Pharmacokinetic Interaction between Efavirenz and Itraconazole in HIV- infected Patients

Kritsana Kongklao

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Thesis Title	Pharmacokinetic Interaction between Efavirenz and Itraconazole in HIV- infected Patients
Author	Miss Kritsana Kongklao
Major Program	Pharmacology

Major Advisor:

……
(Assoc. Prof. Werawath Mahatthanatrakul, M.D.)

Co-advisor:

……
(Prof. Sutep Jaruratanasirikul, M.D.)

……
(Assoc. Prof. Malinee Wongnawa)

Examining Committee:

…….Chairperson
(Assoc. Prof. Dr. Wichittra Tassaneeyakul)

…….....
(Assoc. Prof. Werawath Mahatthanatrakul, M.D.)

…….……...
(Prof., Sutep Jaruratanasirikul, M.D.)

…….…….....
(Assoc. Prof. Malinee Wongnawa)

……
(Assoc. Prof. Dr. Payom Wongpoowarak)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Pharmacology

..…….……
(Prof. Dr. Amornrat Phongdara)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

_____ Signature

(Assoc. Prof. Werawath Mahatthanatrakul, M.D.)

Major Advisor

_____ Signature

(Kritsana Kongklao)

Candidate

I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

_____ Signature

(Kritsana Kongklao)

Candidate

ชื่อวิทยานิพนธ์ ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่าง Efavirenz และ Itraconazole ในผู้ป่วยติดเชื้อเอชไอวี

ผู้เขียน นางสาวกฤษณา คงเคล้า

สาขาวิชา เภสัชวิทยา

ปีการศึกษา 2555

บทคัดย่อ

Efavirenz เป็นยาต้านไวรัสเอดส์ในกลุ่ม NNRTIs ซึ่งมีฤทธิ์ชักนำเอนไซม์ CYP3A4 และเป็น weak substrate ของ P-glycoprotein ส่วน itraconazole เป็นยาต้านเชื้อราในกลุ่ม triazole ที่ออกฤทธิ์ครอบคลุมเชื้อได้อย่างกว้างขวาง มีวิถีการแปรรูปผ่าน cytochrome P450 โดยมีฤทธิ์ยับยั้งเอนไซม์ CYP3A4 และ P-glycoprotein วัตถุประสงค์การศึกษาคือ เพื่อศึกษาการเกิดปฏิกิริยาระหว่าง efavirenz และ itraconazole ในผู้ป่วยติดเชื้อ HIV ผู้ป่วยจำนวน 3 รายเข้าร่วมในการศึกษานี้ ระยะแรกผู้ป่วยจะได้รับ itraconazole ขนาด 200 มิลลิกรัมวันละหนึ่งครั้งเป็นเวลา 14 วัน ส่วนระยะที่สองผู้ป่วยจะได้รับยาต้านไวรัสคือ efavirenz ขนาด 600 มิลลิกรัมวันละหนึ่งครั้งร่วมกับยากลุ่ม NRTIs อีก 2 ชนิดตั้งแต่วันที่ 15 ถึง 28 และระยะที่สามผู้ป่วยจะได้รับ itraconazole ขนาด 200 มิลลิกรัมร่วมกับ efavirenz ขนาด 600 มิลลิกรัมวันละครั้งตั้งแต่วันที่ 29 ถึง 42 ทำการศึกษาเภสัชจลนศาสตร์ของ Itraconazole และ efavirenz ในวันที่ 14 28 และ 42 ผลการศึกษาพบว่า efavirenz มีผลให้ความเข้มข้นสูงสุดของ itraconazole ในพลาสมาลดลงอย่างมีนัยสำคัญถึง 92.47% ส่วนความเข้มข้นต่ำสุดของยา itraconazole ในพลาสมาและพื้นที่ใต้กราฟระหว่างความเข้มข้นของยาและเวลาระหว่าง 0-24 ชั่วโมงลดลง 10 และ 13 เท่าตามลำดับ ส่วน itraconazole มีผลต่อเวลาที่มีความเข้มข้นสูงสุดของ efavirenz ในพลาสมาลดลงอย่างมีนัยสำคัญ การศึกษานี้สรุปได้ว่ายา efavirenz มีฤทธิ์กระตุ้นวิถีการแปรรูปของ itraconazole โดยผ่านการกระตุ้นเอนไซม์ CYP3A4 ส่วน itraconazole นั้นมีผลต่อการดูดซึมของ efavirenz โดยผ่านการยับยั้ง P-glycoprotein ดังนั้นแพทย์ควรเพิ่มขนาดของ itraconazole เมื่อให้ร่วมกับ efavirenz และควรติดตามผลการรักษาอย่างใกล้ชิดหรือเปลี่ยนยาต้านเชื้อราเป็นชนิดอื่นเพื่อให้มีประสิทธิภาพในการรักษา

Thesis Title Pharmacokinetic Interaction between Efavirenz and Itraconazole in HIV- infected Patients

Author Miss Kritsana Kongklao

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ABSTRACT

Efavirenz, a non-nucleoside reverse transcriptase inhibitors (NNRTIs), has been shown to be an inducer of CYP3A4 and weak substrate of P-glycoprotein. Itraconazole, a broad spectrum triazole antifungal agent, metabolized via cytochrome P450 isozyme system and also a potent inhibitor of CYP3A4 and P-glycoprotein. The aim of this study was to investigate the drug interaction between efavirenz and itraconazole in HIV-infected patients. Three patients participated in the study. In phase one (day 1-14), the patients received 200 mg of itraconazole once daily. In phase two (days 15–28), the patients were administered 600 mg of efavirenz once daily in combination with two NRTIs. In phase three (days 29–42), all patients were administered 200 mg of itraconazole with 600 mg of efavirenz once daily. Itraconazole and efavirenz pharmacokinetic studies were carried out on days 14, 28 and 42. The results indicated that efavirenz significantly decreased C_{max} of itraconazole by 92.47% and the C_{min} and AUC_{0-24} were decreased by 10 and 13 fold, respectively. However, itraconazole significantly decreased T_{max} of efavirenz. In conclusion, efavirenz has an enzyme induction effect on the metabolism of itraconazole, most likely through CYP3A4 induction. However, itraconazole slightly increased the absorption of efavirenz probably by inhibition of P-glycoprotein but its inhibitory activity on metabolism of efavirenz could not be demonstrated in this study. Therefore, the dosage of itraconazole should be increased and its therapeutic outcome be closely monitored when these two agents are concomitantly administered or considered alternative antifungal agents especially in patients who are on a long-term therapy in order to get the optimum result.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	=	acquired immunodeficiency syndrome
AUC	=	area under the concentration-time curve
BUN	=	blood urea nitrogen
CD_4^+	=	cluster of differentiation
C_{max}	=	maximum plasma concentration
C_{min}	=	minimum plasma concentration
Cl/f	=	oral clearance
Cr	=	creatinine
%CV	=	percentage of coefficient of variation (standard deviation x100/mean)
CYP	=	cytochrome P450
DNA	=	deoxyribonucleic acid
FDA	=	food and drug administration
g	=	gram
h	=	hour
HAART	=	highly active antiretroviral therapy
HIV	=	human immunodeficiency virus
HPLC	=	high performance liquid chromatography
kg	=	kilogram
L	=	liter
μg	=	microgram
μl	=	micro liter
mg	=	milligram
min	=	minute
ml	=	milliliter
mm^3	=	cubic milliliter
MW	=	molecular weight
ng	=	nanogram
NRTIs	=	nucleoside reverse transcriptase inhibitors

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

NNRTIs	=	non-nucleoside reverse transcriptase inhibitors
OIs	=	opportunistic infections
OPC	=	oropharyngeal candidiasis
PIs	=	protease inhibitors
PCP	=	pneumocystis pneumonia
r	=	correlation coefficient
RNA	=	ribonucleic acid
rpm	=	round per minute
SD	=	standard deviation
sec	=	second
SGOT	=	serum glutamic-oxaloacetic transaminase
SGPT	=	serum glutamic-pyruvic transaminase
TID	=	three times a day
T_{max}	=	time to maximal serum concentration
$T_{1/2}$	=	half-life
V_d	=	volume of distribution
v/v	=	volume by volume
%	=	percent
®	=	tread name
∞	=	infinite

CHAPTER 1

INTRODUCTION

1.1. Background and Rationale

The acquired immunodeficiency syndrome (AIDS) is caused by retrovirus known as human immunodeficiency virus (HIV). HIV is a lentivirus (member of the retrovirus family) which produces a progress immunosuppression by destruction of CD_4^+ T-lymphocytes and macrophages, resulting in falling of the immune system and leading to life-threatening opportunistic infections, neurological and neoplastic diseases, and death (Portegies *et al.,* 2007). AIDS was first described in 1981. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk. HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unprotected sexual intercourse, contaminated needles, breast milk and transmission from an infected mother to her baby at birth (vertical transmission). Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world (Kilmarx, 2009). According to the AIDS epidemic update 2011 by UNAIDS/WHO, released on July 2012, 34 million people were living with the human immunodeficiency virus (HIV) and 1.5 million have died from HIV/AIDS infection in 2011(UNAIDS/WHO, 2011). An estimated number of 1.58 million were infected in ASEAN and 480,000 in Thailand at the end 2011 and 307 have died from the infection.

HIV is a member of the genus lentivirus, part of the family of retroviridae. Lentiviruses have many common morphologies and biological properties. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period (Douek *et al.,* 2009). Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry of the target cell, the viral RNA genome is converted to double-stranded DNA by a virally encoded reverse transcriptase that is present in the virus particle. This viral DNA is then integrated into the cellular DNA

by a virally encoded integrase, along with host cellular co-factors. So that the genome can be transcribed. After the virus has infected the cell, two pathways are possible: either the virus become latent or the infected cell continues to function, or the virus becomes active and liberated that can then infect either cell.

The primary goal of antiretroviral therapy is to reduce HIV-associated morbidity and mortality. This is best accomplished by using antiretroviral therapy to maximally inhibit HIV replication, as measured by consistent plasma HIV RNA (viral load) values below the level of detection using commercially available assays. Additional benefits of antiretroviral therapy, supported by accumulating evidence, are reduction in HIV-associated inflammation and its associated complications and reduction in HIV transmission (Sungkanuparph *et al.,* 2008). In the pharmacological treatment of HIV infections using highly active antiretroviral strategy. Considered a very effective therapy, HAART (highly active antiretroviral therapy) decreased HIV viral load and leads to increased of CD_4^+ T-lymphocyte counts, causing on improvement of immunity and decreased in the incidence of opportunistic infection (DHHS, 2012; Sun *et al.,* 2012).

Antiretroviral therapy for treatment of human immunodeficiency virus type 1 (HIV-1) infection has improved steadily since the advent of potent combination therapy in 1996. New drugs have been approved that offer new mechanisms of action, improvements in potency and activity even against multidrug-resistant viruses, dosing convenience, and tolerability. Antiretroviral therapy should be initiated in treatment naïve patients with a history of an AIDS-defining illness or with a CD_4^+ cell counts <350 cells/mm^3 in all asymptomatic individuals. The most extensively preferred combination antiretroviral regimens for treatment-naïve patients generally consist of NNRTIs-based has been based on a combination of efavirenz plus 2-NRTIs (Waters *et al.,* 2006; UNAIDS/WHO, 2011; DHHS, 2012). Since nevirapine is more hepatobility toxic than efavirenz (Leth *et al.,* 2003) and nevirapine can cause severe rash (Leth *et al.,* 2005). Moreover the availability of efavirenz, the first antiretroviral drugs to be asministered as single daily dose, enhanced and improved quality of life (Maggiolo, 2007). These factors make efavirenz an important component of NNRTIs-based instead of nevirapine (Waters *et al.,* 2006).

Several clinical studies have reported a reduction in the plasma levels of other cytochrome P450 (CYP) 3A4 substates when they are coadminisered with efavirenz (Falloon *et al.,* 2000; Clarke *et al.,* 2001; Aarnoutse *et al.,* 2002). Efavirenz caused a concentration-dependent CYP3A4 induction and activation of the human pragnane X receptor (hPXR), a key transcriptional regulator of CYP3A4, *in vitro* (Hariparsad *et al.,* 2004). However, efavirenz did not appear to induce intestinal CYP3A4 or intestinal P-glycoprotein (Mouly *et al.,* 2002; Berruet *et al.,* 2005).

Opportunistic infections (OIs), which have been defined as infections that are more frequent or more severe because of immunosuppression in HIV-infected patients. OIs resulting from depletion of the immune system are major causes of morbidities and mortalities among HIV-infected patients throughout the global (Ramaiah *et al.,* 2010). The widespread use of potent combination antiretroviral therapy (ART) has reduced the incidence of OIs for certain patients with access to care. Prophylaxis and early treatment of OIs have been clearly shown to prolong and improve the quality of life for people living with HIV-infected patients. However, some patients do not have access to care and have OIs (Brooks *et al.,* 2009). OIs were the principal cause of morbidity and mortality in this patient. Fungal OIs are common and some can be life-threatening for those with advanced HIV infection. Candidiasis is one of the most frequently occurring fungal diseases in persons with HIV infection of the mouth, throat, or vagina, caused by the *candida albicans*, oropharyugeal and esopharyngeal candidiasis are common (Banerjee, 2005). *Cryptococcus neoformans*, can cause infections of the skin, lungs, and meninges. The most common form of cryptococcal infection in AIDS patients is meningitis. Aspergillosis is caused by *aspergillus*, can cause serious lung problems and can also spread to other organs, including the kidneys, liver, skin, bones, and brain. HIV-positive people with CD_4^+ cell counts below 150 cells/mm^3 are at the highest risk of developing histoplasmosis, caused by the *histoplasma capsulatum*, especially in the Mississippi and Ohio River and South America (Kauffman, 2007). It's can cause more serious problems, including respiratory distress, kidney and liver failure, and brain damage. Over 85% of people with HIV would eventually develop pneumocystis pneumonia (PCP), almost always affects the lungs (Chaisson *et al.,* 1998).

