon after the start of TB therapy some patients experience a paradoxical worsening of symptoms or the onset of new symptoms which manifests as fever, rapidly enlarging lymph nodes, worsening pulmonary infiltrates, development of new plural effusions, appearance of a miliary pattern on chest x-rays and worsening central nervous system lesions. This worsening of the signs and symptoms of TB characterized by exaggerated inflammatory disease is called immune

reconstitution inflammatory syndrome (IRIS) (Narita et al 1998; Michailidis et al 2005; Lawn et al 2008). The initiation of ART stimulates the reconstitution of immune responses to mycobacteria, and cytokine secretion, particularly of interferon (IFN)-γ is boosted. It is believed that IRIS may be a result of this sudden boosting of previously diminished immune function (Schluger et al 2002; Elliott et al 2009).

There are two types of IRIS presentation. The most common form, reported in 8 to 43% of patients, occurs in patients on TB treatment who soon after starting ART experience a deterioration of the clinical manifestations of TB. The second form of IRIS is called “unmasking” of subclinical TB which occurs as immune function is reinstated by ART (Lawn et al 2008). Risk factors for IRIS include starting ART within the first two months of TB treatment, extra-pulmonary or disseminated TB; low $CD4^+$ cell counts at the start of ART, a viral load greater than 10^5 $\log_{10}$ copies/mL and a good immunological and virological response during HAART. Death from IRIS is rare and management with short-term corticosteroids (without interrupting TB therapy) suppresses the enhanced immune response (Colebunders et al 2006).

1.4.3. Malabsorption of anti-Tuberculosis drugs

The malabsorption of anti-TB drugs, particularly RMP has been reported in TB patients with and without HIV infection (Peloquin et al 1993; Choudhri et al 1996; Peloquin et al 1996; Sahai et al 1997; Gurumurthy et al 2004; Perlman et al 2005; Tappero et al 2005; McIlleron et al 2006; Pinheiro et al 2006; McIlleron et al 2012). In some

populations sub-optimal RMP concentrations have been reported in 70-80% of co-infected patients (van Crevel et al 2002; Tappero et al 2005). Lower rifamycin exposure may contribute to lower effectiveness of anti-TB treatment and acquired rifamycin resistance (ARR) (Spradling et al 2002; Li et al 2005; Weiner et al 2005a; Burman et al 2006).

There is evidence to suggest that the absorption of rifamycins is affected by certain disease states. In Indonesian patients with TB and type 2 diabetes, RMP exposure was decreased by 53% compared to patients with TB alone (Nijland et al 2006). Low plasma concentrations of RMP were also observed in patients with cystic fibrosis and mycobacterial disease (Gilljam et al 1999). In TB-HIV co-infected patients, lower plasma RMP concentrations are associated with advancing HIV disease (evidenced by lower $CD4^+$ cell counts) (Sahai et al 1997; Gurumurthy et al 2004). Low bioavailability of anti-TB drugs has been associated with various factors such as alcohol use (Kimerling et al 1998), race (Tappero et al 2005), age (McIlleron et al 2006), gender (Sahai et al 1997; Ray et al 2003; McIlleron et al 2012), hypoalbuminaemia (Tappero et al 2005) and dose per kg of body weight (McIlleron et al 2006). HIV-associated alterations in gastro-intestinal structure and function may also be responsible for the altered absorption of anti-TB drugs (Smith et al 1992; Lambi et al 1996).

1.4.4. Drug-drug interactions

Pharmacokinetic (PK) drug-drug interactions occur when drugs that share a common metabolic or elimination pathway are administered together. In humans, approximately 50% of all drugs are metabolized by cytochrome 450 (CYP450) isoenzymes. CYP 450 is located in the gut wall (enterocyte) of the small intestine and in the liver. Cytochrome 3A4 (CYP 3A4) is the most prominent form of CYP 450 involved in drug metabolism. Other forms of CYP 450 involved in drug metabolism include CYP2D6, 2C9, 2C19, 1A2, 2B6, 2E1 and 2A6 (Bachmann et al 1993; Diliger et al 1999). Cytochrome 450 metabolizes various drugs, including antiretrovirals (ARVs). Nevirapine and all PIs are metabolized predominantly by CYP3A4 (Erickson et al 1999; Riska et al 1999). Efavirenz is metabolized predominantly by hepatic CYP2B6 and partially by CYP3A4 and CYP2A6 (Ward et al 2003; Desta et al 2007). Rifampicin is a strong inducer of CYP3A4 and CYP2B6 (Rae et al 2001). Rifampicin increases the expression of CYP 3A4 and CYP2B6 by binding with the nuclear receptors, pregnane X receptor (PXR) or constitutive androstane receptor (CAR) which then form a heterodimer with the retinoid X receptor (RXR). The heterodimer interacts with the regulatory region of the CYP3A4 and CYP2B6 genes thereby increasing the synthesis of CYP3A4 and CYP2B6. Rifampicin also induces the activity of phase II enzymes (Diliger et al 1999; Gallicano et al 1999) and membrane transporters such as P-glycoprotein (PGP) (Greiner et al 1999) via activation of PXR.

1.5. Study Objectives and Scope of the thesis

The general aim of this study was to investigate the pharmacokinetics of rifabutin (RBT) given alone and in combination with ART in African TB-HIV co-infected patients with advanced immunosuppression. The specific aims were to:

1. Describe the pharmacokinetics of standard doses of RBT (300mg) in TB-HIV co-infected African patients with advanced immunosuppression

2. Investigate the pharmacokinetic interaction between two different doses of RBT and NNRTIs, viz. EFV and nevirapine (NVP)

3. Investigate the pharmacokinetic interaction between two different doses of RBT and boosted lopinavir (LVP/r)

4. Assess the frequency and severity of adverse drug events associated with the combination of RBT and ART

5. Investigate the frequency of SNPs of SLCO1B1 in African TB patients co-infected with HIV and their influence on RBT exposure

Section 2 Literature Review

1.6. Discovery Of Rifabutin

Rifabutin is a member of the rifamycin family of antibiotics. The rifamycins are closely related bactericidal antibiotics. During a routine screening exercise for new antibiotics by staff of the Lepetit Research Laboratories (Milan, Italy) in 1957, an organism was isolated from a pine forest in France. Culture of this organism (initially believed to be

Streptomyces but later classified as *Amycolatopsis)* produced a supernatant containing several substances with activity against gram-positive bacteria and mycobacteria. Further studies on the individual components of the mixture revealed substances that were highly active against mycobacteria and gram-negative bacteria. Following collaborative research with Ciba-Geigy (Basel, Switzerland) to produce an anti-mycobacterial agent with good oral bioavailability and potent activity against Mycobacterium tuberculosis, RMP was developed, followed by RBT and rifapentine (RPT).

The first step in the discovery of the rifamycins was the identification of a substance (ME/83) with antibiotic activity against Gram-positive bacteria, produced by cultures of the organism (Sensi 1983). Biochemical analysis identified a mixture of 5 microbiologically active substances collectively named rifamycins (Margalith and Pagani 1961). Further biochemical analysis led to the production of rifamycin B, which possessed extremely low toxicity, but was poorly active against gram-positive bacteria and converted spontaneously to rifamycin SV. Rifamycin SV had higher antibacterial activity; therefore a decision was taken to investigate chemical modifications of rifamycin B. Rifamycin SV showed high antibacterial activity *in vitro*, particularly against gram-positive bacteria and *Mycobacterium tuberculosis* (*M. tuberculosis)* as well as low toxicity. Thus Rifamycin SV was the first rifamycin to be used clinically and was administered intravenously for the treatment of infections due to gram-positive bacteria, infections of the biliary tract, TB and leprosy. Rifamycin SV was not effective

in the treatment of TB because therapeutic drug levels in blood were not achieved as the drug was concentrated in the liver and rapidly excreted in bile (Sensi 1966).

A new rifamycin was sought with better oral absorption, slower biliary excretion and better activity against *M. tuberculosis* than Rifamycin SV. Modifications of the aliphatic bridge, glycolic chain and chromophoric nucleus of rifamycin B were studied. The *N,N*-disubstituted hydrazone derivatives of rifamycin B exhibited the highest antitubercular activity and were chosen for more extensive evaluation. All the derivatives of this class showed high *in vivo* activity against staphyloccal infection in mice, possessed good oral absorption as well as low biliary excretion. The most active derivative of this class was the hydrazone with *N*-amino-*N'*-methylpiperazine which was named RMP (Sensi 1966). Further biochemical modifications of the side chain at carbon-3 in rifamycin S produced 4-N-isobutylspiropiperidylrifamycin S (Sanfilippo et al 1980). This derivative of rifamycin S, RBT (initially named LM 427), showed remarkable activity against *M. tuberculosis in vitro* and *in vivo.* Further research by the Centre for Disease Control in Atlanta (USA) showed that LM 427 inhibited growth of a number of clinical strains of RMP-resistant *M. tuberculosis* and displayed potent activity against strains of *Mycobacterium avium intracellulare* complex (MAC) (Della Bruna et al 1983).

1.7. Structure Of Rifabutin

The rifamycins have closely similar chemical structures. A common feature of all rifamycins is a chromophoric naphthohydroquinone group spanned by a long aliphatic

bridge and an acetyl group at position 25 (Sensi et al 1966). The structural difference between the three rifamycins is due to a different functional group at Carbon 3 (shown by arrows in Figure 1.2). Although the rifamycins have similar chemical structures, they have different physio-chemical and biological properties.

Rifabutin is a spiro-piperidyl rifamycin and has a unique structure in which positions 3 and 4 are incorporated into an imidazoline ring bearing a spiro-piperidyl group while RMP and RPT have a piperazinyl iminomethyl group (Figure 1.2). The piperazinyl iminomethyl group is recognized by receptors on enzymes that catalyze the deacetylation of RMP and RPT. The absence of this group in the chemical structure of RBT gives RBT a more rigid ring structure that imparts steaic hinderance to the binding of RBT to receptors on enzymes that catalyze the deacetylation of the rifamycins and is probably the reason why the metabolism of RBT does not occur predominantly by deacteylation (Nakajima et al 2011).

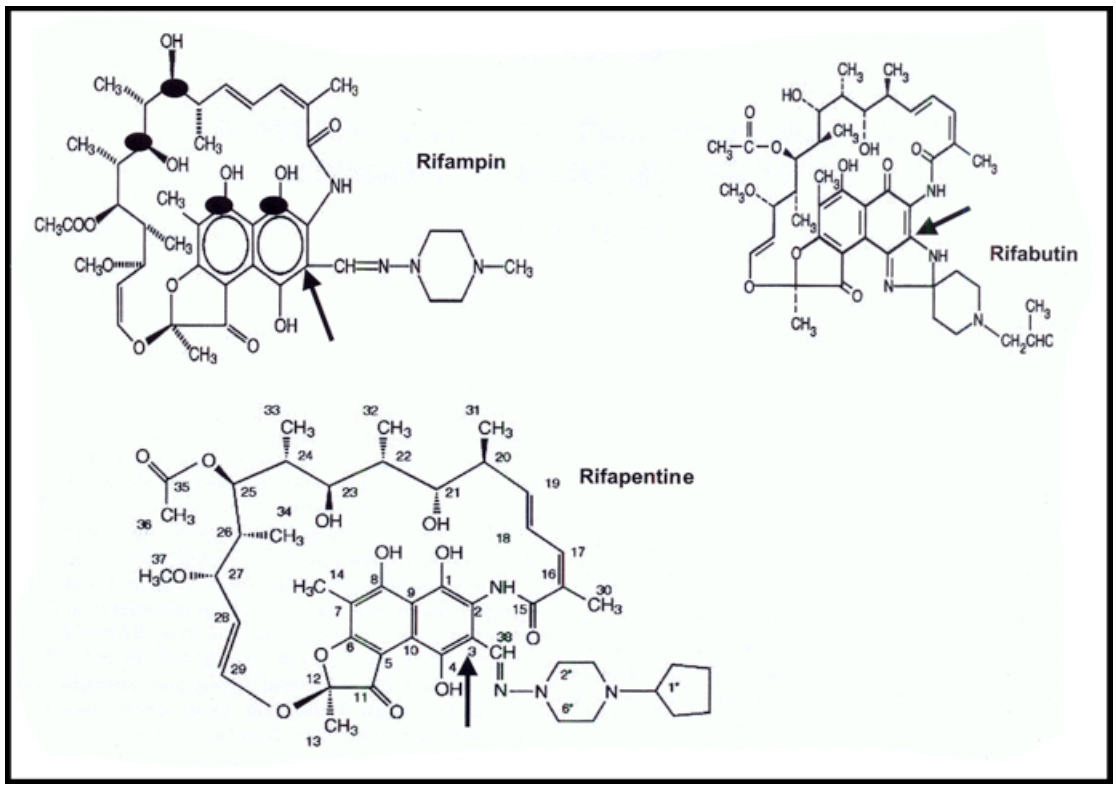

Figure 1.2. Chemical structures of the rifamycins (Vernon 2004)

1.8. Physio-chemical Properties of Rifabutin

The molecular formula of RBT is $C_{46}H_{62}N_4O_{11}$ and its molecular weight is 847.02. The pKa of RBT is 6.9, hence it is highly lipid soluble. It is absorbed well after oral ingestion and distributes extensively in tissues. Rifabutin penetrates easily into polymorphonuclear leukocytes, lymphocytes and macrophages. High concentrations of RBT are attained in the liver and lung (Brogden and Fitton 1994).

At physiological pH, RBT is mostly unionized while RMP exists as a mixture of zwitterionic and anionic species (Acocella et al 1978; Narang et al 1995). On the basis

of their different ionization states, RBT and RMP possess different distribution coefficients. Rifabutin has a higher octanol / water partition coefficient (logP) and lower water solubility than RMP. The solubility of RBT is pH dependant (stable at pH range of 2-8), therefore dissolution occurs in the stomach and absorption occurs in the small intestine where pH is higher. Rifabutin is also more lipophilic than RMP; therefore uptake of RBT into intracellular tissues and macrophages is greater than that of RMP (Acocella et al 1978; Narang 1995).

1.9. Mode Of Action

The predominant biological activity of the rifamycins is the inhibition of gram-positive bacteria and mycobacteria. They exhibit selective toxicity against these pathogens, exerting their antibacterial activity by blocking the synthesis of bacterial RNA but not interfering with host RNA. The macrocyclic ring of the rifamycin binds with bacterial RNA forming a complex that inactivates RNA polymerase (Wehrli and Staehelin1971). Rifabutin also has a unique ability to inhibit bacterial DNA synthesis (Della Bruna et al. 1983). This unique characteristic of RBT might be responsible for its effects against RMP-resistant strains (Olliaro et al 1995).

1.10. Antimicrobial Actions Of Rifabutin

Rifabutin is a broad-spectrum antibiotic, effective against both Gram-positive and Gram-negative bacteria. However, its antibacterial effects are most profound against

Mycobacteria. Initial *in vitro* studies (Sanfilippo et al 1980) showed that the spiro-piperidyl rifamycins demonstrated potent activity against *M. tuberculosis*, which increased as the number of carbon atoms in the linear side chain increased. In comparison to RMP, these rifamycins displayed better gastrointestinal absorption, lower plasma levels and higher concentrations in tissues. Further *in vitro* studies on the minimum inhibitory concentration (MIC) of RBT showed that RBT possessed greater anti-mycobacterial activity than RMP and that 31% of *M. tuberculosis* strains resistant to RMP were susceptible to RBT (Woodley et al 1982; Dickinson and Mitchison 1987).

The MIC of RBT for *M. tuberculosis* was initially reported to be 0.003-0.06 mg/L in liquid media (eg. Bactec), 0.03 - 0.08 mg/L in solid media (eg. 7H10, 7H11), and 0.25-1 mg/L in Loewenstein-Jensen and the MBC/MIC ratio for *M.tuberculosis* is 4 (Heifets et al 1988). A study by Breda et al (1992) in healthy volunteers showed that plasma concentrations of RBT above the MICs for RMP-resistant strains of *M.tuberculosis* are maintained for 12-24 hours with 150-300 mg daily. Recently, Rastogi et al (2000) compared the MICs of RMP, RBT and RPT in 7H11 agar medium and Bactec broth against members of the *M.tuberculosis* complex. The MIC90 of RBT was 0.063 mg/L and 16.0 mg/L in Bactec and 7H11 against drug-susceptible and RMP-resistant isolates of *M.tuberculosis* respectively. When compared with RMP and RPT, the inhibitory activity of RBT was greater than RMP and comparable to RPT (Table 1.2).

Table 1.2. The minimum inhibitory concentrations of rifabutin, rifampicin and rifapentine in Bactec and 7H11 against members of the *M.tuberculosis* complex (from Rastogi et al 2000)

	Rifabutin		**Rifampicin**		**Rifapentine**	
Species	Bactec	7H11	Bactec	7H11	Bactec	7H11
*M.tuberculosis**	0.063	0.063	0.25	0.25	0.063	0.125
*M.tuberculosis***	16.0	16.0	>32.0	>64.0	>32.0	64.0
M.africanum	0.063	0.125	0.125	0.50	0.031	0.25
M.bovis	0.125	0.125	0.125	0.125	0.063	0.125

* = drug susceptible; ** = RMP-resistant

1.11. Activity Of Rifabutin In Animal Models

In *in vivo* studies of experimental mice TB, RBT was 2-3 times more potent than RMP (Sanfilippo et al 1980). In addition, RBT was effective against RMP-resistant *M.avium intracellulare* (Woodley et al 1982). Further studies by (Luna-Herrera et al 1995) on the *in-vitro* and intracellular activity of RBT on drug-susceptible and multi drug resistant (MDR) tubercle bacilli showed that the in-vitro activity of RBT was comparable to or greater than the activity of RMP as determined by the MIC, minimal bactericidal concentration (MBC), MBC/MIC ratios as well as continuous exposure and post-antibiotic effect studies. The MICs of RBT with RMP susceptible strains were much lower than those of RMP (MIC_{90} for RBT was 0.06 mg/L and 0.25 mg/L for RMP). Intracellular activity of RBT in macrophages showed that RBT displayed equally high potency against *M. tuberculosis* (strain H37Rv) as well as RMP-resistant strains (strain 2227). This study also showed that RBT activity against tubercle bacilli persisted even

after the drug was removed indicating a prolonged post-antibiotic effect of RBT against intracellular and extracellular organisms. In a murine model of TB (BCG-vaccinated and *M. tuberculosis* infected immunocompetent mice) comparing the pharmacokinetics and antimicrobial activities of RMP, RBT and RPT, Ji et al (1993) have shown that RBT displayed the lowest C_{max} but RBT was more bactericidal than RMP and comparable to RPT. The activity of RMP was significantly reduced when dosage was reduced from daily to TPW whereas the bactericidal activity of RBT was strongly maintained at 10 mg/kg twice weekly. In another study by Jabes et al (1994), the efficacies of 12-week regimens of daily RBT or INH or intermittent combination of the two drugs were evaluated in a murine model of TB. Daily doses of RBT at 10mg/kg was highly effective as by 8 weeks of treatment all spleens were sterilized and the number of bacilli was significantly reduced in the lungs and bacilli were completely eradicated from the spleens and lungs of mice after 12 weeks of treatment. Furthermore, the combination of RBT 10 mg/kg plus INH 25mg/kg twice weekly was almost as effective as RBT daily but daily INH and once-weekly RBT were less effective. Thus data from this study showed that RBT given alone, daily or twice weekly in combination with INH was effective in the treatment of TB.

The efficacy of an anti-bacterial is determined by the concentration of the drug, or the length of time bacteria is exposed to the drug. Consequently, antibiotics are usually classified as concentration-dependent or time-dependent (Craig 1998). The efficacy of a dose is determined by pharmacokinetic / pharmacodynamic (PK / PD) parameters such as the ratio of the peak concentration to minimum inhibitory concentration (C_{max}/

MIC), the ratio of 24-hour area under the curve to MIC (AUC_{0-24} / MIC) or time above MIC (Craig 2001). Presently, rifamycin PK/PD studies in humans are limited and there is controversy over whether the AUC_{0-24} / MIC or C_{max}/ MIC ratio correlates best with rifamycin anti-bacterial activity. Early studies in guinea pigs (Mitchison and Dickinson 1971) and mice (Verbist 1969) suggested that RMP displayed concentration-dependent anti-bacterial activity, which corresponded with a C_{max}/ MIC ratio. Subsequently evidence from a mouse model of TB by Jayaram et al (2003) suggested that the antimicrobial activity of RMP correlated best with the AUC / MIC ratio and Gumbo et al (2007) confirmed this in an *in vitro* hollow fiber system. Gumbo et al also demonstrated that the prevention of resistance was related to C_{max}/ MIC.

Recently, Diacon et al (2007) investigated the effect of increasing doses of RMP on early bactericidal activity (EBA) in South African patients. Their results showed that as the dose of RMP increased, AUC, C_{max} and EBA also increased. Clinical studies comparing higher doses of RMP to standard doses in pulmonary TB patients by Ruslami et al (2007) reported that increasing the RMP dose from 10 to 13 mg/kg was associated with increases in the mean AUC and C_{max} of 65% and 49% respectively. However these studies were unable to clearly determine if the AUC and C_{max} were correlated with the EBA.

1.12. Pharmacokinetics of Rifabutin

1.12.1. Dosage and Administration

Rifabutin is available as 150 mg capsules and can be administered as a single daily dose of 300 mg, independent of meals, for the treatment of newly diagnosed pulmonary TB, chronic RMP-resistant pulmonary TB, atypical *Mycobacterial* infections and/or prophylaxis of MAC in HIV-infected patients with advanced immunosuppression (Pharmacia Upjohn Company).

1.12.2. Absorption and Bioavailability

Rifabutin was rapidly absorbed after administration of four oral doses (300, 600, 900 and 1 200 mg/day) to HIV-infected male patients (Skinner et al 1989) and a single 270 mg dose to healthy volunteers (Battaglia et al 1990). Maximum concentration in plasma (C_{max}) was attained in 2-3 hours after drug ingestion and increased approximately proportionally as the dose increased (Skinner et al 1989). Peak plasma concentration (C_{max}) of RBT after a 600mg oral dose is 0.4 – 0.6 µg/mL, which is approximately 10-fold lower than the C_{max} of a 600mg dose of RMP (Blaschke et al 1996). At the currently licensed dose of 300mg, the C_{max} of RBT is reported to be 0.2 to 0.9 mg/L in HIV-infected patients. Mean trough plasma concentrations (C_{min}) obtained 24h after ingestion of 300 or 600mg doses were 0.05 mg/L (Skinner et al., 1989). The ingestion of RBT with food does not influence the C_{max} but slows the rate of absorption. Thus AUC and C_{max} are unaffected but T_{max} is increased (Narang et al 1992).

In one study (Skinner et al 1989), the AUC of RBT on the first day of administration was significantly higher than the AUC after 28 days (at steady state) for each of the four doses. This indicates that auto induction of RBT metabolism occurs. The reported bioavailability in this study was low (compared to that of RMP) and showed substantial inter-patient variability. Average bioavailability was approximately 20% and ranged from 3% to 42%. However, the factors influencing RBT bioavailability were not investigated. A subsequent study on the influence of gender on the pharmacokinetics of RBT by Colborn et al (1993) has shown that the extent of RBT absorption may be slightly greater in females due to its high lipophilic nature and given that females have a higher body fat composition compared to males.

A formal study on the effect of HIV infection on the pharmacokinetics of RBT has to date not been reported. An initial report on pooled data from 5 Phase I studies on the prophylactic use of RBT for MAC showed that RBT absorption was not associated with the severity of immunosuppression in HIV-infected patients (Narang et al 1996). Subsequently (Gatti et al 1999), it was showed that RBT $AUC_{0\text{-}24}$ and C_{max} were similar in HIV-infected patients with and without wasting syndrome. A population pharmacokinetic study of RBT in HIV-infected patients confirmed this. The study did not show that RBT exposure was influenced by severity of disease or gastrointestinal function (Gatti et al 1998). However, a review on RBT pharmacology by Narang (1995) reported higher inter-patient variability among the HIV-infected patients and a subsequent study by the same author (Narang et al 1996) confirmed that the variability

of RBT AUC in HIV-infected patients was approximately double that observed in healthy volunteers.

1.12.3. Distribution

The AUC curves of RBT show a clear distribution phase (Skinner et al 1989; Gatti et al 1998) with a distribution half-life of approximately 2-3 hours. The large differences between peak and trough plasma concentrations on the first day and after chronic administration also indicate the presence of a distribution phase. The mean volume of distribution determined in five HIV-positive patients who were receiving RBT intravenously was 9.3 L/kg (Skinner et al 1989).

The extent of RBT binding to plasma proteins is reported to be 71% (Skinner et al 1989) and the predicted free serum peak concentration is 0.13 mg/L (Burman et al 2001). Rifabutin penetrates across clinically un-inflamed meninges and concentrations between 30 and 70% of those in plasma have been recorded in cerebrospinal fluid from HIV-infected patients treated with doses of 300 to 900 mg/day. Concentrations of RBT in bile and urine are reported to be more than 100 times greater than concentrations in plasma and tissue concentrations are approximately 8.5 times greater than plasma 5-12 hours after administration of a single 150 or 300mg dose to HIV-infected patients (Brogden and Fitton 1994).

1.12.4. Elimination

The primary pathway of RBT elimination is metabolism. Systemic clearance is reported to be 0.81L/h/kg in healthy volunteers after a single 150mg dose and the mean plasma elimination half-life varies between individuals and studies, ranging from 32 to 67 hours (Narang et al 1992; Brogden and Fitton 1994). In humans, RBT is metabolized extensively and eliminated by both urinary and fecal excretion (Battaglia et al 1990). Approximately 5% of the dose is excreted as unchanged drug in urine (Strolin Benedetti et al 1990). The major lipophilic metabolites identified in human urine are 25-O-desacetyl-rifabutin (d-RBT), 31-hydroxyrifabutin and 32-hydroxy-rifabutin, 20-hydroxyrifabutin and 27-*O*-desmethylrifabutin (Battaglia et al 1990; Utkin et al 1997; Iatsimirskaia et al 1997). The plasma concentration of d-RBT is approximately 5% that of the parent drug and its antibacterial potency is similar to the parent drug (Brogden and Fitton 1994; Blaschke and Skinner 1996).

Studies on the enzyme systems involved in the metabolism of RBT were undertaken in by Iatsimirskaia et al (1997), Jamis-Dow et al (1997), and Trapnell et al (1997) and more recently by Prasad and Singh (2012). The study by Iatsimirskaia et al. (1997) showed that RBT was readily metabolized by CYP3A4 in both human enterocytes and liver microsomes with similar metabolic patterns and kinetic pathways. Furthermore, in both enterocytes and liver microsomes, RBT metabolism was inhibited by clarithromycin, fluconazole and ketoconazole, which are known inhibitors of CYP3A4, and unaffected by azithromycin. The inhibition of RBT metabolism was more pronounced in enterocyte microsomes than in liver microsomes indicating that much

interaction occurs in the intestine during concurrent drug absorption. In contrast, the study by Trapnell et al. (1997) employing human liver microsomes and recombinant human CYP450 demonstrated that RBT was not metabolized by CYP3A4 and that CYP3A4 was only responsible for the biotransformation of RBT to d-RBT. However, the recent study by Prasad and Singh establishes that RBT is extensively metabolized by CYP3A4. Prasad and Singh incubated RMP, RBT and RPT with recombinant CYP450 isoforms, CYP1A2, CYP2C19, CYP2D6 and CYP3A4. The metabolism of RBT by CYP3A4 was extensive (60-70%) compared to that of RMP and RPT (< 20%). CYP1A2, CYP2C19 and CYP2D6 had no significant effect of the metabolism of all three rifamycins.

Studies by Iatsimirskaia (1997) and Jamis-Dow (1997) have also shown that RBT is deacetylated to d-RBT by microsomal cholinesterase (β-esterase). Cholinesterases are also located in the liver and intestinal mucosa as well as in the circulation. However, RBT was not deacetylated when incubated with human serum or whole blood by Iatsimirskaia et al. indicating that the metabolism of RBT occurs only in the liver and intestine. Consequently, in patients with impaired liver function the RBT AUC and half-life were significantly increased (Benedetti et al 1993), whereas a case report of RBT pharmacokinetics in a haemodialysis patient by Bassilios et al (2002) shows that the pharmacokinetics of RBT were not altered by renal insufficiency and that dialysis did not significantly remove RBT.

Rifabutin also induces its own metabolism after repeated administration, similar to that of RMP and RPT. Strolin-Benedetti et al (1990) studied the auto induction of RBT metabolism in 7 healthy male volunteers. Subjects were given RBT 450mg orally after fasting for 10 hours on day 1. From day 6 onwards they were given the same daily dose for 10 days. Plasma concentrations of RBT, d-RBT and 31-hydroxyrifabutin were measured after the single and last dose using HPLC with UV detection. Rifabutin AUC_{0-24} after the last (10^{th}) dose was significantly lower than the AUC_{0-24} of the first dose. However, there was no significant change in the elimination half-life between the first and 10^{th} doses. The C_{max} of d-RBT decreased significantly after repeated administration while, the C_{max} of 31-hydroxyrifabutin increased by about 54%. It is believed that the auto induction of RBT is stimulated by intestinal CYP 3A4.

1.13. Pharmacogenomics

Previous pharmacokinetic studies on RBT and RMP in healthy volunteers, TB patients and TB-HIV co-infected patients have observed a wide distribution of drug concentrations (Narang 1995; Skinner et al 1989; Peloquin et al 1997; Peloquin et al 1999; Wilkins et al 2008). Pharmacokinetic variability has been associated with the emergence of resistance in patients (Weiner et al 2005a) and model systems (Strivastava et al 2012). At the other extreme, higher exposure to RBT has been associated with increased toxicity. However, the factors determining such variation in drug exposure are not completely understood.

Pharmacogenomic studies in HIV-infected individuals have identified genetic differences and profiles that affect drug bioavailability and responses to ARVs (Haas et al 2005). For example, the ABCB1 (MDR 1) gene encodes PGP (an ABC transporter). P-glycoprotein is known to act on a broad range of drugs, therefore a number of studies have investigated the functional consequences of variation in the ABCB1 gene particularly the ABCB1 C3435T polymorphism. Studies have shown an association with this variant and serum drug levels, and with responses to a number of drugs (Loeuillet et al 2007). Plasma concentrations of the PIs have been associated with the T allele at the MDR1 C3435T locus (Saitoh et al 2005). In addition LPV and RTV are substrates, and may also inhibit and / or induce ABCB1. Similarly, genetic differences in the MDR1 gene may also affect the exposure and disposition of anti-TB drugs. Rifampicin is also a substrate of PGP and a recent study (Davies et al 2008) has shown that in MDR1 C3435T CC homozygotes, the bioavailability of RMP was reduced by 33% but remained unchanged in the TT homozygotes. There are ethnic differences in the allele frequencies of MDR1. Various studies have shown that Africans have a higher frequency of the CC genotype (and lower frequency of the TT phenotype) compared to Caucasians (Ameyaw et al 2001; Kim et al 2001; Schaeffeler et al 2001).

The organic anion transporting polypeptide 1B1 (OATP1B1) is an uptake transporter. The SLCO1B1 gene encodes OATP1B1 (Hagenbuch et al 2003) and SNPs have been identified in the SLCO1B1 gene, which may result in significant alteration of drug pharmacokinetics by decreasing the uptake activity of the corresponding OATP protein. Furthermore, the allele frequencies of SLCO1B1 polymorphisms vary markedly

between different populations (Pasanen et al 2008). A recent study has shown that RMP concentrations are lower in African American patients and in these patients the SLCO1B1 SNP rss11045819 (C463A) has been associated with reduced RMP exposure (Weiner et al 2010). In another South African study a different polymorphism, rs4149032 SNP, was reported in 70% of individuals and patients heterozygous and homozygous for this polymorphism had reductions of 18% and 28% respectively, in the bioavailability of RMP (Chigutsa et al 2011).

Although a few studies have been done on the pharmacogenomics of RMP as cited above there are no studies reporting on the pharmacogenomics of RBT. Information on the pharmacogenomics of RBT may help with the design of treatment regiments for the treatment of TB-HIV co-infection especially in African populations.

1.14. Human Studies on Rifabutin

In humans, initial studies on RBT focused on the treatment of disseminated MAC infection in HIV infected patients (O'Brien et al1987). Two randomized double blind multi-center trials administered 300mg RBT or placebo daily to HIV infected patients with advanced immunosuppression ($CD4^+$ cell count < 200 cells/mm^3). In the first trial, MAC bacteremia developed in 17% of patients assigned the placebo and in 8% of patients assigned RBT. In the second trial, 18% of patients in the placebo group and 9% of patients in the RBT group developed MAC bacteremia. Patients in the RBT group also showed a significant gain in well-being and longer survival (Nightingale et al

1993). Rifabutin administered to HIV infected patients at doses of 150 – 600 mg/day as prophylaxis for MAC was well tolerated (Olliaro et al 1993) hence RBT has been recommended as prophylaxis to prevent disseminated MAC in HIV infected patients with advanced immunosuppression.

Clinical data on the efficacy of RBT in the treatment of pulmonary TB in HIV co-infected patients is limited. Data from 5 randomized control trials (RCTs) (Hong Kong Chest Service/British Medical Research Council 1992, Gonzalez-Montaner et al 1994, McGregor et al 1996, Schwander et al 1995) that assessed the efficacy of RBT in TB disease show that RMP and RBT are equally effective. However, TB-HIV co-infected patients were under-represented in the RCTs, comprising only 5% of the randomized patients.

In a pilot study in Uganda, that compared the efficacy of a RBT-based regimen against a RMP-based regimen for the treatment of pulmonary TB, RBT and RMP showed comparable efficacy. However, patients treated with RBT had significantly more rapid clearance of acid-fast bacilli from sputum at 2 months and over the entire study period than patients treated with RMP (Schwander et al 1995). Another multi-centre clinical trial compared the efficacy of RBT- and RMP-based SCC in the treatment of TB-HIV co-infected patients. Newly diagnosed smear positive TB patients were treated with RBT 150mg daily, RBT 300mg daily or RMP 600mg daily and sputum smear conversion rates of 94%, 92% and 89% respectively were produced (Gonzalez-

Montaner et al 1994). Both these clinical trials have shown that RBT is effective in the treatment of pulmonary TB, however the trials enrolled a small number of patients and only one trial was conducted in HIV-positive patients. Collectively these trials provide no evidence that RBT is inferior to RMP, but none of them were sufficiently powered to show superiority of one rifamycin over another. A recent review on the use of RBT for the treatment of pulmonary TB suggests that there is insufficient data on the efficacy of RBT in the treatment of TB in HIV-co-infected patients and more studies are required (Davies et al 2007).

1.15. Clinical Pharmacology of Rifabutin

1.15.1. Drug Interactions With Non-ARVs

HIV-positive patients are administered cotrimoxazole (CTX) prophylactic therapy according to WHO guidelines. Therefore many patients who require anti-TB therapy and are prescribed RBT will be taking RBT and CTX concurrently. Colborn et al (1996) have investigated the interaction between CTX and standard doses of RBT in 12 HIV-infected patients. At steady state, RBT and d-RBT $AUC_{0\text{-}24}$ and C_{max} were not altered by concomitant CTX.

Breda et al (1992) investigated the effects of RBT on INH pharmacokinetics and metabolism in 6 healthy volunteers. This study found no significant effect on the pharmacokinetics of INH or its metabolism by concomitant RBT, including patients that were rapid acetylators. Breda et al (1999) also investigated the effects of concomitant

RBT on the pharmacokinetics of EMB in healthy volunteers. No statistically significant differences were found in the AUC, C_{max}, T_{max}, half—life and clearance of EMB.

Due to the metabolism of RBT through CYP3A4, inhibitors of this enzyme are predicted to produce significant increases in RBT bioavailability. Jordan et al (2000) investigated the pharmacokinetics of RBT in the presence of fluconazole alone, clarithromycin alone, or both agents together in HIV-infected patients. Rifabutin AUC_{0-24} increased by 76% when combined with either fluconazole or clarithromycin and 152% when both drugs were given together with RBT. Plasma concentrations of d-RBT were also significantly increased (fivefold higher) in the presence of combined RBT, fluconazole and clarithromycin. The incidence of leukopenia was 38% in patients receiving the drug combination and 19% in patients receiving RBT alone.

In a phase I open label, crossover trial of concomitant RBT and fluconazole, the plasma concentrations of both RBT and d-RBT were significantly increased in 12 HIV-infected patients (Trapnell et al 1996). Rifabutin AUC_{0-24} was increased by 82% and that of the metabolite was increased by 216%. A study by (Hafner et al 2001) reported no significant changes in the pharmacokinetics of either drug when RBT and azithromycin were co-administered.

1.15.2. Drug Interactions Between Rifabutin and ARVs

1.15.2.1. NRTIs

An initial study of 16 HIV-infected patients receiving concomitant zidovudine (ZDV) and RBT (either 300mg or 450mg), reported that concomitant RBT decreased ZDV C_{max} and AUC by 48% and 32% respectively; and increased ZDV clearance by 43% (Narang et al 1992). However, a subsequent study found that the only significant interaction observed by the concomitant administration of RBT 300mg daily and ZDV was a 28% decrease in the terminal half-life of ZDV (Gallicano et al 1995). Therefore RBT can be used concurrently with ZDV in the treatment of HIV-positive patients. Sahai et al (1995) investigated the pharmacokinetic interaction between RBT (either 300 or 600mg) and didanosine (ddI) in HIV-infected patients and found no significant pharmacokinetic interactions between the 2 drugs; and there were no adverse events associated with the combined administration of both drugs.

1.15.3.1. Drug Interactions between Rifampicin and Nevirapine

Studies from Africa, India, South-Asia and Europe have reported significant reductions in plasma NVP concentrations in the presence of RMP leading to sub-therapeutic NVP trough concentrations in a large proportion of patients. Reductions of 31 and 21% in NVP AUC_{0-12} and C_{12} respectively, have been reported in TB-HIV co-infected Spanish patients treated with concomitant NVP and RMP (Ribera et al 2001). In a South African study, NVP trough plasma concentrations were decreased to below the recommended level of 3.4 mg/L in 38% of patients (Cohen et al 2008). In Malawians and Ugandan patients, sub-therapeutic plasma NVP concentrations were reported in approximately

60% of patients in the lead in phase of treatment with NVP in the presence of RMP (van Oosterhout et al 2007; Lamorde et al 2010).

In an Indian study, NVP C_{max}, C_{12} and AUC_{0-12} were decreased by 42%, 53% and 46% respectively in the presence of RMP and the C_{12} of NVP was sub-therapeutic in 8/13 patients (Ramachandran et al 2006). In Thai HIV-infected patients, mean trough plasma NVP concentrations decreased by 17.7 % in patients taking RMP and NVP concomitantly (Manosuthi et al 2006) and subsequently increased by 16.7% after discontinuation of RMP therapy (Manosuthi et al 2007).

In another study, Thai patients were investigated to see whether RMP co-administration in clinical practice led to clinically relevant decrease of NVP plasma concentrations. They compared NVP pharmacokinetics in 74 patients receiving concomitant NVP and RMP with a control group matched for age, weight, gender, height and body mass index (BMI), receiving only NVP. In the group taking RMP, NVP C_{12} concentration was significantly lower in 10% of patients compared with the control group (Autar et al 2005).

A population pharmacokinetic study has suggested increasing the NVP dose to 600mg daily to counteract the reductions in NVP plasma concentrations in the presence of RMP (Elsherbiny et al 2009) another study has shown that increasing the NVP dose to 600mg in Indian patients compensated for the increased metabolism of NVP in the

presence of RMP (Ramachandran et al 2006). However, a study in Thai patients comparing the effect of RMP on the pharmacokinetics of NVP 400mg and 600mg daily reported that while the NVP AUC_{0-12}, C_{max} and C_{12} for the 400mg dose was 26%, 25% and 31% lower respectively, compared with the 600mg dose, a hypersensitivity rash occurred in 25% of patients in the 600mg group, which was associated with high NVP trough concentrations. Four patients in the 600mg group discontinued the study prematurely due to NVP hypersensitivity (Avihingsanon et al 2008).

1.15.3.2. Drug Interactions between Rifabutin and Nevirapine

Current WHO ART guidelines recommend the combination of NVP (400 mg daily) with RBT (300mg daily) for the treatment of TB-HIV co-infected patients (World Health Organization 2010 revision), but clinical data supporting this recommendation are limited. In contrast to the many studies on the pharmacokinetic interaction between NVP and RMP, there is only one report (an abstract by Maldonado et al 1999) in the literature on the pharmacokinetic interactions between RBT and NVP. Maldonado et al (1999) investigated the pharmacokinetics of RBT and NVP in an open-label single arm trial of RBT alone and in combination with NVP. Nineteen HIV-infected patients with CD4 counts > 100 cells/mm^3 were enrolled and given RBT 300mg for two weeks. After a 2-week lead in period of NVP 200 mg daily, patients were given NVP 200 mg twice daily for another 2 weeks. At steady state, RBT co-administration resulted in only a slight enhancement of NVP clearance. Nevirapine AUC, C_{max} and C_{min} were reported to be 54.0 µg.h/mL, 5.8 µg/mL and 3.8 µg/mL respectively. The only adverse event reported was Pneumococcal pneumonia, which was deemed unrelated to study

medication. This study did not report on the effect of NVP on RBT pharmacokinetics. More studies in HIV co-infected patients on the pharmacokinetic interactions between NVP and RBT are needed, particularly African patients, to evaluate the combination of RBT and NVP for the treatment of TB-HIV co-infection.