Itraconazole is a broad-spectrum triazole antifungal with clinically useful activity against both superficial and systemic mycoses. Itraconazole inhibits the synthesis of fungal ergosterol. HIV- infected patients more than 15,000 that infection with Candida species, a major problem in AIDS (Vazquez, 2000). A prospective, randomized trial in HIV-positive and AIDS patients with oropharyngeal candidiasis (OPC) compared itraconazole oral solution 200 mg/day and fluconazole 100 mg/day for 14 days Clinical response rates were 97% for itraconazole and 87% for fluconazole, with few adverse effect in both groups. However, approximately 50% of the patients in both groups experienced relapses at the one month follow-up evaluation (Graybill *et al.,* 1998). The frequency of mucosal and cutaneous fungal infections is increasing worldwide in HIV-infected patients. The drug is drug of choice that has been in clinical use for over a decade.

Itraconazole is primarily metabolised in the liver (Grant and Clissold, 1989). It is metabolized by CYP3A4 (Isoherranen *et al.,* 2004). Hydroxy-itraconazole is a major metabolite accounting for 1-5% of the dose of itraconazole (Beule and Gestel, 2001). Itraconazole has similar drug interactions as ketoconazole due to the inhibition of the same CYP3A4 enzyme system and is also a potent inhibitor of CYP3A4 (Tapaninen *et al.,* 2011). The primary mechanism of action of itraconazole and azoles, in general, is the inhibition of sterol 14-α denethylase, a microsomal cytochrome P450-dependent enzyme system (Pinjon *et al.,* 2005).

Thus, the possibility of itraconazole and efavirenz co-administration tends to have a chance to occur in clinical practice and may lead to failure of treatment with itraconazole and efavirenz.

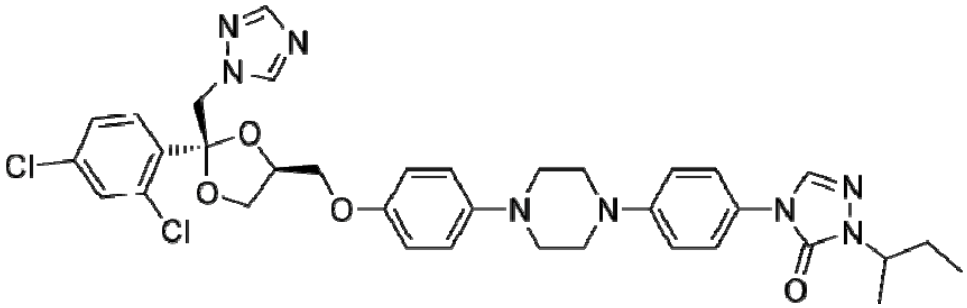
1.2. Objective of the study

To study drug interactions between itraconazole and efavirenz in HIV-infected patients.

CHAPTER 2

REVIEW OF LITURATURE

2.1. Itraconazole



MW = 705.64

Figure 1 The chemical structure of itraconazole

Itraconazole is a synthetic triazole derivative antifungal agent with a broad spectrum of activity. The structure formular of itraconazole is shown in Figure 1. It has five-membered ring structures containing three nitrogen atoms (Korting *et al.,* 2009). Itraconazole is a highly lipophilic compound. While it achieves high concentrations in fatty tissues and purulent exudates, its penetration into aqueous fluids remains very limited. Gastric acidity and food influence the absorption of oral formulation (Bailey *et al.,* 1990).

2.1.1. The mechanism of action

Itraconazole inhibits 14-α demethylase, a microsomal cytochrome P450 enzyme, in the fungal membrane. The 14-α demethylase is necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial

forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Consequently, the accumulation of 14-α methylsterols leads to the impairment of membrane permeability and membrane-bound enzyme activity and to the arrest of fungal cell growth (Pinjon *et al.*, 2005). The mechanism is shown in Figure 2.

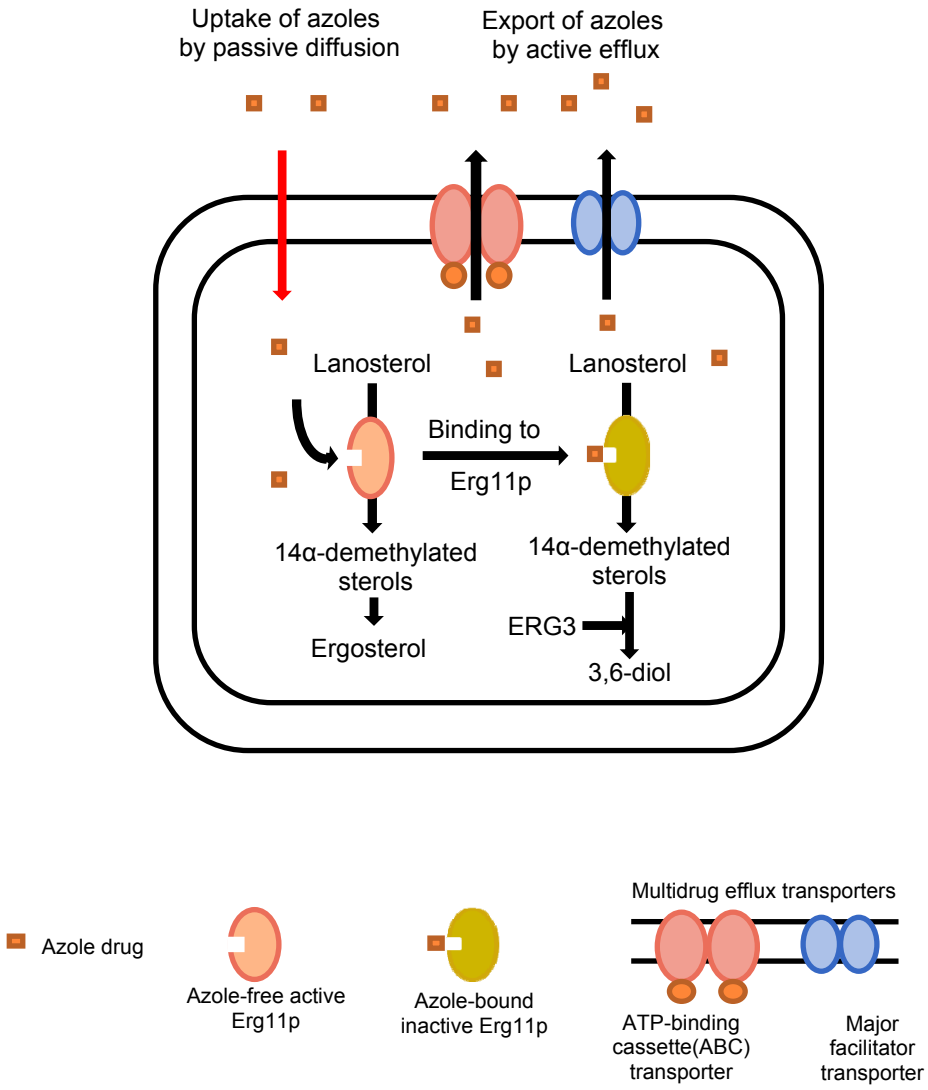


Figure 2 Model for mechanisms of action of itraconazole (adepted from Sanglar and Odds, 2002)

2.1.2. Pharmacokinetics

Itraconazole displays non-linear pharmacokinetics and the oral bioavailability can be markedly decreased by concurrent use of antacids. The capsule is poorly absorbed, while the solution (dissolved in cyclodextrin) has improved absorption irrespective of gastric pH. This drug has a long half-life and may take up to 14 days or more to achieve steady-state levels.

- Absorption

Itraconazole is frequently poorly absorbed, with water solubility being the rate-limiting step in itraconazole absorption from the gastrointestinal tract. Itraconazole is a weak base (pKa = 3.7) (Grant and Clissold, 1989) that is highly ionized and poorly absorbed at the pH of gastric juice, the absolute oral bioavailability of itraconazole is 55% and it was reduced by 40% when administrated under fasting conditions (Heykants *et al.,* 1989). The coadministration of an acidic beverage (cola beverage, orange juice) with itraconazole were increased the absorption of drug (Lange *et al.,* 1997). In addition, the absorption of itraconazole was reduced when administered with omeprazole (Jaruratanasirikul and Sriwiriyajan, 1998). HIV infection may be a factor which can affect the absorption of itraconazole as shown by a 50% reduction of the absorption of itraconazole capsules in AIDS patients compared with healthy people (Smith *et al.,* 1991, Willems *et al.,* 2001) but the bioavailability of itraconazole is increased in fasted HIV-infected patients when itraconazole is coadministered with a cola beverage (Lange *et al.,* 1997).

- Distribution

Plasma protein binding of itraconazole is 99%, mainly to albumin, leaving only 0.2% of the drug unbound (Heykants *et al.,* 1989). Itraconazole have a large volume of distribution (11 L/kg). Tissues such as lung, kidney, liver, bone, stomach, spleen and muscle accumulate large concentrations of itraconazole (Figure 3) (Heykants *et al*., 1989; Templeton *et al*., 2008). Itraconazole also accumulates in tissues that are prone to fungal infections, such as the skin, nails and female genital tract (Grant *et al*., 1989), while in the cerebrospinal fluid, urine and sputum contain at a low concentrations. Tissue concentrations are 5 to 10 times higher than in plasma (Beule and Gestel, 2001).

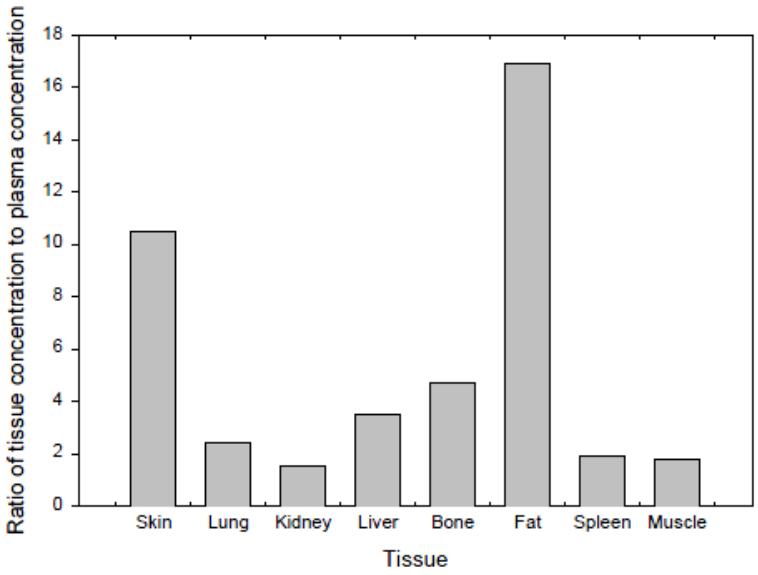


Figure 3 Peak ratio of tissue concentration to plasma concentration of itraconazole in different tissues after administration of a single dose of itraconazole 200 mg (adepted from Willems *et al.*, 2001).

- Biotransformation

Itraconazole is primarily metabolized in the liver by a large number of pathways to produce more than 30 metabolites (Grant and Clissold, 1989). The major metabolite, hydroxy-itraconazole itraconazole, reaches higher plasma concentrations than the parent compound accounting (1-5% of the dose) (Beule and Gestel, 2001). Itraconazole is metabolized by CYP3A4 sequentially to three metabolites (Figure 4) (Kunze *et al*., 2006) such as hydroxyitraconazole (OH-ITZ), keto-itraconazole (keto-ITZ) and N-desalkyl-itraconazole (ND-ITZ) which are all present in plasma following administration of itraconazole, because keto-ITZ and ND-ITZ are formed sequentially from itraconazole via OH-ITZ (Figure 5) (Isoherranen *et al*., 2004).

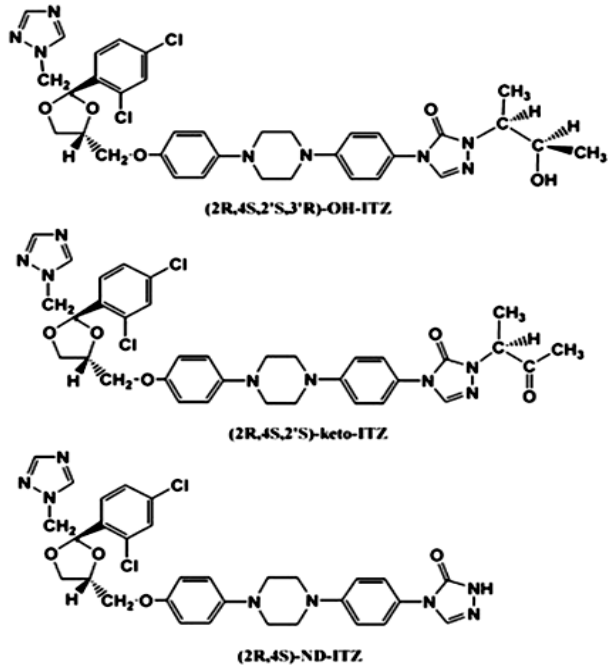


Figure 4 Structures of the metabolite of itraconazole

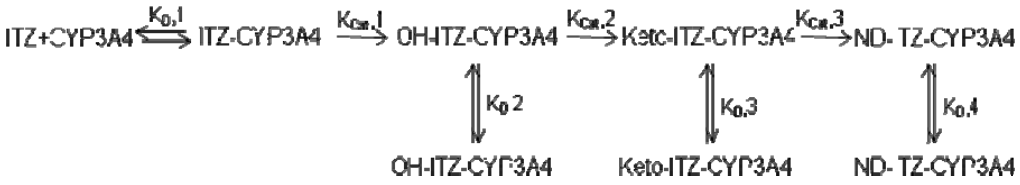


Figure 5 CYP3A4-catalased metabolic pathways for itraconazole

The drug is both a potent inhibitor and a substrate for the cytochrome P450 including CYP3A4 and P-glycoprotein, caused significant the drug-drug interactions when combination with other CYP3A4 substrates (Bacman *et al.,* 1998; Neuvonen *et al.,* 1998; Cronin and Chandrasekar, 2009). The metabolites are also potent inhibitors of CYP3A4 in *in vitro*, ND-ITZ is more potent inhibitor than OH-ITZ and the parent compound in human microsome (Isoherranen *et al*., 2004).

- Elimination

Elimination of itraconazole is biphasic, with a terminal half life of approximately 20-24 h after a single dose (Beule and Gestel, 2001) and increase approximately 25-42 h after a multiple dose (Hardin *et al*., 1988). At the steady state, the terminal half-life was over to 30 h, indicating that the itraconazole excretion mechanism is a saturated a clinical dose. Most metabolites are excreted through the bile approximately 54% of dose and approximately 35% of dose through the urine (Beule, 1996). Moreover, it is not excreted unchanged into the urine, only a small fraction (3-18%) is excreted unchanged in the feces (Isoherranen *et al*., 2004; Poirer and Cheymol, 1998). However, in rat and dogs have been excreted this metabolite through the urine and the feces (Heykant *et al.*, 1989).

2.1.3. Clinical use

Administration of itraconazole may therefore be inappropriate in the seriously ill patient (Cronin and Chandrasekar, 2009). Itraconazole has a broader spectrum of activity, effective and indicated for the treatment of a number of localized and systemic fungal infections in adults, irrespective of their immune status (Haria *et al.,* 1996). These properties have resulted in shorter treatment times in vaginal candidiasis, dermatomycosis and onychomycosis, as well as effective oral treatment of several deep mycoses, including aspergillosis, candidiasis, cryptococcosis, histoplasmosis and several endemic mycoses such as sporotrichosis, paracoccidioidomycosis and chromoblastomycosis (Vladimir and Krcmery, 2005). Itraconazole capsules can be used as maintenance therapy in HIV-infected patients and as prophylaxis before expected neutropenia, but as absorption is often impaired, blood monitoring should be performed and if necessary, the dose should be increased. There are inadequate data on itraconazole capsules in children and the elderly for their use to be recommended in these special patient populations.

2.1.4. Dosage and administration

Itraconazole was shown to be extremely effective in a wide range of superficial and more serious deep fungal infections when administered once or twice daily. Itraconazole is used at doses of 100-400 mg daily by oral route. At 400 mg/day, better levels are achieved with twice a day dosing. Intravenous itraconazole is administered at a dose of 200 mg twice a day for two days, followed by 200 mg/day.

2.1.5. Contraindications

Considerable concern remains regarding adequate oral absorption and oral itraconazole is not recommended in seriously ill patients or patients who have life-threatening disease. Dose adjustment is not indicated when the oral formulation of itraconazole is used in patients who have renal insufficiency or those receiving hemodialysis or continuous ambulatory peritoneal dialysis and the half life of itraconazole is prolonged in patients who have hepatic dysfunction (Beule and Gestel, 2001). Itraconazole is contraindicated in patients with known hypersensitivity to the drug and should not be administered for the treatment of onychomycosis in patients with evidence of ventricular dysfunction such as congestive heart failure (CHF) or a history of congestive heart failure (CHF). Concomitant administration of itraconazole and quinidine, dofetilide or disopyramide may increase plasma concentrations of quinidine or dofetilide which could result in serious cardiovascular events. Concomitant administration of digoxin and itraconazole has led to increased plasma concentrations of digoxin (Partanen *et al.,* 1996).

2.1.6. Adverse effect

The major drawbacks of itraconazole therapy are from occasionally seen adverse reaction (Amichai and Grunwald, 1998; Denning *et al.,* 1996; US.FDA, 2009).

(1) Gastrointestinal effects

The most common side effects are related to the gastrointestinal system in patients receiving itraconazole. Side effect are dose dependent, including a nausea (11%), diarrhea (11%), vomiting (7%), abdominal pain or discomfort

(6%), dyspepsia (4%), flatulence (4%), gingivitis (3%), constipation (3%), ulcerative stomatitis (3%), gastritis (2%), increased appetite (2%), gastroenteritis (2%), dysphagia (less than 2%), hemorrhoids (less than 2%), dysgeusia (less than 2%), and anorexia (1%).

(2) Dermatologic effects

Dermatologic side effects have included rash (up to 9%), pruritus (up to 5%), increased sweating (up to 3%), unspecified skin disorder (2.3%), and erythematous rash (1% to 2%). Rarely, isolated reports of skin disease related to itraconazole therapy have been described, including acute generalized exanthemic pustulosis, photo allergic reaction and urticaria.

(3) Hepatic effects

Hepatic side effects have included bilirubinemia (6%), abnormal liver function (3%), increased SGPT/ALT (3%), jaundice (2%), increased SGOT/AST (2%), increased gamma-glutamyltransferase (1-2%), and hepatitis (less than 2%). Tucker et al (1990) reported the serum transaminases was increased about 5% of patients and 10.9% in invasive fungal infections (IFI) patients from China (Aixia *et al.,* 2006).

(4) Metabolic effects

Metabolic side effects have included hypertriglyceridemia (11%), hypokalemia (9%), increased alkaline phosphatase (2%), hypomagnesemia (2%), increased lactate dehydrogenase (2%), hypophosphatemia (1-2%), hyperglycemia (<2%), dehydration (<2%), decreased weight (<2%), fluid overload (1%), and hypocalcemia (1%).

(5) Nervous system effects

Nervous system side effects have included headache (up to 10%), dizziness (4%), hypoacusis (3.3%), tremor (1-2%), insomnia (<2%), tinnitus (<2%), vertigo (1%), and somnolence (1%). An elderly patient experienced visual hallucinations, confusion, and weakness after receiving itraconazole. The symptoms reappeared following accidental itraconazole doses 7 and 10 days later.

(6) Respiratory effects

Respiratory side effects have included rhinitis (9%), upper respiratory tract infection (8%), sinusitis (7%), coughing (4%), pneumonia (2%), increased sputum (2%), dyspnea (2%), pharyngitis (2%), pulmonary infiltration (1-2%), and pharyngolaryngeal pain (1%).

(7) Cardiovascular effects

Cardiovascular side effects have included hypertension (3.2%), vein disorder (3%), abnormal electrocardiogram (1.4%), hypotension (1%), orthostatic hypotension (1%), vasculitis (1%), sinus bradycardia (1%), and tachycardia (1%).

(8) Genitourinary effects

Genitourinary side effects have included cystitis (3%), urinary tract infection (3%), albuminuria (<2%), hematuria (<2%), gynecomastia (<2%), male breast pain (<2%), bacteriuria (1.4%), impotence (1%), and menstrual disorders (infrequent).

(9) Endocrine effects

In a reversible adrenal insufficiency have been reported during treatment with high doses (600 mg/day) of itraconazole. Gynecomastia has been described in 1% of patients tread with itraconazole 400 mg/day (Tuker *et al.,* 1990).

(10) Immunologic effects

Immunologic side effects have included Pneumocystis carinii infection (2%), herpes zoster (2%), and unspecified infection (<2%).

(11) Hematologic effects

Thrombocytopenia was reported in 0.5% of patients treated with itraconazole (Tuker *et al.,* 1990).

(12) Musculoskeletal effects

Musculoskeletal side effects have included myalgia (up to 3%), bursitis (3%), and back pain (1.2%).

(13) Hypersensitivity effects

Hypersensitivity side effects have included rash and pruritus in up to 5% of treated patients (may be more likely in immunocompromised patients).

2.1.7. Drug interaction

Itraconazole is mainly metabolised through CYP3A4. It's also a potent inhibitor of CYP3A4 and P-glycoprotein (Venkatakrishnan *et al*., 2000; Cronin and Chandrasekar, 2009). Concomitant administration of and certain drugs metabolized by the cytochrome P450 3A4 isoenzyme system (CYP3A4) may result in increased plasma concentrations of those drugs, leading to potentially serious and/or life-threatening adverse events. The liver is the primary site of drug metabolism mediated by the cytochrome P450 system, but CYP3A4 is also present in the enterocytes of the small intestine, placenta, kidney, brain and liver.

- Budesonide

Azole antifungals may inhibit the CYP3A4-mediated metabolism of inhaled glucocorticoids, resulting in their increased serum concentrations and effects. Ten healthy subjects in a randomized, double-blind, 2-phase crossover study, treated with 200 mg of itraconazole and received 1000 μg of inhaled budesonide after the last dose of itraconazole. Itraconazole increased the mean total area under the plasma concentration-time curve (AUC) and the peak plasma concentration of inhaled budesonide, 4.2-fold and 1.6-fold (p<0.01) respectively, compared with placebo. Moreover, the mean terminal half-life of inhaled budesonide was prolonged from 1.6 to 6.2 h (p<0.001) by itraconazole. The suppression of cortisol production after inhalation of budesonide was significantly increased when compared with placebo, about 43% reduction in the area under the plasma cortisol concentration–time curve from 0.5 to 10 h (p<0.001) and a 12% decrease in the cortisol concentration measured 23 h after administration of budesonide (Raaska *et al.,* 2002). In case reports of Cushing's syndrome have been documented during use of inhaled corticosteroids and itraconazole. Itraconazole markedly increased the systemic effects (decreased cortisol AUC) of inhaled budesonide (Bolland *et al.,* 2004).

- Buspirone

Buspirone is highly protein bound and may displace, or be displaced by, other protein bound drugs. It is metabolised by CYP3A4 and may interact with other drugs metabolised by this isoenzyme. Eight young healthy volunteers in a randomized, double-blind, double-dummy crossover study, treated either 1.5 gm/day erythromycin, 200 mg/day itraconazole, or placebo orally for 4 days. On day 4, 10 mg

buspirone was administered orally. Itraconazole increased the mean $AUC_{0-\infty}$ 19-fold (p<0.01), respectively, compared with placebo. The mean C_{max} of buspirone was increased about 13-fold (p<0.01) by itraconazole (Kivistö *et al.,* 1998).

- Dexamethasone

In a randomized, double-blind, placebo-controlled crossover studied in eight healthy subjects. They found that itraconazole markedly increased the plasma concentrations of dexamethasone (substrate of CYP3A4) and enhances its adrenal-suppressant effect showed that itraconazole decreased the systemic clearance of IV administered dexamethasone by 68% (p<0.01), increased the $AUC_{0-\infty}$ 3.3-fold (p<0.01) and prolonged the elimination half-life about 3.2-fold (p<0.01). The $AUC_{0-\infty}$ of oral dexamethasone was increased 3.7-fold (p<0.01) the peak plasma concentration 1.7-fold (p<0.01) and the elimination half-life 2.8-fold (p<0.01) by itraconazole (Varis *et al.,* 2000).

- Loperamide

Loperamide is a synthetic opioid analogue which acts as an antidiarrhoeal agent. Loperamide slows intestinal motility, and may delay absorption of other drugs. Itraconazole may inhibit the metabolism of loperamide via CYP3A4 and P-glycoprotein inhibition. Coadministration of itraconazole and loperamide in 12 healthy subjects resulted in 2.9-fold and 3.8-fold increase in the C_{max} and AUC of loperamide, respectively. The half-life of loperamide was also prolonged (Niemi *et al.,* 2006).

- Methylprednisolone

Ten healthy volunteers in this double-blind, randomized, 2-phase crossover study, received either 200 mg itraconazole or placebo orally once a day for 4 days. On day 4, each subject ingested a dose of 16 mg methylprednisolone. Total AUC, C_{max} and elimination half life of methylprednisolone was increased 3.9-fold, 1.9-fold and 2.4-fold by itraconazole (Varis *et al.,* 1998).

- Morphine

Twelve healthy volunteers, randomized crossover study, once daily 200 mg itraconazole or placebo for 4 days. On day 4, 1 h after the last pretreatment dose, the subjects ingested 0.3 mg/kg morphine. They found that itraconazole moderately increases the plasma concentrations of oral morphine. The AUC_{0-9},

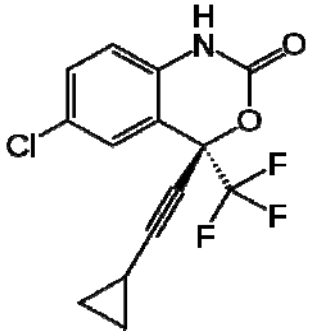
$AUC_{0–48}$ of morphine was increased by 29% and 22% respectively, probably by enhancing its absorption by inhibiting intestinal wall P-glycoprotein (Heiskanen *et al.,* 2008).

- Quinidine

The study of pharmacokinetics of quinidine in a treatment dose trial in healthy volunteers given before and during treatment with itraconazole .They found that itraconazole increased an average the peak plasma and the AUC of quinidine, 1.6-fold ($p<0.05$) and 2.4-fold ($p<0.01$). The elimination half-life of quinidine was prolonged 1.6-fold ($p<0.001$) and the area under the 3-hydroxyquinidine/quinidine ratio-time curve decreased to one-fifth ($p<0.001$) and the renal clearance was decreased about 50% ($p<0.001$) by itraconazole (Kaukonen *et al*., 1997).

2.2. Efavirenz

Efavirenz is non-nucleoside reverse transcriptase inhibitors (NNRTIs) and is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus type 1 (HIV-1) (Adkins *et al.,* 1998). Efavirenz was approved by the FDA in 1998 for use in combination with other antiretrovirals in adults and children with HIV infection. Approval was based on studies showing that initial therapy consisting of efavirenz plus 2 nucleoside analogues or a protease inhibitors, was at least as effective in achieving viral load suppression as were regimens consisting of a protease inhibitor and 2 nucleoside analogues (DHHS, 2004; Micromedex, 2004). The chemical designation is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. It is empirical formula is $C_{14}H_9C_lF_3NO_2$ is shown in Figure 6. Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.68 g/mol. It is practically insoluble in water (<10 μg/ml) (Adkins *et al.,* 1998).



MW = 315.68

Figure 6 The chemical structure of efavirenz

2.2.1. The mechanism of action

Efavirenz is an oral medication that is used for the treatment of infections with the human immunodeficiency virus type 1 (HIV-1). It falls in the non-nucleoside reverse transcriptase inhibitors (NNRTIs) class of antiretroviral drugs. Both nucleoside and non-nucleoside reverse transcriptase inhibitors act at the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. During infection with HIV, the HIV virus multiplies within the

body's cells. The newly-formed viruses then are released from the cells and spread throughout the body where they infect other cells. In this manner, the infection continually spreads to new, uninfected cells that the body is continually producing, and HIV infection is perpetuated. When producing new viruses, the HIV virus must manufacture new DNA for each virus. Reverse transcriptase is the enzyme that the virus uses to form this new DNA. Efavirenz directly inhibits the activity of reverse transcriptase and blocks the production of DNA and new viruses (Hsian *et al.,* 1996; Maurin *et al.,* 2002).