1.15.3.3. Drug Interactions between Rifampicin and Efavirenz

There is substantial evidence of drug-drug interactions between RMP and EFV in Caucasian patients. Reduced plasma EFV concentrations have been reported in healthy volunteers (Benedek et al 1998) and in TB-HIV co-infected patients (Lopez-Cortes et al 2002; Matteelli et al 2007). These two very similar studies in Italian and Spanish patients compared the plasma concentrations of EFV 600 and 800mg in combination with RMP and found significant reductions in plasma EFV concentrations for the 600mg dose in the presence of RMP (Lopez-Cortes et al 2002; Matteelli et al 2007). A subsequent study (Stohr et al 2008) investigated the factors influencing plasma EFV concentrations in 339 patients and also found that concomitant RMP substantially decreased plasma EFV concentrations in Caucasians. This study showed clear associations between EFV concentrations and ethnicity. Plasma EFV concentrations were not significantly reduced by concomitant RMP in African, Thai and Indian patients. Further studies in these population groups support this finding. In a small study of TB-HIV co-infected South African patients taking RMP-based anti-TB therapy and EFV 600mg, trough EFV concentrations were highly variable in the presence of RMP (CV 157%) compared to after RMP discontinuation (CV 58%) (Friedland et al 2006). Another South African study found no significant increases or

decreases in EFV concentrations in patients taking 600 or 800mg EFV in the presence or absence of RMP and similar virological outcomes in both the groups (Orrell et al 2011). In a randomized controlled trial of 84 Thai HIV-infected patients with active TB, plasma EFV levels after 600- and 800mg EFV daily in the presence of RMP were compared. Mid-dosing plasma EFV concentrations for both the 600 and 800mg groups were not significantly different and immunological and virological outcomes assessed at 48 weeks after initiating HAART in both groups were comparable (Manosuthi et al 2005). An observational study from India (Patel et al 2004) also reported comparable immunologic responses in patients taking 600mg EFV with and without concomitant RMP.

1.15.3.4. Drug Interactions between Rifabutin and Efavirenz

An initial study on the pharmacokinetic interaction between RBT and EFV (Benedek et al 1988) reported that EFV reduced the AUC_{0-24} and C_{max} of RBT by 38% and 32% respectively, while EFV pharmacokinetics were not affected in the presence of RBT. This study suggested that a 50% higher dose of RBT might be required for patients co-administered both drugs. Subsequently, treatment guidelines issued by the American Thoracic Society/ Centres for Disease Control (CDC) recommended increasing the RBT dose from 300mg to 450 or 600mg for combined therapy with EFV (American Thoracic Society/ Centres for Disease Control and Prevention 2003).

Weiner et al (2005b) assessed this recommendation in a pharmacokinetic study of TB-HIV co-infected patients with advanced immunosuppression. Fifteen patients were enrolled in the continuation phase of TB therapy while patients were receiving RBT 300mg two times per week and INH. Blood samples for baseline RBT pharmacokinetic parameters were obtained after two weeks of therapy. Antiretroviral therapy was then initiated with EFV 600mg daily and 2 NRTIs. After a further two weeks of therapy, a second series of pharmacokinetic samples were obtained. Efavirenz pharmacokinetics was compared with a historical control group of 35 HIV-infected patients without TB. At steady state, RBT AUC_{0-24} was slightly higher in patients taking RBT 600mg compared with the same patients taking 300mg RBT alone. In TBTC Study 23 (Weiner et al 2005a) it was determined that RBT AUC_{0-24} below 4.5 µg.h/mL was associated with treatment failure or ARR. In this study RBT AUC_{0-24} was below 4.5 µg.h/mL in 5/15 patients and 11/15 patients administered RBT 600mg plus EFV and RBT 300mg without EFV, respectively. Rifabutin C_{max} values were significantly higher in patients taking EFV co-therapy compared to patients taking RBT alone. The AUC_{0-24} d-RBT was significantly lower in the presence of EFV compared with RBT alone. There were no significant changes in EFV pharmacokinetics between the group of HIV-infected patients that took RBT and the group that took only EFV.

The higher dose of RBT was well tolerated with grade 3 or 4 adverse events reported in only 3 patients. One case each of neutropenia and elevated pancreatic enzymes was reported which were probably associated with study drug although drug levels in these patients were not high compared with concentrations in other patients. Favorable

virological and immunological outcomes were also achieved in this study. The HIV viral load decreased by 2.6 log_{10} copes/mL and the median $CD4^+$ T cell count increased by 41% after 21 days of EFV-based ART. This study demonstrated that RBT 600mg with standard doses of EFV was suitable for the treatment of TB-HIV co-infected patients with advanced immunosuppression. However, this approach has not been confirmed with a daily treatment regimen in African TB-HIV co-infected patients with advanced immunosuppression.

1.15.3.5. Drug Interactions between Rifampicin and Protease Inhibitors

The drug-drug interactions that occur between RMP and PIs are more severe than those that occur between RMP and NNRTIs because PIs are substrates of both CYP 3A4 and PGP (Burman et al 2001). Trough concentrations of PIs have been associated with viral outcome (Kempf et al 1997) therefore it is essential to maintain trough concentrations of all PIs co-administered with RMP within their therapeutic ranges. Reductions of saquinavir (SQV) trough concentrations of 64.3% and 49.6% have been reported in TB-HIV co-infected patients (Ribera et al 2007). Indinavir (IDV) trough concentrations were reduced by 87% when IDV/r 800/100 mg twice daily in combination with 300 mg RMP was given to HIV-infected patients (Justesen et al 2004). In healthy volunteer studies, reductions of up to 90% in atazanavir (ATV) trough concentrations were reported when ATV/r was combined with RMP (Burger et al 2006; Acosta et al 2007). In both studies sub-therapeutic ATV trough concentrations were observed with the combined dosing of RMP and ATV/r.

The CDC has recommended increasing the dose of RTV to 400mg every 12h in combination with SQV or LPV or doubling the dose of LPV to maintain trough PI concentrations at therapeutic levels in the presence of RMP (Centres for Disease Control & Prevention 2007. Updated December 2007). However, studies in healthy volunteers (la Porte et al 2004; Nijland et al 2008) and HIV-infected patients (L'homme et al 2009; Decloedt et al 2011) have reported sub-therapeutic PI trough concentrations and a high incidence of adverse events with higher doses of SQV/r (Rolla et al 2006) and LPV/r (either 800/200 mg twice daily or 400/400 twice daily) in combination with RMP when compared to the standard dose of LPV/r (400/100 mg twice daily) without RMP. In the presence of RMP, LPV trough concentrations decreased by 90% in patients taking LPV/r 800 / 200 mg and 93% in patients taking LPV/r 400/ 400 mg. Twelve subjects (31%) were prematurely withdrawn due to elevations in hepatic transaminases that occurred only after combining LPV and RMP. L'homme et al (2009) reported similar observations in HIV positive patients. Dose adjustment was associated with inferior exposure to LPV and virological failure, and a high proportion of patients on a recommended increased dose stopped the combination prematurely due to adverse events. In a recent South African study of HIV-infected adults on a LPV/r regimen with concomitant RMP, gradual increase of the LPV dose to twice the baseline dose showed no significant increase in LPV trough concentrations between baseline and double-dose LPV/r. Furthermore, a large proportion of patients reported sub-therapeutic LPV/r trough concentrations. (Decloedt et al 2011). Another option recommended by the CDC is to replace RMP with RBT in patients requiring anti-TB therapy and concomitant PI-based HAART (Centres for Disease Control & Prevention 2007. Updated December 2007).

1.15.3.6. Drug Interactions between Rifabutin and Protease Inhibitors

The pharmacokinetic interaction between RBT and PIs has been studied extensively in healthy volunteers (Polk et al 2001; Hamzeh et al 2003; Kraft et al 2004; Ng et al 2009; Sekar et al 2010). The combination of boosted protease inhibitors and RBT in healthy volunteers has resulted in significantly large increases in RBT exposure and more profound increases in the bioavailability of d-RBT. A high incidence of neutropenia was consistent in all of these studies and some studies were prematurely terminated due to the high incidence of adverse events. The relationship between d-RBT exposure and neutropenia in healthy volunteers is as yet uncertain.

Narita and colleagues reported that RBT could be combined with PIs for the successful treatment of TB-HIV co-infected patents. In their study, TB-HIV co-infected patients were treated with standard TB treatment (replacing RMP with RBT 300 mg) co-administered with IDV and/or nelfinavir (NFV). Nelfinavir was dosed at either 800mg, 1000mg or 1200mg 8 hourly or 1200mg twice daily and IDV was dosed at 1000mg or 1250mg twice daily, depending on treatment guidelines or adjustments for drug levels. Two weeks after the initiation of PIs, plasma RBT concentrations were significantly increased and approached the published therapeutic range while plasma NFV and IDV levels were not significantly decreased. Plasma concentrations of the PIs were maintained within therapeutic concentrations. HIV viral loads of < 500 copies/mL were obtained by 77% of patients after 15.6 weeks of ART. There was no increase in RBT-

related adverse events such as uveitis and neutropenia. The investigators have however not reported on specific pharmacokinetic data for RBT and the PIs in this study (Narita et al 2000).

Another study in HIV-infected patients evaluated the plasma levels of RBT and d-RBT during concomitant therapy of intermittent RBT dosing regimens with a combination of RTV and SQV. All patients were receiving 400mg of RTV and SQV twice daily as part of standard ART and were randomized to receive RBT 300mg every 7 days or 150mg TPW. At steady state RBT C_{max} was significantly decreased by 37% and trough levels were significantly increased in the group taking 150mg TPW compared with the group taking 300mg every 7 days. The co-administration of RBT with RTV and SQV resulted in increases in AUC and C_{max} of both PIs but the differences from baseline levels were not statistically significant. However, there was a significant increase in RTV (27%) and SQV (39%) trough levels at 12 hours. Overall, the effect of RBT on the pharmacokinetics of both RTV and SQV was consistent at 4 and 8 weeks of concurrent therapy. The only adverse events reported in this study were two incidences of rash probably caused by RBT (Gallicano et al 2001).

The pharmacokinetic interaction between SQV and RBT was investigated in fourteen TB-HIV co-infected patients. Patients enrolled into the study received RBT 300mg daily for 10 days. After a 14 day wash-out period, patients were randomized to receive either SQV 1200mg three times daily for 10 days or SQV 1200mg three times daily plus RBT 300mg daily for 10 days. There were no reports of serious adverse events during the

study. Two patients were withdrawn prior to completion. One patient reported headache, pyrexia, nausea and vomiting during treatment and the other reported abdominal pain, arthralgia and night sweats. A higher incidence of adverse events was reported during the combined treatment of RBT with SQV. The most commonly reported adverse events were headache, diarrhea and nausea. Most of these were considered to be mild. The AUC and C_{max} of SQV decreased by 47% and 39% respectively when co-administered with RBT. In contrast, RBT AUC, C_{max} and C_{min} were increased by 19.6%, 20.5% and 86.6% respectively (Moyle et al 2002).

Benator et al (2007) investigated the pharmacokinetic interaction between NFV and an intermittent dose of RBT in TB-HIV co-infected patients. Patients were administered RBT 300mg two times per week and HAART consisting of two 2NRTIs plus NFV 1250 mg twice daily. In the presence of NFV, RBT AUC increased by 22% while C_{max} remained unchanged. Rifabutin had no effect on the plasma concentrations of NFV. Two patients developed toxicity that was attributed to TB therapy. One patient developed grade 3 transaminase elevations two weeks after starting ART. This patient had very high plasma concentrations of RBT in the presence of NFV. The other patient developed grade 4 neutropenia two months after the start of ART. This patient had a moderate increase in plasma RBT concentration in the presence of NFV.

Boulanger and colleagues investigated the pharmacokinetics of RBT administered in combination with LPV/r in TB- HIV co-infected patients. Patients were first given RBT

300mg TPW. After two weeks of treatment, steady state RBT concentrations were measured (Visit 1). Thereafter, patients were switched to RBT 150mg TPW and LPV/r 400/100mg twice daily with tenofovir and emtricitabine. New steady state assessments were done after another week of treatment (Visit 2) as well as rapid RBT analysis 4h and 8h post-dose. If RBT C_{max} values were below the published reference ranges, RBT was increased to 300mg TPW and steady state assessments were repeated two weeks later (Visit 3) (Boulanger et al 2009).

The published reference C_{max} for RBT is 300-900 ng/ml according to Peloquin et al (2002). The RBT C_{max} values obtained in this study for RBT given on its own or in combination with LPV/r were below this range. At visit 1, when patients were given 300mg RBT TPW, the median RBT C_{max} value was at the low end of this range and in 5 patients it was below the range. After changing the RBT dose to 150mg TPW plus LPV/r (Visit 2) 7/10 patients had decreased C_{max} values and only 3 patients had C_{max} values greater than 450 ng/mL.

In a previous study (Weiner et al 2005a), patients with RBT AUC_{0-24} values lower than 4 500 ng.h/mL were associated with ARR. When RBT 300mg was given TPW, the median and maximum RBT AUC_{0-24} values obtained were close to the values associated with ARR in the study by Weiner and colleagues. Similar low RBT AUC_{0-24} was observed when the RBT dose was changed to 150mg TPW in the presence of LPV/r. Thus the authors concluded that standard starting doses of RBT 300mg TPW

and the standard adjusted dose of RBT 150mg TPW with LPV/r are inadequate for HIV-positive patients.

In addition to the studies detailed above, there are two short reports on the pharmacokinetic interaction between RBT and LPV/r in TB-HIV co-infected patients. The first report by (Bonora et al 2003) investigated the pharmacokinetics of RBT 150 mg daily and LPV/r in five TB-HIV co-infected patients. The AUC and C_{max} of RBT were below the reference range recommended by Peloquin et al (2002) in 4/4 and 3/4 patients respectively. Khachi et al (2009) investigated the pharmacokinetic interactions between RBT 150mg TPW and LPV/r (400/100mg) in five TB-HIV co-infected patients. RBT plasma concentrations were found to be below the reference range in all 5 patients. In two patients who deteriorated clinically, the RBT dose was increased to 300mg TPW resulting in therapeutic RBT plasma concentrations in only one patient. Furthermore, relapse has been reported in 3 TB-HIV co-infected patients treated with an intermittent RBT regiment and ART (Jenny-Avital and Joseph 2009).

1.16. Adverse Events Associated With Rifabutin

Rifabutin is well tolerated in HIV-infected patients in doses ranging from 300 to 1 200 mg / day. However, adverse events such as uveitis, corneal deposits, neutropenia, arthralgias and skin discoloration have been reported in patients taking high doses of RBT (Torseth et al 1989) or low doses of RBT in combination with CYP3A4 inhibitors

such as macrolides, azole antifungals and protease inhibitors (Cato et al 1996; Benson et al 2000).

An arthritis – arthralgia syndrome was associated with doses of 1 200 mg / day, in HIV-infected (Torseth et al 1989). Neutropenia has been reported in healthy volunteers taking 300mg daily in clinical trials (Apseloff et al 2003). and in patients taking 600-900mg RBT daily (Torseth et al 1989). Uveitis has been reported in patients taking RBT as prophylaxis for MAC, usually if RBT was given in combination with a macrolide (Havlir et al 1996).

1.17. Clinical Use Of Rifabutin

Current WHO and CDC guidelines recommend that all TB-HIV co-infected patients, irrespective of CD4 cell-count, should be started on ART as soon as possible after starting TB treatment (as early as 2 weeks or within 8 weeks) (World Health Organization 2010 Revision). Two NRTIs and EFV are recommended as first-line treatment for TB-HIV co-infected patients. In the event of first-line treatment failure or toxicity, the WHO recommends the use of RBT 150 mg TPW with LPV/r and two NRTIs as second-line treatment. If RBT is not available RMP is recommended with super-boosted PIs, LPV/r or ATV/r.

Presently, the recommendation of using EFV in combination with anti-TB therapy for the treatment of TB-HIV co-infected patients is not suitable for all co-infected patients

as EFV is contra-indicated for pregnant women and women of child-bearing age. Furthermore, in patients that require second-line therapy, RMP is poorly tolerated with PIs and super-boosted PIs have an additional risk of hepatotoxicity. Rifabutin is presently recommended at a dose of 150mg TPW in combination with PIs, but there are concerns that this dose may be sub-optimal in TB-HIV co-infected patients and current recommendations may require revision.

The use of RBT is currently limited to developed countries mainly because it is more expensive than RMP and will be too costly to sustain in TB programs in high burden countries. However, the need for RBT-based therapy is increasing in resource-limited countries. A recent review forecasted the need for RBT in the top 21 TB-HIV burden countries to be between 10 000 and 18 000 courses in 2012, representing a 20 -30% increase from 2010. Of these 21 countries, South Africa represented half of the projected need (Loelinger et al 2011).

Chapter 2. Materials and Methods

2.1. Study design

An open-label, three-period, crossover drug interaction study was undertaken to investigate the pharmacokinetics of RBT in African TB patients co-infected with HIV. A total of 48 HIV-positive patients with newly diagnosed pulmonary TB and who met all inclusion requirements were recruited. All enrolled patients commenced trial medication by replacing RMP with RBT 300mg daily in combination with INH, PZA and EMB. After 4 weeks of RBT therapy, serial blood samples were obtained in a dosing interval for assessment of plasma RBT concentrations. Patients were then randomized to one of three arms according to computer-generated sequence and received ART in combination with RBT at two different doses. The doses of RBT administered in combination with ART to patients in each of the 3 arms are discussed in more detail in section 2.3 below. Pharmacokinetic sampling took place after 4 weeks of ART treatment with each dose of RBT. The cross-over design in which all patients received 3 full pharmacokinetic assessments with RBT alone and at two different RBT doses in combination with ART, allowed us to formally compare the two different dosing strategies of RBT in combination with ART, with the recommended TB treatment dose. The Biomedical Research Ethics Committees of the Universities of Kwa-Zulu Natal and Cape Town as well as the South African Medicines Control Council approved the study.

2.2. Patient recruitment and Inclusion Criteria

Patients were recruited from local TB clinics in Kwa-Zulu Natal (KZN), South Africa. Eligibility requirements were provision of written informed consent, a diagnosis of

pulmonary TB confirmed by microscopy or culture, HIV infection with CD4 lymphocyte count ≥ 50 and ≤ 200 cells/mm^3, weight ≥ 50kg or a BMI ≥ 18, a Karnofsky score Q ≥ 80% and no grade 3 or 4 clinical or laboratory findings according to DAID tables (AIDS Clinical Trials Group 2004). Only patients who completed and adhered to 6 weeks of standard intensive phase TB chemotherapy and had not received ART in the preceding 3 months were enrolled. Patients with a previous TB episode within 3 years prior to the current episode, a history of prior treatment for MDR TB, concomitant opportunistic infection requiring additional anti-microbial treatment, a formal contraindication to any trial medication, diabetes mellitus requiring treatment, recreational drug or alcohol abuse, interrupted TB therapy for more than a week or less than 90% adherent to the first six weeks of intensive phase chemotherapy, mental illness, total neutrophil count < 1200 cells/L, hemoglobin < 6.8 g/dL, or liver function tests > grade 2 according to DAID tables and pregnant or lactating women or women unwilling to use appropriate contraception were excluded.

2.3. Treatments under study

At enrollment, six weeks after starting standard intensive phase TB chemotherapy, RMP was switched to RBT 300 mg daily for two weeks. Then PZA and EMB were stopped and patients commenced the continuation phase of treatment with RBT 300 mg daily in combination with INH 300mg. After two more weeks, patients were admitted to the pharmacokinetic facility for the first pharmacokinetic evaluation (PK1). After PK1, patients were randomized to one of three arms comprising of two different

RBT dose sequences together with daily doses of INH and ART (Refer to Figure 2.1 below) comprising of 600mg EFV daily, 400mg NVP daily (after a lead-in dose of 200mg daily) or LPV/r (400/100 mg) plus lamivudine (150mg bd) and stavudine (30mg bd).

Half the patients on the LPV/r arm received RBT 150 mg TPW for 4 weeks before being switched to RBT 150 mg daily after a second pharmacokinetic evaluation (PK2). They remained on this dose of RBT until completion of TB treatment. The remaining patients received the two RBT doses in a reverse sequence, starting with the 150 mg daily dose of RBT and switching after PK2 to the TPW doses of RBT for the rest of the continuation phase. The final pharmacokinetic evaluation (PK3) took place after 4 weeks on the second RBT dose. Patients randomized to the EFV arm received either 450mg RBT (low dose) first followed by 600mg RBT (high dose) or 600mg first followed by the 450mg dose. Patients randomized to receive NVP-based ART received either 300mg RBT first or 450mg RBT with NVP, stavudine and lamivudine. After 4 weeks of therapy on the first RBT dose and PK2, patients were switched to the alternate dose of RBT.

Physical examinations and laboratory investigations were done at every pharmacokinetic evaluation and at the penultimate trial visit to assess safety and adverse events. Pfizer (South Africa) supplied the RBT (Mycobutin®) 150mg capsules for oral administration. Stockrin® was donated by MSD (South Africa). Nevirapine, stavudine and lamivudine were purchased from Aspen (South Africa) and the new film-

coated tablet formulation of LPV/r, Aluvia® was purchased from Abbott Laboratories (USA).

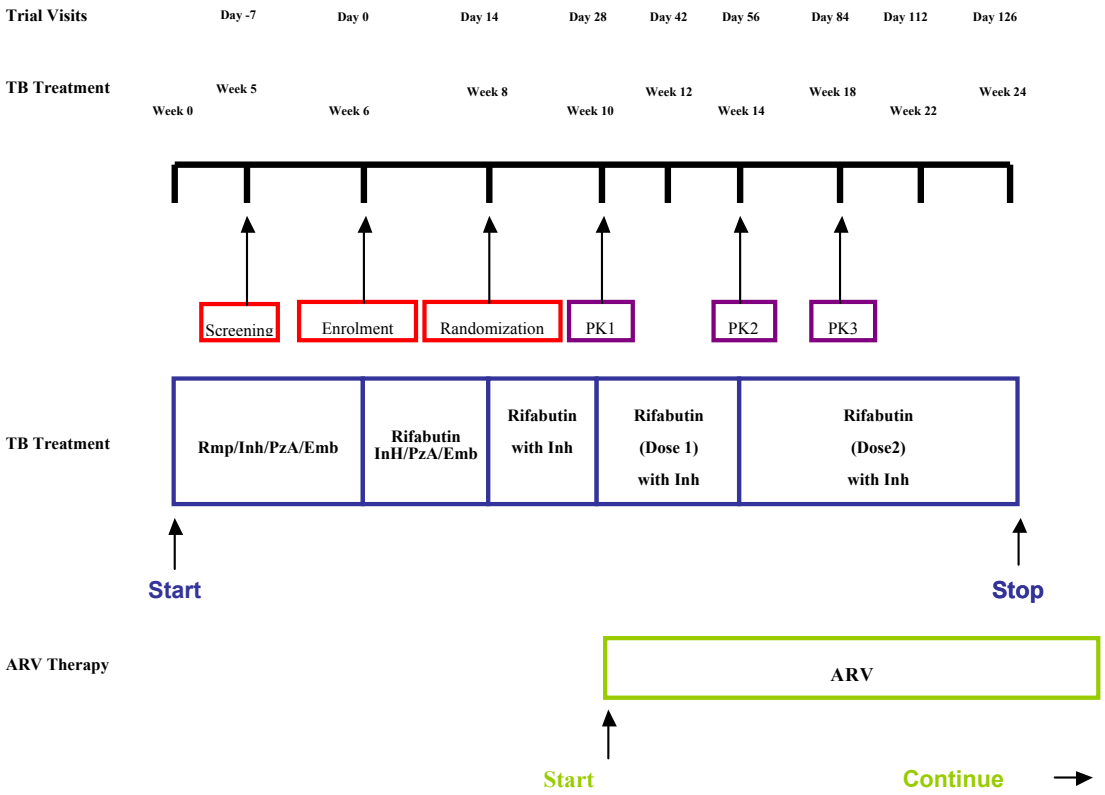

Figure 2.1. Figure 1: Diagram showing the timing of the trial procedures relative to trial visits and treatments (Rmp = rifampicinn, Inh = isoniazid, PzA = pyrazinamide, Emb =ethambutol).

2.3. Sample size

The sample size was calculated on the basis that 16 enrolled participants (per arm) would result in a minimum of 12 evaluable subjects. Based on the variability of AUC_{0-24} for RBT determined in previous studies it was estimated that a sample size of 12 participants has a power of 80% to detect a 20% difference between the mean AUC_{0-24} for the participants taking RBT without ART and the AUC_{0-24} for RBT when combined with ART.

2.4. Pharmacokinetic sampling

All patients were admitted to the pharmacokinetic facility the night before each pharmacokinetic occasion and were fasted from midnight. On the morning of the pharmacokinetic sampling day, an indwelling catheter was inserted into the forearm of each patient via which serial blood samples were obtained. The first blood sample (0h) was drawn prior to administration of study drugs. All study drugs were administered with water by a clinical trial nurse and none of the patients vomited their doses. Subsequent blood draws were done at 2, 3, 4, 5, 6, 8, 12, 24 and 48h after drug ingestion. A standard hospital breakfast (oatmeal porridge) was served immediately after the 2h blood was drawn.. The samples were placed on ice immediately and centrifuged at 3000 rpm at 40^0C for 10 minutes within 30 minutes of collection. Separated plasma was transferred to polypropylene tubes and stored immediately at -70^0C until analysis.

2.5. Drug Analysis

The extraction of RBT, EFV, NVP and LPV from plasma samples obtained from trial participants and the quantification of the plasma concentrations of each of these drugs were performed at the Division of Clinical Pharmacology, University of Cape Town. The laboratory participates in the International Interlaboratory Control Program of Stichting Kwaliteitsbewaking Klinische Geneesmiddelanalyse en Toxicologie (KKGT; Hague, The Netherlands) on an ongoing basis.

2.5.1. Rifabutin

Rifabutin and d-RBT were analyzed with a validated LC/MS/MS assay developed by the Division of Clinical Pharmacology, University of Cape Town. The samples were processed with a protein precipitation extraction method, using 50 μL plasma and 300 μL acetonitrile. Rifaximin was used as internal standard, and was spiked into the precipitation solvent at a concentration of 100 ng/mL. Gradient chromatography was performed on a Phenomenex, Luna 5 μm PFP(2), 100 Angstroms, 50 mm × 2 mm analytical column, using acetonitrile and 0.1% formic acid as mobile phase, and was delivered at a flow rate of 500 μL /min. An AB Sciex API 3200 mass spectrometer was operated at unit resolution in the multiple reaction monitoring (MRM) mode, monitoring the transition of the protonated molecular ions at m/z 847.4 to the product ions at m/z 95.1 for RBT, the protonated molecular ions at m/z 805.4 to the product ions at m/z 95.1 for d-RBT, the protonated molecular ions at m/z 786.3 to the product ions m/z 151.1 for the internal standard. The accuracies for RBT and d-RBT were between 99.1% and 109.0% at low, medium and high QC levels during inter-batch validation.

The precision (%CV) for RBT and d-RBT during inter-batch validation was less than 9.2% at low, medium and high QC levels. The calibration range for RBT was between 3.91 ng/ml and 1000 ng/ml, and for d-RBT the calibration range was between 0.780 ng/ml and 200 ng/ml.

2.5.2. Antiretrovirals

Plasma concentrations of NVP were determined by a validated LC/MS/MS method, which has been described previously by Chi et al (2002) and EFV and LPV were determined by a method previously described by Chi et al (2003).

Nevirapine was extracted by addition of 50 μL acetonitrile containing 1 mg/L internal standard to 20 μL of each sample or control to precipitate protein. Samples were vortexed for 30s, centrifuged for 5 min at 750g and 2 μL of the supernatant was injected onto the column. The standard curve was linear in the range 0.2-20 mg/L. The lower limit of quantification (LLQ) was set at 0.2 mg/L. A Waters alliance 2690 HPLC coupled to a Waters/Micromass ZMD single quadropole mass spectrometer was used for mass spectral analyses. The mobile phase consisted of 50% acetonitrile in 4mM ammonium acetate and 0.1% trifluoroacetic acid. A 20 by 2.1mm Hypersil Gold C18 column (Thermo) was used at a flow rate of 0.3 mL/min. Neostigmine served as internal standard. Detection in positive ionization mode of NVP was at 276.2 (m/z) and neostigmine at 223.2 (m/z).

Lopinavir and EFV were extracted from thawed plasma samples from trial participants, quality control (QC) samples and calibration standards by transferring 100 μL aliquots to 1.5 ml microcentrifuge tubes. 200 μL of internal standard solution was added to each tube and mixed gently, followed by 400 μL acetonitrile. Each tube was vortexed for 20 seconds at high speed. The tubes were then centrifuged at 12 000 x g (11,000 rpm) for 5 minutes. The precipitated proteins formed a pellet at the bottom of the tube leaving a clear supernatant. Aliquots of supernatant were transferred to vial insets and placed in the autosampler tray and loaded on the LC column. The supernatant was eluted through the LC column with a linear gradient of 10 mM ammonium formate buffer (pH 4.10) and acetonitrile.

An AB Sciex API 3200 mass spectrometer was used for mass spectral analyses. The calibration curve was linear over the range from 0.05 to 20 mg/L. If the LPV or EFV concentration of a particular sample was determined to be > 20 mg/L, the sample was diluted with drug-free plasma and re-analyzed. The LLQ of the LPV assay was 0.05 mg/L. The LPV calibration curve was linear over the range from 0.05 to 20 mg/L. Accuracy ranged from 94.3% to 103.0%. The intraday and interday precisions ranged from 0.14% to 4.72% and from 1.61% to 4.22%, respectively. The calibration curve for EFV was linear over the range 0.1-15mg/L. Accuracy ranged from 98.3% to 103.7%. The intraday and interday precisions ranged from 0.97% to 3.27% and from 2.28% to 4.98%, respectively. Any sample with a LPV or EFV concentration below the LLQ was reported as 0.5 X LLQ and treated as such in the analysis.

2.6. Pharmacokinetic Analysis

Concentration-time curves were plotted for each series of drug assays for each participant. The main pharmacokinetic measures for RBT, d-RBT, NVP, EFV and LPV/r were derived by noncompartmental analysis using Stata (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP). The peak concentration (C_{max}), and time to C_{max} (T_{max}) were obtained directly from the concentration-time profiles. Drug concentrations at the end of a dosing interval (trough) are reported as C_{24} for RBT and d-RBT and as C_{12} for each of the ARVs. Pre-dose (0h) concentrations on the day of pharmacokinetic evaluation are reported as C_0. The steady-state AUC from time 0h to the last quantifiable sample within 24h (AUC_{0-24}) for RBT and d-RBT and at 12h (AUC_{0-12}) for each of the ARVs were calculated by the linear trapezoidal method. The apparent total oral clearance of RBT from plasma at steady state (CL/F) was calculated by dose/AUC.

2.7. Statistics

The steady state pharmacokinetic parameters of RBT and d-RBT were determined at each of the 3 pharmacokinetic evaluations and the pharmacokinetic parameters of EFV, NVP and LPV/r were determined after the second and third pharmacokinetic evaluations.

For statistical analysis and to test for an effect of sequence on RBT AUC_{0-24}, the AUC_{0-24} was log- transformed. A linear mixed model of log AUC with 3 levels of dose (baseline, high and low) and sequence effect (high-low and low-high) was used where

id (patient identifier) was nested within sequence in order to adjust for the repeated measurements on individuals. Day was not included in the model because day was a function of sequence and dose. Using this model, a significant sequence effect was not obtained for the EFV and NVP arms therefore data for high and low RBT doses were pooled. However a significant sequence effect ($p = 0.04$) was obtained for the LPV/r arm. There were significant differences in baseline AUC_{0-24} values between patients randomized to high-low and low-high sequences in the LPV/r arm ($p = 0.01$), which could have accounted for the significant sequence effect. To test this, a linear mixed model with two doses (high and low), day (2 and 3), sequence and log AUC at baseline and id nested within sequence was used. In this model sequence was no longer significant ($p= 0.2$) confirming that baseline differences were driving the sequence effect. Therefore the two arms were pooled and the model was used to test the dose effect after adjusting for baseline differences. Inter-patient variability for each of the dosing periods per arm was measured by co-efficient of variation (%CV) which was calculated as $\{100 \text{ X } (e(\text{var est}) -1)^{1/2}\}$.

To calculate geometric mean ratios (GMR) for AUC, log means and 90% confidence limits were back transformed and presented in their original units as geometric means. Geometric mean ratios for the AUC of RBT were computed for each arm as follows :

a. EFV arm: 450mg daily with EFV / 300mg daily; 600mg daily with EFV/ 300mg daily and 450mg daily with EFV / 600mg daily with EFV

b. NVP arm: 300mg daily with NVP / 300mg daily; 450mg daily with NVP / 300mg daily and 300mg daily with NVP / 450mg daily with NVP

c. LPV arm: 150 mg daily with LPV/r / 300mg daily; 150 mg TPW with LPV/r 300mg daily and 150 mg daily with LPV/r / 150 mg TPW with LPV/r

Paired t-tests were used to compare the AUC_{0-24} of two doses of RBT given with ART with the AUC_{0-24} of RBT 300mg given alone to the same patients in each of the three arms. In the LPV/r arm, the AUC_{0-48} of RBT150mg TPW was also compared with 2 x RBT AUC_{0-24} because the dosing interval for the TPW dose is 48 hours. Demographic data, baseline and final log viral loads and $CD4^+$ counts were also compared using paired t-tests. A P-value < 0.05 was considered significant.

A stepwise regression model was used to select factors associated with the AUC_{0-24} and C_{max} and variables significant at the 0.05 level were included and then factors such as sex and weight which have been shown in the literature to be associated were included in the model.

2.8. Pharmacogenomics

2.8.1. Study participants and Sample Acquisition

Of the 48 patients who were recruited for the main pharmacokinetic study as described in section 2.2.2., 42 patients provided additional written informed consent for the pharmacogenetic study. An additional blood sample was collected in EDTA-coated

tubes. Whole blood was aliquoted into 2ml samples and frozen immediately at -70^{o}C. Genotyping was done at the Department of Molecular and Clinical Pharmacology, University of Liverpool.

2.8.2. Genotyping

Total genomic DNA was isolated from whole blood using the QIAamp DNA mini kit according to manufacturer's instructions. Purity was assessed following the extraction, by comparing the A260 and A280 ratio and the DNA was then normalized to 20 ng/μL. Genotyping for SLCO1B1 rs4149032, rs2306283, rs4149056 and rs11045819 was performed by real-time P.C.R. allelic discrimination by standard methodology (95˚C for 15 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min). The Applied Biosystems assay IDs for the first three SNPs were C_1901709_10, C_1901697_20 and C_30633906_10, respectively. For rs11045819, the forward primer, reverse primer, VIC probe and FAM probe were 5′CAGTGATGTTCTTACAGTTACAGGTATTCTAA3′, 5′GAAGACTTTTTACTGTCAATATTAATTCTTACCTTTTCC3′, 5′-ACTATCTCAGGTGATGCT-VIC, and 5′-CACTATCTCAGTTGATGCT-FAM, respectively.

2.8.3. Non-compartmental analysis

Rifabutin AUC_{0-24} and C_{max} for each patient were computed as described in 2.6 above. Genomic data for each patient was matched with pharmacokinetic data and grouped

according to genotype. To achieve normality the AUC_{0-24} and C_{max} was log transformed. The AUC_{0-24} and C_{max} between the different genotype groups were compared, respectively using t-tests. Simple linear regression was used to determine the association between each SNP. Due to us not finding more than one significant relationship with an SNP, multiple regressions was not pursued.

2.8.3. Statistics

The Shapiro-Wilk test was used to test for normality. AUC was analyzed on th e log scale. ANOVA was used to determine whether AUC and Cmax differed between the three genotype categories. T-tests were used for pairwise comparisons.

Chapter 3. The Pharmacokinetics of Rifabutin 300mg in African TB-HIV co-infected patients with advanced immunosuppression

Summary

The pharmacokinetics of RBT 300mg was investigated in 44 African TB-HIV co-infected patients with advanced immunosuppression. The median RBT AUC_{0-24}, C_{max}, C_{24} and CL/F obtained were 2 790.8 ng.h/mL, 284 ng/mL, 48.0 ng/mL and 107.5 L/h respectively. Peak plasma concentration was attained 3 hours post-dose. Large interpatient variability (CV = 36%) was observed for RBT AUC_{0-24}. The AUC_{0-24}, of d-RBT was approximately 10% of the parent drug and the drug concentration-versus-time profile of d-RBT mimicked the profile of RBT.

In comparison to previous Phase I studies in healthy volunteers (3390ng.h/mL- 4659 ng.h/mL) the AUC_{0-24} of RBT obtained in this study (2 790.8ng.h/mL) is lower (Table 3.1). However, the median AUC_{0-24} of RBT obtained in this study is comparable to the median AUC_{0-24} of RBT obtained in previous studies in TB-HIV co-infected patients (Table 3.2). The AUC_{0-24} of d-RBT obtained in this study was slightly higher than in healthy volunteers and lower than that previously reported in a study in TB-HIV co-infected patients (Boulanger et al 2009).

3.1. Introduction

Rifabutin is currently prescribed for the treatment of TB and MAC at 300mg daily. However, the concentrations required for effective RBT therapy are only partially known and detailed PK-PD data from human studies are limited. A recent pharmacokinetic study reported that a RBT AUC_{0-24} of < 4.5 ug.h/mL was associated with treatment failure and ARR (Weiner et al 2005a) and TDM studies of anti-TB drugs recommend that peak RBT concentrations should fall between 300 and 900 ng/mL for the treatment of *M tuberculosis* (Peloquin et al 2002). Previous healthy volunteer (Table 3.1) and treatment studies (Table 3.2) show large variability in RBT AUC_{0-24} and C_{max} concentrations. The objective of this chapter is to describe the enrollment of patients into the clinical trial and the clinical pharmacokinetics of RBT 300mg daily in African patients with TB-HIV co-infection. The pharmacokinetic analysis of RBT in combination with ARVs in these patients is described in subsequent chapters.

Table 3.1. Pharmacokinetic parameters of rifabutin 300mg daily obtained from previous studies in healthy volunteers

Reference	n	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)
Hamezh, 2003	17	3 980	470.0
Sekar, 2010	15	4 659	565.2
Polk, 2001	24	3 390	380.0

Table 3.2. Pharmacokinetic parameters of rifabutin 300mg daily obtained from previous studies in HIV-infected patients

Reference	n	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)
Sahai, 1995	9	5 023.0	525.0
Jordan, 2000	10	3 109.0	244.9
Trapnell, 1996	12	3 025.0	-
Blaschke and Skinner, 1996	75	5 324.0	381.0
Colborn, 1996	12	2 663.0	352.0
Narang, 1996	48	4 054.0	348.0
Narang, 1995	8	2 539.0	265.0
Moyle, 2002	14	3 007.0	308.0
Ford, 2008	22	3 050.0	313.0
Boulanger, 2009	10	2 700.0	280.0
Hafner, 1998	34	4 000.0	430.0
Li, 1996	8	3 400.0	400.0
Weiner, 2005a	102	5 220.0	450.0

- UNK = unknown

3.2. Patient Enrollment Information

In total, 176 patients were screened of which 128 patients failed screening, mainly because they did not fulfill all inclusion criteria. The first 48 patients to pass screening were enrolled and treated. These patients formed the intent-to-treat (ITT) population and form the group of patients in whom the safety analysis is based (Chapter 6). The ITT population is defined as all the patients that were enrolled into the trial and received trial medication; and includes patients that were non-compliant on trial medication and patients that were prematurely withdrawn as well as treatment failures. Forty-six patients completed pharmacokinetic analysis for RBT 300mg without ART. Forty-three patients completed all 3 pharmacokinetic visits. Three patients were

withdrawn after PK1, due to uveitis, elevated transaminase and TB meningitis. The data of 2 patients were excluded from the pharmacokinetic analysis. One patient reported non-compliance to trial medication and the other patient was hospitalized for cryptococcal meningitis and administered fluconazole intravenously.

3.3. Trial Completion and Subject Withdrawal

Of the total of 48 enrolled patients, sixteen were randomized to arm 1(EFV), fourteen were randomized to arm 2 (NVP) and sixteen were randomized to arm 3 (LPV/r) as described in section 2.3. All enrolled patients received 4 weeks of RBT before randomization to one of three ARV regimens, Two enrolled patients were not randomized. One patient died before reaching the randomization visit and another patient started ART at a local clinic prior to his randomization visit. Five patients discontinued the trial prematurely. The reasons for discontinuation were uveitis experienced after day 28 (arm 3), elevated transaminases experienced on day 42 (NVP arm), meningitis diagnosed after day 42, MDR TB confirmed after day 56 (EFV arm) and neutropenia diagnosed on day 84 (EFV arm). These are described in more detail in Chapter 6 (Safety).