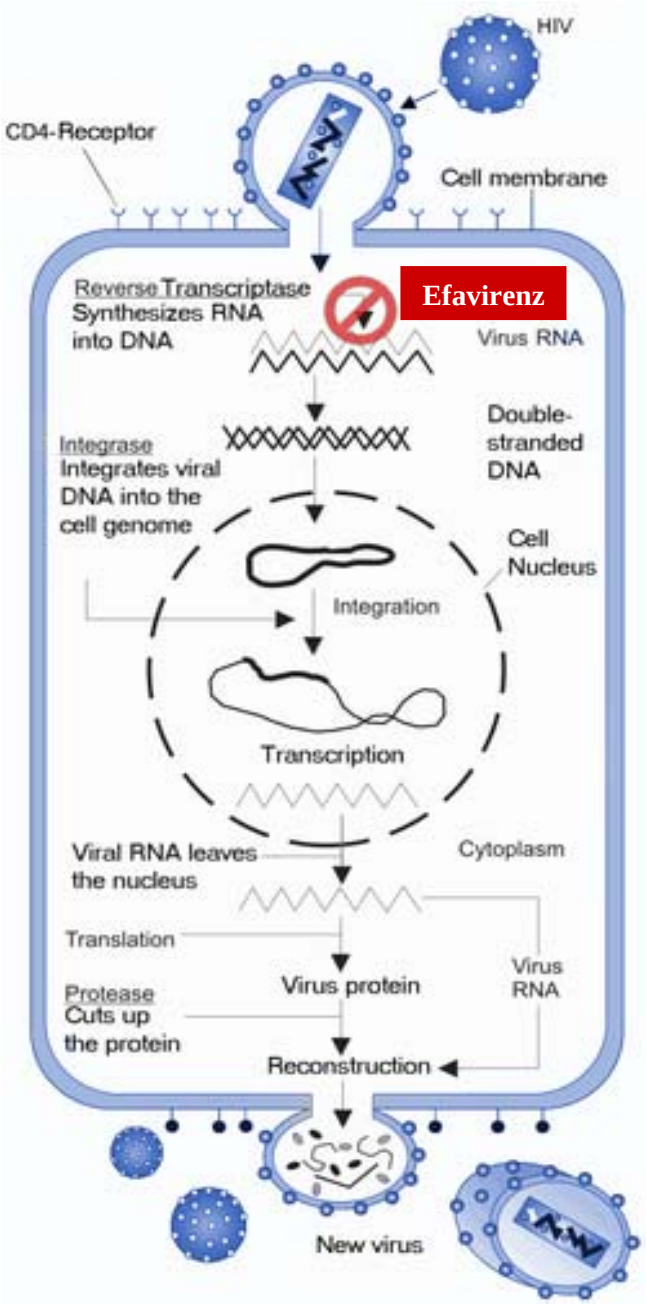


Figure 7 Efavirenz inhibit the reverse transcriptase enzyme and therefore the replication of new viruses (adapted from Boyle and Cohen, 2006)

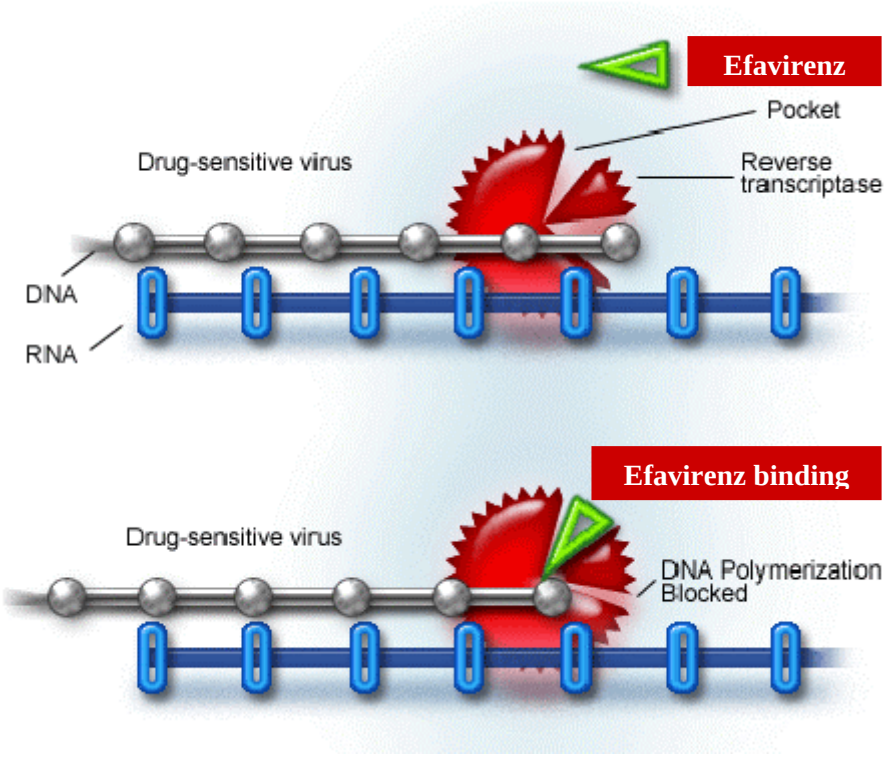


Figure 8 Mechanism of Action of NNRTIs (adapted from Boyle and Cohen, 2006)

2.2.2. Pharmacokinetics

- Absorption

Efavirenz is a class II drug (low solubility, high permeability) according to the biopharmaceutical classification system guidance by the Food and Drug Administration (Kasim *et al.*, 2004; Takano *et al.*, 2006). Highly permeable, poorly soluble drugs often demonstrate poor gastrointestinal (GI) absorption due to inadequate drug solubility in GI fluids (Aungst *et al.*, 2002). Peak efavirenz plasma concentrations are reached by 5 h following single oral doses in uninfected volunteers. The time to peak plasma concentrations is about 3-5 h and steady-state plasma concentrations of efavirenz are reached in 627 days. The bioavailability of a single 600 mg dose of efavirenz hard capsules in uninfected volunteers is increased by 17-22% by food. Efavirenz is highly bound (99.5% - 99.75%) to human plasma proteins, predominantly albumin (Almond *et al*., 2005; Maggiolo, 2009).

- Distribution

Efavirenz is very highly bound (approximately 99.5-99.75%) to human plasma proteins, predominantly albumin. In HIV-1 infected patients who received efavirenz 200 to 600 mg once daily for at least one month, cerebrospinal fluid concentrations ranged from 0.26 to 1.19% (mean 0.69%) of the corresponding plasma concentration. This proportion is approximately three-fold higher than the nonprotein-bound (free) fraction of efavirenz in plasma (Adkins *et al.,* 1998).

- Biotransformation

Efavirenz is principally metabolised by the cytochrome P450 system to hydroxylated metabolites with subsequent glucuronidation of these hydroxylated metabolites. These metabolites are inactive against HIV-1. CYP2B6 and CYP3A4 are the major isozymes responsible for efavirenz metabolism. Efavirenz has been shown to induce P450 enzymes, resulting in the induction of its own metabolism (Smith *et al.,* 2001; Zanger *et al.,* 2008).

- Elimination

Efavirenz has a terminal half-life of at least 52-76 h after single doses and 40-55 h after multiple doses. Approximately 14% - 34% of a radiolabelled dose of efavirenz is recovered in the urine and <1% of the dose is excreted in urine as unchanged efavirenz (Almond *et al*., 2005). The half-life of efavirenz appears to be shorter (~24 h) when it is given in combination with didanosine and emtricitabine (Molina *et al.,* 2004), but this combination is effective and well tolerated in long-term therapy (Molina *et al.,* 2007). The long half-life of efavirenz makes it suitable for once-daily dosing. The recommended dosage in adults is 600 mg once daily. Genotypic testing for variants of the CYP2B6 could detect individuals at increased risk of neuropsychiatric adverse events but this is not routine practice.

2.2.3. Clinical use

HIV belongs to a family of retroviruses or viruses that replicate through the use of the reverse transcriptase enzyme, or the enzyme needed to convert RNA to DNA for replication. The DNA genes allow the virus to replicate. HIV targets human CD_4^+ cells called T-helper cells by entering through blood or mucous membranes. HIV enters the T-helper cell attached to the CD_4^+ T-cell receptor site and one of two co-receptors (CCR5 or CXCR4) on the T-helper cell surface. This attachment is needed before it fuses into the key target cell surface proteins (glycoprotein; gp120). After the virus membrane and CD_4^+ cell receptor fuse, the HIV core passes into the cell to uncoat the viral RNA enzyme.To continue replication, the reverse transcriptase enzyme transcripts the RNA into the double-stranded DNA. The newly formed DNA enters into the host cell nucleus where an enzyme, integrase, allows the CD_4^+ cell DNA and the HIV DNA to hide. This integrated HIV DNA is called provirus DNA. When the provirus is active, it then transcribes into messenger RNA. HIV messenger RNA leaves the nucleus through nuclear pores and then is assembled into HIV proteins, HIV protease. The HIV protease then packages itself into a virion, also known as a "bud" so it can continue to replicate by infecting other CD^{4+} T-cells (Waters *et al.,* 2007).

Efavirenz is used with other antiretroviral agents to treat human immunodeficiency virus (HIV) infection in patients with or without acquired immunodeficiency syndrome (AIDS). Efavirenz is in a class of medications called non-nucleoside reverse transcriptase inhibitors (NNRTIs). Efavirenz works by slowing the spread of HIV in the body. Efavirenz does not cure HIV infection and may not prevent you from developing HIV-related illnesses. Efavirenz does not prevent you from spreading HIV to other people (Maggiolo, 2009). Efavirenz has been combined successfully with nucleoside backbones consisting of lamivudine or emtricitabine plus abacavir, didanosine, stavudine, tenofovir, or zidovudine to achieve virologic suppression in a high percentage of recipients (Boyle and Cohen, 2006).

2.2.4. Dosage and administration

The recommended dosage of efavirenz is 600 mg orally, once daily, in combination with a protease inhibitor and/or nucleoside analogue reverse transcriptase inhibitors (NRTIs). It is recommended that efavirenz be taken on an empty stomach, preferably at bedtime. The increased efavirenz concentrations observed following administration of efavirenz with food may lead to an increase in frequency of adverse reactions. Dosing at bedtime may improve the tolerability of nervous system symptoms (Boyle and Cohen, 2006). Table 1 describes the recommended dose of efavirenz for pediatric patients 3 years of age or older and weighing between 10 and 40 kg. The recommended dosage of efavirenz for pediatric patients weighing greater than 40 kg is 600 mg, once daily (Sustiva, 2007).

Table 1 Pediatric dose to be administered once daily

Body Weight		**Efavirenz Dose (mg)**
kg	**lbs**	
10 to less than 15	22 to less than 33	200
15 to less than 20	33 to less than 44	250
20 to less than 25	44 to less than 55	300
25 to less than 32.5	55 to less than 71.5	350
32.5 to less than 40	71.5 to less than 88	400
at least 40	at least 88	600

2.2.5. Contraindications

Efavirenz is contraindicated in patients with known hypersensitivity to the drug. Coadministration of efavirenz and midazolam or trizolam is contraindicated due to the risk of excessive sedation and confusion. Coadministration of the drug and astemzole, or cisapride is contraindicated due to the risk of potentially fetal cardiac arrest (Micromedex, 2004).

2.2.6. Adverse effect

The major drawbacks of efavirenz therapy are from occationally seen adverse reactions (Sustiva, 2007; US. FDA, 2009).

(1) General effects

The most significant side effects associated with efavirenz have included nervous system symptoms, psychiatric symptoms, and rash. The most common side effects of at least moderate severity have included rash, dizziness, nausea, headache, fatigue, insomnia, and vomiting in greater than 5% of patients treated with efavirenz in combination with lamivudine, zidovudine or indinavir.

(2) Central nervous system effects

Central nervous system adverse effects have been commonly associated with efavirenz. In clinical trials, 54% of patients taking efavirenz reported central nervous system adverse effects compared with 27% of patients receiving control regimens. These symptoms included, but were not limited to, dizziness 28.1%, insomnia 16.3%, impaired concentration 8.3%, somnolence 7.0%, abnormal dreams 6.2% and hallucinations 1.2%. These symptoms were mild in 33.3%, moderate in 17.4%, and severe in 2% of patients.

Nervous system symptoms generally begin the first or second day of therapy and often resolve after 2 to 4 weeks. Dosing at bedtime may improve the tolerability of these effects (Deeks, 1998; Micromedex, 2004).

(3) Gastrointestinal effects

Abdominal discomforts are the most commonly reported side effects with efavirenz and may occur earlier on in therapy. Common patient complains included diarrhea 14%, nausea 10% and/or vomiting 6%, dyspepsia 4%, abdominal pain 3% and anorexia 2%. Pancreatitis has been reported, although causality was not determined. Burning mouth syndrome has also been reported. Constipation and malabsorption have been reported during postmarketing experience.

(4) Psychiatric effects

Psychiatric symptoms generally begin the first or second day of therapy and often resolve after 2 to 4 weeks. These symptoms are more tolerable if the drug is taken just prior to bedtime (Deeks, 1998; Micromedex, 2004).

(5) Dermatologic effect

Dermatologic effects of moderate or severe intensity have included rash (includes erythema multiforme, rash, erythematous rash, follicular rash, maculopapular rash, petechial rash, pustular rash, urticaria, macules, papules, erythema, redness, inflammation, allergic rash, welts, hives, and itchy; 16%) and pruritus (9%). Skin rash (26.3%). Severe skin rash effects includes Stevens-Johnson syndrome, toxic epidermal necrolysis, necrosis requiring surgery, exfoliative dermatitis; 0.1%. Treatment was discontinued in 1.7% of patients due to rash.

(7) Hepatic effects

Hepatic side effects have included increased ALT, AST and GGT. During clinical trials, elevations in ALT and AST to greater than five times ULN occurred in 20% and 13%, respectively, of patients seropositive for hepatitis B and/or C treated with efavirenz. Treatment was discontinued in 3% of coinfected patients due to liver or biliary system disorders. Hepatic failure, hepatic enzyme increase, and hepatitis have been reported during postmarketing experience.

(8) Other effects

Adverse effect moderate or severe intensity have included pain (13%) and fatigue (8%). Elevated amylase (greater than 2 times ULN; 6%), asymptomatic increases in serum amylase levels, and vitamin D deficiency have been reported.

2.2.7. Drug interaction

Efavirenz has been shown *in vivo* to induce CYP3A4. Other compounds that are substrates of CYP3A4 may have decreased plasma concentrations when coadministered with efavirenz. In *in vitro* studies have demonstrated that efavirenz inhibits or inducts 2C9, 2C19 and 3A4 isozymes in the range of observed efavirenz plasma concentrations. Coadministration of efavirenz with drugs primarily metabolized by these isozymes may result in altered plasma concentrations of the coadministered drug. Therefore, appropriate dose adjustments may be necessary for these drugs (Cohen *et al.,* 2002; Young, 2005; Clarke *et al.,* 2008).

- Amprenavir

Amprenavir is a protease inhibitor. Due to high affinity for CYP3A4, the potential exists for significant drug interactions. Efavirenz may induce the CYP3A4-mediated metabolism of amprenavir, resulting in decreased serum concentrations of amprenavir and possibly antiviral effects. Several studies have reported decreases in amprenavir serum concentrations and AUC to subtherapeutic levels. Monitor the patient for signs of reduced antiviral effects and increase the dose of amprenavir/fosamprenavir accordingly (Falloon *et al*., 2000; Wintergerst *et al*., 2000; Xavier *et al*., 2000; Wire *et al*., 2004; Dailly *et al*., 2008)

- Atazanavir

Atazanavir is a protease inhibitor. Due to high affinity for several CYP450 isoenzymes, the potential exists for significant drug interactions. Efavirenz (a CYP3A inducer) increases the metabolism of atazanavir (CYP3A substrate), resulting in decreased serum concentrations of atazanavir. If atazanavir is to be coadministered with efavirenz, for treatment naïve patients, it is recommended that atazanavir 300 mg with ritonavir 100 mg be coadministered with efavirenz 600 mg. efavirenz should be taken 2 h after atazanavir and ritonavir, which are taken with food. Atazanavir without ritonavir should not be coadministered with efavirenz. Dosing recommendations for treatment-experienced patients have not been established (LeTiec *et al*., 2005; Dailly *et al*., 2006)

- Atorvastatin/Simvastatin/ Pravastatin

Atorvastatin, simvastatin and pravastatin belongs to the class of HMG-CoA reductase inhibitors, class of lipid lowering medicines (statins) metabolised by CYP3A4. Efavirenz (600 mg daily) has been shown to decrease the AUC of atorvastatin, simvastatin (acid) and provastatin by 43%, 58% and 40%, respectively, in an interaction study. This could be a result of efavirenz inducing the CYP3A4-mediated metabolism of HMG-CoA reductase inhibitors that are primarily eliminated via this route. Monitor for decreased lipid-lowering efficacy and increase the dose of statins accordingly (Gerber *et al*., 2005).

- Clarithromycin

. Clarithromycin is a semisynthetic macrolide antibiotic, metabolised by CYP3A and may interact with other drugs metabolised by this enzyme. Concurrent use of efavirenz and clarithromycin may increase the risk of dermatological reactions. A study reported that 46% of subjects developed a rash during concurrent therapy. In the same study, it was also observed that efavirenz induced the CYP3A4-mediated metabolism of clarithromycin, and resulted in a 39% reduction in clarithromycin AUC (Benedek *et al.,* 1998; Kuper *et al*., 2000).

- Ethinyl estradiol

Coadministration of efavirenz 400 mg once daily with a single 50 mg dose of ethinyl estradiol resulted in a 37% increase in ethinyl estradiol AUC (Adkins *et al.,* 1998; Joshi *et al.,* 1998).

- Fluconazole

Fluconazole at a dose of 200 mg once a day increased efavirenz AUC by approximately 16%, which is not considered clinically significant. Efavirenz at a dose of 400 mg once a day did not alter the steady-state pharmacokinetic parameters of fluconazole. Fluconazole and efavirenz may be co-administered without adjusting the dose of either drug (Sustiva, 2007).

- Indinavir

Indinavir is a protease inhibitor. Due to high affinity for CYP3A4 the potential exists for significant drug interactions. Efavirenz (CYP3A4 inducer) may increase the metabolism of indinavir (CYP3A4 substrate), resulting in decreased C_{max} and AUC of indinavir by 16% and 31%. The dose of indinavir may need to be

increased to 1,000 mg TID or 800 mg twice daily if boosted with ritonavir (Aarnoutse *et al.,* 2002; Boyd *et al.,* 2003; DiCenzo *et al.,* 2003; Lee *et al.,* 2004).

- Ketoconazole

12 HIV-infected patients received 400 mg of ketoconazole as a single oral dose on day 1 and received 600 mg of efavirenz once daily on days 2 to 16. On day 16, 400 mg of ketoconazole was added to the regimen as a single oral dose. Efavirenz significantly increased the clearance of ketoconazole by 201%. C_{max} and AUC were significantly decreased by 44% and 72%, respectively. The $T_{1/2}$ was significantly shorter by 58% (Sriwiriyajan *et al.,* 2007).

- Lopinavir/ritonavir

Lopinavir is a protease inhibitor. Due to high affinity for CYP3A4 the potential exists for significant drug interactions. Efavirenz may induce the CYP3A4-mediated metabolism of lopinavir, resulting in decreased lopinavir plasma concentrations to subtherapeutic levels. Lopinavir/ritonavir capsules or oral solution, a dose increase to 533/133 mg twice a day taken with food is recommended when used in combination with efavirenz 600 mg once daily in 45 HIV-infected patients. Efavirenz increased the mean the steady state elimination rate constant of lopinavir by 36% (Bergshoeff *et al.,* 2003; Cvetkovic *et al.,* 2003; Dailly *et al.,* 2005; Hsu *et al.,* 2005).

- Methadone

Methadone is metabolised by CYP450 (mainly CYP3A4) and may interact with other drugs metabolised by CYP450. Efavirenz may induce the hepatic metabolism of methadone, resulting in the significant reductions in the plasma levels and effects of methadone. Coadministration with efavirenz 600 mg once a day for 7 days in 11 HIV-infected patients attending on stable methadone maintenance therapy. Efavirenz decreased the AUC and C_{max} of methadone by 52% and 45% (Clarke *et al.,* 2001; Eap *et al.,* 2004; Bruce *et al.,* 2006).

- Nelfinavir

Nelfinavir is a protease inhibitor. It may interact with other drugs metabolised by CYP3A and CYP2C19. Nelfinavir 750 mg TID administered with efavirenz 600 mg once a day decreased the mean clearance of nelfinavir metabolite (M8) by 43% (Labbe *et al.,* 2005).

- Nevirapine

Nevirapine is a nonnucleoside reverse transcriptase inhibitor. It is metabolised by CYP3A4 and CYP2B6 and may interact with other drugs metabolised by these enzymes. For coadministration, nevirapine has also been shown to decrease the AUC and C_{max} of efavirenz by 22% and 17% and increased incidence of adverse effects (such as clinical hepatitis, elevated liver enzymes, rash and CNS toxicity). Efavirenz dose may need to be increased to 800 mg once a day (Veldkamp *et al.,* 2001; Kappelhoff *et al.,* 2005).

- Rifabutin

Rifamycin is an antimycobacterial agent. Rifabutin may interact with other hepatically metabolised drugs. Efavirenz may induce the CYP3A4-mediated metabolism of rifabutin, resulting in reduced serum concentration and effects of rifabutin, Advised that the dose of rifabutin should be increased by 50% (450 mg once daily) when combined with efavirenz (Spradling *et al.,* 2002; Edelstein *et al.,* 2004; Weiner *et al.,* 2005).

- Rifampin

Rifampicin may reduce the serum concentration of efavirenz which may lead to decreased systemic exposure to efavirenz, coadministrated of rifampin 600 mg once a day with efavirenz 600 mg resulted in a statistically significant decrease in efavirenz AUC (26%), C_{max} (24%) and C_{min} (25%) (Sustiva, 2011).

- Ritonavir

Twenty four normal volunteers received 1,875 mg of nelfinavir plus ritonavir 200 mg once a day with a 300-kcal snack for 10 days. During day 11-20 efavirenz 600 mg once a day was added to the regimen. Decreases were observed in ritonavir AUC_{0-24} (-20%), C_{max} (-24%) and C_{min} (-12%) after the addition of efavirenz to the regimen, due to induction effect of efavirenz (Porte *et al.,* 2004).

- Saquinavir

Concomitant administration of efavirenz (600 mg once daily) and saquinavir soft gel capsules (1,200 mg TID) enhances the metabolism of saquinavir resulting in a reduction of saquinavir AUC and C_{max} by approximately 62% and 50%, respectively. Because of the reduction in saquinavir levels, saquinavir should not be

used as the sole protease inhibitor in combination with efavirenz (Adkins *et al.,* 1998; Micromedex., 2004; Jamois *et al.,* 2009).

- Voriconazole

Voriconazole is a broad spectrum triazole antifungal agent. It may interact with drugs metabolised by CYP450 isoenzymes. Efavirenz may induce the metabolism of voriconazole, resulting in decreased voriconazole serum concentration. In a study in healthy subjects, efavirenz decreased the AUC and C_{max} of voriconazole by 80% and 66%, respectively. In the same study, voriconazole increased the AUC and C_{max} of efavirenz by 43% and 37%, respectively (Liu *et al.,* 2005; Liu *et al.,* 2007; Gerzenstein *et al.,* 2008).

2.3. Cytochrome P450 System

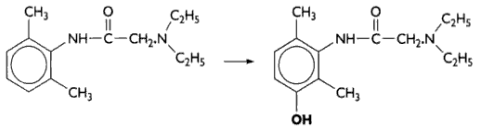
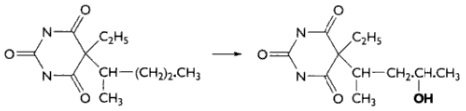
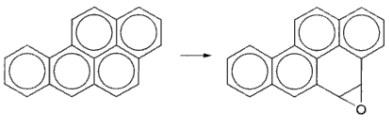
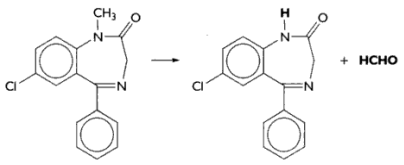
2.3.1. Cytochrome P450

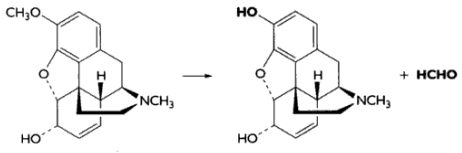
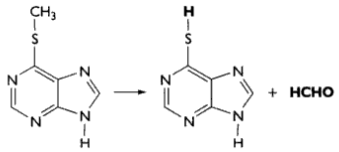
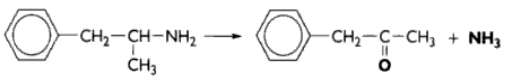
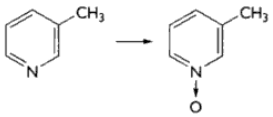
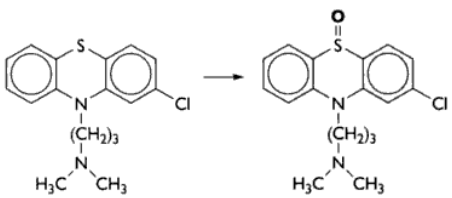
Pharmacological effect or the drugs and chemicals toxicity that may from substrate or metabolites from metabolism of substance when act in the body. Metabolism happen of liver mainly because of enzyme of source that are essential to this process, related enzyme with the metabolism process are many types such as cytochrome P450 (CYP), which the oxidation accelerate and transferases, which use the conjugation reaction. These enzymes of inhibition or induction may be by external chemical or other drugs that have been together. Makes the pharmacological effect or toxicity that has changed.

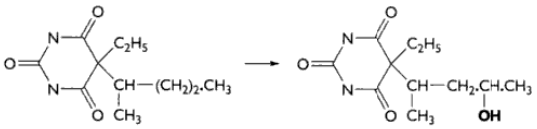
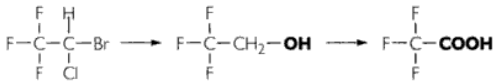
The liver is responsible for concentrating, metabolizing, and eliminating the majority of drugs and toxins that are introduced into the body. Drug metabolism is the process by which drug molecules are chemically altered, usually to more polar metabolites that exhibit increased water solubility to allow elimination in urine or bile and/or increased access to excretory transporters. Enzymes in the liver serve as catalysts for chemical reactions which change the drugs into other substances which are called metabolites.

Most drugs are lipid-soluble and this makes them difficult to excrete. The overall aim of hepatic drug metabolism is to produce a more water soluble compound to facilitate the excretion of the drug in body fluids such as urine and bile, the primary routes of drug excretion. Two important detoxification mechanisms in normally functioning livers are Phase I and Phase II metabolic reaction. Phase I reactions drug metabolism enzymes make compounds more hydrophilic and add functional groups (-OH, -NH_2, SH, or –CO_2H) necessary for the completion of Phase II drug metabolism by oxidation, reduction or hydrolysis. Some example of Phase I reactions are presented in Table 2. The biactivation of drug candidates metabolites are detoxified by Phase II conjugation reaction with cellular macromolecules such as glucuronide, sulfate, acetate or animo acid as shown in figure 9. Some examples of phase II reaction are presented in figure 10. Phase II preceding Phase I reaction, although less common, can also occur. Thease reaction, either alone or in concert, is responsible for generation of readily excretable metabolite (Yan *et al.,* 2001).