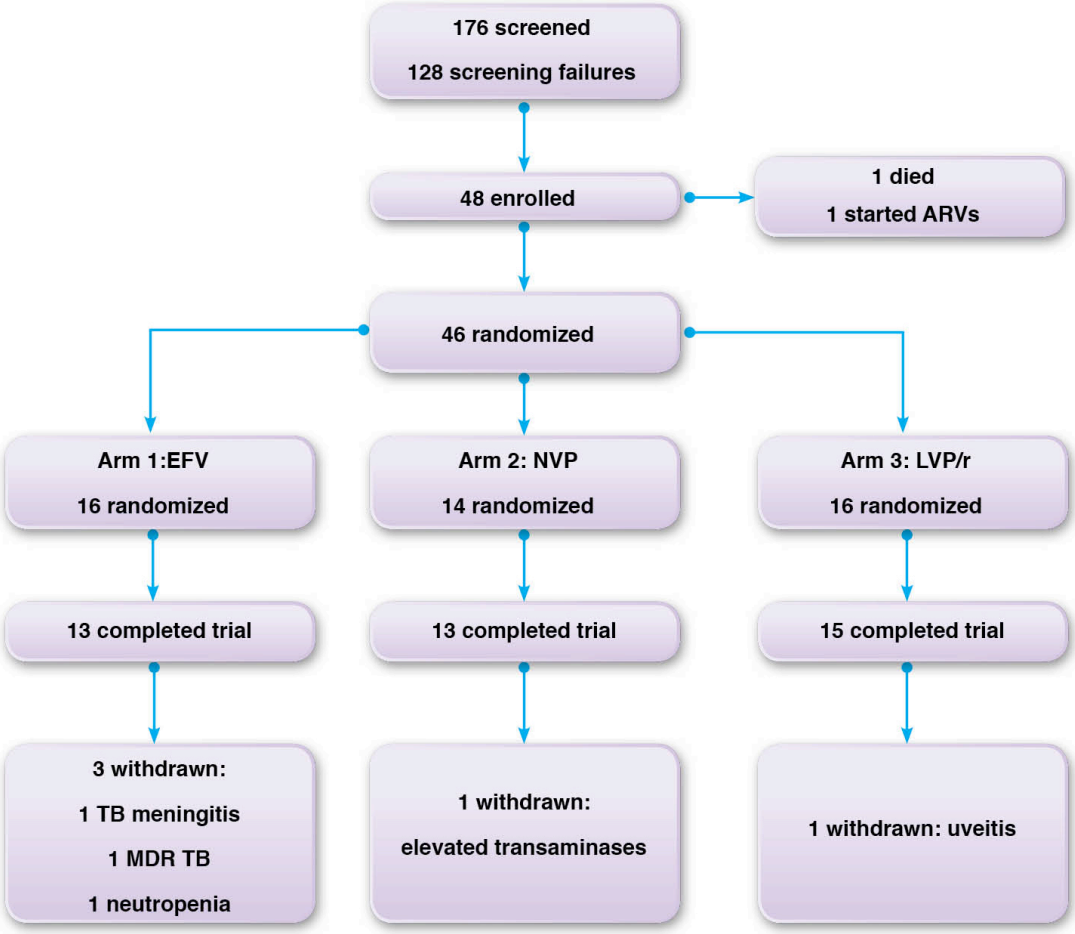

Figure 3.1. Screening and enrolment

3.3. Patient Demographics and Baseline Characteristics

The baseline mean and standard deviation of the demographic data for the ITT population is displayed in table 3.3 below.

Table 3.3. Baseline demographic data of the ITT population. Values are the mean with standard deviation in brackets.

Parameter	All n = 48*	Arm 1 n = 16	Arm 2 n = 14	Arm 3 n = 16
Age at screening, years	33.1 (5.8)	31.8 (4.7)	36.1 (6.6)	31.6 (5.5)
Height, cm	159.8 (7.6)	159.1 (8.4)	159.5 (7.5)	160.1 (7.1)
Weight, kg	58.3 (9.0)	59.4 (9.8)	57.2 (8.2)	59.0 (9.4)
BMI, kg/m^2	23.0 (3.6)	23.7 (5.2)	22.4 (2.8)	22.9 (2.6)
Gender, n (%)				
Female	18.0 (34.5)	6.0	6.0	6.0
Male	30.0 (65.5)	10.0	8 .0	10.0
$CD4^+$ cell count, $cells/mm^3$	123.2 (43.5)	118.8 (47.1)	105.6 (29.4)	147 (43)
Viral load, log_{10} copies/mL	5.3 (0.1)	5.2 (0.84)	5.3 (0.68)	5.4 (0.68)

***2 patients were not randomized**

There were no significant differences between the three randomized arms with regard to any of the demographic parameters. All patients were from the Black race group. Thirty were male (65.5%). All patients had a mean (sd) Karnofsky Q score of 100 (100).

Physical examination at screening did not reveal any relevant differences between the three arms. An overview of specific symptoms and active disorders at screening is presented in Tables 3.4 and 3.5 respectively. The most commonly reported diseases active at screening were related to the respiratory system (reported in 7 patients in the EFV arm, 13 patients in the NVP arm 2, 11 patients in the LPV arm) and the lymphatic

system (reported in 11 patients each in EFV and NVP arms and 14 patients in the LPV arm.

Table 3.4. Specific symptoms present at screening. Conditions were graded according to DMID tables and Grade 0 corresponds to the absence of the condition

Symptom	Arm 1	Arm 2	Arm 3
Cough			
Grade 0	0	2	2
Grade 1	9	6	5
Grade 2	7	6	9
Chest Pain			
Grade 0	13	10	12
Grade 1	2	4	4
Grade 2	2	0	0
Haemoptysis			
Grade 0	13	11	14
Grade 1	3	3	2
Grade 2	0	0	0
Night sweats			
Grade 0	3	4	6
Grade 1	10	9	5
Grade 2	3	1	5
Weight Loss			
Grade 0	13	13	11
Grade 1	3	1	1

Grade 2	3	1	5
Eye Pain			
Grade 0	13	11	14
Grade 1	3	3	1
Grade 2	0	0	1
Red Eye			
Grade 0	14	12	14
Grade 1	2	2	2
Grade 2	0	0	0
Light Sensitivity			
Grade 0	13	12	14
Grade 1	3	2	2
Grade 2	0	0	0
Blurred vision			
Grade 0	15	12	15
Grade 1	1	2	1
Grade 2	0	0	0
Joint Pain			
Grade 0	13	12	11
Grade 1	1	1	2
Grade 2	2	1	3
Muscle Pain			
Grade 0	16	13	16
Grade 1	0	1	0
Grade 2	0	0	0

Table 3.5. Active Concomitant Disease (by Body System Class) at Screening

Body System	Arm 1		Arm 2		Arm 3	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
ENT	16	0	14	0	15	1
Eyes	14	2	12	2	15	1
Cardiovascular	16	0	14	0	15	1
Chest & lungs	9	7	1	13	5	11
Abdomen	16	0	12	2	12	4
Nervous sys	16	0	13	1	15	1
Musculo-skeletal	13	3	10	4	13	3
Skin and Mucosa	11	5	9	5	9	7
Periph Vasc	16	0	13	1	15	1
Lymphatic	5	11	3	11	2	14

Of the 46 randomised patients, only 8 patients were taking any concomitant medication at screening. The most frequently used concomitant medication was pyridoxine 25mg daily (4 patients). The concomitant medication taken by all enrolled patients is tabulated in Table 3.6 below. Only three patients were taking cotrimoxazole at screening. All patients enrolled in the trial were given cotrimoxazole.

Table 3.6. Concomitant non-TB medication taken by all enrolled patients at screening

Drug	Arm 1	Arm 2	Arm 3
No concomitant medication	13	11	14
Pyridoxine (25 mg od)	1	3	0
Allergex 2 (4mg bd)	1	0	0
Co-trimoxazole (2T daily)	1	0	2
Total	16	14	16

3.4. Response to treatment

Of the 48 patients enrolled there were 6 withdrawals at weeks 1, 6, 8 (two patients) 13 and 18. Episodes of poor adherence were documented in two patients. Both patients were from the LPV/r arm. The microbiology, virology and immunology results of patients are shown below.

3.4.1. Microbiology Results

The microbiology results for all patients enrolled in the trial are shown in Table 3.7. Patients were enrolled after 6 weeks of standard intensive phase chemotherapy. After 2 weeks of RBT substitution, a sputum sample was provided for drug sensitivity test (DST) Of the 48 patients enrolled, *M. tuberculosis* was isolated from 5 patients at visit 2 and from 2 patients at visit 7. Only 2% were culture positive at visit 7. One patient was found to be MDR on DST at visit 2, although the results only became available after visit 7. A second patient was diagnosed with MDR TB from the visit 7 DST result. MDR from this patient had not been detected at visit 2. The two patients who were

diagnosed with MDR were the only two patients who remained sputum culture positive at visit 7.

Table 3.7. Microbiology results for all enrolled patients

	Visit 2	Visit 7
Smear		
Positive	10/46	2/46
MIGIT		
MTB isolated (n)	8/46	2/46
7H11 (n, %)		
MTB isolated	3.0 (6.3)	2.0 (4.30
Drug resistance (n, %)		
Rifampicin	1.0 (2.1)	2.0 (4.3)
Isoniazid	1.0 (2.1)	2.0 (4.3)
Ethambutol	1.0 (2.1)	1.0 (2.1)
Streptomycin	1.0 (2.1)	1.0 (2.1)

3.4.2. Viral Loads

There was a significant decrease in viral load in 42/46 patients after 16 weeks of ART ($p < 0.05$). The baseline mean (sd) viral loads for these patients was 5.3 (0.73) $\log_{10}$ copies/mL and decreased by 2.97 $\log_{10}$ copies/mL to 2.37 (1.1) $\log_{10}$ copies/mL.

At the end of TB treatment, there were 7 patients who had a viral load of >1000. A genotypic resistance test was carried out on samples from each of these patients. Resistance mutations were discovered in only one patient (from the NVP arm). The mutations detected in viral DNA from this patient were V901, K103N and G190AG. This patient was male and baseline genotypic results were not available.

3.4.3. CD4 counts

There was a significant increase in the mean $CD4^+$ counts of 42/46 patients at the end of TB therapy ($p < 0.05$). In these patients, the mean baseline $CD4^+$ count increased from 125.6 (43.4) cells/mm^3 at screening to 209.7 (119.2) cells/mm^3 at the end of TB treatment.

3.5. Pharmacokinetics

The pharmacokinetic analysis of RBT 300mg (displayed in Tables 3.8 and 3.9) is based on the pharmacokinetic data of 44 patients prior to the administration of ARVs. Of the 46 enrolled patients, one patient reported non-compliance to trial medication therefore his pharmacokinetic data was excluded from the data set. Another patient was hospitalized for meningitis and administered oral fluconazole 200mg daily. The patient was discharged on fluconazole prophylaxis therapy. Although the patient did not interrupt her trial medication, her pharmacokinetic data was excluded from the analysis. The main pharmacokinetic parameters for RBT and d-RBT for 44 patients are summarized in Table 3.8 below and according to arm in Table 3.9.

Table 3.8. Pharmacokinetic parameters of Rifabutin 300mg daily and d-RBT for 44 patients from non-compartmental analysis

Rifabutin (n = 44)		
Parameter	**Median**	**IQR**
AUC_{0-24} (ng.h/mL)	2 790.8	2 171.2 - 3324.9
C_{max} (ng/mL)	284.0	198.5 - 372.5
T_{max} (h)	3.0	3.0 - 4.0
C_0 (ng/mL)	65.0	49.2 - 80.5
C_{24} (ng/mL)	48.0	39.5 - 62.6
CL/F (L/h)	107.5	90.3 – 138.5
25-Desacetyl-Rifabutin (n = 44)		
Parameter	**Median**	**IQR**
AUC_{0-24} (ng.mL/h)	232.3	191.8 - 317.9
C_{max} (ng/mL)	25.4	18.8 - 37.8
T_{max} (h)	3.0	3.0 - 3.0
C_0 (ng/mL)	5.0	3.5 - 7.7
C_{24} (ng/mL)	3.6	2.9 - 5.0
CL/F (L/h)	1291.8	944.8 – 1564.3

Table 3.9. Pharmacokinetic parameters of rifabutin 300mg daily and 25-O-deacetylrifabutin according to arm from non-compartmental analysis. Data are presented as medians and IQR

Rifabutin	Arm 1 (n = 14)	Arm 2 (n= 14)	Arm 3 (n = 16)
AUC_{0-24} (ng.h/mL)	2647.6 (2065 - 3510.4)	2487.5 (2007.1 - 3120.1)	3052.85 (2650.2 - 3431.5)
C_{max} (ng/mL)	245.5 (194.0 – 336.0)	246.0 (193.0 - 414.0)	291.5 (250.0- 377.0)
T_{max} (h)	3.0 (3.0 - 4.0)	3.0 (3.0 - 3.0)	3.0 (3.0 - 4.0)
C_0 (ng/mL)	62.7 (44.1 – 82.8)	73.6 (63.1 – 83.2)	59.0 (36.4 - 78.6)
C_{24} (ng/mL)	25.2 (38.3 – 58.0)	45.8 (37.6 – 56.0)	60.7 (40.6 - 68.8)
CL/F (L/h)	113.8 (85.5 - 145.3)	120.0 (96.1 - 149.5)	98.3 (87.4 - 113.2)
d-RBT			
AUC_{0-24} (ng.h/mL)	216.9 (182.9 - 339.2)	193.7 (154.0 – 236.9)	273.3 (235.7- 344.1)
C_{max} (ng/mL)	22.4 (18.9 – 45.5)	21.6 (16.7 – 28.8)	32.5 (25.2 - 37.7)
T_{max} (h)	3.0 (3.0 – 3.0)	3.0 (3.0 – 4.0)	3.0 (3.0 – 4.0)
C_0 (ng/mL)	4.4 (4.0 – 7.1)	5.8 (3.4 – 7.9)	5.1 (2.7 - 6.6)
C_{24} (ng/mL)	3.5 (2.8 – 4.7)	3.2 (2.2 -4.2)	5.0 (3.4 - 5.8)
CL/F (L/h)	1386.4 (884.5 - 1640.3)	1548.9 (1213.6 - 1949.0)	1098.6 (871.9 - 1272.6)

d-RBT = 25-O-deacetylrifabutin; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; IQR= interquartile range; GM = geometric mean; CL/F = oral clearance

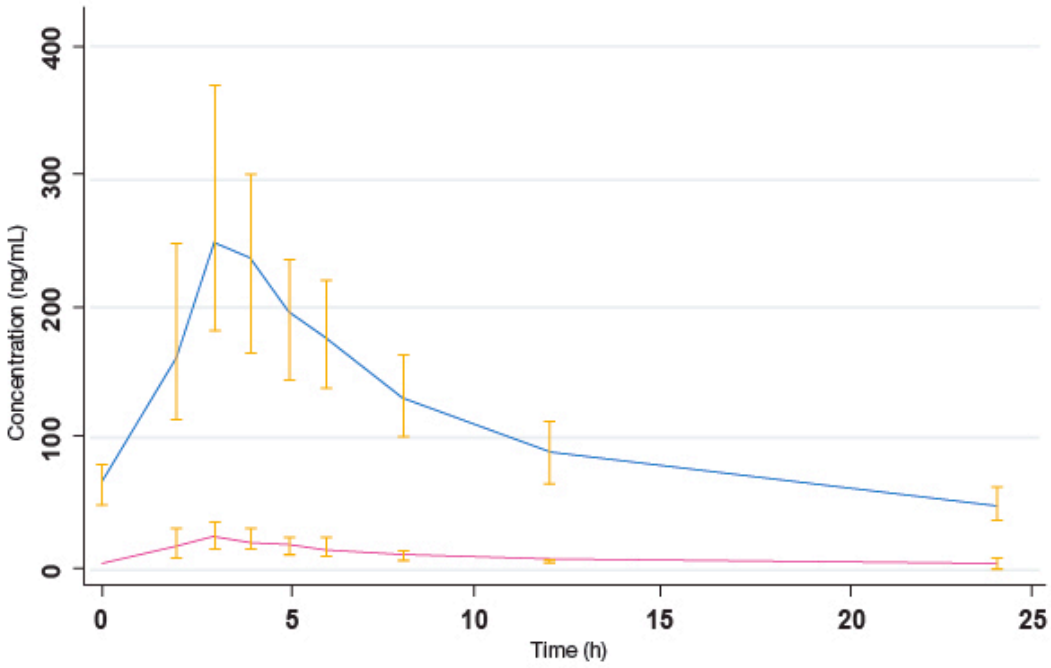

Figure 3.2. Median (IQR) concentration-time profile of rifabutin 300mg daily (blue) and 25-O-desacetylrifabutin (red) for 44 patients

The area-under-the-plasma-concentration vs time curve (AUC) (Figure 3.2) shows that an oral dose of RBT 300mg reaches a median peak plasma concentration of 284.0 ng/mL (IQR, 198.5 - 372.5 ng/mL) in African patients co-infected with TB-HIV. Peak plasma concentration was obtained 3 hours after the dose was taken. Median trough concentration (C_{24}) attained was 48.0 ng/mL (IQR, 39.5- 62.6 ng/mL). Trough concentration was 6-fold lower than peak concentration indicating a large volume of distribution. The median RBT AUC_{0-24} obtained in this study was 2 790.8 ng.h/mL. Individual AUC_{0-24} concentrations ranged from 1 088.5 ng.h/mL to 5 892.9 ng.h/mL. Inter-patient variability for AUC_{0-24} and C_{max} was 36 and 43% respectively. Individual AUC_{0-24} plots of the 14 and 16 patients enrolled to the NVP, EFV and LPV arms respectively, (Figure 3.3 A,B&C) shows heterogeneous absorption. Peak concentration was observed approximately 3 hours post-dose and in some patients lower secondary

peaks occurred. For 5 patients with C_{max} values below the published reference (range 108 - 207 ng/mL), 2-hour and 6-hour post-dose concentrations were roughly the same indicating the possibility of delayed absorption (Peloquin et al 2002).

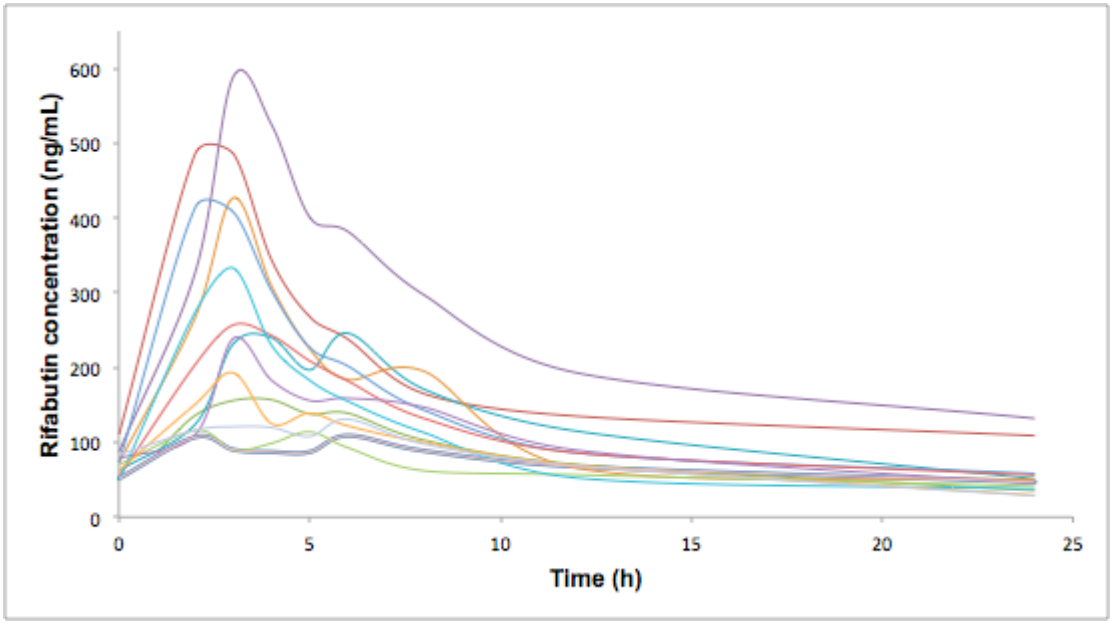

Figure 3.3 A. Individual concentration-time profiles of rifabutin 300mg for 14 patients randomised to the nevirapine arm

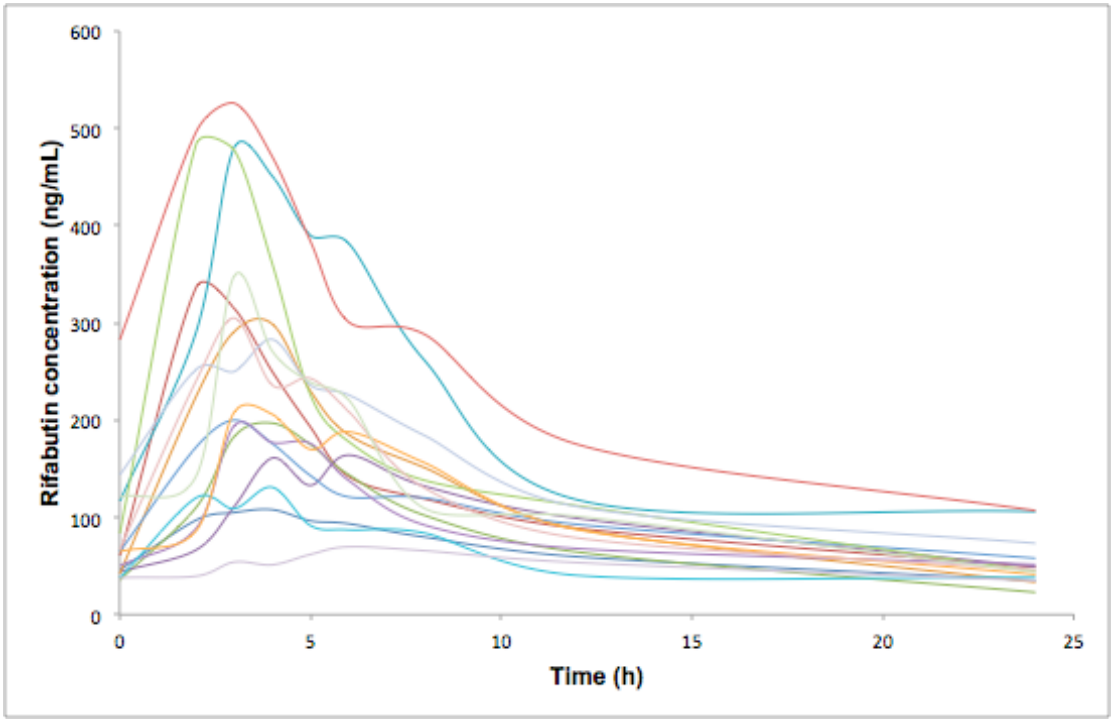

Figure 3.3 B. Individual concentration-time profiles of rifabutin 300mg for 16 patients randomised to the efavirenz arm

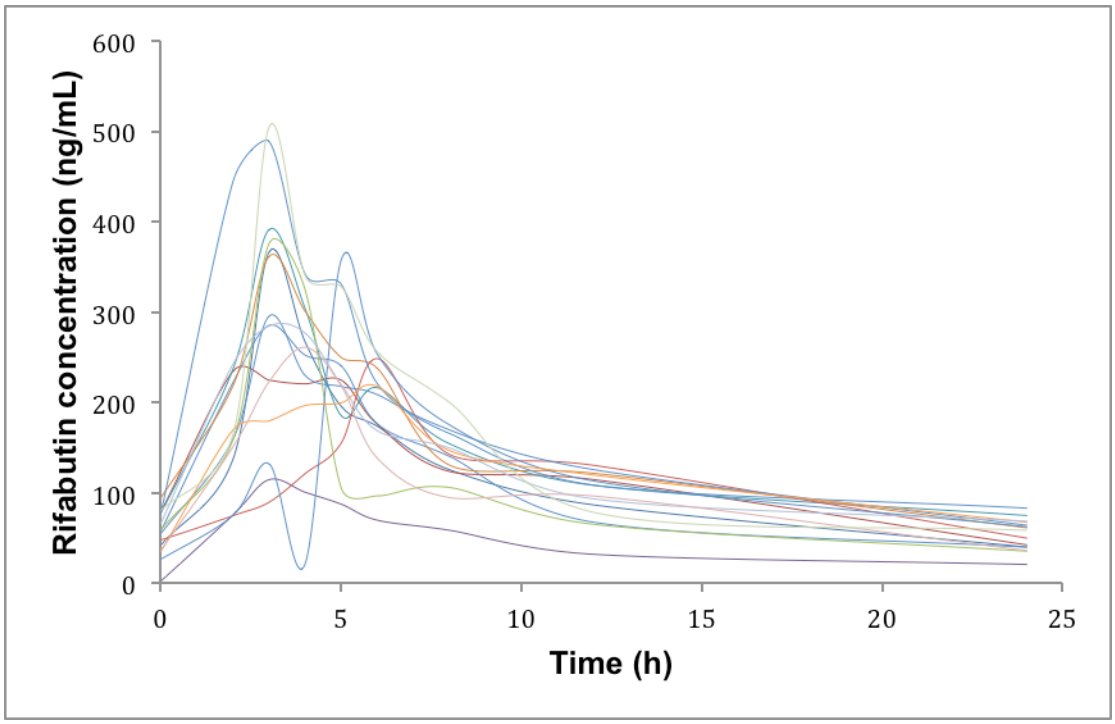

Figure 3.3 C. Individual concentration-time profiles of rifabutin 300mg for 16 patients randomised to the lopinavir arm

The median AUC_{0-24} and C_{max} of d-RBT was 232.3 ng.h/mL and 25.4 ng/mL respectively. Maximum plasma concentrations of the metabolite were attained in 3 hours. The ratio of the AUC_{0-24} of parent drug to metabolite was 12 : 1. The metabolite reached peak concentrations of approximately 10% of the parent drug and demonstrated a similar plasma decay curve. The main pharmacokinetic parameters for 25-O-desacetylrifabutin are displayed in Table 3.7.

Based on previously published reference ranges there are 41/44 (93%) and 26/44 (59%) patients with low AUC_{0-24} and C_{max} values, respectively. To identify the determinants of RBT plasma concentrations, the effects of weight, height, gender, $CD4^+$ count, hemoglobin, neutrophil count, platelets, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were tested in a univariate and multivariate logistic regression model. Rifabutin AUC_{0-24} concentrations were log transformed prior to analysis. Mean (sd) values for RBT AUC_{0-24}, log AUC_{0-24}, C_{max}, C_{24} and all covariates tested in the model are displayed in Table 3.10.

Table 3.10. Mean (sd) values for rifabutin AUC_{0-24}, log AUC, C_{max}, C_{24} and various covariates for 44 patients on rifabutin 300mg

Variable	n	mean	sd	median	min	max
AUC_{0-24} (ng.h/mL)	44	2859.5	1021.0	2790.8	1088.5	5892.9
C_{max} (ng/mL)	44	294.8	127.1	284.0	108	589.0
C_{24} (ng/mL)	44	54.2	23.6	48.0	21.2	132.0
Weight (kg)	44	60.7	8.7	58.3	46.8	80.5
Height (cm)	44	159.7	7.7	160.1	146	176.0
Hemoglobin (g/dL)	44	11.6	1.7	11.5	7.5	14.8
Neutrophil count (cells/L)	44	2.2	1.3	1.9	0.6	6.5
Platelets (x 10^9/L)	44	304.5	152.0	281.5	100.0	745.0
AST (units/L)	44	49.3	22.0	43.0	24.0	129.0
ALT (units/L)	44	29.1	16.1	25.0	11.0	91.0
$CD4^+$ count (cells/mm^3)	44	126.1	43.9	129.5	50.0	199.0
log AUC_{0-24} (ng.h/mL)	44	7.9	0.3	7.9	7.0	8.7

In univariate analysis female sex, weight and $CD4^+$ count were significantly associated with $AUC_{0\text{-}24}$ and female sex, hemoglobin and weight were significantly associated with C_{max}. However, with multivariate analysis only female sex and weight were significantly associated with $C_{max.}$ As body weight increased RBT C_{max} decreased. There were no significant associations observed for C_{24} and covariates with univariate and multivariate analysis.

Table 3.11. Determinants of rifabutin serum concentrations by univariate and multivariate analysis

	Univariate (n=44)		**Multivariate (n = 44)**	
	β coefficient (95%CI)	P value	β coefficient (95%CI)	P value
log AUC_{0-24}				
Female sex	0.205132 (-0.00523 - 0.415496)	0.05	0.148507 (-0.06207 - 0.359086)	0.16
Weight	- 0.01353 (-0.0252 - -0.00185)	0.02	- 0.00944 (-0.02148 - 0.002608)	0.12
CD4 count	0.002245 (-0.00012 - 0.004608)	0.06	0.001876 (-0.0004 - 0.004157)	0.1
C_{max}				
Female sex	125.756 (55.61095-195.901)	0.001	104.1372 (33.08654 - 175.1879)	0.01
Weight	-5.81287 (-9.97138 - -1.65436)	0.007	- 4.03424 (-8.04543 - -0.02305)	0.04
Hemoglobin	- 22.9306 (-45.6179 - -0.24322)	0.04	5.7 (-18.6 – 30.0)	0.6

3.6. Discussion

Low bioavailability of anti-TB drugs has been reported frequently in patients co-infected with TB and HIV (van Crevel et al 202; Choudhri et al 1996; Peloquin et al 1996; Sahai et al 1997; Gurumurthy et al 2004; Perlman et al 2005; Tappero et al 2005; McIlleron et al 2006) and these studies report that of the commonly used anti-TB drugs, RMP is the most common drug with reduced bioavailability. Although initial studies reported no association between HIV and plasma RBT concentrations (Narang et al 1996; Gatti et al 1998; Gatti et al 1999), there is more recent evidence that RBT absorption is also decreased in TB-HIV co-infected patients and contributes to ARR (Weiner et al 2005a; Holland et al 2009). The median AUC_{0-24} and C_{max} values for RBT 300mg obtained in this study are comparable to values obtained in a previous study of RBT pharmacokinetics in TB-HIV co-infected patients by (Boulanger et al 2009). However, in comparison to a Phase I single dose study of RBT 300mg daily in HIV-infected patients without TB (Skinner et al 1989), the bioavailability of RBT in our study is lower. Furthermore, the RBT AUC_{0-24} and C_{max} values in this study are also lower than those obtained in healthy volunteers studies (Strolin-Benedetti et al 1990, Narang et al 1992, Polk et al 2001, Hamezh et al 2003, Sekar et al 2010). In contrast, the AUC_{0-24} d-RBT was higher than values previously reported in healthy volunteers but lower than values reported by Boulanger et al. This may indicate increased metabolism of RBT in TB-HIV co-infected patients.

Rifabutin also undergoes autoinduction, which may account for its decreased oral bioavailability, and steady-state is attained after 6 days of repeated dosing of RBT

300mg. In a study by Strolin-Benedetti et al (1990) in healthy volunteers, RBT and d-RBT AUC_{0-24} was significantly decreased after 10 consecutive doses whereas half-life remained unchanged. This pattern of elimination is characteristic of drugs with a high hepatic extraction ratio (Rowland and Towser 1995). However, the hepatic extraction ratio of RBT is presently unknown due to a lack of pharmacokinetic studies following i.v. administration of RBT to healthy volunteers. Strolin-Benedetti et al (1990) have estimated the hepatic extraction ratio of RBT from available i.v data in HIV-infected patients to be 0.16. Consequently, RBT is not a high hepatic extraction ratio drug suggesting that RBT is metabolized extensively in the gut after oral dosing leading to its low oral bioavailability.

Analysis of individual time-concentration graphs suggest that delayed absorption accounted for the decreased exposure of RBT in a very small proportion of patients, suggesting that the bioavailability of RBT may be determined by various other factors. The bioavailability of anti-TB drugs have been associated with various factors such as alcohol use (Kimerling et al 1998), race (Tappero et al 2005), age (McIlleron et al 2006), gender (Sahai et al 1997; Ray et al 2003; McIlleron et al 2006), hypoalbuminaemia (Tappero et al 2005) and dose per kg of body weight (McIlleron et al 2006). In this study peak RBT plasma concentration was associated with body weight and patients who were males by multivariate logistic regression analysis. Males had lower C_{max} than females ($p = 0.06$). This is in agreement with a previous study comparing RBT pharmacokinetics in males and females. Females had higher bioavailability than males. Rifabutin is highly lipophilic therefore more uptake and

distribution in tissues is expected to take place in females who have a higher fat content than males (Colborn et al 1993). Approximately 66% of the patients in this study were males, which may have contributed to the overall low bioavailability. A recent South African study conducted in a similar cohort to this study reported that male patients have lower RMP bioavailability (McIlleron et al 2012).

A significant difference in RBT bioavailability was shown between male and female patients. Females showed higher RBT bioavailability, consistent with a previous study (Colborn et al 1993). Gender differences in RMP pharmacokinetics has been reported recently in a study of TB-HIV co-infected patients recruited in Kwa-Zulu Natal (McIlleron et al 2012). In this study, females showed greater RMP bioavailability compared to males. Differences in drug pharmacokinetics between males and female may also be due to differences in the activity of drug metabolizing enzymes and drug transporters. Studies in normal human liver samples have suggested that women have higher CYP3A4 and 2-fold lower PGP activity compared to men (Schuetz et al 1995; Meibhom et al 2002). However, hepatic PGP and CYP3A4 work together to increase drug elimination from the liver. Therefore, in women, intracellular accumulations of PGP substrates occur, which consequently increase competitive drug interactions between PGP and CYP3A4, ultimately delaying drug elimination (Chiou et al 2000; Lan et al 2000).

Physiological differences such as generally lower body weight and organ size, higher percentage of body fat and different gastric motility in women compared to men could also account for the differences in bioavailability (Meibhom et al 2002). Greater body fat in women may account for greater volume of distribution of lipophilic drugs such as RBT and oral bioavailability of CYP3A substrates are higher in women compared to men (Schwartz 2003).

Significant weight loss is one of the first signs and a symptom of TB and HIV infection and malnutrition is common in TB-HIV co-infected patients (Harries et al 1985; Onwubalili et al 1988). Reduced RMP bioavailability has been reported in patients with malnutrition (Polasa et al 1984) and there is evidence from patients without TB that malabsorption of antimycobacterial drugs increases with advancing HIV disease (as determined by decreasing CD4 cell counts) (Sahai et al 1996). Low serum albumin levels have also been reported in TB patients (Onwubalili et al 1988; Tappero et al 2005). Low serum albumin levels reduce intravascular oncotic pressure, which could lead to edema, thickening of the intestinal wall, and impaired drug absorption. Rifabutin exhibits high affinity for plasma proteins. The extent of RBT binding to plasma proteins is reported to be 70 - 90% (Skinner et al 1989; Brogden and Fitton 1994). Therefore when serum albumin levels are low, less RBT is bound with plasma proteins and more is available in the circulation thereby increasing hepatic clearance and decreasing bioavailability. However, in this study there were no malnourished patients as the mean BMI and weight was 23kg/m^2 and 58.3 kg respectively.

Gastro-intestinal malfunction is common in patients with HIV-infection (Smith et al 1992; Lambi et al 1996). Rifabutin absorption may be decreased in TB-HIV patients by the presence of gastrointestinal disorders, which reduce the intestinal absorptive area or alter the absorptive processes. The D-Xylose tolerance test is used in clinical practice to assess small intestine function (Rolston et al 1989). A low D-Xylose level indicates poor mucosal absorption or the presence of bacteria in the small intestine that metabolize the xylose before it can be absorbed. Altered D-Xylose kinetics was observed in patients infected with HIV (Ehrenpreis et al 1991; Carlson et al 1994) and who had severe weight loss and diarrhoea in the absence of intestinal pathogens and histologic abnormalities, suggesting that lymphatic obstruction produces the malabsorptive state (Ehrenpreis et al 1992). Two previous studies that investigated the absorption of anti-TB drugs in TB patients with HIV infection and the concurrent absorption of D-Xylose in the same patients found that the AUC of D-Xylose correlated with the reduced AUC of RMP and PZA (Choudhri et al 1996; Sahai et al 1997). Thus there is evidence of altered drug absorption taking place in the small intestines of HIV-infected patients.

Differences in RBT bioavailability among patients can also be due to different rates of metabolism. Rifabutin is metabolized to d-RBT by β-esterases (Jamis-Dow et al 1997) in the liver and by CYP3A4 in the gastrointestinal tract and liver (Iatsimirskaia et al 1997). However, there is presently no data on the effect of β-esterase gene polymorphisms on RBT pharmacokinetic parameters. Various studies have shown a

10-fold variation among individuals in the extent to which RMP induces CYP3A4 expression in humans (Watkins 1989; Kolars 1992). Whether such variation in induction of CYP3A4 expression by RBT also exists is unknown; and likewise variable induction of CYP3A4 by RBT may account for the inter-individual variation in RBT bioavailability. In addition, various physiological and environmental factors such as hormonal homeostasis, disease status, age and diet are also responsible for the individual variability in CYP3A4 induction (Tang et al 2005).

The bioavailability of RBT 300mg is low in TB-HIV co-infected patients in this study. However, the metabolite is also microbiologically active and the combination of parent drug and metabolite may provide adequate antimicrobial activity in TB-HIV co-infected patients. More studies are required to determine whether a revision of RBT dosing in African TB-HIV co-infected patients is required.

Chapter 4: The Pharmacokinetic Interaction between Rifabutin and Non-nucleoside Reverse Transcriptase Inhibitors

Summary

The combination of RBT 300mg daily plus NVP maintains RBT C_{max} and AUC_{0-24} in line with currently recommended ranges for the treatment of M. tuberculosis and the prevention of ARR respectively. When RBT 600mg is combined with EFV, the AUC_{0-24} and C_{max} of RBT is similar to the AUC_{0-24} and C_{max} of RBT 300mg daily in the same patients. The administration of EFV and NVP in combination with RBT to TB-HIV co-infected patients was well tolerated by patients in both treatment arms. There was one report of grade 4 hepatotoxicity in the NVP arm, one report each per arm of grade 4 neutropenia and one patient in the NVP arm reported grade 4 uveitis after 24 weeks of TB treatment.

4.1. Introduction

Treatment options for TB-HIV co-infected patients living in resource-limited countries are few because of limited access to ART and the high cost of treatment. Efavirenz is currently prescribed with RMP-based ART, according to WHO ART treatment guidelines, on the basis of evidence of greater drug interactions occurring between RMP and NVP. Pozniak et al (1999) have suggested that when TB is diagnosed in patients taking NVP, NVP should be stopped and the patients switched to an alternative regiment. However, in resource-limited countries NVP is the more widely used NNRTI because it is cheap and safe to take during pregnancy. Rifabutin is an

alternate rifamycin recommended for the treatment of TB-HIV co-infected patients requiring PI-based therapy but clinical data on the pharmacokinetic interactions between RBT and first line ART are limited. This study investigated the pharmacokinetic interactions between standard doses of EFV and NVP administered with two different daily doses of RBT in severely immunocompromised African patients co-infected with TB-HIV.

4.2. Patient enrolment

In total 14 patients were randomized to the NVP arm and 16 to the EFV arm. Three randomized patients were excluded from the pharmacokinetic analysis, leaving 14 patients in the EFV arm and 13 patients in the NVP arm. One patient in the EFV arm was withdrawn after completing one pharmacokinetic visit due to TB meningitis. Another patient was hospitalized for cryptococcal meningitis where she was administered fluconazole and discharged on secondary prophylaxis with fluconazole. One patient in the NVP arm developed grade 4 elevated transaminases two weeks after starting NVP therapy and was subsequently withdrawn from the trial.

4.3. Results

4.3.1. Baseline patient demographics

The baseline demographic data of patients randomized to the EFV and NVP arms are comparable (Table 4.1). The mean age, weight, height, BMI, $CD4^+$ counts and viral load of patients randomized to the EFV arm was 31.6 years, 56.05 kg, 161.0 cm, 22.8

kg/m^2, 117.6 cells/mm^3 and 5.2 $\log_{10}$ copies. In the NVP arm the mean age, weight, height, BMI, $CD4^+$ counts and viral load was 34.8 years, 54.4 kg, 158.6 cm, 22.3 kg/m^2, 99.0 cells/mm^3 and 5.3 $\log_{10}$ copies.

Table 4.1. Baseline demographic data for patients randomized to the efavirenz and nevirapine arms. Data presented are means with standard deviation in brackets

Parameter	EFV Arm (n = 16)	NVP Arm (n = 14)
Age at screening, years	31.8 (4.7)	36.1 (6.6)
Height, cm	159.1 (8.4)	159.5 (7.5)
Weight, kg	59.4 (9.8)	57.2 (8.2)
BMI, kg/m^2	23.7 (5.2)	22.4 (2.8)
Gender, n (%)		
Female	6.0 (3.5)	6.0 (43)
Male	10.0 (62.5)	8 .0 (57)
$CD4^+$ cell count, cells/mm^3	117.6 (44.4)	107.3 (30.0)
Viral Load	5.2 $\log_{10}$	5.3 $\log_{10}$
Karnofsky Q score	100 (100)	100 (100)

4.3.2. Response to treatment

At randomization there were a total of 6 patients who were AFB sputum smear positive, one of whom was also sputum culture positive for *M. tuberculosis*. At the end of TB treatment, 2 patients remained sputum culture positive for *M. tuberculosis* one each from the EFV and NVP arms. Both these patients' drug susceptibility testing (DST) results at randomization, available after completion of pharmacokinetic visits, revealed multi-drug resistant (MDR) *M. tuberculosis*. In addition there was one patient on the EFV arm who remained sputum smear positive but sputum culture negative at the penultimate trial visit. Both patients diagnosed with MDR-TB were initiated on standardized second-line MDR-TB treatment according to South African Department of Health guidelines. All patients in both arms reported 100% compliance to trial medication.