Table 2 Oxidation reactions performed by the microsomal mixed-function oxidase system (Gibson, 2001)

Type of reaction	Example substrate
Aromatic Hydroxylation	Lignocaine 
Aliphatic hydroxylation	Pentobarbitone 
Epoxidation	Benzo[*a*]pyrene 
Dealkylation	*N*-Dealkylation: Diazepam 

Type of reaction	Example substrate
Dealkylation	*O*-Dealkylation: Codeine  S- Dealkylation: 6-methylthiopurine 
Oxidative deamination	Amphetamine 
N-Oxidation	3-methylpyridine 
S-Oxidation	Chlorpromazine 

Type of reaction	Example substrate
Phosphothionate oxidation	Parathion 
Dehalogenation	Halothane 

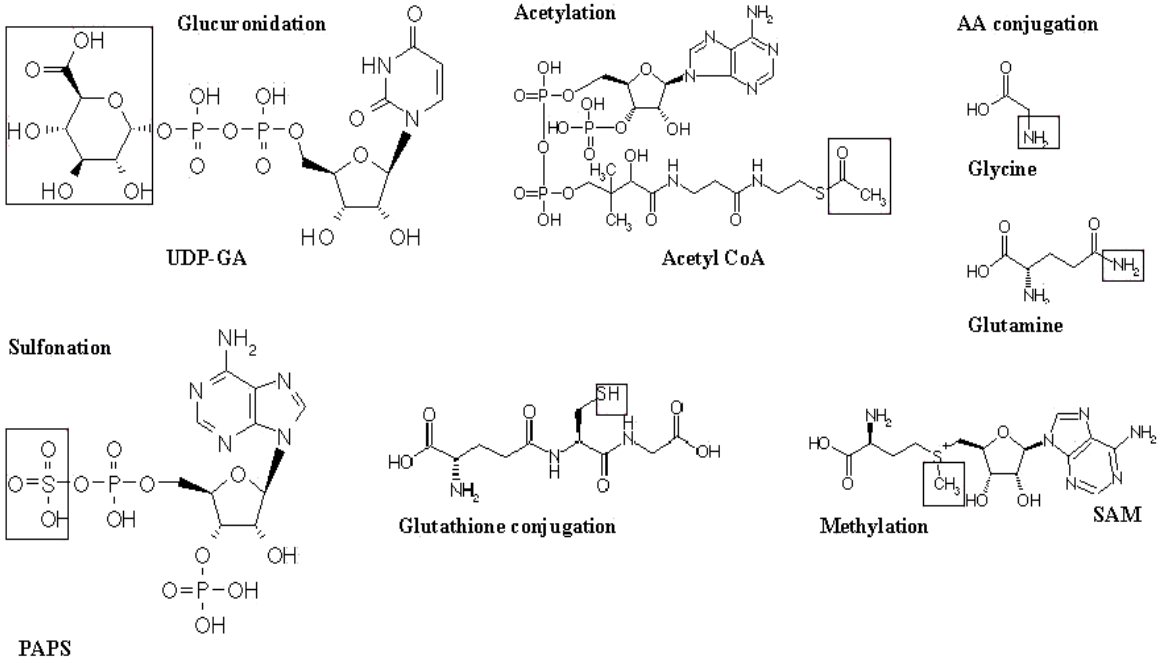


Figure 9 Structures of cofactors for phase 2 biotransformation (Jassal, 2004)

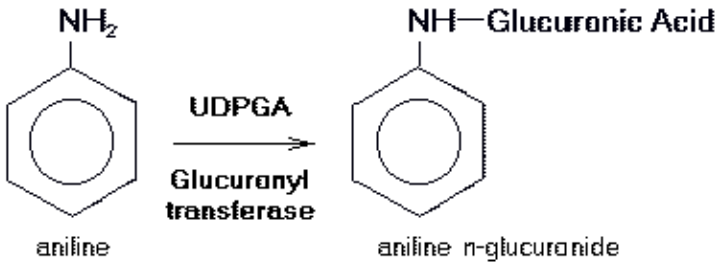


Figure 10 Reaction of glucuronide conjugation of aniline (Monosson, 2008)

Human CYPs are involved in the metabolism of xenobiotic (drug and carcinogens) and endogenous compound such as steroids, fatty acid and other important lipids such as prostacyclins and thromboxane A2 (Shimada *et al.,* 1994). Human have been estimated more than 2,400 CYP sequences know and in human these gene can be group in to 18 families of CYP genes and 43 subfamilies. At least 53 different CYP genes and 24 pseudogenes (Nelson, 1999). Mainly enzymes from the CYP1, CYP2 and CYP3 families metabolise xenobiotics in humans. The CYP1 family includes 3 subfamilies (3 genes and 1 pseudogene); CYP2 family, 13 subfamilies (16 genes and 16 pseudogenes) and CYP3 family, 1 subfamily (4 genes and 2 pseudogenes). Most P450s are hepatic and few are expressed exclusively extrahepatically. Also, the most important site for metabolism of exogenous compounds in human is the liver. About 70% of liver P450s are accounted for by the CYP1A2, CYP2A6, CYP2C, CYP2D6, CYP2E1 and CYP3A enzymes (Shimada *et al.,* 1994). These P450 enzymes are responsible for the metabolism of 90% of all drugs (Bertz and Granneman, 1997; Rendic and DiCarlo, 1997).

The notable diversity of CYP enzymes has given rise to a systematic classification of individual forms into families and subfamilies. The protein sequences within a given gene family are at least 40% identical (such as CYP2A6 and CYP2B6), and the sequences within a given subfamily are > 55% identical (such as CYP2A6 and CYP2A7) (Nelson *et al.,* 1996). The italicized names refer to genes, e.g. CYP2A13. There are 17 different families currently known in humans. The enzymes in the families 1-3 are mostly active in the metabolism of xenobiotics, whereas the

other families have important endogenous functions (Table 3). Inactivating mutations in the CYPs with physiological functions often lead to serious diseases, whereas similar mutations in xenobiotic-metabolizing CYPs rarely do, although they affect the host's drug metabolism and susceptibility to some diseases, without directly causing disease (Nelson, 1999).

Table 3 Human CYP families and their main functions

(Gonzalez, 1992; Nelson *et al*., 1996; White *et al*., 1997; Nelson, 1999)

CYP family	Main functions
CYP1	Xenobiotic metabolism
CYP2	Xenobiotic metabolism Arachidonic acid metabolism
CYP3	Xenobiotic and steroid metabolism
CYP4	Fatty acid hydroxylation
CYP5	Thromboxane synthesis
CYP7	Cholesterol 7α-hydroxylation
CYP8	Prostacyclin synthesis
CYP11	Cholesterol side-chain cleavage Steroid 11β-hydroxylation Aldosterone synthesis
CYP17	Steroid 17α-hydroxylation
CYP19	Androgen aromatization
CYP21	Steroid 21-hydroxylation
CYP24	Steroid 24-hydroxylation
CYP26	Retinoic acid hydroxylation
CYP27	Steroid 27-hydroxylation
CYP39	Unknown
CYP46	Cholesterol 24-hydroxylation
CYP51	Sterol biosynthesis

The liver is the major site of drug biotransformation. Oxidative Phase I enzymatic reactions like hydroxylation terminate the biological activity of drugs in one or more steps, especially that of the most important phase I enzymes or

cytochrome P450 (CYP) enzymes, is an important complicating factor in many areas of pharmacology and toxicology, in drug development, preclinical toxicity studies, clinical trials, drug therapy, environmental exposures and risk assessment (Pelkonen *et al.,* 2008). The cytochrome P450, a cellular chromophore, was first named in 1961, because the pigment (P) has a 450-nm spectral peak when reduced and bound to carbon monoxide (Nebert *et al.,* 2002). The cytochrome P450 mixed function monooxygenases are located on the smooth endoplasmic reticulum (SER) of cells throughout the body, but the highest concentrations are found in the liver (hepatocytes) and small intestine (SI). CYP450 comprise a superfamily of haemoproteins which contain a single iron protoporphyrin IX prosthetic group. This superfamily is subdivided into families and subfamilies that are defined solely on the basis of amino acid sequence homology (Lin and Lu, 1998). Information about the CYP superfamily has increased exponentially in recent years, and it has been subclassified into families and subfamilies, based on the overlap of shared amino acid sequences. Families are designated by an Arabic numeral, subfamilies by a capital letter, and each individual isozyme (or its gene) by a numeral, so that each isozyme is represented in the format CYPnXm, like CYP2D6 (Goshman *et al,.* 1999). The CYP superfamily consisted of more than 7,000 named sequences in animals, plants, bacteria and fungi. There are approximately 55 different CYP genes in the human genome, and these are further divided into families and subfamilies based on their sequence homology. There have been identified 16 different CYP gene families and 29 subfamilies in humans up to date. Members of one gene family have more than 40% amino acid identity. Members of one subfamily have more than 55% amino acid identity. Proteins belonging to CYP families 1, 2, 3 and 4 metabolize xenobiotics, including the majority of small molecule drugs currently in use. A typical feature of these CYP is broad and overlapping substrate specificity (Guengerich *et al.,* 2005). Approximately 70% of human liver CYP is accounted for by CYP1A2, CYP2A6, CYP2B, CYP2C, CYP2D6, CYP2E1 and CYP3A enzymes. Amomg these, CYP3A and CYP2C are the most abundant subfamilies, accounting for 30% and 20%, respectively (Lin and Lu, 1998).

In the CYP3A subfamily, an important regulatory pathway controlling expression of these enzymes in the liver and gut has been reported.

Evidence suggests that drugs of diverse structure can induce members of this family, and that the capacity of a particular compound to induce CYP3A enzymes varies between species (Nebert *et al.,* 2002). CYP3A4 is the most abundant CYP 450 expressed in liver and intestine, and it contributes to metabolism of approximately 50% of all used drugs in humans. Hepatic and intestinal CYP3A4 can be induced by several widely used drugs, such as rifampicin, dexamethasone, carbamazepine and phenytoin. CYP3A4 catalyzes the metabolism of a wide variety of commonly prescribed drugs, such as the psychotropic drugs buspirone, alprazolam, midazolam, and triazolam, the HMG-CoA reductase inhibitors atorvastatin, lovastatin, and simvastatin, the calcium-channel blockers felodipine, nifedipine, and verapamil, and the gastroprokinetic cisapride (Dresser *et a.,l* 2000). The CYP3A4 enzyme has two substrate binding sites and is allosterically regulated. The drug interactions caused by inhibition of CYP3A4 exhibit substrate dependency (Wang *et al*., 2000), and one should be careful in extrapolating drug-drug interactions studied for one CYP3A4 substrate to another substrate.

Activities of cytochrome P450 (CYP) enzymes are affected by numerous genetic, endogenous host, and environmental factors, making drug metabolism exceedingly variable and even individualistic. This variability has important repercussions to drug development, clinical drug therapy and in general to sensitivity to chemicals foreign to the body, i.e. xenobiotics. Among environmental factors, compounds causing inhibition and induction are amongst the most important ones, or at least the most researched. Table 4 illustrates the relative abundance of individual CYP forms in the liver, and lists some examples of substrates, inhibitors and inducers.

Table 4 Major hepatic CYP enzyme involved in drug metabolism including substrate, inhibitors and inducers of each isoform

CYP	Drug substrate	Marker substrate/ reaction	Inhibitor	Inducer
1A2	Paracetamol (acetaminophen), caffeine, ondansetron, phenacetin, tacrine, tamoxifen, theophylline	Phenacetin *O*-de-ethylation	Furafylline	Smoking, charred food
2A6	Coumarin, nicotine	Coumarin 7-hydroxylation	Ditiocarb sodium (diethyldithio-carbamate)	
2C9	Diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (*S*)-warfarin	Tolbutamide methyl hydroxylation	Sulfaphenazole	Barbiturates, rifampicin (rifampin)
2C19	Diazepam, (*S*)-mephenytoin, omeprazole, pentamidine, propranolol, (*R*)-warfarin	(*S*)-mephenytoin 4′-hydroxylation		
2D6	Bufuralol, codeine, debrisoquine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	Bufuralol 1′-hydroxylation	Quinidine, ajmaline	
2E1	Paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	Chlorzoxazone 6-hydroxylation	Ditiocarb sodium	Alcohol (ethanol), isoniazid
3A4	Benzphetamine, clarithromycin, codeine, cyclosporin, dapsone, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	Testosterone 6β-hydroxylation	Gestodene, troleandomycin, L-754,394, ketoconazole, itraconazole	Barbiturates, rifampicin, dexamethasone, carbamazepine

2.3.2. Mechanisms of inhibition of CYP

The catalytic cycle of CYP consists of at least 6 discrete steps:

(1) Binding of substrate to the enzyme, sometimes accompanied by a spinstate change of the iron, to afford an enzyme-substrate adducts.

(2) Reduction of the ferric cytochrome P450 by an associated reductase with an NADPH-derived electron to the ferrous cytochrome P450.

(3) Binding of molecular oxygen to the ferrous heme to produce a ferrous cytochrome P450-dioxygen complex 3, similar to the situation in oxymyoglobin.

(4) A second one-electron reduction and protonation to arrive at the Fe(III)-hydroperoxy complex.

(5) Protonation and heterolytic cleavage of the O-O bond in (4) with concurrent production of a water molecule to form a reactive iron-oxo intermediate.

(6) Oxygen-atom transfer from this iron-oxo complex (5) to the bound substrate to form the oxygenated product complex. Product dissociation completes the cycle.

Although impairment of any one of these steps can lead to inhibition of CYP enzyme activity, Step (1), (3) and (6) are particularly vulnerable to inhibition.

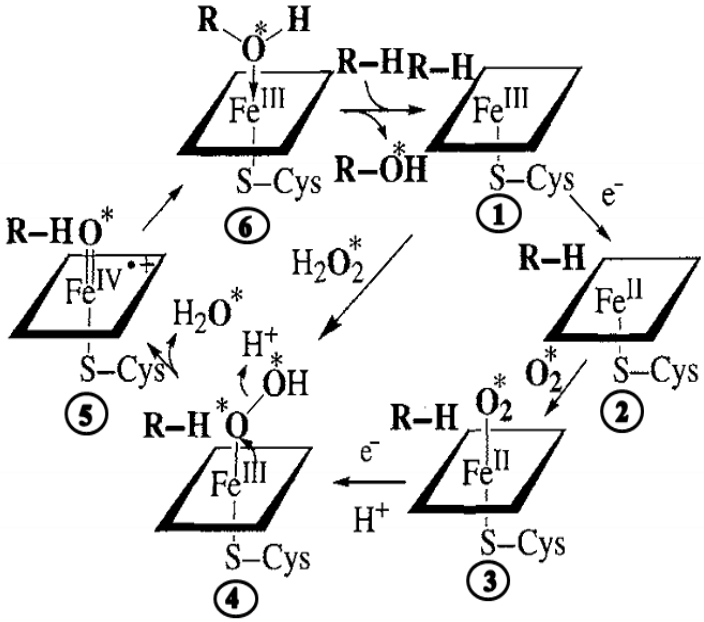


Figure 11 The catalytic cycle of cytochrome P450

The inhibition of drug metabolism is the most important mechanism for drug interactions because it can lead to an increase in plasma drug concentration, increased drug response, and toxicity. Inhibition can lead to increased bioavailability of the parent compound normally subject to extensive first-pass elimination or to decreased elimination of compounds dependent on metabolism for systemic clearance (Lasser *et al*,. 2002).

The mechanisms of CYP inhibition can occur via the formation of metabolite intermediate complexes or via the strong, covalent binding of reactive intermediates to the protein or heme of the CYP. Mechanism-based inhibition can be divided grossly into three categories: reversible inhibition, quasi-irreversible inhibition and irreversible inhibition. Among these, reversible inhibition is probably

the most common mechanism responsible for the documented drug interactions (Lee *et al.,* 2006).

Reversible inhibition of CYP

Many of the potent reversible CYP inhibitors are nitrogen-containing drugs, including imidazoles, pyridines and quinolines. These compounds can not only bind to the prosthetic heme iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. Reversible inhibition can be competitive or non-competitive (Badyal and Dadhich, 2001). A drug may inhibit the CYP isoenzyme whether or not it is a substrate for that isoenzyme. If the two drugs are substrates for the same CYP isoenzyme then metabolism of one or both the drug may be delayed (Lin and Lu, 1998). Erythromycin and midazolam both are substrates for 3A4 isoenzyme so, there is competition for enzyme sites and metabolism of midazolam is inhibited (Olkkola *et al.,* 1994). These drugs are converted through multiple CYP dependent steps to nitroso derivatives that bind with high affinity to the reduced form of CYP enzymes. Thus CYP enzymes are unavailable for further oxidation and synthesis of new enzyme is therefore the only means by which activity can be restored and this may take several days (Murray, 2006).

Quasi-Irreversible Inhibition of CYP

A large number of drugs, including methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines undergo metabolic activation by CYP enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic haem of CYP, called metabolic intermediate (M1) complex, so that the CYP is sequestered in a functionally inactive state. M1 complexation can be reversed and the catalytic function of ferric CYP can be restored by in vitro incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site. Other in vitro methods by which the ferrous complex can be disrupted include irradiation at 400-500 nm or oxidation to the ferric state by the addition of potassium ferricyanide. Dissociation or displacement of the M1 complex results in the reactivation of CYP functional activity. However, in *in vivo* situations, the M1 complex is so stable that the CYP involved in the complex is unavailable for drug metabolism and synthesis of new enzymes is the only means

by which activity can be restored. The nature of the M1 complexation, therefore, considered to be quasi-irreversible (Lin and Lu, 1998).

Irreversible Inactivation of CYP

Drugs containing certain functional groups can be oxidised by CYP to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site. Because metabolic activation is required for enzyme inactivation, these drugs are classified as mechanism-based inactivators or suicide substrates (Silverman, 1988). The mechanism based inactivation of CYP may result from irreversible alteration of heme or protein, or a combination of both. In general, modification of the heme group invariably inactivates the CYP, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer, and oxygen activation, are modified. Several α-ethinyl substituted steroids such as ethinylestradiol, gestodene, and levonorgestrel are report to cause mechanism-based inhibition (Guengerich, 2000).

2.3.3. Mechanisms of induction of CYP

Induction of CYP enzymes can lower the plasma concentrations and effects of some substrates of CYP enzymes, but not all, are inducible. Human CYP1A1, CYP2C9, CYP2E1 and CYP3A4 are known to be inducible. CYP induction is a slow regulatory process that can reduce drug concentrations in plasma, and may compromise the efficacy of the drug in a time-dependent manner. An increase in enzyme activity, due to activation, is not usually included under the term induction, although the functional outcome is similar. The mechanism of the induction of protein synthesis by the nuclear receptors CAR, PXR, and PPAR is essentially similar (Niemi, 2001). An inducer binds to CAR, PXR, or PPAR, and the inducer-receptor complex forms a heterodimer with the retinoid X receptor (RXR). This heterodimer binds to a DNA response element and enhances DNA transcription and eventually protein synthesis (Waxman, 1999). These include phenobarbital, other anticonvulsants and rifampin. One of the more serious recent examples is the induction of CYP3A4 by rifampin in HIV patients using protease inhibitors. Addition of rifampin decreases the AUC (area under the curve) of saquinavir by 80%, and of ritonavir by 35%.

Several clinical studies have reported a reduction in the plasma levels of the other cytochrome P450 (CYP) 3A4 substrate when concomitanted with 600 mg once daily of efavirenz (Falloon *et al.,* 2000; Clarke *et al.,* 2001; Aarnoutse *et al.,* 2002). Futuremore, the mechanism may be efavirenz increase CYP3A4 activity. Recent studies suggest that the human pregnane X receptor of the CYP3A4 gene and the transactivation of this receptor leads to upregulation of CYP3A4. In the presence of an activating ligand, PXR forms a heterodimer with the retinoid X receptor (RXRα). This heterodimer binds to the xenobiotic response element in the promoter sequence of CYP3A4, leading to increased gene transcription (Honkakoskl *et al.,* 2000). Efavirenz is an efficacious activator of hPXR (Hariparsad *et al.,* 2004).

2.4. The HIV life cycle

The virus that causes Acquired Immunodeficiency Syndrome (AIDS) is called the Human Immunodeficiency Virus or HIV, is a retroviruses. They are microscopic germs that are unable to reproduce (replicate) by themselves. Instead they need to find and infect a cell that will act as a host in which new viruses can be made. These newly minted viruses then go on to infect other cells. Without treatment, expert estimate that up to 10 billion copies of HIV may be made every day (Alfano and Poli, 2004).

There are two main types of HIV, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 is the most common type of strains, which affects the populations all across the globe and begins showing symptoms within 5 year of infection. HIV-2 which results in a less severe disease and is limited to the areas of West Africa. HIV-1 is the most common and pathogenic strain of the virus, divided HIV-1 into four sub-group based on the sequences of *gag* and *env* genes: the "major" group M, the "outlier" group O and two new groups, N and P (non-M/O). These four groups may represent four separate introductions of simian immunodeficiency virus into humans.

2.4.1. The structure of HIV

HIV (human immunodeficiency virus) is composed of two strands of RNA, 15 types of viral proteins, and a few proteins from the last host cell it infected, all surrounded by a lipid bilayer membrane. Together, these molecules allow the virus to infect cells of the immune system and force them to build new copies of the virus. Each moleculein the virus plays a role in this process, from the first steps of viral attachment to the final process of budding (Chan *et al.,* 1997). The structure of HIV as shown in Figure 12.

- The viral envelope

HIV is different in structure from other retro-viruses, is roughly spherical and has a diameter of 1/10,000 of a millimeter. The outer coat of the virus, known as the viral envelope, is composed of two layers of fatty molecules called phospholipids, taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Evidence indicates that HIV may enter and exit cells

through special areas of the cell membrane known as lipid rafts. These rafts are high in cholesterol and glycolipids and may provide a new target for blocking HIV. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein called Env, which protrudes through the surface of the virus particle. The entry of HIV into host cells is mediated by the viral envelope glycoproteins, which are organized into oligomeric, probably trimeric spikes displayed on the surface of the virion. These envelope complexes are anchored in the viral membrane by the gp41 transmembrane envelope glycoprotein. The surface of the spike is composed primarily of the exterior envelope glycoprotein, gp120, associated by non-covalent interactions with each subunit of the trimeric gp41 glycoprotein complex.

-The viral core

Within the viral envelope have the core is composed of the p24 capsid protein (bullet-shaped core), made up of 2,000 copies of the viral protein and the p17 matrix protein, maintained viral structure. The capsid protein (p24) surrounds two single strands of HIV RNA, each of which has a complete copy of the virus's genes. HIV has three structural genes (*gag*, *pol*, and *env*) that contain information needed to make structural proteins for new virus particles. The *gag* gene codes for a precursor protein that can be cleaved by the viral protease into four smaller proteins, p24 (capsid), p17 (matrix), p7 (nucleocapsid) and p6. The *pol* gene codes for a precursor protein that contain four enzymes, protease, integrase, RNase H, and reverse transcriptase. The *env* gene codes for a protein called gp160 that is broken down by the viral protease to form gp120 and gp41, the components of Env. HIV has six regulatory genes (*tat, rev, nef, vif, vpr, and vpu*) that contain information needed to produce proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease.

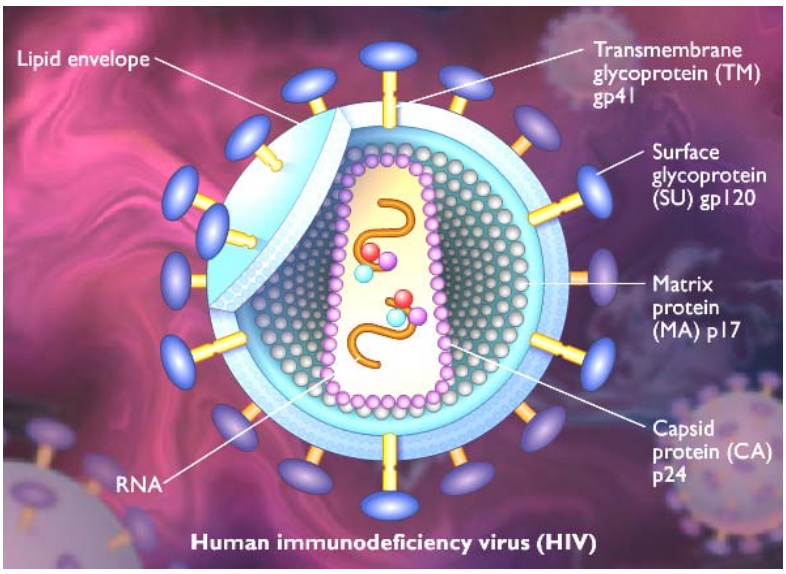


Figure 12 The structure of HIV (adepted from Miller, 2002)

2.4.2. The HIV life cycle

Then the virus begins a cycle of attacking cells of the immune system by incorporating its genetic material into the cells, using the immune cells' machinery to make more viruses from the incorporated genetic material, and then breaking the cells apart (killing them) so that the new viruses can infect more cells. In this manner, the immune system is weakened, so that the body can no longer defend itself against the pathogens that it encounters every day. Finally, patients typically die within a few years of showing symptoms of AIDS. The HIV life cycle as shown in Figure 13 (Miller, 2002; Schwa, 2004; Bryntesson, 2009).

1. Entry of HIV into cells

To establish infection, HIV must first attach to its host cell. Attachment by interaction between gp120 on the surface of virus and the CD_4^+ antigen receptor on the surface of the host cell. In addition on CD_4^+ receptor, there must also be a co-receptor on the host cell. The co-receptor differs for different host cell types. In T-lymphocyte, the co-receptor is called CXCR-4, whereas on macrophage, the co-receptor is called CCR-5. After the attachment, the viral envelope and host cell membrane fuse, resulting in entry of the virus into the cell.

2. Viral DNA is formed by reverse transcription

In the cytoplasm of the cell the reverse transcriptase, an enzyme (protein) that's part of the human immuno-deficiency virus reads the sequence of viral RNA nucleic acids that have entered the host cell and transcribes the sequence into a complementary DNA sequence.

3. Viral DNA is transported across the nucleus and integrates into the host DNA

The newly made HIV DNA moves to the cell's nucleus, where it is spliced into the host's DNA with the help of the integrase, an enzyme found in retroviruses. HIV DNA that enters the DNA of the cell is called a provirus. Several drugs that target the integrase enzyme are in the early stages of development and are being investigated for their potential as antiretroviral agents.

4. New viral RNA is used as genomic RNA and to make viral proteins

For a provirus to produce new viruses, RNA copies must be made that can be read by the host cell's protein-making machinery. These copies are called messenger RNA (mRNA), and production of mRNA is called transcription, a process that involves the host cell's own enzymes.

5. New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms

After HIV mRNA is processed in the cell's nucleus, it is transported to the cytoplasm. The protein encoded by the HIV's rev gene is critical to this process. Without the rev protein, structural proteins are not made. In the cytoplasm, the virus co-opts the cell's protein-making machinery including structures called ribosomes, to make long chains of viral proteins and enzymes, using HIV mRNA as a template. This process is calletranslation.

6. The virus matures by protease releasing individual HIV proteins

When viral RNA is translated into a polypeptide sequence, that sequence is assembled in a long chain that includes several individual proteins (reverse transcriptase, protease, integrase). Before these enzymes become functional, they must be cut from the longer polypeptide chain. The protease cuts the long chain into its individual enzyme components, which then facilitate the production of new

viruses. Finally, viral RNA and associated proteins are packaged and released from the host cell surface, taking with them a swatch of cell membrane containing viral surface proteins. These proteins will then bind to the receptors on other immune cells facilitating continued infection.