There were significant increases in the $CD4^+$ counts of patients randomized to both arms, by the end of TB treatment. Baseline $CD4^+$ count increased from 110.5 cells/mm^3 to 182.5 cells/mm^3 ($p < 0.01$) in the EFV arm and from 99.0 cells/mm^3 to 202.8 cells/mm^3 ($p = < 0.01$) the NVP arm. There was also a significant decrease in log viral load in both arms. In the EFV arm, mean viral load decreased significantly from 5.3 log_{10} copies to 1.9 log_{10} copies ($p < 0.01$) and from 5.3 log_{10} copies to 2.3 log_{10} copies ($p < 0.01$) in the NVP. This corresponded to a mean change of 3.3 log_{10} in the EFV arm and 2.9 log_{10} in the NVP arm. After 16 weeks of ART in combination with RBT, there were two patients with viral loads greater than 1 000, both from the NVP arm. One had a viral load of 17 600 copies/mL and the other a viral load of 188 000

copies/mL. Only the latter patient had genetic evidence of NNRTI resistance. Genotyping of viral DNA showed the presence of V90I, K103KN and G190AG mutations.

4.3.3. Pharmacokinetic analysis

The median AUC_{0-24} of RBT 300mg was comparable between the EFV and NVP arms (Table 4.2 and Table 4.5). The AUC_{0-24} of RBT 300mg was 2 647.6 ng.h/mL in the EFV arm and 2 487.5 ng.h/mL in the NVP arm. The median C_{max} values of RBT 300mg were similar between the two arms, 245.5 ng/mL in the EFV arm and 246.0 ng/mL in the NVP arm. However, RBT trough concentration (C_{24}) in the NVP arm was almost double the value obtained in the EFV arm. There were no significant differences in the oral clearance of RBT between patients randomized to the two arms.

Large inter-patient variability was noted for AUC_{0-24} and C_{max} in both arms. In the EFV arm, RBT AUC_{0-24} ranged from 1 483.1 ng.h/mL to 5764.5 ng.h/mL and C_{max} ranged from 108.0 ng/mL to 526.0 ng/mL. Similar interpatient variability in RBT AUC_{0-24} and C_{max} values as in the EFV arm were observed in the NVP arm. Rifabutin AUC_{0-24} ranged from 1 572.4 ng.h/mL to 5 892.9 ng.h/mL and C_{max} ranged from 116.0 ng/mL to 589.0 ng/mL. The inter-patient variability expressed as coefficient of variation (%CV) for AUC_{0-24} and C_{max} was 42 and 48% respectively in the EFV arm and 43% and 50%, respectively in the NVP arm.

The main pharmacokinetic parameters of RBT given alone and in combination with EFV are displayed in Table 4.2 and the median concentration-time profile of RBT before and after EFV-based ART is shown in Figure 4.1. The median RBT AUC_{0-24} for 300mg RBT daily given alone and 450mg RBT daily plus EFV in the same patients was 2 647.6 and 1 967.8 ng.h/mL respectively, representing a decrease of 26%. However, the median AUC_{0-24} of RBT 300mg daily was similar to the median AUC_{0-24} of RBT 600mg plus EFV in the same patents. The median AUC_{0-24} of RBT 600mg plus EFV was 2 567.7 ng.h/mL compared to 2 647.6 ng.h/mL for RBT 300mg given alone in the same patients. The difference in AUC_{0-24} between the two dosing periods was 3%.

The median C_{max} of RBT 450mg decreased in the presence of EFV while that of RBT 600mg increased in comparison to 300mg RBT given alone, but the differences were not significant ($p = 0.6$ and 0.5 respectively). Large inter-patient variation in RBT C_{max} was observed for all three dosing periods. The inter-patient variability (%CV) was equal for 300mg RBT given alone and 600mg RBT plus EFV, 42% and 43% respectively, and 52% for 450mg RBT plus EFV. There were no significant changes in median T_{max} values between the three dosing periods indicating that absorption of RBT was not affected by the presence of EFV. However the oral clearance of RBT 450mg plus EFV was significantly higher when compared to the clearance of RBT 300mg given alone ($p = 0.01$). The clearance of RBT 600mg was not increased in the presence of EFV.

Table 4.2. Rifabutin pharmacokinetic parameters before and after efavirenz-based ART

Treatment Period	Parameter	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{24} (ng/mL)	CL/*F* (L/h)
Period 1	Mean	2 901.4	279.6	3.4	81.3	54.0	118.9
RBT300mg	SD	1 211.3	135.0	1.0	65.5	25.2	44.1
	Median	2 647.6	245.5	3.0	62.7	25.2	113.8
	IQR (25)	2 065.0	194.0	3.0	44.1	38.3	85.5
	IQR (75)	3 510.4	336.0	4.0	82.8	58.0	145.3
	GM	2 697.5	250.8	3.3	67.2	49.4	111.2
	Min	1 483.1	108.0	2.0	35.7	22.9	52.0
	Max	5 764.5	526.0	6.0	282.0	107.0	202.3
	CV%	42	48	29	81	47	37
Period 2	Mean	2 057.4	257.2	3.1	42.7	31.8	195.3
RBT450mg	SD	1072.5	150.3	0.8	33.9	21.5	115.0
+EFV	Median	1967.8	209.0	3.0	34.3	26.6	152.5
	IQR (25)	1091.6	137.0	2.0	18.6	19.0	103.3
	IQR (75)	2903.5	406.0	3.0	59.4	43.9	274.8
	GM	1788.4	216.5	3.0	28.8	26.0	167.8
	Min	676.8	78.1	2.0	2.0	7.3	76.2
	Max	3936.0	544.0	5.0	113.0	81.8	443.3
	CV%	52	58	26	79	68	59
Period 3	Mean	2 655.8	328.3	2.9	55.6	43.7	145.2
RBT600mg	SD	1148.5	183.0	1.1	20.1	18.0	89.3
+EFV	Median	2567.7	309.5	3.0	51.2	43.1	117.0
	IQR (25)	1977.8	171.0	2.0	45.1	26.9	79.7
	IQR (75)	3765.5	426.0	3.0	73.4	54.0	151.7
	GM	2378.2	273.7	2.8	51.8	40.0	126.1
	Min	866.5	71.1	2.0	23.4	18.4	72.2
	Max	4154.7	662.0	6.0	85.1	76.0	346.2
	CV%	43	56	38	36	41	62

RBT = rifabutin; EFV = efavirenz; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

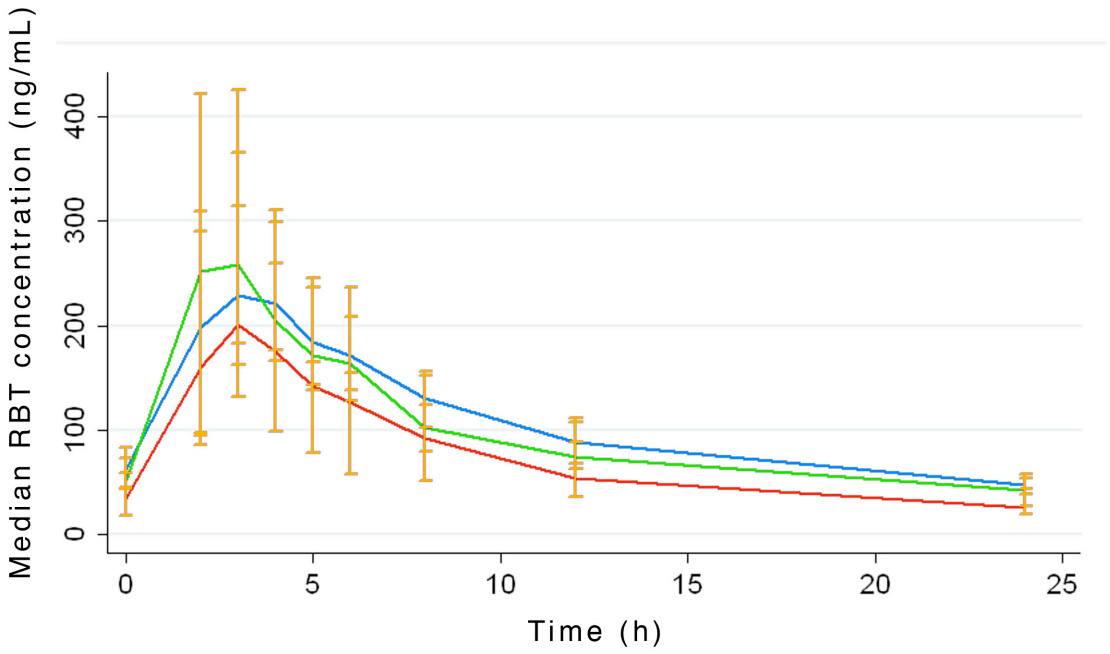

Figure 4.1. Median (IQR) concentration-time profiles for rifabutin (RBT) 300mg (blue), rifabutin 450mg daily plus efavirenz (red) and rifabutin 600mg daily plus efavirenz (green)

The pharmacokinetic parameters and median concentration-time profile of d-RBT are displayed in Table 4.3 and Figure 4.2 respectively. The median AUC_{0-24} and C_{max} of d-RBT when 300mg RBT was given alone was 216.9 ng.h/mL and 22.4 ng/mL respectively. However, when 450mg RBT was given in combination with EFV in the same patients, the AUC_{0-24} and C_{max} were 139.0 ng.h/mL and 18.3 ng/mL respectively, which corresponded with a 1.5-fold decrease in d-RBT exposure in the presence of EFV. In contrast, median AUC_{0-24} and C_{max} values of d-RBT were similar for RBT 300mg given alone and RBT 600mg plus EFV. Median AUC_{0-24} and C_{max} of d-RBT

were 216.9 ng.h/mL and 22.4 ng/mL and 222.1 ng.h/mL and 24.6 ng/mL for RBT 300mg and RBT 600mg plus EFV respectively. Oral clearance of d-RBT was higher for RBT 450mg and 600mg plus EFV compared to RBT 300mg given alone in the same patients, but only significant for the 450mg dose (p = 0.01). The median combined exposure of the active moiety (parent plus metabolite) was highest for RBT 300mg (2 859.3 ng.h/mL) compared to the other dosing periods (2 107.2 ng.h/mL for 450mg RBT plus EFV and 2 796.5 for 600mg RBT plus EFV).

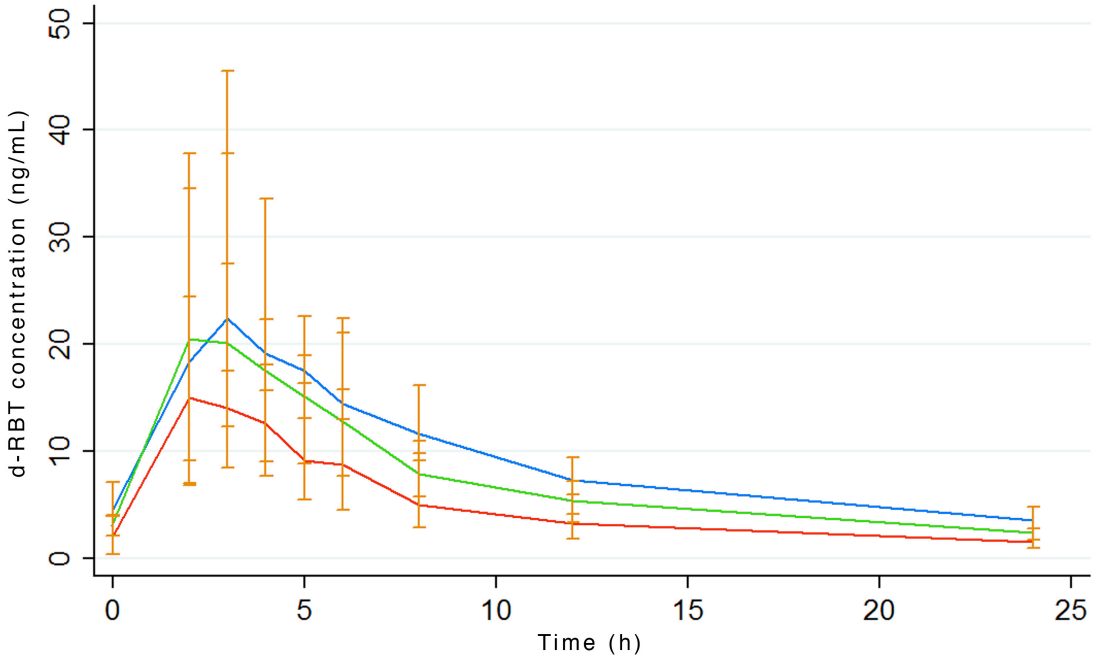

Figure 4.2. Median (IQR) concentration-time profiles of 25-O-deacetylrifabutin (d-RBT) for rifabutin 300mg daily (green), rifabutin 450mg plus efavirenz (blue) and rifabutin 600mg plusefavirenz (red)

Table 4.3. Pharmacokinetic parameters of 25-O-desacetylrifabutin before and after efavirenz based antiretroviral therapy

Treatment Period	Parameter	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{24} (ng/mL)	CL/*F* (L/h)
Period 1	Mean	316.6	34.0	3.1	8.7	4.7	1228.4
RBT 300mg	SD	224.9	24.8	0.9	11.4	3.5	474.6
	Median	216.9	22.4	3.0	4.4	3.5	1386.4
	IQR (25)	182.9	18.9	3.0	4.0	2.8	884.5
	IQR (75)	339.2	45.5	3.0	7.1	4.7	1640.3
	GM	270.8	28.1	3.0	6.0	3.9	1108.0
	Min	173.7	14.5	2.0	3.1	1.8	319.1
	Max	940.1	98.8	6.0	46.6	13.7	1727.6
	CV%	71	73	29	131	74	39
Period 2	Mean	160.0	21.4	3.1	3.4	2.0	3262.4
RBT 450mg	SD	118.5	15.4	1.0	4.0	2.0	2734.5
+ EFV	Median	139.0	18.3	3.0	1.9	1.6	2161.2
	IQR (25)	66.6	8.4	3.0	0.4	0.9	1530.3
	IQR (75)	196.0	28.1	3.0	4.0	1.7	4501.8
	GM	122.9	16.5	3.0	1.8	1.4	2441.6
	Min	32.0	4.4	2.0	0.4	0.4	670.8
	Max	447.2	56.5	6.0	13.3	7.2	9370.6
	CV%	74	73	32	118	100	84
Period 3	Mean	205.6	27.3	4.4	3.5	2.6	2228.9
RBT 600mg	SD	119.5	17.4	5.7	2.5	1.5	1780.4
+ EFV	Median	222.1	24.6	3.0	3.1	2.4	1350.7
	IQR (25)	124.7	13.9	2.0	2.1	1.7	1235.3
	IQR (75)	242.9	37.8	3.0	3.9	2.8	2405.4
	GM	170.4	21.0	3.2	2.9	2.2	1760.9
	Min	43.8	2.7	2.0	0.5	0.4	689.4
	Max	435.2	58.7	24	9.4	6.4	6852.3
	CV%	58	64	133	71	58	80

RBT = rifabutin; EFV = efavirenz; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

The pharmacokinetic parameters of EFV administered with two different doses of RBT are displayed in Table 4.4 and the corresponding median concentration-time profile of EFV is displayed in Figure 4.3.

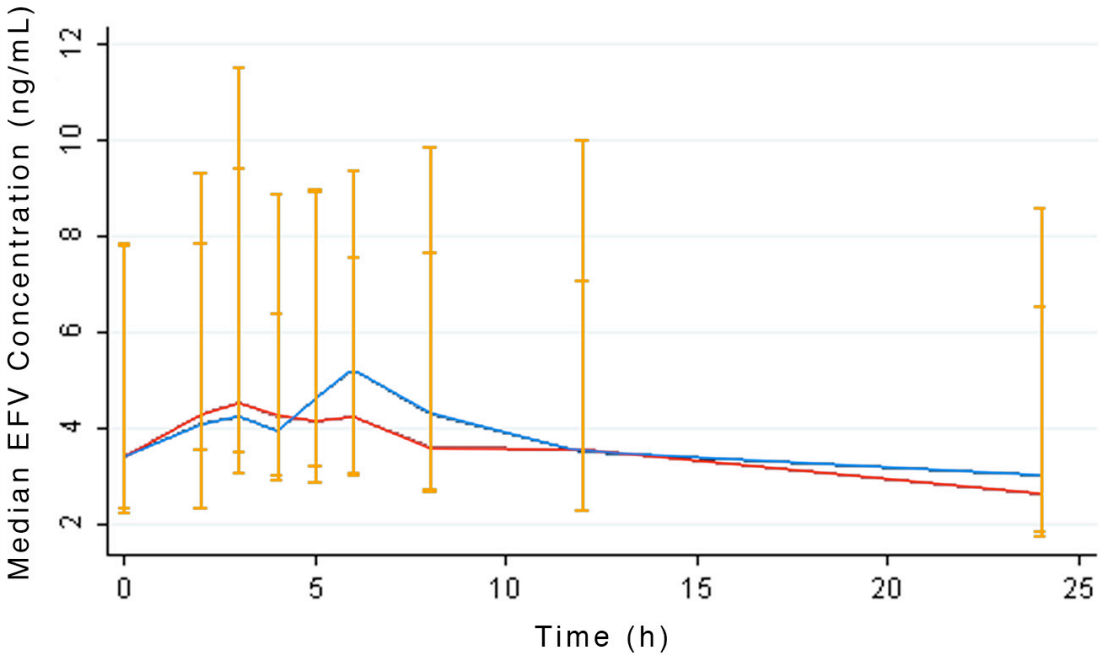

Figure 4.3. Median (IQR) concentration-time profile of efavirenz (EFV) in the presence of rifabutin 450mg (blue) and 600mg (red) daily

The median EFV AUC_{0-12} when EFV was given with 450mg RBT was similar to the median EFV AUC_{0-12} when EFV was given with 600mg RBT (86.3 ng.h/mL). Comparison of the median EFV trough (C_{12}) concentrations for the two dosing periods showed no differences (3.0 ng/mL and 2.7 ng/mL for the 450mg and 600mg doses of RBT respectively). However, inter-patient variability of EFV trough concentrations was very high (%CV = 113% and 93 % for 450 and 600mg dose of RBT respectively).

Trough concentrations ranged from 1.3 ng/mL to 24.4 ng/mL when patients took 450mg RBT plus EFV and from 1.1 ng/mL to 16.5 ng/mL when the same patients took 600mg RBT plus EFV. In this study there were no patients with EFV trough levels below 1.0 ng/mL, however 5 and 4 patients had EFV trough concentrations above 4 ng/mL for the 450 and 600mg doses of RBT, respectively. Trough EFV concentrations were notably high in one patient (24.2 ng/mL for 450mg RBT plus EFV and 16.5 ng/mL for 600mg RBT plus EFV). However, adverse events such as CNS toxicity, which have been associated with such high EFV concentrations (Marzolini et al 2002) were not reported in this study.

In most patients, secondary peaks were observed for EFV in combination with RBT 450 and 600mg approximately 6 hours after the ingestion of EFV. The second peak was significantly higher for the 450mg dose of RBT.

Table 4.4. Summary pharmacokinetics for efavirenz in the presence of rifabutin 450mg and 600mg daily

Treatment Period	Parameter	AUC_{0-12} (h.ng/L)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{12} (ng/mL)
	Mean	142.6	7.4	5.6	5.5	5.5
EFV +	SD	133.4	6.0	5.9	5.2	6.2
RBT	Median	86.3	5.2	3.0	3.4	3.0
450mg	IQR (25)	62.3	4.1	3.0	2.3	1.8
	IQR (75)	235.1	11.7	6.0	7.9	8.6
	GM	103.7	5.9	4.2	3.9	3.6
	Min	35.7	2.4	2.0	1.1	1.3
	Max	524.0	24.2	24	20.6	24.2
	CV%	94	81	105	95	113
EFV +	Mean	123.4	6.7	4.1	5.3	4.5
RBT	SD	97.4	4.9	3.0	5.0	4.2
600mg	Median	86.3	4.9	3.5	3.5	2.7
	IQR (25)	57.6	3.6	2.0	2.3	1.9
	IQR (75)	178.8	9.6	5.0	7.8	6.6
	GM	97.6	5.5	3.8	3.8	3.3
	Min	32.8	1.9	0.0	0.7	1.1
	Max	381.4	20.2	12.0	20.22	16.5
	CV%	79	73	73	94	93

RBT = rifabutin; EFV = efavirenz; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{12}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

The pharmacokinetic parameters of two different doses of RBT in the presence of NVP were compared with the pharmacokinetics of RBT 300mg given without NVP. The pharmacokinetic parameters of RBT before and after NVP-based ART are displayed in Table 4.5 and the corresponding median concentration-time profile is displayed in Figure 4.4.

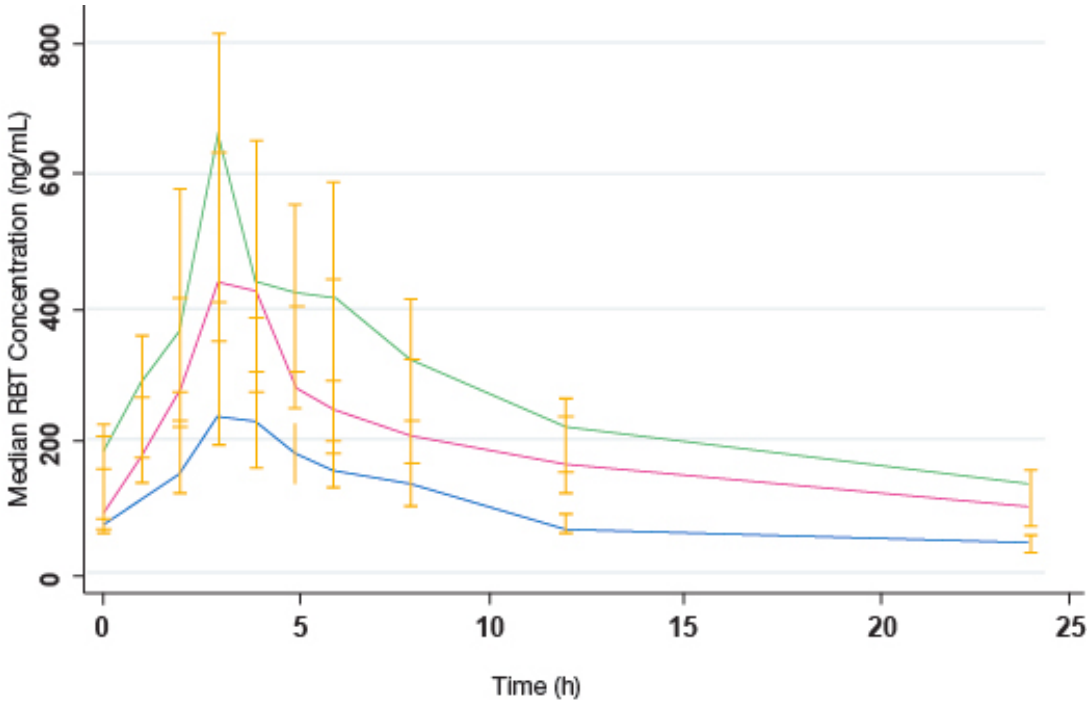

Figure 4.4. Median (IQR) concentration-time profiles for rifabutin (RBT) 300mg daily (blue), rifabutin 300mg daily plus NVP (red) and rifabutin 450mg daily plus nevirapine (green)

The RBT AUC_{0-24} of RBT 300 and 450mg plus NVP was significantly higher than the AUC_{0-24} of RBT 300mg given alone in the same patients (p = 0.003 and 0.0002 respectively). The median RBT AUC_{0-24} was 2 487.5 ng.h/mL for 300mg RBT alone, increased by 46% to 4 443.3 ng.h/mL for the same dose in the presence of NVP and increased further by 60% to 6 214.0 ng.h/mL with the 450mg dose. One patient in the NVP arm had significantly higher RBT AUC_{0-24} values compared to concentrations of the remaining patients. Her RBT AUC_{0-24} was 2760.5 ng.h/mL for 300mg RBT, 8 373.5 ng.h/mL for 300mg plus NVP and 9550 ng.h/mL for 450mg RBT plus NVP respectively. This patient developed grade 4 uveitis after 26 weeks of RBT therapy.

The C_{max} of RBT 300 and 450mg plus NVP were also significantly higher than the C_{max} of RBT 300mg given alone (p = 0.004 and 0.002 respectively). A median C_{max} value of 500.0 ng/mL was obtained with RBT 300mg in combination with NVP. Increasing the RBT dose to 450mg achieved a higher C_{max} value of 668.0 ng/mL. However the difference in C_{max} for high and low doses of RBT were not statistically significant (p= 0.2). There were no significant changes in T_{max} between baseline and high and low doses of RBT in the presence of NVP. However, oral clearance of RBT 300mg and 450mg was significantly lower in the presence of NVP (p = 0.01) compared to 300mg RBT given alone.

Table 4.5. Rifabutin pharmacokinetic parameters before and after nevirapine based ART

Treatment Period	Parameter	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{24} (ng/mL)	CL/*F* (L/h)
Period 1	Mean	2 848.5	291.9	3.4	74.9	55.4	120.0
RBT 300mg	SD	1 213.1	146.9	1.3	16.1	30.7	40.4
	Median	2 487.5	246.0	3.0	73.6	45.8	120.6
	IQR (25)	2 007.1	193.0	3.0	63.1	37.6	96.2
	IQR (75)	3 120.1	414.0	3.0	83.2	56.0	149.5
	GM	2 655.3	259.6	3.2	73.3	49.8	113.0
	Min	1 572.4	116.0	2.0	50.3	28.6	50.9
	Max	5 892.9	589.0	6.0	111.0	132.0	190.8
	CV%	43	50.3	38	21	55	34
Period 2	Mean	5 350.8	550.2	3.3	133.2	116.9	68.2
RBT 300mg	SD	2444.7	277.3	0.9	76.9	67.8	31.3
+NVP	Median	4 443.2	500.0	3.0	91.8	103.0	67.5
	IQR (25)	3 937.8	356.0	3.0	69.4	71.1	40.9
	IQR (75)	7 338.0	642.0	3.0	208.0	155.0	76.2
	GM	4 853.8	493.6	3.2	112.6	102.5	61.8
	Min	2 438.8	196.0	3.0	46.8	46.8	28.9
	Max	10 370.5	1 270.0	6.0	263.0	299.0	123.0
	CV%	46	50	27	58	58	46
Period 3	Mean	6 579.5	621.1	3.6	204.8	140.5	53.3
RBT 450mg	SD	2 475.9	254.0	1.0	101.5	70.2	24.3
NVP	Median	6 214.0	668.0	3.0	181.0	136.0	48.3
	IQR (25)	4 980.3	440.0	3.0	157.0	91.4	35.8
	IQR (75)	8 387.5	841.0	4.0	228.0	157.0	60.2
	GM	6 116.2	564.6	3.5	183.5	124.5	49.1
	Min	2 713.2	198.0	3.0	84.8	42.1	28.9
	Max	10 385	935.0	6.0	395.0	280.0	110.6
	CV%	37	41	28	50	50	46

RBT = rifabutin; NVP = nevirapine; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

The AUC_{0-24} and C_{max} of d-RBT increased significantly in the presence of NVP compared to when RBT 300mg was given alone. The AUC_{0-24} of d-RBT increased by four- and five-fold when RBT 300mg and 450mg was combined with NVP respectively, compared to when RBT 300mg was given alone. The pharmacokinetic parameters of d-RBT in the presence and absence of NVP are displayed in Table 4.6 and the corresponding median concentration-time profile of d-RBT is displayed in Figure 4.5 below. The combined median exposure of the active moiety (parent plus metabolite) was 2 677 ng.h/mL for 300mg RBT, 5 182.2 ng.h/mL for RBT 300mg plus NVP and 7 334.2 ng.h/mL for RBT 450mg plus NVP.

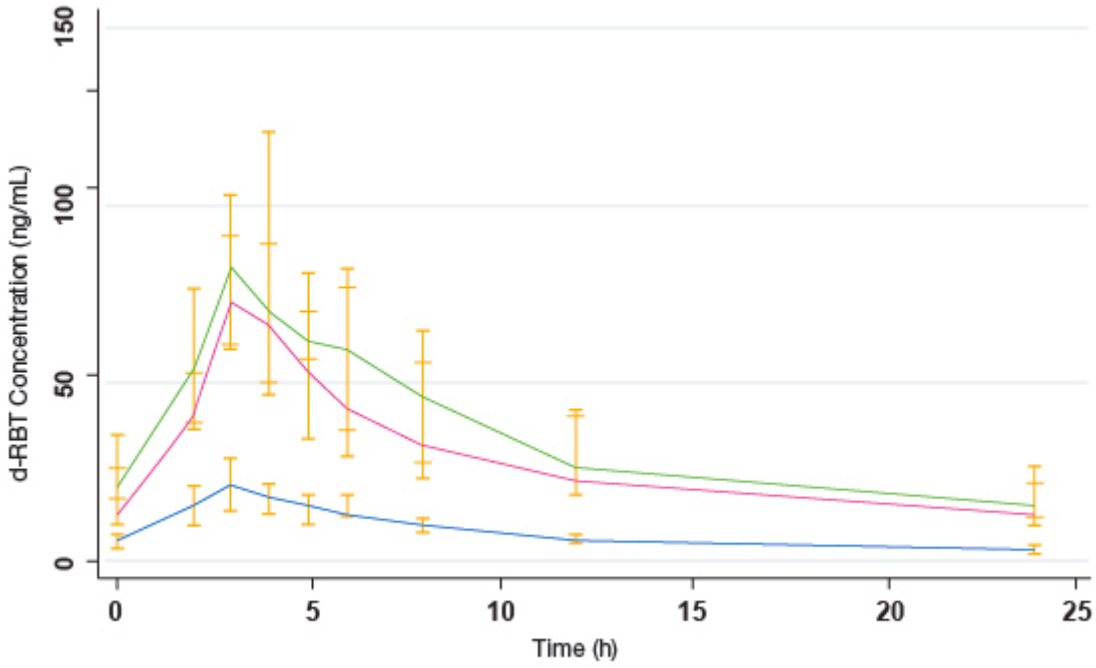

Figure 4.5. Median (IQR) concentration-time profiles of 25-O-deacetylrifabutin (d-RBT) for rifabutin 300mg daily (blue), rifabutin 300mg daily plus NVP (red) and rifabutin 450mg daily plus nevirapine (green)

Table 4.6. Pharmacokinetic parameters of 25-O-desacetylrifabutin before and after nevirapine based antiretroviral therapy

Treatment Period	Parameter	AUC_{0-24} (h.ng/L)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{24} (ng/mL)	CL/F (L/h)
Period 1	Mean	262.0	28.9	3.5	6.1	4.5	1501.5
RBT 300mg	SD	209.9	22.2	1.2	2.8	4.6	579.6
	Median	193.7	21.6	3.0	5.8	3.2	1548.9
	IQR (25)	153.9	16.7	3.0	3.4	2.2	1266.6
	IQR (75)	236.9	28.8	4.0	7.9	4.2	1949.0
	GM	221.2	23.8	3.4	5.4	3.4	1355.9
	Min	120.5	12.3	2.0	2.6	1.3	328.1
	Max	914.2	91.5	6.0	10.0	18.7	2490.5
	CV%	80	77	34	46	102	37
Period 2	Mean	945.0	92.5	3.3	23..5	20.6	466.2
RBT 300mg	SD	640.9	51.0	0.9	21.0	21.4	293.1
+NVP	Median	739.0	85.3	3.0	12.4	13.2	406.0
	IQR (25)	528.3	59.7	3.0	10.4	10.1	250.3
	IQR (75)	1 198.8	123.0	3.0	26.3	21.8	567.9
	GM	775.9	79.7	3.2	16.8	14.7	386.7
	Min	289.6	27.7	3.0	4.7	3.9	122.3
	Max	2 454.0	123.0	6.0	67.5	83.8	1035.9
	CV%	68	55	27	89	103	63
Period 3	Mean	1 081.6	92.4	3.9	28.6	30.5	361.0
RBT 450mg	SD	643.3	42.4	1.3	20.05	39.3	176.0
+NVP	Median	910.3	82.5	3.0	20.4	15.2	329.6
	IQR (25)	627.1	73.0	3.0	17.1	12.4	267.8
	IQR (75)	1 120.2	91.1	4.0	35.4	26.7	478.4
	GM	939.1	84.4	3.8	23.6	19.2	319.5
	Min	404.1	36.7	3.0	7.6	4.4	118.6
	Max	2 530.2	181.0	6.0	78.9	151.0	742.3
	CV%	59	46	33	70	128	49

RBT = rifabutin; NVP = nevirapine; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

The median AUC_{0-12} and C_{max} of NVP was higher in the presence of 300mg RBT compared to 450mg RBT but the difference was not statistically significant. The main pharmacokinetic parameters of NVP in the presence of two different doses of RB are displayed in Table 4.7 and the corresponding median time-concentration profile is shown in Figure 4.6. The median NVP trough concentration (C_{12}) for the 300mg and 450mg RBT doses was 5.46 ng/mL and 5.35 ng/mL respectively. There was only one patient with a NVP trough concentration less than 3.1 ng/mL for both doses of RBT. His NVP C_{12} was 1.65 ng/mL when he was on the 300mg dose and 1.31 ng/mL when he took the 450mg dose of RBT with NVP. This patient also had a detectable viral load of 188 000 cells/mL after 16 weeks of ART and genotyping of viral DNA showed the presence of NNRTI resistance mutations. For most patients, secondary peaks were observed 5 h after the ingestion of NVP as has been described in previous studies (van Heeswijk et al 2000).

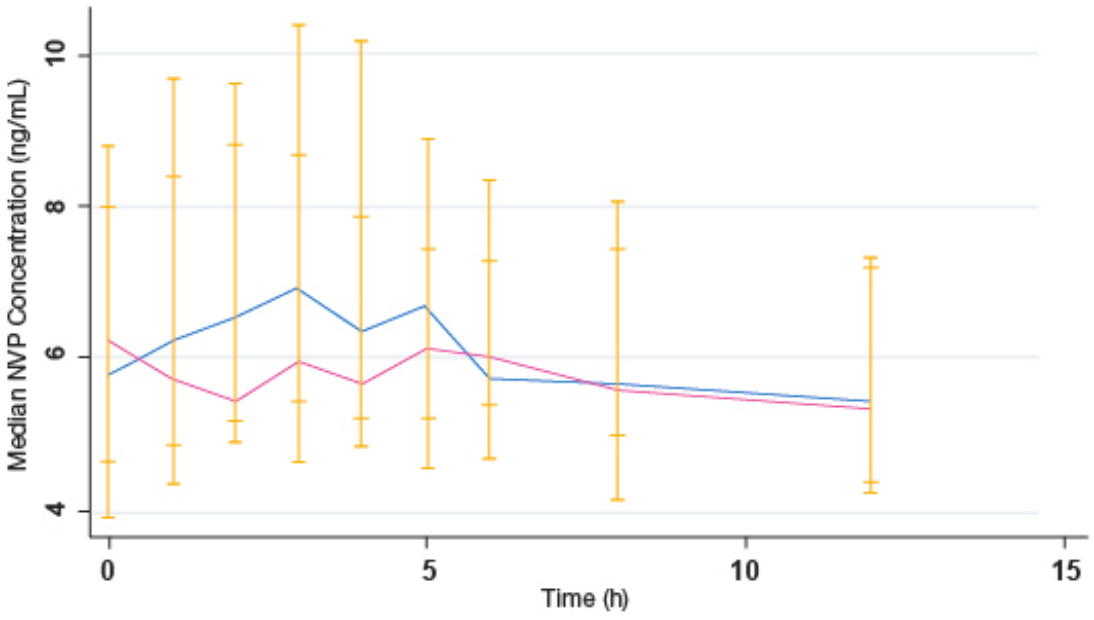

Figure 4.6. Median (IQR) concentration-time profile of nevirapine (NVP) in the presence of rifabutin 300mg (blue) and 450mg (red) daily

Table 4.7. Summary pharmacokinetics for nevirapine in the presence of rifabutin 300mg and 450mg daily

Treatment Period	Parameter	AUC_{0-12} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{12} (ng/mL)
NVP +	Mean	95.3	10.6	2.8	8.0	7.3
300mg	SD	81.5	9.3	1.5	8.7	7.2
RBT	Median	72.4	8.0	3.0	5.8	5.5
	IQR (25)	62.6	5.8	2.0	4.7	4.4
	IQR (75)	107.3	11.5	4.0	8.8	7.2
	GM	76.2	8.2	2.7	5.0	5.7
	Min	20.7	2.4	0.0	0.1	1.7
	Max	347.2	35.	5.0	35.6	30.2
	CV%	86	88	54	109	99
NVP +	Mean	80.2	8.1	3.6	6.7	6.1
450mg	SD	53.4	5.8	3.1	5.4	3.9

RBT	Median	69.6	7.0	2.0	6.3	5.4
	IQR (25)	55.1	5.2	2.0	3.9	4.2
	IQR (75)	96.4	8.8	5.0	8.0	7.3
	GM	66.4	6.6	3.1	4.5	5.1
	Min	12.6	1.3	0.0	0.1	1.3
	Max	232.2	25.1	12.0	21.7	16.8
	CV%	67	72	86	81	64

RBT = rifabutin; NVP = nevirapine; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

Table 4.8 Comparison of geometric mean ratios (GMR) for rifabutin when rifabutin 300 or 450mg is administered with nevirapine vs 300mg daily without rifabutin and when rifabutin 450 or 600mg is administered with efavirenz vs 300mg daily

	GMR (90% CI)	
	RBT 300mg plus	**RBT 450mg plus**

	NVP/RBT 300mg daily	**NVP/RBT 300mg daily**
AUC_{0-24}	1.8 (0.9 – 3.0)	2.3 (1.3 – 2.1)
	RBT 450mg plus EFV/RBT 300mg daily	**RBT 600mg plus EFV/RBT 300mg daily**
AUC_{0-24}	0.7 (1.2 - 2.6)	0.9 (1.2 – 1.9)

RBT = rifabutin; NVP = nevirapine; EFV = efavirenz; AUC = area under the concentration-time curve; GMR = geometric mean ratio; 90% CI = 90% confidence interval

4.4. Discussion

Although previous studies have shown that RBT is as effective as RMP in the treatment drug susceptible TB (Schwander et al 1995; Gonzalez-Montaner 1994), the RBT concentrations required for effective therapy are only partially known and detailed PK-PD data from human studies are lacking (Davies et al 2007). Peloquin et al. (2002) recommend that peak RBT concentrations should fall between 300 to 900 ng/mL in the treatment of *M. tuberculosis* and in TBTC study 23 RBT $AUC_{0-24} < 4.5$ ug.h/mL was associated with ARR (Weiner et al 2005a).

In this arm of the study, the median AUC_{0-24} and C_{max} for RBT 300mg administered to 27 HIV-infected TB patients before the start of ART was considerably lower than the AUC_{0-24} and C_{max} of RBT 300mg reported previously, and is discussed in detail in Chapter 3. However, in the presence of NVP, the AUC_{0-24} and C_{max} of RBT 300 and 450mg is significantly higher than 300mg RBT given alone. Similar respective increases in the bioavailability of d-RBT were also observed in the presence of NVP. The increases in RBT and d-RBT bioavailability may be due to a reduction of their metabolism in the presence of NVP. Rifabutin and its metabolite are metabolized by intestinal and hepatic CYP3A4 (Strolin-Benedetti et al 1990; Trapnell et al 1997; Prasad and Singh 2012). In humans, CYP3A4 is responsible for the metabolism of a vast majority of drugs and particular classes of drugs such as PIs and NNRTIs modulate their activity. Efavirenz and NVP stimulate the activity of CYP3A4 (Mouly et al 2002; Back et al 2003) thus accelerating the metabolism of drugs that are substrates of CYP3A4 in a combined regiment. Previous studies report that NVP induction of

CYP3A4 activity has reduced plasma concentrations of concomitantly administered CYP3A4 substrates, indinavir, nelfinavir, saquinavir and methadone by 20-60% (Merry et al 1998; Barry et al 1999; Altice et al 1999; Piscitelli et al 2001). However, in this study of HIV-infected TB patients, NVP has inhibited the metabolism of concomitantly administered RBT. Similar inhibition of CYP3A4 by concomitant NVP has been reported in previous studies in HIV-infected patients (Mouly et al 2006; Kredo et al 2011). Whether the alteration in CYP3A4 activity with regard to NVP is a consequence of HIV-infection; or gender and race related is not known. Large interindividual variability in hepatic CYP3A4 activity in HIV-infected patients has been reported previously (Slain et al 2000; Mouly et al 2006). Significantly lower CYP3A4 activity in HIV-infected Caucasian males compared to females, before the administration of HAART has been reported by Eap et al (2004) and Fellay et al (2005). Previous studies have also shown race and gender differences in CYP3A4 activity in African (Mouly et al 2006) Caucasian (Min et al 2000) and Asian (Zhu et al 2003) HIV-infected patients and healthy volunteers.

Nevirapine plasma concentrations obtained in the presence of RBT were comparable to data from previous studies in patients taking NVP with (Maldonado et al 1999) and without RBT (Manosuthi et al 2009). There was only one patient with NVP trough concentrations < 3.1mg/L for both dosing periods. A sample from this patient showed evidence of NNRTI resistance mutations.

Previous studies on RMP and NVP interaction have reported significant decreases in NVP trough concentrations in African (Cohen et al 2008; Lamorde et al 2010), Indian (Ramachandran et al 2006) and Thai patients (Autar et al 2005; Avihingsanon et al 2008). While increasing the NVP dose to 600mg daily has been suggested (Ramachandran et al 2006; Elsherbiny et al 2009) to compensate for the decreased NVP exposure in the presence of RMP, a study comparing the pharmacokinetics and efficacy of NVP 400mg daily versus 600mg daily in HIV-co-infected patients receiving concomitant RMP, reported that 25% of patients on 600mg NVP developed NVP-associated rash which was correlated with high NVP trough concentrations (range 5.6 -11.02 mg/L) and there was no significant difference in the efficacy of viral suppression between the two doses after 24 and 48 weeks of treatment (Avihingsanon et al 2008). The use of RBT in combination with NVP would therefore be a more rational option in the treatment of TB-HIV co-infected patients.

In contrast to NVP, plasma concentrations of RBT and d-RBT were significantly reduced when 450mg RBT was combined with EFV. This interaction between RBT and EFV has been reported previously (Benedek et al 1998), as EFV is an inducer of CYP3A4 and consequently accelerated the metabolism of RBT and d-RBT, which are also CYP3A4 substrates. However, increasing the RBT dose to 600mg in combination with EFV resulted in higher RBT and d-RBT concentrations, which were similar to concentrations obtained with 300mg RBT given alone. These observations are in agreement with a previous study by Weiner et al. (2005b). Thus increasing the dose of

RBT to 600mg in combination with EFV as per current treatment guidelines might be appropriate in TB-HIV co-infected African patients with advanced disease.

Efavirenz plasma concentrations are subject to substantial inter-patient variability (Csajka et al 2003) and consistently higher plasma EFV concentrations have been observed in female and non-Caucasian patients (Burger et al 2005). The currently proposed range of acceptable EFV trough concentrations is 1- 4 mg/L. Concentrations below 1 mg/L are associated with virological failure and concentrations above 4 mg/L are associated with CNS toxicity (Csajka et al 2003; Marzolini e al 2001). There were no patients in this study with trough EFV concentrations below 1 mg/L. We also observed high interpatient variability in trough efavirenz concentrations (CV 113% for 450mg and CV 93% for 600mg RBT). However, there were no patients with virological failure in the EFV arm nor were there any reports of EFV-associated CNS toxicity in this arm. The second peak in EFV concentrations may be due to delayed absorption or as a consequence of circadian variations in gastric acid secretion, gastric emptying time and gastrointestinal and hepatic blood flow (Bruguerolle 1998). These findings imply that it may be possible to substitute RBT 600mg for RMP in treatment regimens incorporating EFV. This may not be difficult to implement in a clinical setting as may resource-limited countries use EFV-based ART regimens to treatment HIV-infected TB patients.

Chapter 5. Pharmacokinetics of Different Rifabutin Doses in African HIV-infected Tuberculosis Patients on Lopinavir/ritonavir-based Antiretroviral Therapy

Summary

The pharmacokinetics of RBT was evaluated at two different doses in HIV co-infected African patients before and after the introduction of LPV/r (tablet formulation 400/100 mg 12 hourly)-based ART. Serial RBT and d-RBT concentrations were measured during a dose interval after 4 weeks of RBT 300 mg daily, after 4 weeks of 150 mg RBT daily with ART and after 4 weeks of RBT 150 mg 3 times a week (TPW) with ART. The median AUC_{0-48} and C_{max}, of RBT in patients taking 150mg RBT TPW was significantly reduced when compared to the other treatment arms. 86% of patients on this intermittent RBT arm had an $AUC_{0-24} < 4.5$ µg.h/mL, which has been associated with ARR. A daily 150 mg dose of RBT in combination with LPV/r safely maintained RBT plasma concentrations in line with those shown to prevent ARR.

5.1. Introduction

Rifabutin is an alternative rifamycin, preferred in patients requiring PIs. Despite recent studies suggesting that the current recommended dose of RBT in combination with LPV/r is suboptimal, there are insufficient pharmacokinetic data evaluating the interaction in patients treated with the film-coated tablet formulation of LPV/r. The present study was therefore undertaken to compare the bioavailability of two doses of

RBT (150mg TPW and 150mg daily) in HIV-positive TB African patients initiating ART with LPV/r.

5.2. Patient enrolment information

A total of 16 patients received LPV/r therapy with RBT. One patient was withdrawn from the study due to uveitis, five days after the initiation of ART. Another patient was withdrawn from the pharmacokinetic analysis due to documented non-compliance on trial medication. Thus the pharmacokinetic evaluation was based on 14 patients. All patients were Black South Africans and (64%) were male.

5.3. Results

5.3.1. Patient Demographics

The evaluable subjects' mean (SD) age was 31.5 (5.8) years, weight was 59.9 (9.7) kg, height was 160 (7.7) cm, BMI was 23.3 (2.6), Karnofsky score Q was 100 % (100) and CD4+ lymphocyte count was 150.9 (12.1) cells/mm^3.

5.3.2. Response to TB/HIV Treatment

Amongst the 14 patients, 3 were sputum culture positive after 2 months of TB therapy and all of these patients were culture negative for TB at the end of therapy. The mean final $CD4^+$ count assessed at the end of TB therapy was 253.8 (42.4) cells/mm^3, which

was significantly higher (p = 0.03) than the mean CD4 count of 150 (12.1) cells/mm^3 at baseline. Before the initiation of ART the mean (sd) viral load was 5.4 (0.7) log_{10} copies. Of the fourteen patients who participated in the pharmacokinetic analysis, 8 patients had viral loads > 500 cells/L. Genotypic testing was performed on isolates from all 8 patients. None of these isolates demonstrated HIV protease mutations with resistance. After fourteen weeks of ART the mean (SD) viral load dropped significantly (p < 0.001) by 2.7 log_{10} copies to 2.9 (1.3) log_{10} copies.

5.3.3. Pharmacokinetic Analysis

5.3.3.1. Rifabutin

The steady state pharmacokinetics of RBT and d-RBT were evaluated when RBT was given alone and at two different doses in combination with LPV/r The main pharmacokinetic parameters for RBT and d-RBT for each of the dosing periods are summarized in Tables 5.1 and 5.2 respectively.

The AUC_{0-24} of RBT 150 mg daily with LPV/r was significantly higher when compared to the AUC_{0-24} of RBT 300 mg daily in the absence of LPV/r in the same patients (P = 0.004). In contrast, the AUC_{0-24} of RBT 150 mg TPW with LPV/r was lower than the AUC_{0-24} of RBT 300 mg daily (P = 0.2) and the AUC_{0-24} of RBT 150mg daily with LPV/r (P = 0.0001). The differences in the AUC_{0-48} were more pronounced between the three groups. The AUC_{0-48} of RBT 150 mg TPW with LPV/r was significantly lower than the AUC_{0-48} of RBT 300 mg daily (p = 0.0001) and the AUC_{0-48} of RBT 150mg daily was significantly higher than the AUC_{0-48} of RBT 150mg TPW (p = 0.0001)

Wide inter-patient variability was observed in RBT AUC for all three doses (Table 5.2). The %CV was 24% for the 300mg dose, 46% for RBT 150 mg daily plus LPV/r and 52% for RBT 150mg TPW plus LPV/r.

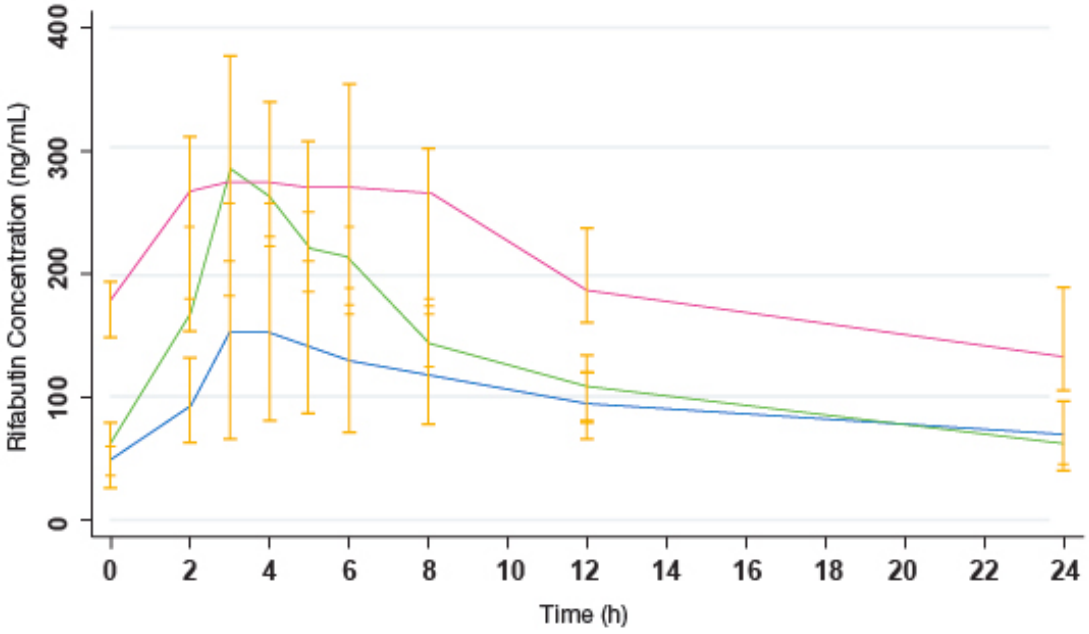

Figure 5.1. Median (IQR) concentration-time profiles for rifabutin 300mg (green), rifabutin 150mg thrice weekly plus lopinavir/ritonavir (blue) and rifabutin 150mg daily plus lopinavir/ritonavir (red)

The C_{max} of RBT 150 mg TPW with LPV/r was also significantly lower when compared to the 150 mg daily dose with LPV/r (P = 0.01) and the 300 mg daily dose without LPV/r (P = 0.01). The median C_{12} for RBT 300mg was 60.7 ng/mL (IQR, 40.6-68.8 ng/mL). The C_{12} values increased in the presence of LPV/r with daily dosing of RBT to 133 ng/mL (IQR, 105-191 ng/mL) but dropped significantly with the RBT 150 mg TPW

dose. For the 150 mg TPW dose, the median C_{24} and C_{48} were 70.7 ng/mL (IQR, 45.7-96.6 ng/mL) and 37 ng/mL (IQR, 26.6-70 ng/mL) respectively. Rifabutin clearance was significantly reduced in the presence of LPV/r (p= 0.001 for daily and p = 0.002 intermittent RBT dosing respectively) compared to 300mg RBT given alone.

Table 5.1. Main pharmacokinetic parameters median (interquartile range) for rifabutin derived by non-compartmental analysis for each of the study treatments: rifabutin 300mg daily, rifabutin 150 mg thrice per week and rifabutin 150mg daily

Treatment period	Rifabutin 300mg (n = 14)	Rifabutin 150 mg TPW plus LPV/r (n=14)	Rifabutin 150 mg daily plus LPV/r (n=14)
AUC_{0-24} (ng.h/mL)	3052.9 (2650.2-3431.5)	2307.5 (1767.5-3884.0)	4766.0 (3950.5-6099.5)
AUC_{0-48} (ng.h/mL)	6105.8 (5300.4-6863.0)*	3402.1 (2809.2-6092.0)	9532.0 (2238.2-22 425.4)*
C_{max} (ng/mL)	291.5 (250.0-377.0)	167.5 (87.8-294.0)	311.0 (258.0-376.0)
T_{max} (h)	3.0 (3.0-4.0)	3.5 (3.0-5.0)	3.0 (3.0-4.0)
C_0 (ng/mL)	59.0 (36.4 - 78.6)	49.1 (27.7-58.9)	176.5 (149.0 – 195.0)
C_{min} 24h (ng/mL)	60.7 (40.6 -68.8)	70.7 (45.7 – 96.6)	133.0 (105.0 – 191.0)
C_{min} 48h (ng/mL)	-	37.0 (26.6-70.0)	-
CL/F (L/h)	98.3 (87.4 – 113.2)	65.2 (38.6 – 85.0)	31.5 (25.0 – 38.0)
AUC_{0-24} (ng.h/mL) (Rifabutin +Metabolite)	3402.3 (2900.3 -3717.2)	3937.2 (2424.6 – 6772.7)	8753.0 (7771.7 – 11 505.0)

* calculated by 2 X AUC_{0-24}

RBT = rifabutin; LPV/r = lopinavir/ritonavir; TPW = three times per week; AUC = area under the curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum plasma attained; CL/F = clearance; C_0 = pre-dose concentration; C_{min} = trough concentration

Table 5.2. Main pharmacokinetic parameters for 25-O-desacetylrifabutin before and after lopinavir/ritonavir-based antiretroviral therapy

Dose	Parameter	AUC_{0-24} (ng.h/L)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{24} (ng/mL)
Period 1	Mean	316.7	34.2	3.7	5.3	5.2
300mg	SD	129.7	15.4	1.3	3.0	2.4
	Median	273.3	32.5	3.0	5.1	5.0
	IQR (25)	235.7	25.2	3.0	2.7	3.4
	IQR (75)	344.1	37.7	4.0	6.6	5.6
	GM	297.7	31.6	3.6	4.2	4.8
	Min	193.6	17.9	3.0	0.4	2.6
	Max	677.3	77.1	6.0	11.7	11.4
	CV%	41	45	35	57	46
Period 2	Mean	1712.4	88.5	6.6	47.7	67.0
RBT 150mg	SD	877.0	44.4	5.6	29.2	33.9
TPW	Median	1565.5	77.2	5.0	44.6	63.9
+ LPV/r	IQR (25)	1105.5	58.6	4.0	31.7	42.7
	IQR (75)	2567.3	128.0	6.0	68.9	101.0
	GM	1454.7	76.0	5.3	25.6	58.1
	Min	345.9	19.7	2.0	0.4	16.7
	Max	2888.7	152.0	24.0	105.0	126.0
	CV%	51	50	85	61	51
Period 3	Mean	3904.0	210.8	4.1	165.3	136.4
RBT 150mg	SD	1731.2	80.3	2.0	73.2	78.0
daily + LPV	Median	4118.0	236.5	4.0	186.0	155.0
	IQR (25)	2678.2	159.0	3.0	115.0	53.6
	IQR (75)	5405.5	274.0	3.0	232.0	206.0
	GM	3327.6	187.6	4.1	138.6	106.3
	Min	589.5	33.7	0.0	17.1	22.7
	Max	5761.5	321.0	8.0	244.0	239.0
	CV%	44	38	49	44	57

RBT = rifabutin; LPV/r = lopinavir/ritonavir; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

Table 5.3. Summary pharmacokinetics for lopinavir/ritonavir in the presence of rifabutin 150mg three times per week and 150mg daily

Dose	Parameter	AUC_{0-12} (ng.h/L)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	C_{12} (ng/mL)
LPV	Mean	129.5	14.2	2.6	8.4	7.4
RBT	SD	45.0	4.3	0.9	5.9	3.4
150 mg	Median	139.5	15.8	2.0	9.8	7.4
TPW	IQR (25)	103.7	12.9	2.0	3.5	4.5
	IQR (75)	163.9	17.1	3.0	14.0	10.0
	GM	118.2	13.3	2.5	4.0	6.2
	Min	26.5	3.9	2.0	0.1	0.8
	Max	185.4	19.1	5.0	15.3	12.0
	CV%	35	30	35	70	46
LPV	Mean	161.8	17.4	2.6	12.0	10.1
RBT	SD	40.1	4.0	0.9	5.1	3.2
150 mg	Median	160.1	18.1	2.0	11.4	9.4
daily	IQR (25)	129.1	14.5	2.0	9.9	7.2
	IQR (75)	181.9	19.6	3.0	15.2	11.6
	GM	157.5	17.0	2.5	10.9	9.6
	Min	102.5	10.6	2.0	3.6	6.1
	Max	25.8	27.4	5.0	23.1	15.5
	CV%	25	23	35	43	32

RBT = rifabutin; LPV/r = lopinavir/ritonavir; AUC = area under the concentration-time curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum concentration in plasma attained; C_0 = pre-dose concentration; C_{24}= trough concentration; SD = standard deviation; IQR= interquartile range; GM = geometric mean; CV = co-efficient of variation

Table 5. 4. Comparison of geometric mean ratios (GMR) for rifabutin when rifabutin 150 mg is administered three times per week (TPW) or daily with lopinavir/r vs. rifabutin 300 mg daily without lopinavir/ritonavir and comparison of rifabutin 150mg TPW with loinavir/ritonavir vs rifabutin 150mg daily with lopinavir/ritonavir

	GMR (90% CI)		
	RBT 150 TPW with ART/RBT 300 mg daily without ART	**RBT 150 mg daily with ART/RBT 300 mg daily without ART**	**RBT 150 mg daily with ART/ RBT 150 mg TPW with ART**
AUC_{0-24}	0.8 (0.7 – 0.9)	0.6 (0.5 – 0.7)	0.4 (0.5 – 0.5)
AUC_{0-48}	0.6 (0.5 – 0.7)	0.6 (0.7 - 0.5)	0.38 8(0.36 – 0.40)

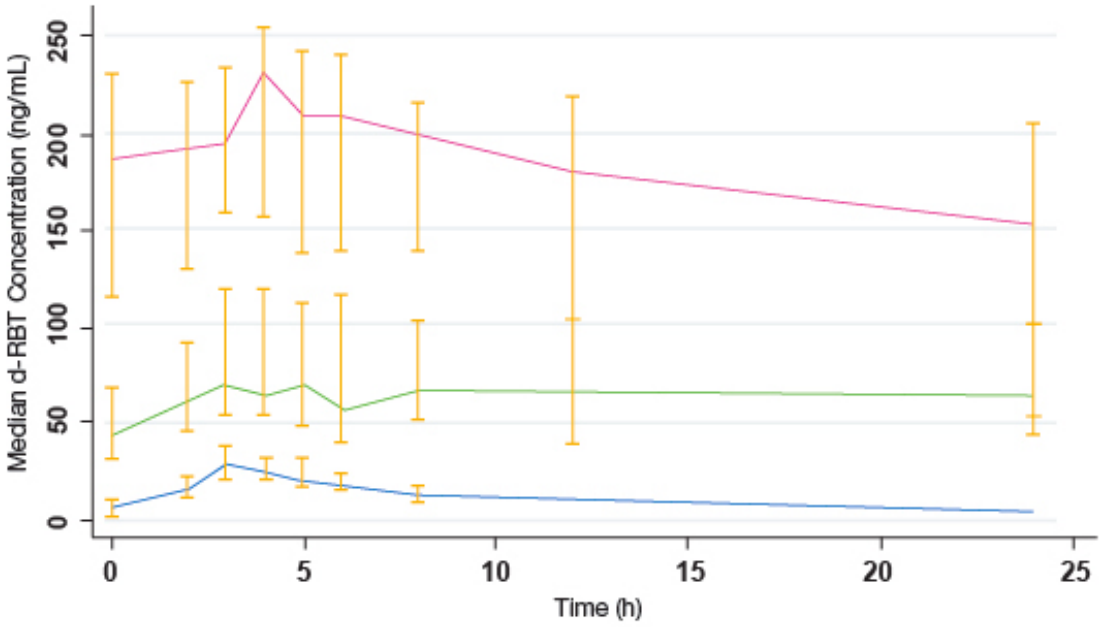

Figure 5.2. Median (IQR) concentration-time profiles of 25-O-deacetylrifabutin (d-RBT) rifabutin 300mg (blue), rifabutin 150mg thrice weekly plus lopinavir/ritonavir (green) and rifabutin 150mg daily plus lopinavir/ritonavir (red)

In the absence of LPV/r, d-RBT concentrations were approximately 11 % of the parent drug. In the presence of LPV/r, plasma d-RBT concentrations increased 5-fold with intermittent RBT dosing and 15-fold with daily doses of RBT (Figure 5.2). The total antimicrobial moiety (combined $AUC_{0\text{-}24}$ of RBT and d-RBT) for RBT at 150mg TPW was 1.2 times greater than that for 300mg RBT and at 150mg daily the total antimicrobial moiety was 2.6 times greater than that at 300mg RBT and 2.2 times greater than that at 150mg TPW. The ratios of parent drug : metabolite were 11:1 at 300mg RBT, 1.5:1 for RBT 150mg TPW and 1.2:1 for RBT 150mg daily.

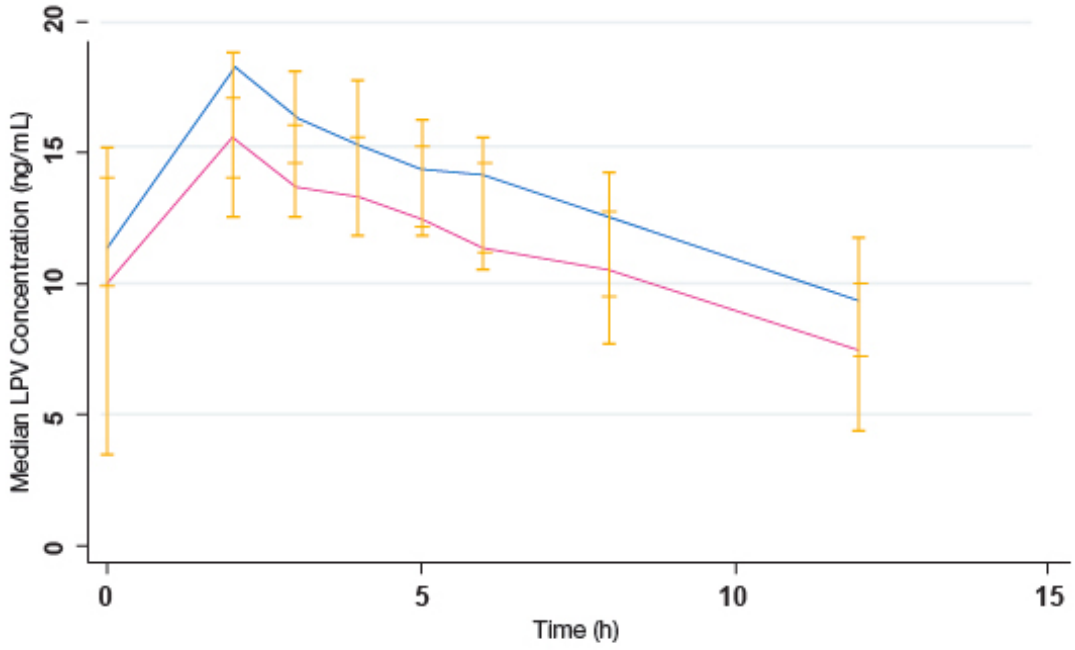

Figure 5. 3. Median (IQR) concentration-time profile of lopinavir (LPV) when lopinavir/ritonavir was administered as part of antiretroviral treatment with rifabutin (150 mg daily (blue) and 150mg thrice weekly (red) and isoniazid

The main LPV pharmacokinetic measures are summarized in Table 5.3 and the concentration-time curves are shown in Figure 5.3. In both dosing arms, median LPV trough (C_0) concentrations at steady state were above the recommended lower limit for ART-naïve patients of 1 μg/mL (la Porte 2006). Although there was a trend to higher LPV concentrations with the once daily dosing of RBT compared to the TPW dose, the differences in $AUC_{0\text{-}12}$ and C_{max} between the two doses were not statistically significant. Double peaks were observed in the LPV concentration-time profile with both doses of RBT.

5.4. Discussion

The current recommended dose of RBT for the treatment of pulmonary TB is 300mg daily but there are few pharmacokinetic data from HIV infected African TB patients treated at this dose. The median RBT AUC_{0-24} for patients randomized to receive LPV-based ART in this study was 3 052.9 ng.h/mL (IQR, 2 650.2 – 3 431.5 ng.h/mL) which was comparable to previous studies of RBT 300mg daily in HIV-infected patients (Colborn et al 1996; Li et al 1996: Moyle et al 2002; Boulanger et al 2009). The AUC_{0-24} in these studies ranged from 2 663 ng.h/mL to 5 324 ng.h/mL. Similiarly the C_{max} from the current study was 291.5 ng/mL again comparable to the range of values (244.9 to 525.0 ng/mL) reported from previous studies.

Previous studies of the pharmacokinetic interaction of RBT with LPV/r in HIV infected individuals have been small case series or involved an adaptive design in which only selected patients were exposed to the higher dose of RBT. In addition, no previous studies have reported on the RBT pharmacokinetics in combination with the tablet formulation of RTV boosted LPV (Aluvia), now the most widely used formulation of a PI in global HIV programs. A cross-over design in which all patients received 3 full pharmacokinetic assessments with RBT alone and at two different RBT doses in combination with LPV/r was adopted allowing a formal comparison to be made between the two different dosing strategies of RBT in combination with LPV/r at the recommended TB treatment dose. In this study the AUC_{0-48} and C_{max} of RBT when 150mg was given intermittently with the PI was significantly lower than when 300mg was given alone. In contrast the exposure of the 150mg daily dose in combination with

LPV/r was significantly higher than that obtained with the 300mg daily dose. This confirms previous reports that the 150mg intermittent dose of RBT with LPV results in potentially suboptimal TB therapy. The changes in AUC and CL/F of RBT are completely different according to whether RBT is dosed daily or intermittently (Table 5.1) despite the dose of LPV/r remaining unchanged. This is rather peculiar and may implicate other pathways in the metabolism of RBT besides CYP3A4. Previous studies have shown that the rifamycins are also metabolized by β-esterases. However, the exact role of β-esterases in RBT metabolism are not completely understood.

The pharmacodynamic-pharmacokinetic relationship for RBT has not been comprehensively studied so it is not clear how the reduced bioavailability of the 150mg intermittent dose would impact on TB treatment outcomes. There is still controversy over whether C_{max} or AUC is the critical pharmacodynamic measure for rifamycins in general. Initial studies in guinea pigs by Mitchison and Dickinson (1971) and (Verbist 1969) were consistent with a C_{max}/MIC being the critical pharmacokinetic parameter. Subsequent studies in a murine model (Jayaram et al 203) and a hollow fibre model (Gumbo et al 2007) found that the AUC_{0-24}/MIC ratio of rifamycins was a superior parameter and this has also been found in an early bactericidal activity of RMP in humans (Diacon et al 2007). The only efficacy trial comparing the 300mg and 150mg daily doses of RBT found there was no difference in bacterial conversion rates between the regimens, but the study was in HIV negative patients and was underpowered to assess differences in tuberculosis relapse rates (Gonzalez-Montaner et al 1995).

Although there is a lack of conventional efficacy data in support of a particular dose of RBT, there is convincing evidence that intermittent rifamycin therapy is associated with TB relapse and ARR. Various studies have reported this association of ARR with intermittent RMP and RBT especially in subjects with low CD4 counts (Spradling et al 2002; Nettles et al 2004; Li et al 2005) [28-30]. In TBTC study 23 (Weiner et al 2005a), TB-HIV co-infected patients who had lower plasma concentrations of RBT were at risk of ARR. 83% of patients with an AUC_{0-24} of < 4.5 µg.h/mL who relapsed or failed therapy developed ARR as opposed to 33% who had AUC's above this threshold value. In this study 71% patients on RBT 150mg daily versus 14% on RBT TPW had AUC_{0-24} values > 4.5 µg.h/mL. Similarly the trough values of RBT 48hours after dosing in the intermittent RBT arm in this study are significantly lower than the trough values for either of the other two arms, which may also be a pertinent pharmacokinetic parameter associated with selection of resistance.

The AUC_{0-24} and C_{max} of d-RBT were significantly increased in the presence of LPV/r in keeping with previous treatment studies (Hamzeh et al 2003; Benator et al 2007) and in healthy volunteers (Polk et al 2001; Ford et al 2008; Sekar et al 2010). On daily dosing of RBT, ritonavir caused a 50% increase in parent exposure but a 1000% increase in metabolite exposure. Furthermore on intermittent dosing the parent exposure decreased while the metabolite exposure increased five-fold. Ritonavir is a potent inhibitor of CYP 3A4, therefore the massive increase in metabolite exposure

with daily dosing of RBT is expected. However, the five-fold increase in metabolite exposure with intermittent dosing is unexpected and may imply bidirectional interactions with other CYP450 isoenymes as well as the participation of alternate metabolic pathways. Furthermore, the patients in this study were severely immunocompromised which may have altered the normal functioning of many processes and pathways including those involved in drug metabolism.

25-O-desacetylrifabutin is known to have significant antimycobacterial activity and could contribute to the regimen efficacy. Interestingly when the combined RBT and metabolite AUC_{0-24} are compared between all three arms of the study only the 150mg daily dose with LPV/r was significantly different from the other arms. The therapeutic index of d-RBT is unknown and it is possible that elevations of the metabolite could affect adverse drug reactions. We were unable to show a significant association between plasma RBT and d-RBT concentration and adverse events such as neutropenia or elevated transaminases although the numbers of patients are few. Using a random effects model, no association between AUC_{0-24} and C_{max} of RBT, dRBT and RBT+dRBT and neutrophil levels was found.

In view of the high NVP concentrations observed when NVP is combined with RBT 300mg and 450mg and significant increases in d-RBT when RBT 150mg daily is given with LPV/r, the results of this study should be interpreted cautiously. Althought the

adverse events related to RBT in this study were few, more studies are required to investigate RBT toxicity at the exposures observed in this study.

Various clinical studies involving PIs have shown that their activity is influenced by their concentrations in plasma (Kempf et al 1997). We were therefore keen determine the pharmacokinetics of LPV in our cohort especially as the heat-stable current formulation has not been previously evaluated. The median LPV AUC_{0-12}, C_{max}, C_0 and C_{12} obtained in this study when LPV was administered with two different doses of RBT are comparable to a recent study by Matteelli et al (Matteelli 2010) and consistent with historical control data (Crommentuyn et al 2004). Secondary peaks were observed in the time-concentration profiles of LPV, usually within 4 hours of drug ingestion, similar to patterns observed in other studies. In both dosing arms, median LPV trough (C_0) concentrations at steady state were above the recommended lower limit for ART-naïve patients of 1 μg/mL la Porte et al (2006) and therapeutic LPV trough (C_0) and C_{min} (C_{12}) concentrations were achieved in all patients with both doses of RBT.

This study supports an alteration to the current guidelines for the dosing of RBT in combination with boosted LPV. The high proportion of patients on the 150mg TPW arm who failed to establish a serum drug concentration that can prevent the emergence of drug resistance is very concerning. Although escalating the RBT dose after therapeutic drug monitoring is a viable option in resource rich settings it is impractical in many regions of the world where HIV TB is endemic. Recent WHO Guidelines for the

treatment of HIV-associated TB recommend that treatment is given daily throughout the intensive and continuation phases of TB treatment (World Health Organisation Stop TB Department 2009). Giving RBT as a daily dose is in line with these recommendations. This would also facilitate the important programmatic issue of combining RBT with other anti-TB medications as a fixed-dose combination pill to be taken on a daily basis.

Chapter 6. Safety

Summary

The safety of combining RBT with ART was investigated in this chapter. There were nine reports of SAEs. There were three patients with grade 4 laboratory toxicities. The remaining SAEs were clinical and not related to RBT. One patient died two weeks after enrolment of disseminated TB. There were no grade 4 adverse events in the LPV/r arm. The most common clinical adverse events reported in the LPV arm were skin infections (three patients) and diarrhea (four patients). Uveitis (grade 2) was reported in one patient after four weeks of RBT therapy. Grade 3 neutropenia occurred on six occasions in four patients. In the EFV and NVP arms there were six grade 4 and 10 grade 3 adverse events, equally distributed per arm. The most common adverse event was neutropenia, two grade 4s (1 each per arm) and three grade 3s. There was one grade 4 elevated transaminases in the NVP arm and there were two grade 3 elevated amylase (one per arm). Grade 3 clinical adverse events were MDR TB, headache with associated nausea and vomiting and weight loss. Grade 4 uveitis was reported in one patient in the NVP arm after twenty-four weeks of anti-tuberculosis therapy.

6.1. Introduction

In previous chapters, the pharmacokinetics of RBT in African TB patients with advanced immunosuppression was described. The study went on to investigate the pharmacokinetic interaction between RBT and ART. However, it is also important to

discuss the toxicity of RBT particularly when it is combined with ART in severely immunocompromised patients. The adverse events associated with ART and anti-TB therapy is well documented and their effects may to be compounded in combined treatment regiments. Previous studies on RBT have raised concerns about the association between high RBT plasma levels and neutropenia, uveitis and hepatotoxicity. In this chapter the adverse events associated with combined RBT and ART are described and their association with RBT is discussed.

6.2. Methods

Safety was evaluated by means of adverse event (AE) reporting, clinical laboratory tests, vital signs, screening for specific symptoms and physical examinations. All events were coded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (December, 2004) (AIDS Clinical Trials Group 2004). Type (body system) and incidence of all adverse events (AEs) from screening up to and including trial termination visit are tabulated in Table 6.3 per arm. A separate tabulation of Grade 3 and 4 AEs and drug relationship of the AEs is presented in Table 6.4. The serious adverse events (SAEs) are described below.

6.3. Results

6.3.1. Serious Adverse Events

Serious adverse events were reported according to standard clinical trial procedures and definitions. There were a total of 9 AEs all in different patients and these are summarized in table 6.1. The majority of these were reported as not related to RBT

and were clinical conditions recognized as complications of HIV or TB infection. However there were three that were possibly or probably related to RBT.

Table 6.1. List of Serious Adverse Events

Patient ID	Description of SAE	CD4 count at screening	Relationship to Study Drug	Outcome
144 2a	Elevated transaminases	84	Possible	Withdrawn
157 1b	Cryptococcal meningitis	68	Not related	Resolved
166 3b	Meningitis	147	Not related	Resolved
184 1a	TB Meningitis	66	Not related	Withdrawn
234	Disseminated TB	85	Not related	Death
188 2a	Uveitis	127	Probable	Resolved
250 3a	Pyelonephritis	133	Not related	Resolved
241 1a	Neutropenia	180	Possible	Withdrawn
257 1b	MDR TB	175	Not related	Withdrawn

6.3.1.1 Serious Adverse Events Related to Rifabutin

Patient NHM 144 2a, a 41-year-old male was enrolled in the trial after taking rifampicin for six weeks. Upon enrollment, he commenced RBT 300mg. At a routine trial visit 4 weeks after commencing RBT, the patients' AST and ALT levels were slightly

increased (grade 1). The patient was asymptomatic and clinically well at this visit and commenced ART the next day. He was given stavudine 30mg bd, lamivudine 150mg bd and NVP 200mg daily. The patient returned to the clinic 2 weeks later for dose escalation and routine LFT analysis for patients on the NVP arm. The patient was asymptomatic and clinically well. There was no evidence to suggest liver pathology. There was also no history of alcohol or traditional medication ingestion. The LFTs revealed grade 4 AST and ALT. The LFTs were repeated and the results were confirmed. A hepatitis screen was negative. This clinical presentation of hepatotoxicity after two weeks of initiating ART, and specifically NVP, is well documented and was the most likely cause of the transaminitis in this case. RBT levels were not elevated in this patient.

All study drugs were subsequently withdrawn and the patient withdrawn from the trial. He was hospitalized for further investigations. An abdominal ultrasound revealed no abnormalities. Serial LFTs revealed an improvement in elevated transaminase levels hence he was re-introduced to anti-TB drugs one at a time at weekly intervals with weekly LFT monitoring. As his LFTs returned to baseline values the patient was discharged from hospital with Rifanah, 2 tablets daily and referred to the local ARV clinic for re-commencement of EFV based therapy.

Patient NNL 188 2a, a 35-year-old female progressed well in the trial without any ocular symptoms. Since day 101 of the trial, the patient experienced painful red eyes

with associated light sensitivity and blurred vision that reached maximal intensity on day 106. She referred herself to the Eye Clinic of a local hospital where an ophthalmologist diagnosed viral uveitis. The patient was started on topical steroids and oral acyclovir. At a subsequent follow-up visit at the Eye Clinic, the patient reported a continuation of painful red eyes since the last visit, suggesting drug-related uveitis. The patient was admitted for treatment and RBT was stopped. At this point in the trial the patient had already completed 24 weeks (6 months) of anti-TB treatment.

On admission, pan uveitis and syphilis were diagnosed on the basis of positive treponemal serolgoy. The following treatment was commenced and prescribed for 10 days: penicillin 4mg 6 hourly iv, with prednisone forte 1 drop every hour in both eyes, in addition to atropine 1 drop 8 hourly in both eyes. She was discharged with prednisone 40mg daily orally for two weeks. The patient was seen at subsequent follow up visits at which there were no residual symptoms or signs of severe uveitis. The patient was completely asymptomatic and reported that she was compliant on ART.

Plasma RBT levels for patient NNL, in contrast to patient NHM, was high at all three PK visits. Her RBT concentrations were 2 760.5 ng.h/mL for 300mg RBT alone, 8373.5 ng.h/mL for 300mg RBT plus NVP and 9550 ng.h/mL for 450mg RBT plus NVP. The patients' plasma RBT concentrations in the presence of NVP were above the IQR for both the 300 and 450mg doses. This would suggest that the uveitis was definitely drug related even though she had evidence of treponemal infection.

Patient BBM 241 1a, a 30-year-old male progressed well in the trial but on day 84 was found to have a grade 4 neutropenia (0.39 x 10^9 /L). The test was repeated and the neutropenia confirmed (0.24 x 10^9 /L). Anti-TB medication was stopped immediately while ART was continued. Serial FBCs were subsequently done and when the neutrophil count had normalized, the patient was restarted on anti-TB treatment (RBT 600mg and INH 300mg daily). The patient was reviewed a week later and the FBC revealed a grade 1 neutropenia. The FBC was repeated 4 days later and the grade 1 neutropenia was confirmed. The patient was therefore withdrawn and was referred to the local TB clinic for continuation of TB therapy (RMP and INH) and ART therapy. The recurrence of neutropenia after a re-challenge with RBT would strongly support that neutropenia in this patient was drug related. The RBT AUC_{0-24} and C_{max}, respectively for this patient were 2 472.7 ng.h/mL and 208 ng/mL for 300mg RBT, 1091.6 ng.h/mL and 109 ng/mL for RBT 450mg plus EFV and 1977.8 ng.h/mL and 242 ng/mL for RBT 600mg plus EFV.

6.3.1.2 Serious adverse event NOT related to Rifabutin

Patient MS 257 1b, a 26-year-old male, was found to be 3+ smear positive after 2 months of TB treatment. Sputum smear was repeated one month later and was found to be 1+ positive. Subsequently the initial sputum was found to be culture positive and resistant to INH, RMP, EMB, SM and ethionamide. The patient remained clinically well and was referred to the local MDR treatment centre. He was started on MDR treatment in the form of ofloxacin 800mg daily, amikacin 830mg daily, pyrazinamide 1.5g daily, ethionamide 750mg nocte, and terizidone 750mg daily. The patient was reviewed 2

months later and was found to be clinically stable and adherent to therapy. The onset of MDR-TB was detected at the randomization visit. This was the first visit at which the patients received a sputum drug susceptibility test in the trial and occurred after 2 weeks of trial medication. The trial was designed like this so that the sputum corresponded to the 2 monthly sputum analysis, which is standard in TB control programs, including in South Africa. This patient had no previous drug susceptibility testing as this is not required in smear positive patients, with an initial episode of TB. It is theoretically possible that 2 weeks of trial medication could have resulted in acquired drug resistance, but it is more likely that the patient had MDR-TB at the time of entry into the trial. Of note is that the patient's *M. tuberculosis* isolate was resistant to SM as well as other drugs and SM is not part of the trial treatment regimen.

Patient TS 157 1b a 34-year-old female was hospitalized with crytptococcal meningitis 21 days after enrollment. Crytptococcal meningitis is a common opportunistic infection associated with HIV infection and therefore the event was considered not related to trial medication. The patient was discharged on fluconazole 400mg daily for 10 weeks, thereafter fluconazole 200mg daily for life so her subsequent pharmacokinetic studies could not be included in the study analysis due to the potential drug-drug interactions.

Two further patients were diagnosed with meningitis. Patient WTS 166 3b, a 44-year-old male was hospitalized for bacterial meningitis. He was discharged after 7 days of

treatment and returned to complete the study. Patient SM 184 1a, a 40-year-old male, was also hospitalized with a provisional diagnosis of TB meningitis. The patient was withdrawn from the trial and started on standard RMP based chemotherapy for TB.

Patient PED 234, a 36-year-old male was hospitalized 2 days after enrollment with a problem of respiratory distress. Study personnel visited the patient in hospital and he disclosed that he had not taken any trial medication since his enrolment. He subsequently deteriorated and died. His death certificate indicated disseminated TB as the cause of death, and there was no indication that study medication was implicated in the cause of this SAE.

Patient PNS 250 3a, a 24-year-old female was hospitalized 16 days after enrolment giving a history of acute lower abdominal pain associated with vomiting since the morning of day of admission. Trial medication was continued in hospital and after 5 days of treatment, the patient was completely asymptomatic and was discharged. The condition was diagnosed as pyelonephritis based on urine microscopy and culture and therefore this SAE was considered as not related to trial medication.

6.3.2. Adverse Events

The occurrence of adverse events was reviewed at each study visit and each was graded according to DAIDS tables. A summary of the number of AEs by trial arm is shown in table 6.2. Adverse events (AEs) were reported in 45(94%) trial participants.

Most AEs were grade 1(52%) and grade 2 (33%) in severity. The most common AEs according to body systems were gastro-intestinal (41%) and hematology (20%).

Table 6.2. Summary of Reported Adverse Events per arm

AE Grade	Arm 1 (n = 14)	Arm 2 (n = 16)	Arm 3 (n = 16)	Total
Grade 1	36	42	34	113
Grade 2	27	15	30	72
Grade 3	5	5	12	23
Grade 4	3	4	0	7
Total	71	67	77	217

Table 6.3. Incidence of All Reported Adverse Events by Body System and by Study Arm

Body System Any AE	Arm 1 (n = 14)	Arm 2 (n = 16)	Arm 3 (n = 16)
Gastrointestinal			
Nausea	1	0	1
Diarrhea	1	0	4
Vomiting	0	0	1
Abdominal pain	1	0	0
Loss of appetite	2	0	2
Loss of weight	1	1	1
Haematemesis	1	0	0
Gastroenteritis	0	0	1
Elevated ALP	4	7	2
Elevated ALT	3	5	9
Elevated AST	7	10	13
Elevated amylase	6	2	3
Elevated Bilirubin	1	1	3
Musculoskeletal			
Arthralgia	3	1	3
Myalgia	1	0	0
Painful feet	1	0	0

Nervous System			
Dizziness	2	1	0
Headache	4	2	1
Cryptococcal meningitis	1	0	0
Peripheral Neuropathy	0	1	0
Drowsiness	0	1	0
Lethargy	0	1	0
Dermatology			
Skin infection	0	2	2
Pruritis	0	3	0
Tinea infection	1	1	1
Scabies	0	1	1
Facial Rash	0	0	1
Respiratory			
Dyspnoea	0	1	0
TB	1	1	0
Upper respiratory tract infection	1	1	0
Lower respiratory tract infection	2	0	2
Chest infection	2	0	0
Cough	0	1	0
Nasal congestion	0	1	0

Haematology			
Lymphadenopathy	0	1	0
Anaemia	1	2	3
Decreased Albumin	1	0	2
Thrombocytopenia	2	2	2
Neutropenia	10	11	12
Virological failure	0	0	1
Opthalmology			
Conjunctivitis	0	0	1
Uveitis	0	1	1
Red Eye	0	1	0
Light sensitivity	0	1	0
ENT			
Oral Candidiasis	1	3	0
Otitis Media	0	0	1
Nasal congestion	0	1	0
Itching Ear	1	0	0

Reproductive			
Dysmenorrhea	1	0	0
Vulvo-vaginal warts	1	0	0
Gynaecomastia	1	0	0
Groin abscess	1	0	0
Cardio-vascular			
Peripheral vascular disease	1	0	0
Renal			
Pyelonephritis	0	0	1
Hyponatraemia	1	0	2
Genito-urinary			
Urinary-tract infection	1	0	0
Other			
Night Sweats	1	0	0
Influenza	0	0	1

Grade 3 AEs (10%) were reported in all 3 arms with arm 1 and 2 reporting 5 each and arm 3 reporting 12. Grade 4 AEs were reported in arm 1 and arm 2 only. A full listing of AEs by clinical system is shown in table 6.3. This was largely accounted by the higher occurrence of neutropenia in this group, but the mean changes from baseline were not different between the various arms.

Table 6.4. Grade 3 and 4 Clinical AEs according to body system and relationship to study drug

Body System *Incidence of AE* *Any Grade 3, 4 AE*	**Arm 1**	**Arm 2**	**Arm 3**	**Related to Rifabutin**	**Related to Other Study Drugs**

Gastro-intestinal					
Elevated transaminases					
Grade 3	1	0	2	Possible	Possible
Grade 4	0	1	0	Possible	Probable
Amylase increased					
Grade 3	2	1	2	Possible	No
Loss of weight					
Grade 3	0	1	0	Not related	Probable
Vomiting					
Grade 3	0	0	1	Possible	Probable
Haematology					
Neutropenia					
Grade 3	1	2	6	Possible	Possible
Grade 4	1	1	0	Possible	Probable
Neurology					
Headache					
Grade 3	0	1	1	Possible	Possible
Grade 4	1	0	0	Possible	Probable
Cryptococcal meningitis					
Grade 4	1	0	0	Unrelated	Unrelated

Opthalmology Uveitis Grade 4	0	1	0	Possible	Unrelated
Renal Pyelonephritis Grade 3	0	0	1	Unrelated	Unrelated
Respiratory MDR TB Grade 3	1	0	0	Unrelated	Unrelated

6.3.3. Safety Laboratory Evaluation

6.3.3.1. Treatment Emergent Changes in Transaminases and Neutrophil Count

The co-administration of tuberculosis chemotherapy and ART is associated with a number of overlapping toxicities including hepatotoxicity and neutropenia. Descriptive statistics of the actual laboratory values and changes from baseline are provided in Table 6.5 below. The baseline value was obtained at the screening visit before

initiation of trial medication but at a time when the patients were receiving intensive phase chemotherapy from the South African Department Of Health clinics.

Table 6.5. Baseline and Mean Change from Baseline Over Time for Selected Laboratory Parameters.

Laboratory Parameter	Baseline (Mean; SE)	Day 28 (Mean change; SE)	Day 56 (Mean change; SE)	Day 84 (Mean change; SE)	Day 112 (Mean change; SE)
Hemoglobin (g/dl)					
Arm 1	10.3 (0.4)	1.3 (0.3)	1.5 (0.4)	1.7 (0.5)	1.5 (0.4)
Arm 2	10.7 (0.5)	1.0 (0.4)	1.4 (0.5)	1.4 (0.5)	1.4 (0.5)
Arm 3	10.4 (0.3)	1.1 (0.2)	1.3 (0.4)	1.3 (0.4)	1.1 (0.5)
Neutrophils (x 10^9 /L)					
Arm 1	3.8 (0.4)	-1.3 (0.3)	-1.2 (0.4)	-1.2 (0.5)	-1.2 (0.5)
Arm 2	3.0 (0.5)	-1.1 (0.4)	-1.2 (0.4)	-1.6 (0.5)	-1.1 (0.5)
Arm 3	3.3 (0.4)	-1.0 (0.3)	-1.1 (0.3)	-1.4 (0.3)	-1.1 (0.3)
ALT (units/L)					
Arm 1	22.2 (3.4)	2.4 (3.4)	10.5 (5.0)	14.0 (4.2)	13.9 (3.9)
Arm 2	34.0 (7.0)	-3.7 (7.5)	0.2 (8.0)	0.9 (6.5)	1.9 (7.9)
Arm 3	32.4 (6.8)	4.8 (4.0)	0.1 (4.6)	8.1 (5.1)	28.4 (11.5)
AST (units/L)					
Arm 1	34.9 (3.5)	11.0 (3.9)	15.7 (6.4)	14.3 (4.8)	15.9 (3.9)
Arm 2	51.4 (12.5)	-3.3 (14.6)	-5.2 (13.3)	-10.2 (12.9)	-5.0 (14.2)
Arm 3	55.6 (11.7)	7.3 (5.9)	1.9 (8.3)	12.7 (13.3)	16.7 (8.5)

There was a significant drop in neutrophil count at day 28 compared to baseline values, in all 3 arms. Thereafter, neutrophil count remained constant.

AST levels increased significantly at day 28 in arm 1 and thereafter remained constant. There were no significant changes in AST levels in arm 2. In arm 3, AST levels increased only after day 56 but the increase was not significant.

There was a significant increase in ALT levels in arm 1 at day 56 compared to baseline values. The increase in ALT levels, continued until day 112, but the increase was not significant. In arm 2, ALT levels dropped at day 28 (not significant) and from day 56 onwards increased to match baseline levels. In arm 3, ALT levels increased at day 28 (non significant) dropped at day 56 (not significant) and then increased to above baseline levels (significant only at day 112 of the trial).

6.3.3.2. Laboratory grades

Laboratory tests were evaluated using the DAIDS grading scale. A summary table of the number of subjects with treatment-emergent laboratory results per grade is provided in Table 6.6 below. Most treatment-emergent abnormalities were grade 1 or 2 in severity. Grade 3 or 4 abnormalities occurred in hemoglobin, neutrophils, AST, ALT, amylase and albumin.

Table 6.6. Treatment-Emergent Graded Laboratory Abnormalities (Worst Toxicity Grade) During the Treatment Period.

Parameter	Severity	Arm 1	Arm 2	Arm 3
Hemoglobin	Grade 1	7	16	10
	Grade 2	0	1	1
	Grade 3	1	0	0
Neutrophils	Grade 1	2	3	3
	Grade 2	1	1	1
	Grade 3	1	2	2
	Grade 4	1	0	0
Platelets	Grade 1	1	2	0
	Grade 2	1	1	1
ASAT	Grade 1	7	4	6
	Grade 2	1	2	4
	Grade 3	0	0	2
	Grade 4	0	1	0

ALAT	Grade 1	3	4	6
	Grade 2	0	0	3
	Grade 4	0	1	0
Amylase	Grade 1	4	8	5
	Grade 2	4	1	5
	Grade 3	2	1	2
Bilirubin	Grade 1	1	1	0
	Grade 2	0	0	1
Albumin	Grade 1	1	3	3
	Grade 2	14	10	11
	Grade 3	0	0	1
Alkaline phosphatase	Grade 1	2	5	2
	Grade 2	1	0	1

Sodium Low	Grade 1	14	10	10
Potassium Low Potassium High	Grade 1 Grade 1	0 1	0 0	4 0
Bicarbonate	Grade 1	0	0	1

6.3.4. Cardiovascular Safety

Examination of vital signs included pulse, systolic blood pressure, diastolic blood pressure and respiration rate measurement at every trial visit. No treatment emergent adverse events related to vital signs were reported for any patients for the duration of the trial.

6.3.5. Physical Examination

A Physical examination was performed at all visits throughout the trial. There were no clinically relevant changes over time in physical examination findings.

6.3.6. Specific Symptom screening

Specific symptom screening was performed at every trial visit. Grade 2 red eye was reported in one patient in arm 1 on day 84 of the trial. There was one report of grade 3 red eye, light sensitivity and blurred vision in a patient in arm 2 at day 112 of the trial.

6.4. Discussion

The commonest adverse event reported in all 3 arms of this study was neutropenia. Neutropenia is a common adverse event in patients taking RBT and has been reported predominantly in previous studies on healthy volunteers (Apseloff 2003). There were 2/46 reports of grade 4 neutropenia in this study, one each in the EFV and NVP arms. Although there was a significant fall in the neutrophil count during the course of the trial most of this decline occurred in the first few weeks of RBT therapy prior to the initiation of ART and we did not find a significant association between neutropenia and plasma RBT concentrations. Neutropenia is also the most common side effect of cotrimoxazole therapy in HIV-infected patients (Moh 2005) and all patients in the present study were prescribed cotrimoxazole 960 mg daily as prophylaxis. The majority of the patients started cotrimoxazole at enrollment and this could have contributed to the declining neutrophil count seen in this study. None of the patients interrupted their study medication because of a decline in neutrophil count.

One patient in the NVP arm developed grade 4 uveitis after 24 weeks of RBT therapy. The RBT plasma concentrations of this patient in the presence of NVP were higher than the 75th percentile of the IQR when compared to concentrations of the other

patients in that arm. Another patient in the LPV/r arm developed grade 2 uveitis on day 84 of the trial which coincided with the start of ART. Plasma RBT concentrations of this patient were not significantly higher than concentrations of the other patients. Uveitis is a common ocular complication occurring in up to 80% of HIV-positive patients with advanced disease (Cunningham 2000). . However, in HIV-positive patients taking RBT uveitis has been associated with high plasma concentrations of the drug, usually in the presence of CYP 450 inhibitors such as PIs, and azithromycin (Havlir et al 1996; Lin et al 2007; Bazewicz et al 2011). It is therefore difficult to attribute uveitis uniquely to RBT, although it was treatment emergent in this case. In the absence of a control group it is impossible to determine exactly the increased risk of uveitis in this patient group

There was only one patient with grade 4 elevated transaminases. He was randomized to the NVP arm. Hepatotoxicity is also a common adverse event in patients taking ART with and without concomitant RBT. Therefore the elevation in hepatic transaminases cannot be attributed solely to the use of RBT. Although the numbers in this trial are small, a single grade 4 event would suggest that hepatotoxicity is not going to be a limiting factor in RBT combined with ART when compared to rifampicin.

There has been a reluctance to recommend a higher dose of RBT partly based on early experience with RBT and CYP3A4 inhibitors (Griffith et al 1995; Apseloff et al 1996; Griffith et al 1996; Shafran et al 1996; Shafran et al 1998; Apseloff et al 2003). An advantage of this study is that patients remained on study doses up to six months

allowing a safety evaluation to be made over a longer duration. In this study the combinations of RBT and ART were generally well tolerated with no grade 4 toxicities in the LPV/r arm apart from 2 clinical serious adverse events unrelated to RBT, one report each of grade 4 neutropenia in the NVP and EFV arms and one report of grade 4 elevated transaminases in the NVP arm.

This study is too small to address all concerns about the toxicity of the higher dose RBT with ART, most notably the decrease in neutrophil count, which requires further evaluation. However the higher doses of RBT were well tolerated and the concerns about neutropenia may be abrogated by using an alternative prophylaxis to cotrimoxazole.

Chapter 7. Pharmacogenomics

Summary

This study was undertaken to investigate the frequencies and influence of SNPs of SLCO1B1, on the pharmacokinetics of RBT. Data were analyzed by non-compartmental methods. According to non-compartmental analysis, patients with the rs4149032 CT genotype had significantly higher RBT AUC and C_{max} than patients with TT and CC genotypes.

7.1. Introduction

The pharmacokinetics of RBT shows substantial inter-patient variability in accordance with previous reports (Narang 1995; Skinner et al 1989). Various factors can affect the bioavailability of drugs, viz. age, weight (McIlleron et al 2006), HIV infection (Sahai et al 1997; Perlman et al 2005) and genetic polymorphisms (Davies et al 2008). Polymorphisms in genes encoding drug metabolizing enzymes or drug transporters can affect the absorption, volume of distribution and hepatic clearance of a drug. The effect of various co-variates on the pharmacokinetics of RBT has been studied previously (Gatti et al 1998) but there is currently no data on the pharmacogenomic determinants of RBT exposure. Therefore, the present study was undertaken to investigate the frequencies of SLCO1B1 SNPs rs4149032, rs2306283, rs4149056 and rs 11045819 and their relationships to the pharmacokinetics of RBT in HIV-infected patients with TB and advanced immunosuppression.

7.2. Patient enrolment information

Forty-four patients provided pharmacokinetic data, of which 27 (61%) were male. Of the 44 patients that provided pharmacokinetic data, 6 were unavailable for provision of a sample for genetic analysis, which was only initiated after some patients had completed therapy. For two patients no result was obtained for one polymorphism (rs4149032). All patients were of black African ethnicity.

7.3. Results

7.3.1. Patient Demographics

The patient demographics have been previously described. The mean (sd) weight, height, age, BMI, Karnofsky score and $CD4^+$ count of the patients were 58.01(8.3) kg, 159.7(7.7) mm, 33.1 (6.0) years, 23.0 (3.3), 100 (100) and 126.1 (44.0) cells/mm^3 respectively. In chapter 3 only sex and weight were associated with RBT C_{max}.

7.3.2. Allelic Frequency and Non-compartmental analysis

The allelic frequencies were determined using quantitative rtPCR as described in chapter two. The results are shown in table 7.1. The frequency of the SLCO1B1 rs4149032 polymorphism was high in this study population. Nineteen individuals (53%) were homozygous and 12 (33%) were heterozygous for the reported variant allele, which is a C to T substitution. These were comparable to a previous study from South Africa (Chigutsa et al 2011). Only 1 individual presented with the SLCOIBI rs49056 CT

allele therefore this SNP was not included in the analysis. All polymorphisms were in Hardy-Weinberg equilibrium.

Table 7.1. Allele frequencies among patients with SLCO1B1 SNPs

SNP	Genotype (number of patients, %)			
rs4149032	TT (19, 53)	CT (12, 33)	CC (5, 14)	No data (8)
rs4149056	CC (37)	CT (1)		No data (6)
rs2306283	GG (29, 76)	AG (9, 24)		No data (6)
rs11045819	CC (33, 87)	AC (5, 13)		No data (6)

The results of non-compartmental analysis are displayed in Table 7.2. below. According to non-compartmental analysis, patients with the rs4149032 CC genotype had significantly lower RBT AUC_{0-24} and C_{max} than patients with CT polymorphism .There are 12 patients in the study with the CT polymorphism and in all 12 patients RBT AUC_{0-24} were below the AUC_{0-24} previously associated with ARR (Weiner 2005a). There were no significant associations between AUC_{0-24} and C_{max} for the remaining SNPs.

Table 7.2. Rifabutin pharmacokinetic parameters from non-compartmental analysis among patients with SLCO1B1 rs4149032, rs2306283 and *rs11045819* single nucleotide polymorphisms

SNP	Mean	Standard Deviation	Confidence Interval		P-value
SLCO1B1 rs4149032					
Log AUC_{0-24}					
CC(5)	7.8	0.1	(7.6- 7.2)	mean(CT) vs mean(TT)	0.07
CT (12)	8.0	0.2	(7.9 - 8.1)	mean(CC) vs mean(TT)	0.9
TT (19)	7.8	0.4	(7.6 - 8.0)	mean(CC) vs mean(CT)	**0.01**
C_{max}					
CC (5)	210.2	56.0	(159.3 - 261.1)	mean(CT) vs mean(TT)	0.08
CT (12)	344.5	87.5	(293.2 - 395.8)	mean(CC) vs mean(TT)	0.5
TT (19)	256,8947	148.9	(187.5 - 326.3)	mean(CC) vs mean(CT)	**0.007**
SLCO1B1 rs2306283					
log AUC_{0-24}					
AG (9)	7.8	0.4	(7.5 - 8.1)		
GG (29)	7.9	0.3	(7.8 - 8.0)	mean(AG) vs mean(GG)	0.6
C_{max}					
AG (9)	271.3	116.	(181.8 - 360.9)		
GG (29)	287.0	135.4	(235.5 - 338.5)	mean(AG) vs mean(GG)	0.8
SLCO1B1 rs11045819					
log AUC_{0-24}					
AC (5)	8.1	0.4	(7.5 - 8.7)		
CC (33)	7.8	0.3	(7.7 - 8.0)	mean(AC) vs mean(CC)	0.1
C_{max}					
AC (5)	323.8	164.7	(119.3 - 528.2)		
CC (33)	277.2	125.7	(232.6 - 321.7)	mean(AC) vs mean(CC)	0.5

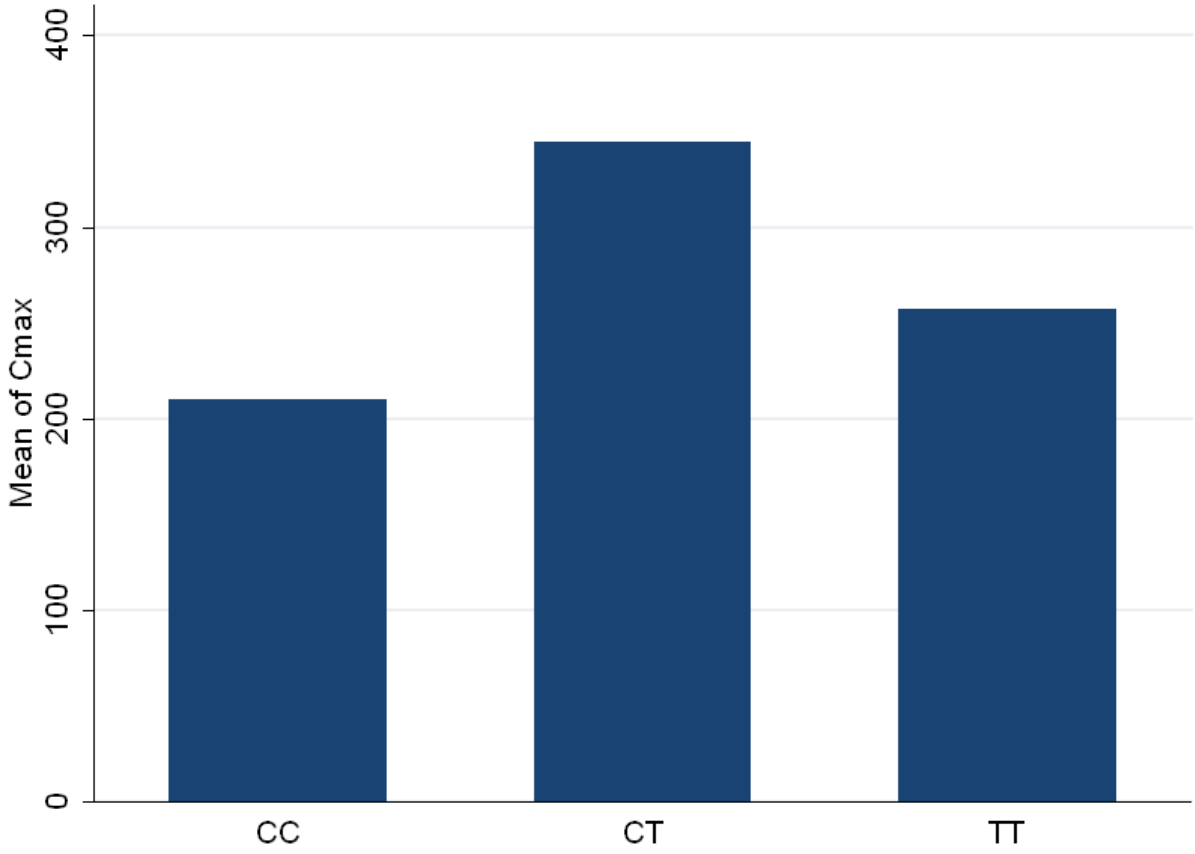

Figure 7.1. Bar graph depicting mean C_{max} across genotype categories

7.4. Discussion

This study shows that the SLCO1B1 rs4149032 polymorphism (C to T) occurs frequently in African patients in Durban. This polymorphism has been reported to occur more frequently in Africans (75%) compared to Caucasians (29%) and Asians (56%) (http://www.ncbi.nlm.nih.gov/progects/SNP/snp_ref.cgi?rs=4149032). A previous South African study has found that thirty-one individuals were homozygous (52%) for the variant allele and 22 (37%) were heterozygous, and 7 (12%) were homozygous for

the common allele. This study showed that both the heterozygous and homozygous variant allele genotypes were associated with reduced RMP bioavailability (Chigutsa et al 2011).

Whilst the allele frequencies are very similar in this study the associations with RMP bioavailability were not found to be statistically significant. Although the AUC_{0-24} and C_{max} of the homozygotes for the variant allele (TT) were lower than the heterozygotes, and close to statistically significant, in line with the study by Chigutsa, The PK parameters of homozygotes for the common allele were also lower and statistically so. This could be due to low numbers and variability in this patient group. Another possibility is linkage disequilibrium with other loci determining RBT bioavailability. None of the other SNPs evaluated in this study were found to be associated with RBT bioavailability.

Another recent study has shown that the presence of SNP rs 11045819 C463A reduced RMP exposure by 36% in a mixed cohort of Africans and Caucasions (Weiner et al 2010). However, this allele was not common in this study population and no significant associations were found with this allele and RBT AUC_{0-24} or C_{max}.

Previous studies on the pharmacogenomic determinants of RMP exposure suggest that a dose increase of RMP should be considered for South African patients. This study has shown that RBT bioavailability is low in South African patients and there may be a genetic association with RBT bioavailability. However, more studies are needed to confirm these findings.

Chapter 8. Conclusion and Future Recommendations

This study investigated the pharmacokinetics, pharmacodynamics and pharmacogenomics of RBT in severely immunosuppressed TB-HIV co-infected patients, in the absence and presence of ART and has provided important information on how the complex management of co-infected patients can be improved.

This study has shown that the bioavailability of RBT is low in African TB patients co-infected with HIV. However, when RBT 150mg daily is co-administered with LPV/r in the same patients, RBT exposure increases and is in line with concentrations required to prevent ARR in co-infected patients. Another interesting observation in this study was the approximately 1000-fold increase in d-RBT exposure with daily dosing of RBT despite the LPV/r dose remaining unchanged. Previous studies have reported that a high incidence of toxicity is associated with high plasma concentrations of d-RBT. However, in this study, adverse events were few and there were no deaths related to RBT. Nevertheless, the results of this study should be interpreted with caution and more studies in a larger cohort are required to confirm theses findings.

The combination of RBT 300mg and 450mg daily with NVP results in significantly higher exposure of RBT compared to 300mg RBT given alone in the same patients. This observation was completely unexpected, as NVP is a CYP 3A4 inducer an in combination with RBT is expected to accelerate RBT metabolism. More studies are required to confirm theses findings and to provide more information on RBT toxicity at

such high exposure in TB-HIV co-infected patients. This study has reported few adverse events but more studies in a larger cohort are required. However, if RBT is well tolerated by severely immunosuppressed co-infected patients at 300mg with NVP then this has implications for current policy on the management of TB-HIV co-infected patients. Presently most ART programs in resource-limited countries use EFV-based ART in the treatment of co-infected patients. Nevirapine is a cheaper ARV and its incorporation into a combined TB-HIV regiment might be more suitable than the EFV-based regiment.

The combination of RBT 450mg with EFV produced RBT exposures lower than the exposure of RBT 300mg given alone in the same patients. Therefore increasing the dose of RBT to 600mg daily with EFV, produces RBT plasma concentrations above and/or equivalent to 300mg RBT daily given alone in the same patients. This confirms current dosing recommendations for the combined use of RBT and EFV

More studies are required to confirm the results of this study. A recent study in Vietnam (NCT 01259219) has compared the pharmacokinetics of RBT 150mg TPW plus LPV/r with RBT 150mg daily plus LPV/r in TB-HIV co-infected patients with $CD4^+$ cell counts $< 200/mm^3$. The results of this study are eagerly awaited, as it will provide further evidence of the most suitable dose of RBT to use in combination with LPV/r in the treatment of TB-HIV co-infected patients with advanced immunosuppression.

The pharmacogenomics study investigated various SNPs and their relationships to RBT exposure. A major limitation of this study was the small sample size. However, this study has shown that there may be a genetic association with RBT bioavailability in African patients. These observations are similar to previous studies that have found a genetic association with RMP bioavailability in South African patients. Thus a larger pharmacogenomic study will be able to provide more evidence on the genetic influence on rifamycin exposure in South African patients particularly when it is believed that the dosing of RMP should be increased in African patients.

This study has generated important new data, which may have implications for clinical practice, however more studies are required to influence policy changes. Data from the Vietnam study are required to confirm the findings of this study and provide conclusive evidence that may possibly change current policies on the treatment of TB patients co-infected with HIV.

References

Abdool Karim, S., Naidoo K, Grobler A, Padyatchi N, Baxter C et al (2010). "Timing of Initiation of Antiretroviral Drugs during Tuberculosis Therapy." N Engl J Med 362: 697-706.

Acocella, G. (1978). "Clinical pharmacokinetics of rifampicin." Clin Pharmacokinet 3: 108-127.

Acosta, E., Kendall MA, Gerber JG, Alston-Smith B, Koletar SL, et al (2007). "Effect of Concomitantly Administered Rifampin on the Pharmacokinetics and Safety of Atazanavir Admiistered Twice Daily." Antimicrob Agents Chemother 51(9): 3104-3110.

AIDS Clinical Trials Group (2004). "Division of AIDS table for grading the severity of adult and pediatric events." http://www.ucdmc.ucdavis.edu/clinicaltrials/documents/DAIDS_AE_GradingTable_FinalDec2004.pdf.

Altice, F., Friedland GH, Cooney EL (1999). "Nevirapine induced opiate withdrawal among injection drug users with HIV infection receiving methadone." AIDS **13**: 957-962.

American Thoracic Society/ Centres for Disease Control and Prevention/ Infectious Diseases Society of America 2003. “Treatment of Tuberculosis”. Am J Respir Crit Care Med **167**: 603-662.

Ameyaw MM., Regateiro F, Li T Liu X, Tariq M, et al (2001).”MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity.” Pharmacogenetics **11**(3): 217-21.

Apseloff, G. (2003). "Severe neutropenia among healthy volunteers given rifabutin in clinical trials." Clin Pharm Therap 74(6): 591-592.

Apseloff, G., Foulds G, LaBoy-Goral L, Kraut E, Vincent J (1996). "Severe neutropenia caused by recommended prophylactic doses of rifabutin." The Lancet 348: 685.

Autar, R., Wit FWNM, Sankote J, Mahanontharit A, Anekthananon T, et al (2005). "Nevirapine plasma concentrations and concomitant use of rifampicin in patients coinfected with HIV-1 and tuberculosis." Antivir Therapy 10: 937-943.

Avihingsanon, A., Manosuthi W, Kantipong P, Chuchotaworn C, Moolphate S, et al (2008). "Pharmacokinetics and 48-week efficacy of nevirapine: 400 mg versus 600 mg per day in HIV-tuberculosis coinfection receiving rifampicin." Antivir Therapy 13: 529-536.

Bachmann, K., Jauregui L (1993). "Use of single sample clearance estimates of cytocrome P450 substrates to characterize human hepatic CYP status in vivo." Xenobiotica 23(3): 307-315.

Back, D., Gibbons S and Khoo S (2003). "Pharmacokinetic Drug Interactions with Nevirapine." JAIDS **34**: S8-14.

Badri, M., Wilson D, Wood R (2002). "Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study." The Lancet 359: 2059-2064.

Barry, M., Mulcahy F, Merry C, Gibbons S, Back D (1999). "Pharmacokinetics and potential interactions amongst anti-retroviral agents used to treat patients with HIV infection." Clin Pharmacokinet **36**: 289-304.

Bassilios, N., Launay-Vacher V, Hamani A, Masias L, Peytavin G, et al (2002). "Pharmacokinetics and dosage adjustment of rifabutin in a haemodialysis patient." Nephrol Dial Transplant 17: 531-532.

Bates, J., Stead WW (1993). "The History of Tuberculosis as a Global Epidemic." Medical Clinics of North America 77(6): 1205-1216.

Battaglia, R., Pianezzola E, Salgarollo G, Zini G and Strolin Benedetti M (1990). "Absorption, disposition and preliminary metabolic pathway of ^{14}C-rifabutin in animals and man." J Antimicrob Chem 26: 813-822.

Bazewicz, M., Fikri J, Martin CH, Libois A, Meunier A, et al (2011). "Drug-induced uveitis in aids patients: two case reports." Bull Soc Belge Ophtamol 318: 19-23.

Beal, S., Sheiner, L.B., Boeckmann, A., & Bauer, R.J. (1989-2009). "NONMEM User's Guides." Icon Development Solutions, Ellicott City, MD, USA.

Benator, D., Weiner MH, Burman WJ, et al (2007). "Clinical Evaluation of the Nelfinavir-Rifabutin Interaction in Patients with Tuberculosis and Human Immunodeficiency Virus Infection." Pharmacotherapy 27(6): 793-800.

Benedek, I., Fiske WD, White SJ, Stevenson D, Joseph JL, Kornhauser DM (1988). "Pharmacokinetic interaction between multiple doses of efavirenz (EFV) and rifabutin in healthy volunteers " The 36th Annual Meeting of the Infectious Diseases Society of America: Abstract no.461.

Benedek, I., Joshi A, Fiske WD, White SJ, Stevenson D, et al (1988). "Pharmacokinetic interaction between efavirenz (EFV) and rifampin (RIF) in healthy volunteers." Int Conf AIDS 12(abstract no. 42280): 829.

Benedetti, M., Edwards DM, Poggesi I, Olliaro P, Divan C, Duchene P (1993). "Pharmacokinetics of rifabutin in hepatic insufficiency." Int Conf AIDS. 1993 Jun 6-11(abstract no. PO-B30-2198): 501.

Benson, C., Williams PL, Cohn DL, Becker S, Hojczyk P, et al (2000). "Clarithromycin of Rifabutin Alone or in Combination for Primary Prophylaxis of Mycobacterium avium Complex Disease in Patients wit AIDS: A Randomized, Double-Blind, Placebo-Controlled Trial." JID 181: 1289-1297.

Blanc, F., Sok T, Laureillard D, Borand L, Rekacewicz C, et al (2011). "Earlier versus Later Start of Antiretroviral Therapy in HIV-Infected Adults with Tuberculosis." N Engl J Med 365: 1471-1481.

Blaschke, T., and Skinner MH (1996). "The Clinical Pharmacokinetics of Rifabutin." CID 22(Suppl 1): S15-S22.

BMRC 1948. “Streptomycin treatment of pulmonary tuberculosis.” British Medical Journal 2: 769-782

Bonnet, M., Pinoges LLP, Varaine FFV, Oberhauser BBO, O'Brien DDO, et al (2006). "Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden." AIDS 20: 1275-1279.

Bonora, S., Boffito M, D'Avolio A, Sciandra M, Aguilar D, et al (2003). "Pharmacokinetics (PKS) Of Rifabutin (RIF) Coadministered With Lopinavir/Ritonavir (LPV/r) in HIV Patients Affected By Tuberculosis (TB)." Antivir Ther 8(Suppl 1): abstract no. 863.

Boulanger, C., Hollander E , Farrelll K , et al (2009). "Pharmacokinetic Evaluation of Rifabutin in Combination with Lopinavir-Ritonavir in Patients with HIV Infection and Active Tuberculosis " CID 49: 1305-1311.

Breda, M., Benedetti MS, Bani M, Pellizzoni C, Poggesi I, et al (1999). "Effect of rifabutin on ethambutol pharmacokinetics in healthy volunteers." Pharmacol Res 40(4): 351-356.

Breda, M., Pianezzola E, Strolin Benedetti M, Efthymiopoulos C, Carpantieri M, et al (1992). "A study on the effects of rifabutin on isoniazid pharmacokinetics and metabolism in healthy volunteers." Drug Metab Drug Interact 10(4): 323-340.

Breen, R., Miller, RF, Gorsuch T, Smith CJ, Schwenk A, et al (2006). "Adverse events and treatment interruption in tuberculosis patients with and without HIV co-infection." Thorax 61: 791-794.

British Medical Research Council (1978). "Controlled Trial of 6-Month and 8-Month Regimens in the Treatment of Pulmonary Tuberculosis." Am Rev Respir Dis 118: 219-227.

British Thoracic and Tuberculosis Association (1976). "Short-Course Chemotherapy in pulmonary Tuberculosis." Lancet: 1102-1104.

Brogden, R., Fitton A (1994). "Rifabutin. A review of its Antimicrobial Activity, Pharmacokinetic Properties and Therapeutic Efficacy." Drugs 47(6): 983-1009.

Buhl, K., Nyboe J (1925). "Epidemiological basis of tuberculosis eradication. Changes in the mortality of Danish tuberculosis patients since 1925." Bull World Health Org 1967(37): 907-925.

Burger, D., Agarwala S, Child M, Been-Tiktak A, Wang Y and Bertz R (2006). "Effect of Rifampin on Steady-State Pharmacokinetics of Atazanavir with Ritonavir in Healthy Volunteers." Antimicrob Agents Chemother 50(10): 3336-3342.

Burger, D., van der Heiden I, la Porte C, van der Ende M, Groeneveld P, et al (2005). "Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race, and CYP2B6 polymorphism." Br J Clin Pharmacol 61(2): 148-154.

Burman, W., Benator D, Vernon A, et al (2006). "Acquired Rifamycin Resistance with Twice-Weekly Treatment of HIV-related Tuberculosis." Am J Respir Crit Care Med 173: 350-356.

Burman, W., Gallicano K and Peloquin C (2001). "Comparative Pharmacokinetics and Pharmacodynamics of the Rifamycin Antibacterials." Clin Pharmacokinet 40(5): 327-341.

Carlson, S., Yokhoo H, Craig RM (1994). "Small intestine HIV-associated enteropathy: evidence for panintestinal enterocyte dysfunction." J Lab Clin Med 124(5): 652-659.

Cato, A., Cavanaugh JH, Shi H, Hsu A, Granneman GR, Leonard J (1996). "Assessment of Multiple Doses of Ritonavir on the Pharmacokinetics of Rifabutin." Int Conf AIDS. 1996 Jul 7-12 11: 89 abstract no. Mo.B1199.

Centres for Disease Control & Prevention (2007. (Updated December 2007)). "Managing drug interactions in the treament of HIV-related tuberculosis." Available from http://www.cdc.gov/tb/TB_HIV_Drugs/default.htm.

Chaisson, R., Clermont HC, Holt EA, Cantave M, Johnson MP ,et al (1996). "Six-Month Supervised Intermittent Tuberculosis Therapy in Haitian Patients With and Without HIV infection." Am J Respir Crit Care Med 154: 1034-1038.

Chi, J., AL Jayewardene, JA Stone, Aweeka FT (2003). "An LC-MS-MS method for the determination of nevirapine, a non-nucleoside reverse transcriptase inhibitor, in human plasma." J Pharm Biomed Anal 31: 953-959.

Chi, J., Jayewardene AL, Stone JA, et al (2002). "Simultaneous determination of five HIV potease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS." J Pharm Biomed Anal 30: 675-684.

Chigutsa, E., Visser ME, Swart EC, Denti P, Pushpakom S, et al (2011). "The SLCO1B1 rs4149032 Polymorpism Is Highly Prevalent in South Africans and Is Associated with Reduced Rifampin Concentrations: Dosing Implications." Antimicrob Agents Chemother 55(9): 4122-4127.

Choudhri, S., Hawken M, Gathua S, Minyiri GO, Watkins W, et al (1996). "Pharmacokinetics of Antimycobacterial Drugs in Patients with Tuberculosis, AIDS and Diarrhea." CID 25: 104-111.

Clinton Health Access Initiative (2009). “President Clinton, Pfizer, and Mylan announce new agreements to lower prices of medicines for patients with drug-resistant HIV in developing countries.” New York, NY, USA. Available at: http://aidsalliance.3cdn.net/3361d31e8019f1447f_ihm6bxsk6.pdf

Cohen, K., van Cutsem G, Boulle A, McIlleron H, Goemaere E, et al (2008). "Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in

South African adults with HIV-associated tuberculosis." J Antimicrob Chem 61: 389-393.

Colborn, D., Lampiris H, Lee B, Lewis R, Sullam P, Narang PK (1996). "Concomitant Cotrimoxazole (CTX) Does Not Affect Rifabutin (RBT) Kinetics in HIV+ Patients." Clin Pharm Therap 59(2): 141.

Colborn, D., Li RC , Narang PK (1993). "Influence of gender on Rifabutin (R) Kinetics." Clin Pharmacol Ther 53: 182.

Colebunders, R., John L, Huyst V, Kambugu A, Scano F, Lynen L (2006). "Tuberculosis immune reconstitution inflammatory syndrome in countries with limited resources." Int J Tuberc Lung Dis 10(9): 946-953.

Craig, W. (1998). "Pharmacokinetic/Pharmacodynamic Parameters: Rationale for Antibacterial Dosing of Mice and Men." CID 26: 1-12.

Craig, W. (2001). "Does the Dose Matter?" CID 33 (Suppl 3): S233-237.

Csajka, C., Marzolini C. Fattinger K, Decosterd LA, Fellay J, et al (2003). "Population pharmacokinetics and effects of efavirenz in patients with immunodeficiency virus infection." Clin Pharm Therap 73: 20-30.

Cunningham, E. (2000). "Uveitis in HIV positive patients." Br J Opthalmol 84: 233-237.

Davies, G., Cerri S, Richeldi L (2007). "Rifabutin for treating pulmonary tuberculosis." Cochrane Database Syst Rev 4: 1-21.

Davies, G., Chierakul N, Saguenwong N, et al (2008). "A factorial study of the effect of HIV, tuberculosis, and pharmacogenetics on the pharmacokinetics, pharmacodynamics of anti-tuberculosis drugs." Conference on Human Retroviruses and Opportunistic Infections Boston 2008: Abstract 0-106.

Dean, G., Edwards SG, Ives NJ, Matthews G, Fox EF, et al (2001). "Treatment of tuberculosis in HIV-infected persons in the era of highy active antiretroviral therapy." AIDS 16: 75-83.

Decloedt, E., McIlleron H, Smith P, Merry C, Orrell C and Maartens G (2011). "Pharmacokinetics of Lopinavir in HIV-Infected Adults Receiving Rifampin with Adjusted Doses of Lopinavir-Ritonavir Tablets." Antimicrob Agents Chemother 55(7): 3195-3200.

Della Bruna C, Schioppacassi G, Ungheri D, Jabes D, Morvillo E and Sanfillipo A (1983). “LM 427, A New Spiropiperidylrifamycin: *In Vitro* and *In vivo* Studies.” J Antibiot XXXVI(11): 1502-1506

Desta, Z., Saussele T, Ward B, Blievernicht J, Li L, et al (2007). "Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro." Pharmacogenomics 8(6): 547-558.

Diacon, A., Patientia RF, Venter A, van Helden PD, Smith PJ, et al (2007). "Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum spears." Antimicrob Agents Chemother 51: 2994-2996.

Dickinson, J., and Mitchison DA (1987). "In vitro activity of new rifamycins against rifampicin-resistant M.tuberculosis and MAIS-complex mycobacteria." Tubercle 68(3): 177-182.

Diliger, K., Greiner B, Fromm MF, Hofmann U, Kroemer HK, Eichelbaum M (1999). "Consequences of rifampicin treatment on propafenone disposition in extensive and poor metabolizers of CYP2D6." Pharmacogenet 9(5): 551-559.

Ducati, R., Ruffino-Netto A, Basso LA, Santos DS (2006). "The resumption of consumption - A review on tuberculosis " Mem Inst Oswaldo Cruz 101(7): 697-714.

Eap, C., Fellay J, Buclin T, Bleiber G, Golay KP, et al (2004). "CYP3A activity measured by the midazolam test is not related to 3435 C>T polymorphism in the multiple drug resistance transporter gene." Pharmacogenet **14**: 255-260.

East African/ British Medical Research Councils (1973). "Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis." Llancet 1(7816): 1331-1339.

Ehrenpreis, E., Gulino SP, Patterson BK, Craig RM, Yokoo H, Atkinson Jr AJ (1991). "Kinetics of D-xylose absorption in patients with human immunodeficiency virus enteropathy." Clin Pharmacol Ther 49(6): 632-640.

Ehrenpreis, E., Patterson BK, Brainer JA, Yokoo H, Rademaker AW, et al (1992). "Histopathologic findings of duodenal biopsy specimens in HIV-infected patients with and without diarrhea and malabsorption." AM J Clin Path 97(1): 21-28.

El-Sadar, W., Perlman DC, Denning E, Matts JP and Cohn DL (2001). "A Review of Efficacy Studies of 6-Month Short-Course Therapy for Tuberculosis among Patients Infected with Human Immunodeficiency Virus: Differences in Study Outcomes." CID 32: 623-632.

Elliott, J., Vohith K, Saramony S, Savuth C, Dara C, et al (2009). "Immunopathogenesis and Diagnosis of Tuberculosis and Tuberculosis-Associated Immune Reconstitution Inflammatory Syndrome during Early Antiretroviral Therapy." JID 200: 17361745.

Elsherbiny, D., Cohen K, Jansson B, Smith P, McIlleron H, Simonsson US (2009). "Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients." Eur J Clin Pharmacol 65(1): 71-80.

Erickson, D., Mather G, Trager WF, Levy RH and Keirns JJ (1999). "Characterisation of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450." Drug Metab Dispos 27(12): 1488-1495.

Fellay, J., Marzolini C, Decosterd L, Powell GK, Baumann P, et al (2005). "Variation of CYP3A4 activiity induced by antiretroviral treatment in HIV-1 infected patients." Eur J Clin Pharmacol **60**: 865-873.

Fenner, L., Forster M, Boulle A, Phiri S, Braitstein P, et al (2011). "Tuberculosis in HIV Programmes in Lower-Income Countries: Practices and Risk Factors." Int J Tuberc Lung Dis 15(5): 620-627.

Fox, W., Ellard, GA, Mitchison, DA (1999). "Studies on the teatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946-1986, with relevant subsequent publications." Int J Tuberc Lung Dis 3(10): S231-279.

Friedland, G., Khoo S, Jack C and Laloo U (2006). "Administration of efavirenz (600 mg/day) with rifampicin results in highly variable levels but excellent clinical outcomes in patients treated for tuberculosis and HIV." J Antimicrob Chem 58: 1299-1302.

Gallicano, K., Khaliq Y, Carignan G, et al (2001). "A pharmacokinetic study of intermittent rifabutin dosing with a combination of ritonavir and saquinavir in patients infected with human immunodeficiency virus." Clin Pharmacol Therap 70: 49-58.

Gallicano, K., Sahai J, Shukla V, Seguin I, Pakuts A, et al (1999). "Induction of zidovudine glucoronidation and amination pathways by rifampicin in HIV-infected patients." Br J Clin Pharmacol 482(2): 168-179.

Gallicano, K., Sahai J, Wick LS, Sequin I, Pakuts A and Cameron DW (1995). "Effect of Rifabutin on the Pharmacokinetics of Zidovudine in Patients Infected with Human Immunodeficiency Virus." CID 21(4): 1008-1001.

Gatti, G., Di Biagio A, De Pascalis CR, Guerra M and Bassetti D (1999). "Pharmacokinetics of rifabutin in HIV-infected patients with or without wasting syndrome." Br J Clin Pharmacol 48: 704-711.

Gatti, G., Papa P, Torre D, Andreoni M, Poggio A, et al (1998). "Population Pharmacokinetics of Rifabutin in Human Immunodeficiency Virus-Infected Ptients." Antimicrob Agents Chemother 42(8): 2017-2023.

Gilljam, M., Bering SE, Peloquin CA, Strandvik B, Larsson LO (1999). "Therapeutic drug monitoring in patients with cystic firosis and mycobacterial disease." Eur Respir J 14: 347-351.

Gonzalez-Montaner LJ, Natal S, Yongchaiyud P, Olliaro P (1994). Rifabutin for the treatment of newly-diagnosed pulmonary tuberculosis: a multinational, randomizes, comparative study versus rifampicin. Rifabutin Study Group." Tubercle Lung Dis **75** :341-347.

Greer, N. (2007). "Posaconazole (Noxafil): a new triazole antifungal agent." Proc (Bayl Univ Med Cent) 20(2): 188-196.

Greiner, B., Eichelbaum M, Fritz P, Kreichgauer H, von Richter O, et al (1999). "The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin." J Clin Invest 104(2): 147-153.

Griffith, D., Brown BA, Girard WA and Wallace RJ Jnr (1995). "Adverse Events Associated with High-Dose Rifabutin in Macrolide-Containing Regimens for the Treatment of Mycobacteium avium Complex Lung Disease " CID 21(3): 594-598.

Griffith, D., Brown BA, Girard WA and Wallace RJ Jnr (1996). "Varying Dosages of Rifabutin Affect White Blood Cell and Platelet Counts in Human Immunodeficiency Virus-Negative Patients Who Are Receiving Mutidrug Regimens for Pulmonary Mycobacterium avium Complex Disease " CID 23: 1321-1322.

Grosset, J. (1978). "The sterilizing value of rifampicin and pyrazinamide in experimental short course chemotherapy." Tubercle 59: 287-297.

Gumbo, T., Louie A, Deziel MR, et al (2007). "Concentration-Dependent Mycobacterium tuberculosis Killing and Prevention of Resistance by Rifampin." Antimicrob Agents Chemother 51(11): 3781-3788.

Gupta, A., Wood R, Kaplan R, Bekker L, Lawn SD (2012). "Tuberculosis Incidence Rates during 8 Years of Follow-Up of an Antiretroviral Treatment Cohort in South Africa: Comparison with Rates in the Community." PLOS One 7(3): e34156.

Gurumurthy, P., Ramachandran G, Kumar AKH, Rajasekaran S, Padmapriyadarshini C, et al (2004). "Decreased Bioavailabilty of Rifampin and Other Antituberculosis Drugs in Patients with Advanced Human Immunodeficiency Virus Disease." Antimicrob Agents Chemother 48(11): 4473-4475.

Haas, D. (2005). "Will Pharmacogenomic discoveries improve HIV therapeutics?" HIV Med 13(3): 90-95.

Hafner, R., Bethel J, Standiford HC, et al (2001). "Tolerance and Pharmacokinetic Interactions of Rifabutin and Azithromycin." Antimicrob Agents Chemother 45(4): 1572-1577.

Hagenbuch, B., Meier PJ (2003). "The superfamily of organic anion transporting polypeptides." Biochem Biophys Acta 1609: 1-18.

Hamzeh, F., Benson C, Gerber J, et al (2003). "Steady-state pharmacokinetic interaction of modified- dose indinavir and rifabutin." Clin Pharmacol Therap 73: 159-169.

Harries, A., Thomas J, Chugh KS (1985). "Malnutrition in African patients with pulmonary tuberculosis " Hum Nutr Clin Nutr 39(5): 361-363.

Havlir, D., Dube MP, Sattler FR, Forthal DN, Kemper CA, et al (1996). "Prophylaxis against disseminated Mycobacterium avium complex with weekly azithromycin, daily rifabutin or both." N Engl J Med 335: 392-398.

Havlir DV, Kendall MA, Ive P, Kumwenda J, Swindells S, et al (2011). “Timing of Antiretroviral Therapy for HIV-1 Infection and Tuberculosis.” N Engl J Med **365**(16): 1482-1491

Hawken, M., Nunn P, Gathua S, Brindle R, Godfrey-Fausseli P, et al (1993). "Increased recurrence of tuberculosis in HIV-1 infected patients in Kenya." Lancet 342: 332-337.

Heifets, L., Lindholm-Levy, PJ, and Iseman, MD (1988). "Rifabutine: minimal inhibitory and bactericidal concentrations for Mycobacterium tuerculosis." Am Rev Respir Dis 137: 719-721.

Holland, D., Hamilton CD, Weintrob AC, Engemann JJ, Fortenberry ER, Peoquin CA, Stout JE (2009). "Therapeutic drug monitoring of antimycobacterial drugs in patients with both tuberculosis and advanced human immunodeficiency virus infection." Pharmacotherapy 29(5): 503-510.

Hong Kong Chest Service/British Medical Research Council. A controlled study of Rifabutin and an uncontrolled study of ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to isoniazid, streptomycin and rifampicin. Tubercle Lung Dis **73**: 59-67

Houlihan, C., Mutevedzi PC, Richard JL, Cooke GS, Tanser, FC, Newell M (2010). "The tuberculosis challenge in a rural South African HIV programme." BMC Infectious Diseases 10(23): Available at http://www.biomedcentral.com/1471-2334/1410/1423.

International Union against Tuberculosis (1964). "An international investigation of the efficacy of chemotherapy in previously untreated patients with pulmonary tuberculosis." Bull Int Union Tuberc 34: 80-191.

Iatsimirskaia, E., Tulebaev S, Storozhuk E, et al (1997). "Metabolism of rifabutin in human enterocyte and liver microsomes: kinetic parameters, identification of

enzyme systems, and drug interaction with macrolides and antifungal agents." Clin Pharmacol Ther **61**(5): 554-562.

Jabes, D., Della Bruna C, Rossi R and Olliaro P (1994). "Effectiveness of Rifabutin Alone or in Combination with Isoniazid in Preventive Therapy o Mouse Tuberculosis." Antimicrob Agents Chemother 38(10): 2346-2350.

Jayaram, R., Gaoakar S, Kaur P, Suresh BL, Mahesh BN, et al (2003). "Pharmacokinetics-Pharmacodynamics of Rifampin in an Aerosol Infection Model of Tuberculosis." Antimicrob Agents Chemother 47(7): 2118-2124.

Ji, B., Truffot-pernot C, Lacroix C, Raviglione MC, O'Brien RJ, Olliaro P, Roscigno G, Grosset J (1993). "Effectiveness of rifampin, rifabutin, and rifapentine for preventive therapy of tuberculosis in mice." Am Rev Respir Dis 148(6 Pt 1): 1541-1546.

Jindani, A., Aber VR, Edwards EA and Mitchison DA (1980). "The Early Bactericidal Activity of Drugs in Patients with Pulmonary Tuberculosis." Am Rev Respir Dis 121: 939-949.

Jindani, A., Nunn AJ, Enarson DA (2004). "Two 8-month regimens of chemotherapy for treatment of newly diagnosed pumonary tuberculosis: international multicentre randomised trial." Lancet 364: 1244-1251.

Jenny-Avital ER, Joseph K (2009). “Rifamycin-resistant Mycobacterium tuberculosis in the highly active antiretroviral therapy era: a report of 3 relapses with acquired rifampin resistance following alternate-day Rifabutin and boosted protease inhibitor therapy”. CID **48**(10): 1471-4

Jordan, M., Polis MA, Kelly G, et al (2000). "Effects of Fluconazole and Clarithromycin on Rifabutin and 25-O-Desacetylrifabutin Pharmacokinetics." Antimicrob Agents Chemother 44(8): 2170-2172.

Justesen, U., Anderson AB, Klitgaard NA, Brosen K, Gerstoft J and Pederson C (2004). "Pharmacokinetic Interaction between Rifampin and the Combination of Indinavir and Low-Dose Ritonavir in HIV-Infected Patients." CID 38(1 February): 426-429.

Kassim, S., Sassan-Morokoro M, Ackah A, Abouya LY, Digbeu H ,et al (1995). "Two-year follow-up of persons with HIV-1-and HIV-2-associated pulmonary tuberculosis treated with short-course chemotherapy in West Africa." AIDS 9: 1185-1191.

Kempf, D., Marsh KC, Kumar G, Rodrigues AD, Denissen JG, et al (1997). "Pharmacokinetic Ehancement of Inhibitors of the Human Immunodeficiency Virus Protease by Coadministration with Ritonavir." Antimicrob Agents Chemother 41(3): 654-660.

Khachi, H., O'Connell, R, Ladenheim, D and Orkin, C (2009). "Pharmacokinetic interactions between rifabutin and lopinavir/ritonavir in HIV-infected patients with mycobacterial co-infection." J Antimicrob Chem 64(4): 871-873.

Khan, F., Minion J, Pai M, Royce S, Burman W, Harries AD and Menzies D (2010). "Treatment of Active Tuberculosis in HIV-Coinfected Patients: A Systematic Review and Meta-Analysis." CID 50(9): 1288-1299.

Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, et al. (2001). “Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther **70**(2): 189-199.

Kimerling, M., Phillips P, Patterson P, Hall M, Robinson CA, Dunlap NE (1998). "Low serum antimycobacterial drug levels in non-HIV-infected tuberculosis patients." Chest 113: 1178-1183.

Koch, R (1882). “Die aetiologie der tuberculose.” Berliner Klinische Wochenschrift **19:** 221-30

Kolars, J., Schmiedlin-Ren P, Schuetz JD, Fang C and Watkins PB (1992). "Identification of Rfampin-inducible P450IIIA4 (CYP3A4) in Human Small Bowel Enterocytes " J Clin Invest 90: 1871-1878.

Kraft, W., McCrea JB, Winchell GA, Carides A, Lowry R, et al (2004). "Indinavir and Rifabutin Drug interactions in Healthy Volunteers." J Clin Pharmacol 44(305): 305-313.

Kredo, T., Mauff K, Van der Walt JS, Wiesner L, Maartens G, et al (2011). "Interaction between Artemether-Lumefantrine and Nevirapine-Based Antiretroviral Therapy in HIV-1-Infected Patients." Antimicrob Agents Chemother **55**(12): 5616-5623.

L'homme, R., Nijland HMJ, Gras L, Aarnoutse RE, van Crevel R, et al (2009). "Clinical experience with the combined use of lopinavir/ritonavir and rifampicin." AIDS 23: 863-867.

la Porte, C., Back DJ, Blaschke T, Boucher CAB, Fletcher CV, et al (2006). "Updated guideline to perform therapeutic drug monitoring for antiretroviral agents." Rev Antivir Ther 3: 4-14.

la Porte, C., Colbers EPH, Bertz R, Voncken DS, Wikstrom K, et al (2004). "Pharmacokinetics of Adjusted-Dose Lopinavir-Ritonavir Combined wih Rifampin in Healthy Volunteers." Antimicrob Agents Chemother 48(5): 1553-1560.

Lambi, B., Federman M, Pleskow D, Wanke CA (1996). "Malabsorption and wasting in AIDS patients with microsporidia and pathogen-negative diarrhea." AIDS 10(739-44).

Lamorde, M., Byakika-Kibwika P, Okaba-Kayom V, Ryan M, Coakley P, et al (2010). "Nevirapine pharmacokinetics when intiated at 200 mg or 400 mg daily in HIV-1 and tuberculosis co-infected Ugandan adults on rifampicin." J Antmicrob Chemother 66: 180-183.

Lawn, S., Badri M and Wood R (2005). "Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort." AIDS 19: 2109-2116.

Lawn, S., Harries AD, Williams BG, Chaisson RE, Losina E, et al (2011). "Antiretroviral therapy and the control of HIV-associated tuberculosis. Will ART do it?" Int J Tuberc Lung Dis 15: 571-581.

Lawn, S., Kranzer K, Wood R (2009). "Antiretroviral therapy for control of the HIV-associated tuberculosis epidemic in resource-limited settings." Clin Chest Med 30(viii): 685-699.

Lawn, S., Myer L, Bekker L and Wood R (2006). "Burden of tuberculosis in an antiretroviral treatment programme in sub-Saharan Africa: impact on treatment outcomes and implications for tuberculosis control." AIDS 20: 1605-1612.

Lawn, S., Wilkinson RJ, Lipman MCI, and Wood R (2008). "Immune Reconstitution and 'Unmasking" of Tuberculosis during Antiretroviral Therapy." Am J Respir Crit Care Med 177: 680-685.

Li, J., Munsiff SS, Driver CR and Sackoff J (2005). "Relapse and Acquired Rifampin Resistance in HIV-Infected Patients with Tuberculosis Treated with Rifampin- or Rifabutin-Based Regimens in New York City, 1997-2000." CIF 41: 83-91.

Lin, H., Lu PL and Chang CH (2007). "Uveitis associated with concurrent administration of rifabutin and lopinavir/ritonavir (Kaletra)." Eye 21: 1540-1541.

Loeliger A, Suthar AB, Ripin D, Glaziou P, O'Brien M, et al. (2011)."Protease inhibitor-containing antiretroviral treatment and tuberculosis: can Rifabutin fill the breach? Int J Tuberc Lung Dis **16**(1): 6-15

Loeuillet, C., Weale M, Deutsch S, et al (2007). "Promoter polymorphisms and allelic imbalance in ABCB1 expression." Pharmacogenet Genomics 17(11): 951-959.

Lopez-Cortes, L., Ruiz-Valderas R, Viciana P, Alarcon-Gonzalez A, Gomez-Mateos J, et al (2002). "Pharmacoknetic Interactions Between Efavirenz and Rifampicin in HIV-Infected Patients with Tuberculosis." Clin Pharmacokinet 41(9): 681-690.

Luna-Herrera, J., Reddy MV, and Gangadharam RJ (1995). "In-vitro and intracellular activity of rifabutin on drug-susceptible and multiple drug-resistant (MDR) tubercle bacilli." J Antimicrob Chem 36: 355-363.

Maldonado, S., Lamson M, Gigliotti M, Pav JW, Robinson P (1999). "Pharmacokinetic (PK) Interaction between Nevirapine and Rifabutin (RFB)." Abstr Intersci Conf Antimicrob Agents Chemother Intersci Antimicrob Agents Chemother Sep 26-29; 39: 21 (abstract no. 341).

Manosuthi W, C. S., Thongyen S, Chaovavanich A and Sungkanuparph S (2006). "Survival Rate and Risk Factors of Mortality Among HIV/Tuberculosis-Coinfected Patients With and Without Antiretroviral Therapy." Acquir Immune Defic Syndr 43(1): 42-46.

Manosuthi, W., Ruxrungtham K, Likanonsakul S, Prasithsirikul W, Inthong Y, et al (2007). "Nevirapine Levels after Discontinuation of Rifampicin Therapy and 60-Week Efficacy of Nevirapine-Based Antiretroviral Therapy in HIV-Infected Patients with Tuberculosis." CID 44: 141-144.

Manosuthi, W., Sugkanuparph S, Thakkinstian A, Vibhagool A, Kiertiburanakul S, et al (2005). "Efavirenz levels and 24-week efficacy in HIV-infected patients with tuberculosis receiving highly active antretroviral therapy and rifampicin." AIDS 19: 1481-1486.

Manosuthi, W., Sungkanuparph S, Thakkinstian A, Rattanasiri S, Chaovavanich A, et al (2006). "Plasma Nevirapine Levels and 24-Week Efficacy in HIV-Infected Patients Receiving Nevirapine-Based Highly Active Antiretroviral Therapy with or without Rifampicin." CID 43: 253-255.

Margalith, P., and Pagani H (1961). "Production of Rifomycin B." App Microb 9: 325-326.

Marzolini, C., Telenti A, Decosterd LA, Greub G, Biollaz J and Buclin T (2001). "Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients." AIDS 15: 71-75.

Matteelli, A., Olliaro P, Signorini L, Cadeo G, Scalzini A, et al (1999). "Tolerability of twice-weekly rifabutin-isoniazid combinations versus daily isoniazid for latent

tuberculosis in HIV-infected subjects: a pilot study." Int J Tuberc Lung Dis 3(11): 1043-1046.

Matteelli, A., Regazzi M, Villani P, De Iaco G, Cusato M, et al (2007). "Multiple-dose pharmacokinetics of efavirenz with and without the use of rifampicin in HIV-positive patients." Curr HIV Res 5(3): 349-353.

Matteelli, A., Villani P, Carvalho AC, El-Hamad I, Cusato M, et al (2010). "Lopinavir pharmacokinetic profiles in HIV-infected patients during rifabutin-based anti-mycobacterial therapy." J Antimicrob Chem.

McIlleron, H., Rustomjee R, Vahedi M, Mthiyane T, Denti P, et al (2012). "Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: implications for international dosing guidelines." Antimicrob Agents Chemother 56(6): 3232-3238.

McIlleron, H., Wash P, Burger A, Norman J, Folb PI and Smith P (2006). "Determinants of Rifampin, Isoniazd, pyrazinamide, and Ethambutol Pharmacokinetics in a Cohort of Tuberculosis Patients." Antimicrob Agents Chemother 50(4): 1170-1177.

McGregor MM, Olliaro P, Wolmarans L, et al. “Efficacy and safety of Rifabutin in the treatment of patoients with newly diagnosed pulmonary tuberculosis.” (1996) Am J Respir Crit Care Med **154**: 1462-1467

Medical Research Council (1955). "Various combinations of isoniazid with streptomycin or with PAS in the treatment of pulmonary tuberculosis." BMJ 1: 435-445.

Meibohm B, Beierle I and Derendorf H (2002). “How Important Are Gender Differences in Pharmacokinetics”. Clin Pharmacokinet **41**(5): 329-324

Merry, C., Barry MG, Mulcahy F, Ryan M, Tjia JF, et al (1998). "The pharmacokinetics of combination therapy with nelfinavir plus nevirapine." AIDS **12**: 1163-1167.

Michailidis, C., Pozniak AL, Mandalia S, Basnayake S, Nelson MR and Gazzard BG (2005). "Clinical characteristics of IRIS syndrome in patients with HIV and tuberculosis." Antivir Ther 10: 417-422.

Min, D., Lee M, Ku YM, Flanigan M (2000). "Gender-dependent racial difference in disposition of cyclosporine among healthy African-American and white volunteers." Clin Pharm Ther **68**: 478-486.

Mitchison, D. (1979). "Basic mechanisms of chemotherpay." Chest 76: 771-781.

Mitchison, D., and Dickinson JM (1971). "Laboratory aspects of intermittnt drug therapy." Postgrad Med J 47: 737-741.

Moh, R., Danel C, Sorho S, Sauvageot D, Anzian A, et al (2005). "Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Cote d'Ivore." Antiviral Ther 10: 615-624.

Morris, M. (2009). "Posaconazole: a new oral antifungal agent with an expanded spectrum of activity." Am J Health Syst Pharm 66(3): 225-236.

Mouly, S., Lown KS, Kornhauser D, Joseph JL, Fiske WD, et al (2002). "Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans." Clin Pharm Ther **72**: 1-9.

Mouly, S., Rizzo-Padoin N, Simoneau G, Verstuyft C, Aymard G, et al (2006). "Effect of widely used combinations of antiretroviral therapy on liver CYP3A4 activity in HIV-infected patients." Br J Clin Pharmacol **62**(2): 200-209.

Moyle, G., Buss NE, Goggin T et al. (2002). "Interaction between saquinavir soft-gel and rifabutin in patients infected with HIV." Br J Clin Pharmacol 54: 178-182.

Mukadi, Y., Maher D and Harries A (2001). "Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa." AIDS 15: 143-152.

Nakajima, A., Fukami T, Kobayashi Y, Watanabe A, Nakajima M, Yokol T (2011). "Human arylacetamide decetylase is responsible for deacetylation of rifamycins: Rifampicin, Rifabutin and rifapentine." Biochem Pharm **82** (11): 1747-1756.

Narang, P. (1995). "Clinical Pharmacology of Rifabutin: A New Antimycobacterial." Rev Contemp Pharmacother 6(129-151).

Narang, P. (1996). "Rifabutin (RBT) Oral Absorption Unaltered With Progressing HIV Disease." Clin Pharmacol Ther 89(2): 141.

Narang, P., Lewis RC, Bianchine JR (1992). "Rifabutin absorption in humans: relative bioavailability and food effect." Clin Pharmacol Ther 52: 335-341.

Narang, P., Nightingale S, Manzone C, Colborn D, Wynne B, et al (1992). "Does rifabutin (RIF) affect zidovudine (ZDV) dispodition in HIV (+) patients? ." VIII International Conference on AIDS/III STD World Congress, Amsterdam, The Netherlands abstract Vol. 2: B239.

Narita, M., Ashkin D, Hollender ES, and Pitchenik AE (1998). "Paradoxical Worsening of Tuberculosis Following Antiretroviral Therapy in Patients with AIDS." Am J Respir Crit Care Med 158: 157-161.

Narita, M., Stambaugh JJ, Hollender ES, et al (2000). "Use of Rifabutin with Protease Inhibitors for Human Immunodeficiency Virus-Infected Patients with Tuberculosis." CID 30: 779-783.

Ng, J., Nada A, Freeman S, Chiu Y, Cohen D, et al (2009). "Pharmacokinetics of Rifabutin 150mg TIW Plus Lopinavir/ritonavir (LPV/r) 400/100 mg BID Administered in Healthy Adult Subjects." 10th Iternational Workshop on Clinical Pharmacology of HIV Therapy, Amsterdam, The Netherlands: Abstract O_21.

Nijland, H., L'homme RFA, Rongen GA, van Uden P, van Crevel R, et al (2008). "High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets." AIDS 22(931-935).

Nijland, H., Ruslami R, Stalenhoef JE, Nelwan EJ, Alisjahbana B, et al (2006). "Exposure to Rifampicin Is Strongly Reduced in Patients with Tuberculosis and Type 2 Diabetes." CID 43(7): 848-854.

Okwera, A., Johnson JL, Luzze H, et al (2006). "Comparison of intermittent ethambutol with rifampicin-based regimens in HIV-infected adults with PTB, Kapala." Int J Tuberc Lung Dis 10: 39-44.

Olliaro, P., Dolfi L, Morelli P, Della Bruna C, Strolin-Benedetti M,, Sassella D (1995). "Rifabutin for prevention and treatment of mycobacterial diseases: a review of microbiology, clinical pharmacolog, efficacy and tolerability data." Eur Respir Rev 5(25): 77-83.

Onwubalili, J. (1988). "Malnutrition among tuberculosis patients in Harrow, England." Eur J Clin Nutr 42(4): 363-366.

Orrell, C., Cohen K, Conradie F, Zeinecker J, Ive P, Sanne I and Wood R (2011). "Efavirenz and rifampicin in the South African context: is there a need to dose increase efavirenz with concurrent rifampicin therapy?" Antivir Therapy 16(4): 527-534.

Pasanen, M., Neuvonen PJ, Niemi M (2008). "Global analysis of genetic variation in SLCO1B1." Pharmacogenomics 9: 19-33.

Patel, A., Patel K, Patel J, Shaah N, Patel B and Rani S (2004). "Safety and Antiretroviral Effectiveness of Concomitant Use of Rifampicin and Efavirenz for Antiretroviral-Naive Patients in India Who Are Coinfected ith Tuberulosis and HIV-1." J Acquir Immune Defic Syndr 37: 1166-1169.

Peloquin, C. (2002). "Therapeutic Drug Monitoring in the Treatment of Tuberculosis." Drugs 62(15): 2169-2183.

Peloquin, C., Jaresko GS, Yong C-L, Keung ACF, Bulpitt AE and Jelliffee W (1997). "Population Pharmacokinetic Modeling of Isoniazid, Rifampin, and Pyrazinamide." Antimicrob Agents Chemother 41(12): 2670-2679.

Peloquin, C., Macphee AA, Berning SE (1993). "Malabsorption of antimycobacterial medications." N Engl J Med 329: 1122-1123.

Peloquin, C., Namdar R, Singleton MD and Nix DE (1999). "Pharmacokinetics of Rifampin Under Fasting Conditions, With Food, and With Antacids." Chest 115: 12-18.

Peloquin, C., Nitta AT, Burman WJ, Brudney KF, Miranda-Massari JR, et al (1996). "Low Antituberculosis Drug Concentrations in Patients with AIDS." Ann Pharmacother 30: 919-925.

Perlman, D., Segal Y, Rosenkranz S, Rainey PM, Remmel RP, et al (2005). "The Clinical Pharmacokinetics of Rifampin and Ethambutol in HIV-Infected Persons with Tuberculosis." CID 41(1638-1647).

Perriens, J., Colebunders RL, Karahunga C, Willame J, Jeugmans J, et al (1991). "Increased Mortality and Tuberculosis Treatment Failure Rate among Human

Immunodeficiency Virus (HIV) Seropositive Compared with HIV Seronegative Patients with Pulmonary Tuberculosis Treated with "Standard" Chemotherapy in Kinshasa, Zare." Am Rev Respir Dis 144: 750-755.

Perriens, J., St Louis ME, Mukadi YB, Brown C, Prignot J, et al (1995). "Pulmonary Tuberculosis in HIV-Infected Patients in Zaire - A Controlled Trial of Treatment for Either 6 or 12 Months." N Engl J Med 332(12): 779-785.

Pharmacia and Upjohn Company "Mycobutin package insert." http://dailymed.nlm.nih.gov/dailymedinfo.cfm?id=39959.

Pinheiro, V., Ramos LM, Monteiro HS, Barroso EC, Bushen OY, et al (2006). "Intestinal permeability and malabsorption of rifampin and isoniazid in active pulmonary tuberculosis." Braz J Infect Dis 10(6): 374-379.

Piscitelli, S., Gallicano KD (2001). "Interactions among drugs for HIV and opportunistic infections." N Engl J Med **344**: 984-996.

Polasa, K., Murthy KJR and Krishnaswamy K (1984). "Rifampicin kinetics in undernutrition." Br J Clin Pharmac 17: 481-484.

Polk, R., Brophy, DF, Israel, DS, Patron, R, Sadler, BM, et al (2001). "Pharmacokinetic Interaction between Amprenavir and Rifabutin or Rifampin in Healthy Males." Antimicrob Agents Chemother 45(2): 502-508.

Pozniak, AK, Miller R, Ormerod LP (1999). “The treatment of tuberculosis in HIV-infected patients.” AIDS 13: 435-445

Rae, J., Johnson MD, Lippman ME, and Flockhart DA (2001). "Rifampin is a selective, pleiotropic inducer of drug metaolism genes in human hepatocytes: studies with

cDNA and oligonucletide expression arrays." J Pharmacol Exper Ther 299(3): 849-857.

Ramachandran G, H. A., Rajasekaram S, Padmapriyadarsini C, Narendran G, et al (2006). "Increasing Nevirapine Dose Can Overcome Reduced Bioavailability Due to Rifampicin Coadministration." J Acquir Immune Defic Syndr 42(1): 36-41.

Rastogi, N., Goh KS, Berchel Mand, A BryskierA (200). "Activity of rifapentine and its metabolite 25-O-desacetylrifapentine compared with rifampicin and rifabutin against Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis and M.bovis BCG." J Antimicrob Chem 46: 565-570.

Ray, J., Gardiner I, Marriott D (2003). "Managing antituberculosis drug therapy by therapeutic drug monitoring of rifampicin and isoniazid." Intern Med J 33(229-234).

Ribera, E., Azuaje C, Lopez RM. Domingo P, Curran A, et al (2007). "Pharmacokinetic interaction between rifampicin and the once-daily combination of saquinavir and low-dose ritonavir in HIV-infected patients with tuberculosis." J Antimicrob Chem 59(4): 690-697.

Ribera, E., Pou L, Lopez RM, Crespo M, Falco V, et al (2001). "Pharmacokinetic Interaction Between Nevirapine and Rifampicin in HIV-Infected Patients With Tuberculosis." JAIDS 28(5): 450-453.

Riska, P., Lamson M, MacGregor T, Sabo J, Hattox S, Pav J and Keirns J (1999). "Disposition and biotransformation of the antiretroviral drug nevirapine in humans." Drug Metab Disp 27: 895-901.

Rolla, V., da Silva VMA, Pereira PD, Lourenco MC, de Jesus CS, et al (2006). "Safety, efficacy and pharmacokinetics of ritonavir 400mg/saquinavir 400mg twice daily

plus rifampicin combined therapy in HIV patients with tuberculosis." CID 26(8): 469-479.

Rolston, D., and Mathan VI (1989). "Xylose transport in human jejunum." Digestive Diseases and Sciences 34(4): 553-558.

Rowland, M., and Towser TN (1995). "Clinical Pharmacokinetics. Concepts and Applications." 3rd edition. Lippincott Williams & Wilkins.

Ruslami, R., Nijland HMJ, Alisjahbana B, Parwati I, van Crevel R and Aaroutse RE (2007). "Pharmacokinetics and Tolerability of a Higher Rifampin Dose versus the Standard Dose in Pulmonary Tuberculosis Patients." Antimicrob Agents Chemother 51(7): 2546-2551.

Sahai, J., Gallicano K, Swick L, Tailor S, Garber G, et al (1997). "Reduced Plasma Concentrations of Antituberculosis Drugs in Patients with HIV Infection " Ann Intern Med 127: 289-293.

Sahai, J., Narang PK, Hawley-Foss N, Li RC, Kamal M and Cameron DW (1995). "A Phase I Evaluation of Concomitant Rifabutin and Didanosine in Symptomatic HIV-infected Patients." J Acquir Immune Defic Syndr 9(3): 274-279.

Sahai, J., Swick L, Tailor S et al (1996). "Reduced oral absorption of tuberculosis drugs in HIV infection." Clin Pharmacol Ther 59(abstract PI 51): 142.

Saitoh, A., Singh KK, Powell CA, et al (2005). "An MDR1-3435 variant is associated with higher plasma nelfinavir levels and more rapid virologic reponse in HIV-1 infected children." AIDS 19(4): 371-380.

Sanfilippo, A., Della Bruna C, Marsili L, Morvillo E, Pasqualucci CR, Schioppacassi G and Ungheri D (1980). "Biological Activity Of A New Class of Rifamycins. Spiro-piperidyl-rifamycins." J Antibiot XXXIII(10): 1193-1198.

Schaeffeler E, Eichelbaum M, Brinkmann U, Penger A, Asante-Poku S, et al. (2001). “Frequency of the C3435T polymorphism of MDR1 gene in African people.” Lancet **358**(9297): 383-4

Schluger, N., Perez D, Liu YM (2002). "Reconstitution of Immune Responses to Tuberculosis in Patients with HIV Infection who Receive Antiretroviral Therapy." Chest 122: 597-602.

Schurmann, D., Bergmann,F ,Jautzke G, Fehrenbach FJ, Mauch H and Ruf B (1993). "Acute and long-term efficacy of antituberculosis treatment in HIV-seropositive patients with tuberculosis: a stuy of 36 cases." J Infection 26: 45-54.

Schwander S, Rusch-Gerdes S, Mateega A, et al. (1995). A pilot study of antituberculosis combinations comparing Rifabutin with rifampicin in the treatment of HIV-1 associated tuberculosis. A single-blind randomized evaluation in Ugandan patients with HIV-1 infection and pulmonary tuberculosis.” Tubercle Lung Dis **76**: 210-218

Sekar, V., Lavreys L, Van de Casteele T, Berckmans C, Spinosa-Guzman S, Vangeneugden TV, et al (2010). "Pharmacokinetics of Darunavir/Ritonavir and Rifabutin Coadministered in HIV-Negative Healthy Volunteers." Antimicrob Agents Chemother 54(10): 4440-4445.

Sensi, P. (1983). "History of the Development of Rifampin." Rev Infect Dis 5(Suppl.5): S402-S406.

Sensi, P., Maggi S, Furasz S and Maffii G (1966). "Chemical Modifications and Biological Properties of Rifamycins." Antimicrob Agents Chemother 6: 699-714.

Shafran, S., Singer J, Zarowny DP, Deschenes J, Phillips P, et al (1998). "Determinants of Rifabutin-Associated Uveitis in Patients Treated with Rifabutin, Clarithromycin, and Ethambutol for Mycobaterium avium Complex Bacteremia: A Mutivariate Analysis." JID 177: 252-255.

Shafran, S., Singer J, Zarowny DP, Phillips P, Salit I, et al. for the Canadian HIV Trials Network Protocol 010 Study Group (1996). "A Comparison of Two Regimens for the Trearment of Mycobacterium avium complex Bacteremia in AIDS: Rifabutin Ethambutol, and Clarithromycin Versus Rifampin, Ethambutol, Clofazimine and Ciprofloxacin." N Engl J Med 335(6): 377-383.

Skinner, M., Hsieh M, Torseth J, Pauloin D, Bhatia G, et al (1989). "Pharmacokinetics of Rifabutin." Antimicrob Agents Chemother 33(8): 1237-1241.

Slain, D., Pakiz A, Israel S, Monroe S, Polk RE (2000). "Variability in activity of hepatic CYP in patients infected with HIV." Pharmacotherpay **20**: 898-907.

Small, P., Schecter GF, Goodman PC, Sande MA, Chaisson RE and Hopewell PC (1991). "Treatment of Tuberculosis in Patients with Advanced Human Immundeficiency Virus Infection." N Engl J Med 324(5): 289-294.

Smith, P., Quin TC, Strober W, Janoff EN, Masur H (1992). "Gastrointestinal infections in AIDS." Ann Intern Med 116: 63-77.

Spradling, P., Drociuk D,McLaughlin S, et al (2002). "Drug-Drug Interactions in Inmates Treated for Human Immunodeficiency Virus and Mycobacterium tuberculosis

Infection or Disease: An Institutional Tuberculosis Outbreak." CID 35: 1106-1112.

Sterling, T., Pham PA, and Chaisson RE (2010). "HIV Infection-Related Tuberculosis: Clinical Manifestations and Treatment." CID 50(S3): S223-S230.

Stohr, W., Back D, Dunn D, Sabin C, Winston A, et al (2008). "Factors influencing efavirenz and nevirapine plasma concentration: effect of ethnicity, weight and co-medication." Antivir Therapy 13: 675-685.

Strivastava, S., Pasipanodya JG, Meek C, Leff R and Gumbo T (2011). "Multidrug-Resistant Tuberculosis Not Due to Noncompliance but to Between-Patient Pharmacokinetic Variability." J Infect Dis 204: 1951-1959.

Tappero, J., Bradford WZ, Agerton TB, Hopewell P, Reingold AL, et al (2005). "Serum Concentrations of Antimycobacterial Drugs in Patients with Pulmonary Tuberculosis in Botswana." CID 41: 461-169.

Thompson, B. (1943). "Survival rates in pulmomary tuberculosis." BMJ 2: 721.

Tirona, R., Leake BF, Merino G and Kim RB (2001). "Polymorhisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans." j Biol Chem 276: 35669-35675.

Torseth, J., Bhatia G, Harkonen S, Child C, Skinner M, et al (1989). "Evaluation of the Antiviral Effect of Rifabutin in AIDS-Related Complex." J Infect Dis 159(6): 1115-1118.

Trapnell, C., Narang PK, Li R, Lavelle JP (1996). "Increased plasma rifabutin levels with concomitant fluconazole therapy in HIV-infected patients." Ann Intern Med 124(6): 573-576.

UNAIDS, World Heath Oranization (2000). “Patent situation of HIV/AIDS-related drugs in 80 countries”. Available at www.who.int/entity/3by5/en/patentshivdrugs.pdf.

van Crevel, R., Alisjahbana B, de Lange WC, Borst F, Danusantoso H, et al (202). "Low plasma concentrations of rifampicin in tuberculosis patients in Indonesia." Int J Tuberc Lung Dis 6(6): 497-502.

van Crevel, R., Alisjahbana B, de Lange WC, Borst F, Danusantoso H, et al (2002). "Low plasma concentrations of rifampicin in tuberculosis patients in Indonesia." Int J Tuberc Lung Dis 6(6): 497-502.

van Heeswijk RPG, Veldkamp AI, Mulder JW, Meenhorst PL, Wit FW, et al (2000). “The steady-state pharmacokinetics of nevirapine during once daily and twice daily dosing in HIV-infected individuals.” AIDS 14(8): F77-82

van Oosterhout, J., Kumwenda JJ, Beadsworth M, Mateyu G, Longwe T, et al (2007). "Nevirapine-based antiretroviral therapy started early in the course of tuberculosis treatment in adult Malawians." Antivir Therapy 12: 515-521.

van Rie, A., Westreich D and Sanne I (2011). "Tuberculosis in patients receiving antiretroviral treatment: incidence, risk factors and prevention strategies." J Acquir Immune Defic Syndr 56(4): 349-355.

Velasco, M., Castilla V, Sanz J, et al (2009). "Effect of Simultaneous Use of Highly Active Antiretroviral Therapy on Survival of HIV Patients With Tuberculosis." J Acquir Immune Defic Syndr 50(2): 148-152.

Verbist, L. (1969). "Rifampicin activity "in vitro" and in established tuberculosis in mice." Acta Tuberc Pneumol Belg 60: 391-408.

Vernon, A. (2004). "Rifamycin antibiotics, with a focus on newer agents." In: Tuberculosis eds: Rom WN and Garay SM 2nd edition. Lipincott Williams & Williams.

Ward, B., Gorski JC, Jones DR, Hall SD, Flockhart DA and Desta Z (2003). "The Cytochrome P450 2B6 (CYP2B6) Is the Main catalyst of Efavirenz Primary and Secondary Metabolism: Implication for HIV/AIDS Therapy and Utilty of Efavirenz as a Substrate Marker of CYP2B6 Catalytic Activity." J Pharmacol Exper Therap 306(1): 287-300.

Watkins, P., Murray SA, Winkelman LG, Heuman DM, Wrighton SA and Guzelian PS (1989). "Erythromycin Breath Test as an Assay of Glucocorticoid-inducible Liver Cytrochromes P-450." J Clin Invest 83: 688-697.

Waksman, SA and Henrici AT (1943). “The Nomenclature and Classification of the Actinomycetes.” J Bacteriol 46 (4): 337-341

Wehrli, W., and Staehelin M (1971). "Actions of the Rifamycins " Bact Rev 35(3): 290-309.

Weiner, M., Benator D, Burman W, Peloquin CA, Khan A, et al (2005a). "Association between Acquired Rifamycin Resistance and the Pharmacokinetics of Rifabutin and Isoniazid among Patients with HIV and Tuberculosis." CID 40: 1481-1491.

Weiner, M., Benator D, Peloquin CA, Burman W, Vernon A, Engle M, Khan A, Zhao Z, and the Tuberculosis Trials Consortium (2005b). "Evaluation of the Drug Interaction between Rifabutin and Efavirenz in Patients with HIV Infection and Tuberculosis." CID 41: 1343-1349.

Weiner, M., Peloquin CA, Burman W, Luo C, Engle M, et al (2010). "Effects of Tuberculosis, Race, and Human Gene SLCO1B1 Polymorphisms on Rifampin Concentrations." Antimicrob Agents Chemother 54(10): 4192-4200.

Wilkins, J., Savic R, Karlsson MO, Langdon G, McIlleron H, et al (2008). "Population Pharmacokinetics of Rifampin in Pulmonary Tuberculosis, including a Semimechanistic Model To Describe Variable Absorption " Antimicrob Agents Chemother 52(6): 2138-2148.

Woodley, C., Kilburn JO (1982). "In vitro susceptibility of Mycobacterium avium coplex and Mycobacterium tuberculosis strains to a spiro-piperidyl rifamycin." Am Rev Respir Dis 126(3): 586-587.

Word Health Organisation (2009a). "Global Tuberculosis Control. Epidemiology, Strategy, Financing." Available at http://whqlibdoc.who.int/publications/2009/9789241563802_eng_doc.pdf.

Word Health Organisation (2009b). "WHO model list of essential medicines, 16th list. Geneva, Switzerland." Available at http://www.who.int/selection_medicines/committees/expert/17/sixteenth_adult_list_en.pdf

World Health Organisation (2004). "Policy on collaborative TB/HIV activities." Available at www.who/htm/tb/2004.330.

World Health Organisation (2010 revision). "Antitretroviral Therapy For HIV Infection In Adults And Adolescents. Recommendations for a public ealth approach." Available at http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf.

World Health Organisation (2011). "Global HIV/AIDS Response. Epidemic update and health sector progress towards Universal Access - Progress Report " Available at www.who.int/hiv/pub/progress_report2011/en/index.html.

World Health Organisation, R. (2011b). "Global Tuberculosis Control." Available at www.who.int/tb/publications/global_report2011/gtbr11_full.pdf.

World Health Organisation Stop TB Department (2009). "Treatment of Tuberculosis: guidelines for national programmes." 4th ed. Geneva: WHO: Available at http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf.

Zhu, B., Liu ZQ, Chen GL, Chen XP, Ou-Lang DS, et al (2003). "The distribution and gender difference of CYP3A activity in Chinese subjects." Br J Clin Pharmacol **55**: 264-269.

Appendix

i. The manuscript below was submitted to Clinical Infectious Diseases and is currently under review:

Pharmacokinetics of Different Rifabutin Doses in African HIV-infected Tuberculosis Patients on Lopinavir/ritonavir-based Antiretroviral Therapy

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Key words: rifabutin, pharmacokinetics, lopinavir, tuberculosis, HIV

Short Title: PK of Rifabutin with Lopinavir/ritonavir

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Summary

The pharmacokinetics of rifabutin at 150mg daily and 150mg three times weekly with LPV/ritonavir were evaluated in TB-HIV co-infected patients. Intermittent rifabutin plus lopinavir/ritonavir results in sub-therapeutic rifabutin drug exposure. Daily rifabutin plus lopinavir/ritonavir produces significantly higher rifabutin bioavailability.

Abstract:

Background: Pharmacokinetic interactions between rifampicin and protease inhibitors complicate the management of HIV-associated tuberculosis. Rifabutin is an alternative rifamycin, preferred in patients requiring protease inhibitors (PIs). Despite recent studies suggesting that the current recommended dose of rifabutin in combination with boosted lopinavir (LPV/r) is suboptimal, there are insufficient pharmacokinetic data evaluating the interaction in patients treated with the film-coated tablet formulation of LPV/r.

Methods and Findings: The pharmacokinetics of rifabutin was evaluated at two different doses in HIV co-infected African patients in Durban, South Africa, before and after administration of LPV/r (tablet formulation 400/100 mg 12 hourly)-based antiretroviral therapy (ART). Serial rifabutin and 25-O-desacetyl rifabutin concentrations were measured during a dose interval after 4 weeks of rifabutin 300 mg daily, after 4 weeks of 150 mg rifabutin daily with ART and after 4 weeks of rifabutin 150 mg 3 times a week (TPW) with ART. The median AUC_{0-48} and C_{max}, of rifabutin in patients taking 150mg rifabutin TPW was significantly reduced when compared to the other treatment arms. 86% of patients on this intermittent rifabutin arm had an AUC_{0-24} < 4.5 μg.h/mL, which has been associated with acquired rifamycin resistance (ARR) in patients on twice weekly doses. Rifabutin was well tolerated at all doses and there were no grade 4 laboratory toxicities. One case of uveitis occurred prior to starting ART, and grade 3 neutropenia was reported in 4 patients.

Conclusions: A daily 150 mg dose of rifabutin in combination with LPV/r safely maintained rifabutin plasma concentrations in line with those shown to prevent ARR.

Introduction

Treating HIV associated tuberculosis remains a formidable challenge. In 2009, 12% of the 9.4 million incident cases of tuberculosis, and 22% of deaths from tuberculosis were in HIV-infected patients [1]. Combining efavirenz-based first-line antiretroviral therapy (ART) with rifampicin based tuberculosis chemotherapy significantly reduces mortality in these patients [2-4] and is safe and efficacious. However, as public sector ART expands, an increasing number of patients are developing virological failure and require second-line ART [5]. However, combining rifampicin and protease inhibitor-based second-line ART is problematic as rifampicin significantly reduces the bioavailability of protease inhibitors by accelerating their metabolism via induction of cytochrome 3A4 (CYP3A4) enzymes. Increasing the dose of the protease inhibitor or co-administering higher doses of a CYP3A4 inhibitor to ameliorate this adverse drug-drug interaction have been thwarted by hepatotoxicity and other problems with tolerance [6].

Rifabutin, a less potent inducer of CYP3A4 [7, 8], is recommended at 300 mg daily as prophylaxis and treatment of Mycobacterium avium complex (MAC) and for the treatment of drug susceptible tuberculosis (Mycobutin package insert). Plasma concentrations of rifabutin are increased in the presence of protease inhibitors [9] therefore dose adjustments are recommended when rifabutin is combined with a protease inhibitor. Current guidelines recommend dosing rifabutin 150 mg three times per week (TPW) in combination with a ritonavir-boosted protease inhibitor [10, 11]. However, recent reports suggested that this dosing in HIV-positive tuberculosis patients may result in inadequate rifamycin concentrations [12, 13], relapse [14] and

acquired rifamycin resistance (ARR) [15]. The present study was therefore undertaken to compare the bioavailability of two doses of rifabutin (150mg TPW and 150mg daily) with the standard 300mg dose in HIV-positive tuberculosis African patients in Durban, South Africa, initiating ART with LPV/r.

Methods

Study Design

An open-label, three-period, crossover drug interaction study was undertaken to investigate the pharmacokinetics of rifabutin with and without PI-based ART. The secondary objective was to assess the tolerability and safety of rifabutin and lopinavir. The study was approved by the Biomedical Research Ethics Committees of the Universities of Kwa-Zulu Natal and Cape Town and the South African Medicines Control Council.

Recruitment

Patients were recruited from local tuberculosis clinics in Kwa-Zulu Natal, South Africa. Eligibility requirements were provision of written informed consent, a diagnosis of pulmonary tuberculosis confirmed by microscopy or culture, HIV infection with CD4 lymphocyte count ≥ 50 and ≤ 200 cells/mm^3, weight ≥ 50kg or a BMI ≥ 18, a Karnofsky score Q ≥ 80% and no grade 3 or 4 clinical or laboratory findings according to DAIDS tables [16]. Only patients who completed and adhered to 6 weeks of standard intensive phase chemotherapy and had not received ART therapy in the preceding three months were enrolled. Patients with a previous tuberculosis episode within three years prior to the current episode, a history of prior treatment for MDR tuberculosis, concomitant

opportunistic infection requiring additional anti-microbial treatment, a formal contraindication to any trial medication, diabetes mellitus requiring treatment, recreational drug or alcohol abuse, , mental illness, total neutrophil count < 1200 cells/L, hemoglobin < 6.8 g/dL, or liver function tests > grade 2, pregnancy or lactating women.

Treatments under study

At enrollment, six weeks after starting standard tuberculosis chemotherapy, rifampicin was switched to rifabutin 300 mg daily. After two weeks of rifabutin, pyrazinamide and ethambutol were stopped and patients continued with daily doses of rifabutin 300 mg in combination with isoniazid 300mg. After two more weeks, the first pharmacokinetic evaluation (PK1) was carried out and patients were randomized to one of two different rifabutin dose sequences together with daily doses of isoniazid and ART comprising LPV/r (400/100 mg) plus lamivudine (150mg bd) and stavudine (30mg bd). Half the patients received rifabutin 150 mg TPW for 4 weeks before being switched to rifabutin 150 mg daily after a second pharmacokinetic evaluation (PK2). A third pharmacokinetic evaluation (PK3) took place after 4 weeks and they remained on this dose of rifabutin until completion of tuberculosis treatment. Half the patients received the two rifabutin doses in a reverse sequence. . Physical examinations and laboratory investigations were done at every pharmacokinetic evaluation and at the penultimate trial visit to assess safety and adverse events. Upon completion of the trial, patients were referred to local antiretroviral clinics for further management. Pfizer (South Africa) supplied the rifabutin (Mycobutin®) 150mg capsules and the new film-coated tablet formulation of LPV/r, Aluvia® was purchased from Abbott Laboratories (USA).

Sample size

The sample size was calculated on the basis that 16 enrolled participants would result in a minimum of 12 evaluable subjects. Based on the AUC_{0-24} for rifabutin determined in previous studies, it was estimated that a sample size of 12 participants had a power of 80% to detect a 20% difference between the mean AUC_{0-24} for rifabutin with and without ART.

Pharmacokinetic Sampling

All patients were admitted before each pharmacokinetic occasion and were fasted from midnight. A standard hospital breakfast was served 2h after drug ingestion. Blood draws were done at 0, 2, 3, 4, 5, 6, 8, 12, 24 and 48h after drug ingestion. The samples were placed on ice immediately and centrifuged at 3000 rpm at 4^0C for 10 minutes. Separated plasma was stored immediately at -70^0C until batch analysis.

Drug Analyses

Rifabutin

Rifabutin and 25-O-desacetylrifabutin were analyzed with a validated LC/MS/MS assay. Rifaximin was used as internal standard at a concentration of 100 ng/ml. Gradient chromatography was performed on a Phenomenex, Luna 5 µm PFP(2), 100 A, 50 mm × 2 mm analytical column, using acetonitrile and 0.1% formic acid as mobile phase, and a flow rate of 500 µl/min. An AB Sciex API 3200 mass spectrometer monitored protonated ions at m/z 847.4 to the product ions at m/z 95.1 for rifabutin, at

m/z 805.4 to the product ions at m/z 95.1 for 25-O-desacetylrifabutin, and at m/z 786.3 to the product ions m/z 151.1 for Rifaximin. Rifabutin and 25-O-desacetylrifabutin accuracies were between 99.1% and 109.0% during inter-batch validation. The co-efficient of variation during inter-batch validation was less than 9.2%. The calibration range for rifabutin was between 3.91 ng/ml and 1000 ng/ml, and for 25-O-desacetylrifabutin between 0.780 ng/ml and 200 ng/ml.

Lopinavir

Plasma lopinavir concentrations were quantified by a validated LCMS/MS method using a modification of the method previously described by Chi et al [17]. The calibration curve was linear over the range from 0.05 to 20 mg/L. Samples with a concentration of > 20 mg/L, were diluted re-analyzed. Any sample below the LLQ was reported as 0.5 X LLQ for analysis. Accuracy ranged from 94.3% to 103.0%. The intraday and interday coefficient of variation (CV) ranged from 0.14% to 4.72% and from 1.61% to 4.22%, respectively.

Pharmacokinetic Analysis

The main pharmacokinetic measures for rifabutin, 25-O-desacetylrifabutin and lopinavir were derived by noncompartmental analysis using Stata (version 11). The peak concentration (C_{max}), and time to C_{max} (T_{max}) were obtained directly from concentration-time profiles. Drug concentrations at the end of a dosing interval are

reported as C_{min} and pre-dose concentrations as $C_{0.}$ The steady-state AUC from time 0h to the last quantifiable sample at 24h (AUC_{0-24}) or 48h (AUC_{0-48}) for rifabutin and 12h (AUC_{0-12}) for LPV/r were calculated by the linear trapezoidal method. The apparent total oral clearance of rifabutin from plasma at steady state (CL/F) was calculated by dose/AUC.

Statistics

For statistical analysis, the AUC was log- transformed and log AUC was normally distributed. A linear mixed model with two doses (high and low), day (2 and 3), sequence and log AUC_{0-24} at baseline and id nested within sequence was used. As there was no significant effect of sequence, the two arms were pooled and the model was used to test the dose effect after adjusting for baseline differences. A paired t-test was used to compare the AUC 150mg daily at 24 hours with baseline at 24 hours and the 150mg TPW at 48 hours with 2 x baseline at 24 hours because the dosing interval is 48 hours for the TPW dose.

To calculate geometric mean ratios (GMR) for AUC, log means and 90% confidence limits were back transformed and presented in their original units as geometric means. Geometric mean ratios for the AUC of rifabutin: 150 mg daily with LPV/r / 300mg daily, and 150 mg TPW with LPV/300mg daily, respectively, were computed. A P-value < 0.05 was considered significant. Inter-patient variability was measured by co-efficient of variation (%CV) which was calculated as $\{100X\ e^{(\text{var est})} -1)^{1/2}\}$. Baseline and final log viral loads and $CD4^+$ counts were compared using paired t-tests.

RESULTS

Patient Demographics

Sixteen patients received LPV/r therapy with rifabutin. Two patients were withdrawn from the pharmacokinetic analysis, one due to uveitis and another due to non-compliance on trial medication. All patients were Black South Africans and (64%) were male. The evaluable subjects' mean (SD) age was 31.5 (5.8) years, weight was 59.9 (9.7) kg, height was 160 (7.7) cm, BMI was 23.3 (2.6), Karnofsky score Q was 100 % (100) and $CD4^+$ lymphocyte count was 150.9 (12.1) cells/mm^3.

Pharmacokinetic Analysis

Rifabutin

The main pharmacokinetic parameters for rifabutin and 25-O-desacetylrifabutin are summarized in Table 1. The AUC_{0-24} of rifabutin 150 mg daily with LPV/r was significantly higher when compared to the AUC_{0-24} of rifabutin 300 mg daily in the absence of LPV/r ($p = 0.004$). In contrast, the AUC_{0-48} of rifabutin 150 mg TPW with lopinavir/r was significantly lower than the AUC_{0-48} of rifabutin 300 mg daily ($p = 0.0001$). Wide inter-patient variability was observed in rifabutin AUC for all three doses (Table 2). The %CV was 24% for the 300mg dose, 46% for rifabutin 150 mg daily plus LPV/r and 52% for rifabutin 150mg TPW plus LPV/r.

The C_{max} of rifabutin 150 mg TPW with LPV/r was also significantly lower when compared to the 150 mg daily dose with LPV/r (P = 0.01) and the 300 mg daily dose without LPV/r (P = 0.01). The median C_{min} for rifabutin 300mg was 60.7 ng/mL (IQR, 40.6-68.8 ng/mL). The C_{min} values increased in the presence of LPV/r with daily dosing of rifabutin but dropped significantly with the intermittent dose. Rifabutin clearance was significantly reduced in the presence of LPV/r (p= 0.001 for daily and p = 0.002 intermittent rifabutin dosing) compared to 300mg rifabutin given alone.

25-O-Desacetylrifabutin

Without lopinavir, 25-O-desacetylrifabutin concentrations were 11 % of the parent drug. Plasma 25-O-desacetylrifabutin concentrations increased 5-fold with intermittent rifabutin dosing and 15-fold with daily doses of rifabutin (Figure 2). The total antimicrobial moiety (combined AUC_{0-24} of rifabutin and metabolite) for rifabutin at 150mg TPW with ART was 1.2 times greater than for 300mg rifabutin and 2.6 times greater than for 150mg daily with ART.

Lopinavir Pharmacokinetics

LPV/r pharmacokinetic measures are shown in Table 3 in Figure 3. Median LPV/r trough (C_0) concentrations were above the recommended lower limit for ART-naïve patients of 1 μg/mL [18]. Although there was a trend to higher LPV/r concentrations with the once daily dosing of rifabutin, the differences in AUC_{0-12} and C_{max} between the

two doses were not significant. Double peaks were observed in the lopinavir concentration-time profile with both doses of rifabutin.

Response to TB/HIV Treatment

Three patients were culture positive after two months of tuberculosis therapy and none culture positive at the end of therapy. The mean final $CD4^+$ count at the end of tuberculosis therapy was 253.8 (42.4) cells/mm^3, and significantly higher ($p = 0.03$) than baseline. The mean (SD) viral load dropped significantly ($p < 0.001$) by 2.7 $\log_{10}$ copies and 8 patients had viral loads > 500 cells/L.

Adverse Events

Adverse events (AE) were analyzed for all sixteen patients. Rifabutin was well tolerated at all doses and there was only one withdrawal because of an adverse event (uveitis). There were two serious adverse events (bacterial meningitis and pyelonephritis), both unrelated to rifabutin. Grade 2 uveitis occurred in one patient after 1 month of rifabutin, coinciding with the start of ART, and resolved with no sequelae after withdrawal. Her $AUC_{0\text{-}24}$ and C_{max} values for rifabutin were within the interquartile range. The commonest laboratory AE was neutropenia. Grade 3 neutropenia occurred on six occasions in 4 patients. There were 2 grade 3 elevations in transaminases and amylase. There were no grade 4 laboratory events.

Discussion

The currently recommended dose of rifabutin for the treatment of pulmonary tuberculosis is 300mg daily but there are few pharmacokinetic data, especially from HIV infected African tuberculosis patients. The median rifabutin AUC_{0-24} and C_{max} values from this study are comparable to previous studies of rifabutin 300mg daily in HIV-infected patients [12, 19-21].

Previous studies of the pharmacokinetic interaction of rifabutin with LPV/r in HIV infected individuals have been small case series or involved an adaptive design in which only selected patients were exposed to the higher dose of rifabutin. In addition, no previous studies have reported on the rifabutin pharmacokinetics in combination with the most widely used tablet formulation of LPV/r (Aluvia). We adopted a cross-over design in which all patients received 3 full pharmacokinetic assessments with rifabutin alone and at two different rifabutin doses in combination with ART allowing us to formally compare the different dosing strategies. We found the AUC_{0-24} and C_{max} of rifabutin when 150mg was given intermittently with ART, to be significantly lower than daily dosing or dosing at 300mg daily without ART.

The reduced bioavailability of intermittent dosing of rifabutin at 150mg with LPV/r could result in suboptimal tuberculosis therapy, although the pharmacodynamic-pharmacokinetic (PKPD) relationship for rifabutin has not been comprehensively studied. It is still uncertain if C_{max} or AUC is the critical pharmacodynamic measure for rifamycins. Mitchison and [22] and others [23] reported the C_{max}/MIC to be the best

PKPD measure, whereas subsequent murine and hollow fibre models [24] [25], and early bactericidal activity studies in humans[26] found that the $AUC_{0\text{-}24}$/MIC ratio was a superior parameter.

Despite a lack of conventional efficacy data in support of a particular dose of RBT, there is convincing evidence that intermittent rifamycin therapy is associated with tuberculosis relapse and ARR, especially in subjects with low CD4 counts [28-30]. In TBTC study 23 [15], TB-HIV co-infected patients who had lower AUC_{0-24} of < 4.5 µg.h/mL plasma concentrations of rifabutin were at risk of ARR. 83% of patients who relapsed or failed therapy developed ARR as opposed to only 33% who had AUC's above this threshold value. We found 71% patients on rifabutin 150mg daily and 14% on rifabutin TPW had $AUC_{0\text{-}24}$ values > 4.5 µg.h/mL.

The $AUC_{0\text{-}24}$ and C_{max} of 25-O-desacetylrifabutin were significantly increased in the presence of LPV/r in keeping with previous studies [9, 31 - 34]. The exposure to the metabolite increased approximately 5- and 15-fold when rifabutin150 mg was given intermittently or daily with LPV/r. 25-O-desacetylrifabutin is known to have significant antimycobacterial activity and could contribute to regimen efficacy. Interestingly, when rifabutin and the metabolite were combined the $AUC_{0\text{-}24}$ on the 150mg daily dose with LPV/r was significantly increased compared to both the other arms. The therapeutic index of 25-O-desacetylrifabutin is unknown and it is possible that elevations of the metabolite could affect adverse drug reactions. A significant association between

plasma rifabutin and 25-O-desacetylrifabutin concentrations and adverse events was not seen although the numbers of patients are few.

There has been a reluctance to recommend a higher dose of rifabutin partly based on early experience with rifabutin and CYP3A4 inhibitors [35-39]. An advantage of this study is that patients remained on study doses up to six months allowing a safety evaluation to be made over a longer duration. In this study the combinations of rifabutin and LPV/r were generally well tolerated with no grade 4 toxicities apart from 2 clinical serious adverse events unrelated to rifabutin. Neutropenia was a common adverse event and has been reported predominantly in previous studies on healthy volunteers, but there were no grade 4 cases. There was a significant fall in the neutrophil count but most of this decline occurred in the first few weeks of rifabutin therapy prior to the initiation of ART and no significant association between neutropenia and plasma rifabutin concentrations was found. Neutropenia is also the most common side effect of cotrimoxazole therapy [40] and all patients in the present study received cotrimoxazole 960 mg daily. The majority of the patients started cotrimoxazole at enrollment and this would have contributed to the declining neutrophil count seen in this study.

We also evaluated the pharmacokinetics of LPV/r in the presence of rifabutin. The median LPV/r $AUC_{0\text{-}12}$, C_{max}, C_0 and C_{12} obtained in this study when LPV/r was administered with two different doses of rifabutin are comparable to those reported by Matteelli et al [42] and consistent with historical control data [43]. Secondary peaks

were observed in the time-concentration profiles of LPV/r, usually within 4 hours of drug ingestion, similar to patterns observed in other studies. In both dosing arms, median LPV/r trough (C_0) concentrations at steady state were above the recommended lower limit for ART-naïve patients of 1 μg/mL [18] and therapeutic LPV/r trough (C_0) and C_{min} (C_{12}) concentrations were achieved in all participants with both doses of rifabutin [41].

In conclusion this study supports an alteration to the current guidelines for the dosing of rifabutin in combination with LPV/r. The high proportion of participants on the 150mg TPW arm who failed to achieve rifabutin concentrations that prevented the emergence of drug resistance when the drug is dosed twice weekly is concerning. Although escalating the rifabutin dose after therapeutic drug monitoring is a viable option in resource rich settings it is impractical in many regions of the world where HIV and TB are endemic. Our study was too small to address all concerns about the toxicity of the higher dose rifabutin with LPV/r, most notably the decrease in neutrophil count, which requires further evaluation. However the 150 mg daily dose was well tolerated and using an alternative prophylaxis to cotrimoxazole may abrogate the concerns about neutropenia.

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References

1. World Health Organization, *Global Tuberculosis Control.* 2010: Available at: http://www.who.int/tb/publications/global_report/2010.

2. Abdool Karim, S., Naidoo K, Grobler A, et al, *Timing of Initiation of Antiretroviral Drugs during Tuberculosis Therapy.* N Engl J Med, 2010. **362**: p. 697-706.

3. Blanc, F., Sok T, Laureillard D, Borand L, Rekacewicz C, et al, *Earlier versus Later Start of Antiretroviral Therapy in HIV-Infected Adults with Tuberculosis.* N Engl J Med, 2011. **365**: p. 1471-1481.

4. Havlir, D., Kendall M, Ive P, Kumwenda J, Swindells S, et al, for the AIDS Clinical Trials Group Study A5221, *Timing of Antiretroviral Therapy for HIV-1 Infection and Tuberculosis.* N Engl J Med, 2011. **365**: p. 1482-1491.

5. World Health Organization. Global HIV/AIDS Response. Epidemic update and health sector progress towards Universal Access. Available at www.who.int/hiv/pub/progress_report2011/en/index.html

6. Burman, W., Gallicano K and Peloquin C, *Comparative Pharmacokinetics and Pharmacodynamics of the Rifamycin Antibacterials.* Clin Pharmacokinet 2001. **40**(5): p. 327-341.

7. Della Bruna, C., Schioppacassi G, Ungheri D, Jabes D, Morvillo E, Sanfilippo A, *LM 427, A new spiropiperidylrifamycin: in vitro and in vivo studies.* J Antibiot, 1983. **XXXVI**(11): p. 1502-1506.

8. Sanfilippo, A., Della Bruna C, Marsili L, Morvillo E, Pasqualucci CR, Schioppacassi G and Ungheri D *Biological Activity Of A New Class of Rifamycins. Spiro-piperidyl-rifamycins.* J Antibiot, 1980. **XXXIII**(10): p. 1193-1198.

9. Polk, R., Brophy, DF, Israel, DS, Patron, R, Sadler, BM, et al, *Pharmacokinetic Interaction between Amprenavir and Rifabutin or Rifampin in Healthy Males.* Antimicrob Agents Chemother, 2001. **45**(2): p. 502-508.

10. Centres for Disease Control & Prevention, *Prevention and treatment of tuberculosis among patients infected with human immunodeficiency virus: principles of therapy and revised recommendations.* MMWR Recomm Rep, 1998. **47**(RR20): p. 1-51.

11. Centres for Disease Control & Prevention, *Updated guidelines for the use of rifamycins for the treatment of tuberculosis among HIV-infected patients taking protease inhibitors or non-nucleoside reverse transcriptase inhibitors.* MMWR, 2004. **53**: p. 37.

12. Boulanger, C., Hollander E , Farrelll K , et al, *Pharmacokinetic Evaluation of Rifabutin in Combination with Lopinavir-Ritonavir in Patients with HIV Infection and Active Tuberculosis* CID, 2009. **49**: p. 1305-11.

13. Khachi, H., O'Connell, R, Ladenheim, D and Orkin, C, *Pharmacokinetic interactions between rifabutin and lopinavir/ritonavir in HIV-infected patients with mycobacterial co-infection.* J Antimicrob Chem, 2009. **64**(4): p. 871-3.

14. Jenny-Avital, E., and Joseph K, *Rifamycin-Resistant Mycobacterium tuberculosis in the Highly Active Antiretroviral Therapy Era: A Report of 3 Relapses with Acquired Rifampin Resistance following Alternate-Day Rifabutin and Boosted Protease Inhibitor Therapy.* CID, 2009. **48**: p. 1471-4.

15. Weiner, M., Benator D, Burman W, Peloquin CA, Khan A, et al, *Association between Acquired Rifamycin Resistance and the Pharmacokinetics of Rifabutin and Isoniazid among Patients with HIV and Tuberculosis.* CID,2005. **40**: p. 1481-91.

16. AIDS Clinical Trials Group, *Division of AIDS table for grading the severity of adult and pediatric events.* 2004. Available at: http://www.ucdmc.ucdavis.edu/clinicaltrials/documents/DAIDS_AE_GradingTable_FinalDec2004.pdf

17. Chi, J., Jayewardene AL, Stone JA, et al, *Simultaneous determination of five HIV potease inhibitors nelfinavir, indinavir, ritonavir, saquinavir and amprenavir in human plasma by LC/MS/MS.* J Pharm Biomed Anal, 2002. **30**: p. 675-684.

18. la Porte, C., Back DJ, Blaschke T, Boucher CAB, Fletcher CV, et al, *Updated guideline to perform therapeutic drug monitoring for antiretroviral agents.* Rev Antivir Ther, 2006. **3**: p. 4-14.

19. Colborn, D., Lampiris H, Lee B, Lewis R, Sullam P, Narang PK, *Concomitant Cotrimoxazole (CTX) Does Not Affect Rifabutin (RBT) Kinetics in HIV+ Patients.* Clin Pharm Therap, 1996. **59**(2): p. 141.

20. Moyle, G., Buss NE, Goggin T et al., *Interaction between saquinavir soft-gel and rifabutin in patients infected with HIV.* Br J Clin Pharmacol, 2002. **54**: p. 178-182.

21. Li, R., Nightingale S, Lewis RC, Colborn DC and Narang PK, *Lack of Effect of Concomitant Zidovudine on Rifabutin Knetics in Patients with AIDS-Related Complex* Antimicrob Agents Chemother, 1996. **40**(6): p. 1397-1402.

22. Mitchison, D., and Dickinson JM, *Laboratory aspects of intermittnt drug therapy.* Postgrad Med J, 1971. **47**: p. 737-741.

23. Verbist, L., *Rifampicin activity "in vitro" and in established tuberculosis in mice.* Acta Tuberc Pneumol Belg, 1969. **60**: p. 391-408.

24. Jayaram, R., Gaoakar S, Kaur P, Suresh BL, Mahesh BN, et al, *Pharmacokinetics-Pharmacodynamics of Rifampin in an Aerosol Infection Model of Tuberculosis.* Antimicrob Agents Chemother, 2003. **47**(7): p. 2118-2124.

25. Gumbo, T., Louie A, Deziel MR, et al, *Concentration-Dependent Mycobacterium tuberculosis Killing and Prevention of Resistance by Rifampin.* Antimicrob Agents Chemother, 2007. **51**(11): p. 3781-3788.

26. Diacon, A., Patientia RF, Venter A, van Helden PD, Smith PJ, et al, *Early bactericidal activity of high-dose rifampin in patients with pulmonary tuberculosis evidenced by positive sputum spears.* Antimicrob Agents Chemother, 2007. **51**: p. 2994-2996.

27. Gonzalez-Montaner, L., Natal S, Yongchaiyud P, Olliaro P, The Rifabutin Study Group, et al, *Rifabutin for the treatment of newly-diagnosed pulmonary tuberculosis: a multinational, randomised, comparative study versus rifampicin* Tuber Lung Dis, 1995. **75**(5): p. 341-347.

28. Spradling, P., Drociuk D,McLaughlin S, et al, *Drug-Drug Interactions in Inmates Treated for Human Immunodeficiency Virus and Mycobacterium tuberculosis Infection or Disease: An Institutional Tuberculosis Outbreak.* CID, 2002. **35**: p. 1106-12.

29. Li, J., Munsiff SS, Driver CR and Sackoff J, *Relapse and Acquired Rifampin Resistance in HIV-Infected Patients with Tuberculosis Treated with Rifampin- or Rifabutin-Based Regimens in New York City, 1997-2000.* CIF, 2005. **41**: p. 83-91.

30. Nettles, R., Mazo D, Alwood Ket al, *Risk Factors for Relapse and Acquired Rifamycin Resistance after Directly Observed Tuberculosis Treatment: A Comparison by HIV Serostatus and Rifamycin Use.* CID, 2004. **38**: p. 731-6.

31. Benator, D., Weiner MH, Burman WJ, et al, *Clinical Evaluation of the Nelfinavir-Rfabutin Interaction in Patients with Tuberculosis and Human Immunodeficiency Virus Infection.* Pharmacotherapy, 2007. **27**(6): p. 793-800.

32. Hamzeh, F., Benson C, Gerber J, et al, *Steady-state pharmacokinetic interaction of modified- dose indinavir and rifabutin.* Clin Pharmacol Ther, 2003. **73**: p. 159-69.

33. Ford, L., Chen Y, Lou Y, et al, *Pharmacokinetic Interaction between Fosamprenavir-Ritonavir and Rifabutin in Healthy Subjects.* Antimicrob Agents Chemother, 2008. **52**: p. 534-538.

34. Sekar, V., Lavreys L, Van de Casteele T, Berckmans C, Spinosa-Guzman S, Vangeneugden TV, et al, *Pharmacokinetics of Darunavir/Ritonavir and Rifabutin*

Coadministered in HIV-Negative Healthy Volunteers. Antimicrob Agents Chemother, 2010. **54**(10): p. 4440-4445.

35. Apseloff, G., *Severe neutropenia among healthy volunteers given rifabutin in clinical trials.* Clin Pharm Therap, 2003. **74**(6): p. 591-592.

36. Apseloff, G., Foulds G, LaBoy-Goral L, Kraut E, Vincent J, *Severe neutropenia caused by recommended prophylactic doses of rifabutin.* The Lancet, 1996. **348**: p. 685.

37. Griffith, D., Brown BA, Girard WA and Wallace RJ Jnr, *Adverse Events Associated with High-Dose Rifabutin in Macrolide-Containing Regimens for the Treatment of Mycobacteium avium Complex Lung Disease* CID, 1995. **21**(3): p. 594-598.

38. Griffith, D., Brown BA, Girard WA and Wallace RJ Jnr, *Varying Dosages of Rifabutin Affect White Blood Cell and Platelet Counts in Human Immunodeficiency Virus-Negative Patients Who Are Receiving Mutidrug Regimens for Pulmonary Mycobacterium avium Complex Disease* CID, 1996. **23**: p. 1321-2.

39. Shafran, S., Singer J, Zarowny DP, Deschenes J, Phillips P, et al, *Determinants of Rifabutin-Associated Uveitis in Patients Treated with Rifabutin, Clarithromycin, and Ethambutol for Mycobaterium avium Complex Bacteremia: A Mutivariate Analysis.* JID, 1998. **177**: p. 252-5.

40. Moh, R., Danel C, Sorho S, Sauvageot D, Anzian A, et al, *Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Cote d'Ivore.* Antiviral Ther, 2005. **10**: p. 615-624.

41. Kempf, D., Marsh KC, Kumar G, Rodrigues AD, Denissen JG, et al, *Pharmacokinetic Ehancement of Inhibitors of the Human Immunodeficiency Virus Protease by Coadministration with Ritonavir.* Antimicrob Agents Chemother, 1997. **41**(3): p. 654-660.

42. Matteelli, A., Regazzi M, Villani P, De Iaco G, Cusato M, et al, *Multiple-dose pharmacokinetics of efavirenz with and without the use of rifampicin in HIV-positive patients.* Curr HIV Res, 2007. **5**(3): p. 349-53.

43. Crommentuyn, K., Mulder JW, Mairuhu ATA, van Garp ECM, Meenhorst PL, et al, *The plasma and intracellular steady-state pharmacokinetics of lopinavir/ritonavir in HIV-1-infected patients.* Antivir Therapy, 2004. **9**: p. 779-785.

Table 1. Main pharmacokinetic parameters median (interquartile range) for rifabutin and 25-*O*-desacetylrifabutin derived by non-compartmental analysis for each of the study treatments: rifabutin 300mg daily, rifabutin 150 mg thrice weekly (TPW) plus lopinavir/ritonavir (LPV/r), and rifabutin 150 mg daily plus LPV/r.

Treatment period	Rifabutin 300mg	Rifabutin 150 mg TPW plus LPV/r	Rifabutin 150 mg daily plus LPV/r
Rifabutin (n=14)			
AUC_{0-24} (ng.h/mL)	3052.9 (2650.2-3431.5)	2307.5 (1767.5-3884.0)	4766.0 (3950.5-6099.5)
AUC_{0-48} (ng.h/mL)	6105.8 (5300.4-6863.0)*	3402.1 (2809.2-6092.0)	9532.0 (2238.2-22 425.4)*
C_{max} (ng/mL)	291.5 (250.0-377.0)	167.5 (87.8-294.0)	311.0 (258.0-376.0)
T_{max} (h)	3.0 (3.0-4.0)	3.5 (3.0-5.0)	3.0 (3.0-4.0)
C_0 (ng/mL)	59.0 (36.4 - 78.6)	49.1 (27.7-58.9)	176.5 (149.0 – 195.0)
C_{min} 24h (ng/mL)	60.7 (40.6 -68.8)	70.7 (45.7 – 96.6)	133.0 (105.0 – 191.0)
C_{min} 48h (ng/mL)	-	37.0 (26.6-70.0)	-
CL/F (L/h)	98.3 (87.4 – 113.2)	65.2 (38.6 – 85.0)	31.5 (25.0 – 38.0)
AUC_{0-24} (ng.h/mL) (Rifabutin +Metabolite)	3402.3 (2900.3 -3717.2)	3937.2 (2424.6 – 6772.7)	8753.0 (7771.7 – 11 505.0)
d-Rifabutin (n = 14)			
AUC_{0-24} (ng.h/mL)	273.3 (235.7-344.1)	1565.5 (1105.5-2567.3)	4118.0 (2678.2-5405.5)
AUC_{0-48} (ng.h/mL)	546.6 (471.4– 688.2)*	2318.2 (1722.9-4685.9)	8236.0 (5356.4-10811.0)*
C_{max} (ng/mL)	32.5 (25.2-37.7)	77.2 (58.6-128)	236.5 (159.0-274.0)
T_{max} (h)	3.0 (3.0-4.0)	5.0 (4.0-6.0)	4.0 (3.0-3.0)
C_0 (ng/mL)	5.1 (2.7-6.6)	44.6 (31.7-68.9)	186.0 (115.0-232.0)
C_{min} 24h (ng/mL)	5.0 (3.4 -5.8	63.9 (42.7-101.0)	155.0 (53.6-206.0)
C_{min} 48h (ng/mL)	-	35.4 (27.7 – 81.0)	-

* calculated by 2 X AUC_{0-24}

RBT = rifabutin; d-RBT = 25-O-deacetylrifabutin; LPV/r = lopinavir/ritonavir; TPW = three times per week; AUC = area under the curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum plasma attained; CL/F = clearance; C_0 = pre-dose concentration; C_{min} = trough concentration

Table 2. Comparison of geometric mean ratios (GMR) for rifabutin when rifabutin 150 mg is administered three times per week (TPW) or daily with lopinavir/r vs. rifabutin 300 mg daily without lopinavir/ritonavir and comparison of rifabutin 150mg TPW with lopinavir/ritonavir vs rifabutin 150mg daily with lopinavir/ritonavir

	GMR (90% CI)		
	RBT 150mg TPW with ART/RBT 300mg daily without ART	RBT 150mg daily with ART/ RBT 300mg daily	RBT 150mg daily with ART/RBT 150mg TPW with ART
AUC_{0-24}	1.3 (1.3 -2.1)	1.6 (0.1 -2.5)	2.04 (1.1 -3.9)
AUC_{0-48}	1.6 (1.0 – 2.6)	1.6 (1.0 -2.4)	2.6 (1.5 -4.5)

RBT = rifabutin; LPV/r = lopinavir/ritonavir; TPW = three times per week; AUC = area under the curve;

Table 3. **Table 3. Lopinavir pharmacokinetic parameters derived from the non-compartmental analysis when lopinavir/ritonavir was given in combination with two different doses of rifabutin.**

	Median (Interquartile range)	
Parameter	**RBT 150mg TPW plus LVP/r**	**RBT 150mg daily plus LPV/r**
AUC_{0-12} (µg.h/mL)	139.5 (103.8 – 163.9)	160.1 (129.1 – 181.9)
C_{max} (ng/mL)	15.8 (12.9 – 17.1)	18.1 (14.5-19.6)
T_{max} (h)	2.0 (2.0 – 3.0)	2.0 (2.0-3.0)
C_0(µg/mL)	9.8 (3.5 – 14.0)	11.4 (9.9 – 15.2)
C_{12} (µg/mL)	7.4 (4.5 – 10.0)	9.4 (7.2 – 11.6)

RBT = rifabutin; LPV/r = lopinavir/ritonavir; TPW = three times per week; AUC = area under the curve; C_{max} = maximum concentration in plasma; T_{max} = time at which maximum plasma attained; C_0 = pre-dose concentration; C_{12} = trough concentration