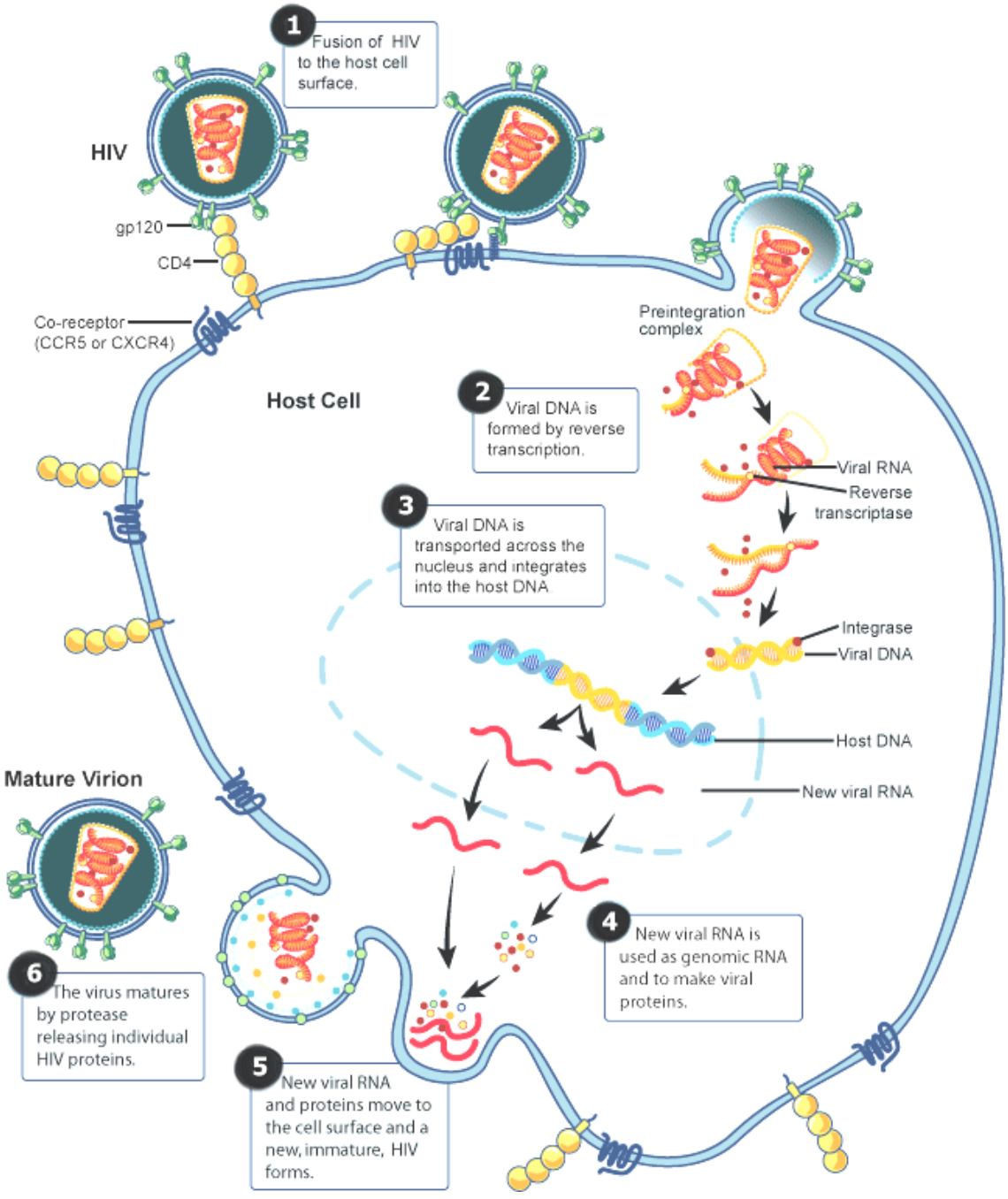


Figure 13 The HIV life cycle (adepted from Miller, 2002)

CHAPTER 3

METHODOLOGY OF STUDY

3.1. Materials

3.1.1. Drugs

Efavirenz (Storcrin®) was donated from MSD, Bangkok Thailand (MERK SHARP & DOHME, AUS).

Itraconazole capsules (Sporal®) were purchased from Olic (Thailand) Limited, under contract with Janssen-Cilag Ltd.

3.1.2 Chemicals and Reagents

The standard efavirenz (Lot No. 030832) was purchased from the Zhejing Jingxin Pharmaceutical company, USA.

The standard itraconazole (Lot No. Stan-9604-007-1) and internal standard itraconazole (R51012) (Lot No. Stan-9404-029-1) were purchased from the Fitzgerald Industrials International Imstitute, USA.

Methanol and Acetonitrile (HPLC grade) were purchased from J.T. Baker NJ, USA.

Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Merck Darmstadt, Germany.

Water was purified for HPLC by Milli Q Water Purification System, Milipore, Milford, MA, USA.

3.2. Equipments

3.2.1. HPLC model

-Waters 2695 Pump, autosampler (Waters Associates, Milford, USA.)

-Waters 2475 Multi λ Fluorescense Detector (Waters Associates, Milford, USA.)

-Water 2487 Dual λ Absorbance Detector (Waters Associates, Milford, USA.)

-Empower software Version 5 (Waters Associates, Milford, USA.)

-μ-Bondapack™ C_{18} column: reverse-phase column C_{18}, 3.9 mm x 300 mm, particle size 10 μm. (Waters Associates, Milford, MA, USA)

-Symmetry® C_{18} column: reverse-phase column C_{18}, 3.9 mm x 150 mm, particle size 5 μm, (Waters Associates, Milford, MA, USA)

-μ-Bondapack™ C_{18} guard column: packed with resolved C_{18} (Waters Associates, Milford, MA, USA.)

3.2.2. Instruments

- Vortex mixer
- Centrifuge machine
- Microcentrifuge machine
- Evaporator machine
- pH meter
- Micropipette (25, 50, 200, and 1,000 μl)
- Pipette tip
- Disposable needle (21-22G)
- Heparin lock
- Test tube with cap
- Disposable syringe (3 ml and 5 ml)
- Eppendorf microcentrifuge tube (1.5 ml)
- PTFE filter, pore size 0.45 μm

3.3. Methodology

3.3.1. Determination of efavirenz in plasma

3.3.1.1. Sample preparation

The 250 μl of plasma sample were added with 50 μl of 8 μg/ml internal standard (trazodone dissolved in 100% methanol) and precipitated with 1,000 μl of ethyl acetate. The mixture was vortexed for 30 seconds and centrifuge at 12,470 g for 15 minutes. The 750 μl of supernatant was evaporated to dryness. The dried residue was reconstituted in 75 μl of mobile phase and 20 μl was injected into the HPLC column.

3.3.1.2. Chromatographic condition

Plasma efavirenz concentrations were determined by HPLC. The assay was modified from Ramachandran et al. (2006) using the following parameters:

Column	:	μ-Bondapack™ C_{18} column: reverse-phase column C_{18}, 3.9 mm x 300 mm, particle size 10 μm.
Guard column	:	μ-Bondapack™ C_{18} pack with resolved C_{18} 125Aº
Mobile Phase	:	Phosphate buffer (KH_2PO_4) adjusted to pH 2.4 with 1 N HCl and acetonitrile (55: 45 % by volume)
Flow rate	:	1.5 ml/min
Injection volume	:	20 μl
Detector	:	Fluorescence, excitation and emission wavelengths of 245 nm.
Temperature	:	Room temperature (~25 ºC)

3.3.1.3. Mobile Phase

The mobile phase consisted of phosphate buffer (KH_2PO_4) adjusted to pH 2.4 with 1 N HCl and acetonitrile (55: 45 % by volume). The mobile phase was freshly prepared daily and filtrated through PTEF filter, pore size 0.45 μm, and degassed in the ultrasonic bath for 30 minutes before using.

3.3.1.4. Standard curves

Standard curves was prepared by diluted working standard solution (16,000 ng/ml) to serial concentrations 250, 500, 1,000, 2,000, 4,000 and 8,000 ng/ml with drug-free plasma. Standard calibration curves were conducted by the least-square linear regression of the efavirenz concentration and peak area ratio of efavirenz. Unknown concentrations of efavirenz in patient's plasma were calculated from the standard curves by reverse prediction.

3.3.1.5. Stock standard and internal standard solution

The stock standard efavirenz solution and internal standard trazodone solution at a concentration of 1,000 µg/ml were prepared in 100% methanol and stored at -80 ºC. The working solution was prepared in 100% methanol. The stock solutions were stable stored at -80 ºC, diluted standard solutions minimally for 6 h at room temperature and daylight (Vybíralova *et al*., 2005). Working standard solution, which was used to preparations of a standard calibration curve, was freshly daily prepared by diluting the solution with drug-free plasma.

3.3.2. Determination of itraconazole in plasma

3.3.2.1. Sample preparation

The 500 µl of plasma was added with 50 µl of 10 µg/ml internal standard (R51012 dissolved in 20% methanol) and precipitated with 500 µl of acetonitrile. The mixture was vortexed for 30 seconds and centrifuged at 12,470 g for 15 minutes. The 200 µl of supernatant was injected into the HPLC system for analysis.

3.3.2.2. Chromatographic condition

Plasma itraconazole concentrations were determined by HPLC. The assay was modified from Badcock (1990) using the following parameters:

Column	:	Symmetry® C_{18} column: reverse-phase column C_{18}, 3.9 mm x 150 mm, particle size 5 µm.
Guard column	:	µ-Bondapack™ C_{18} pack with resolved C_{18} 125Aº
Mobile Phase	:	Mixture of acetonitrile and water (60:40 v/v) was added 300 µl/l of diethylamine and pH was adjusted to 7.8 with 85% H_3PO_4.
Flow rate	:	1.0 ml/min
Injection volume	:	200 µl
Detector	:	Absorbance detector, wavelengths of 263 nm, Gain 10, and AUFS 0.002
Temperature	:	Room temperature (~25 ºC)

3.3.2.3. Mobile Phase

The mobile phase consisted of acetonitrile and water (60: 40 v/v) was added with 300 µl/l of diethylamine and pH was adjusted to 7.8 with 85% H_3PO_4. The mobile phase was freshly prepared daily and filtrated through PTEF filter, pore size 0.45 µm, and degassed in the ultrasonic bath for 30 minutes before using.

3.3.2.4. Standard curves

Standard curves were prepared by diluted working standard solution (10,000 ng/ml) to serial concentrations of 50, 100, 400, 800, 1,600 and 3,200 ng/ml with drug-free plasma. Standard calibration curves were conducted by the least-square linear regression of the itraconazole concentration and peak area ratio of itraconazole. Unknown concentrations of itraconazole in patient's plasma were calculated from the standard curves by reverse prediction.

3.3.2.5. Stock standard and internal standard solution

The stock standard itraconazole solution and internal standard solution (R51012) at a concentration of 1,000 µg/ml were separated in methanol and the working solution was prepared in 20% methanol. Itraconazole methanolic solutions have been reported to be stable at 4°C for at least 12 months and serum samples were stable for >6 months at -20°C (Compas *et al*., 1996; Srivatsan *et al*., 2004). Working standard solution, which was used to preparation of a standard calibration curve, was freshly daily prepared by diluting the solution with drug-free plasma.

3.3.3. Method validation (US FDA, 2001)

3.3.3.1. Recovery study

Analytical recovery of plasma efavirenz and itraconazole was determined by comparing the peak area of precipitated drug in plasma with the peak area of deprecipitated equivalent drug in mobile phase. A good recovery should be more than 90% and percent coefficient of variation (%CV) should be less than 5%.

$$\frac{\text{Peak area ratio of standard efavirenz /Itraconazole in plasma}}{\text{Peak area ratio of standard efavirenz /Itraconazole in mobile phase}} \times 100$$

3.3.3.2. Precision and Accuracy

To determine intra-day precision and accuracy, the standard solution was spike in drug-free plasma at 6,000, 3,000 and 250 ng/ml for efavirenz and at 1,600, 800 and 50 ng/ml for itraconazole. Five replications of each were carried out in one day.

To determine inter-day precision and accuracy, the standard solution was spike in drug-free plasma at 6,000, 3,000 and 250 ng/ml for efavirenz and at 1,600, 800 and 50 ng/ml for itraconazole. Two replications of each were carried out in five days.

The percent of coefficient of variation (%CV) of each concentration should be less than 15%.

Coefficient of variation (%CV) = (Standard deviation / Mean value) x100

Accuracy (%) = (calculated concentration / actual concentration) x 100

3.3.3.3. Lower limit of quantification

Lower limit of quantification was obtain by adding known amount of efavirenz and itraconazole to drug-free plasma (250-8,000 ng/ml, and 50-3,200 ng/ml, respectively) and deprecipitated as above. The peak area of efavirenz and itraconazole were calculated and plotted for correlation between the concentration of efavirenz or itraconazole and peak area ratio. The lowest concentration of efavirenz and itraconazole which was still linearly correlation was regarded as low limit of quantification (LLOQ).

3.3.4. Sample size calculation

The study was conducted to determine drug interaction between itraconazole and efavirenz in HIV-infected patients. However, there was no report on correlation of itraconazole and efavirenz in HIV-infected patients. So, the study of Liu et al. (2007) on the influence of pharmacokinetic interaction between voriconazole and efavirenz at steady state in 34 healthy male subjects was used to calculate sample size. Voriconazole is a novel broad spectrum triazole antifungal agent. It binds the active site of the P450-dependent enzyme lanosterol 14-α demethylase and ligates iron

heme cofactor through a nitrogen atom. This inhibition leads to depletion of ergosterol and accumulation of 14-α methyl sterols such as lanosterol, affecting the integrity and function of the fungal membrane. Results of *in vitro* and *in vivo* studies have shown that voriconazole is primarily metabolized by CYP2C19, CYP2C9, and, to a lesser extent, CYP3A (Hyland *et al.,* 2003), and it also inhibits CYP2C9 and CYP2C19, but has only a mild inhibitory effect on CYP3A4-mediated metabolism (Purkins *et al.,* 2003; Wood *et al.,* 2003; Niwa *et al.,* 2005). They found that $AUC_{0\text{-}12}$ of voriconzole was significantly reduced from 26.3 to 5.71 $\mu g \cdot ml^{-1} \cdot h$ in the presence of efavirenz, p = 0.01 (SD = 9.80 $\mu g.ml^{-1}.h$).

The different AUC of voriconzole (d) = 26.3-5.71 = 20.59 $\mu g \cdot ml^{-1} \cdot h$ Type I error 5 % (α=0.05), Z_α = 1.645, Type II error 10 % (β=0.10),Z_β =1.282.

$$N = \frac{2(Z_\alpha + Z_\beta)^2 (SD)^2}{d^2}$$

$$= \frac{2(1.645+1.282)^2 (9.80)^2}{(20.59)^2}$$

$$= 3.88 \approx 4$$

A total sample size of 4 patients should be enough to detect a significant pharmacokinetic difference in AUC for itraconazole and efavirenz. Therefore four HIV-infected patients who had CD_4^+ cell counts less than 350 cells/mm^3 were enrolled in this study. Unfortunately, one of the four patients was withdrawn from the study due to adverse drug reaction (ADR). The study was a one-sequence and three-periods without washout period pharmacokinetic interaction study.

3.3.5. Pharmacokinetic study

3.3.5.1. Patient selection

Inclusion criteria:

1. HIV-infected patients, who had CD_4^+ T-lymphocyte absolute cell count less than 350 cells/mm^3 (obtained within the preceeding 2 months)
2. Age were over 18 years,

3. Willing to give written informed consents.

Exclusion criteria:

Patients were excluded from the study in the following case:

1. Renal or hepatic impairment.
2. Diarrhea or vomiting during the study period.
3. Currently received the agents known to influence on efavirenz or itraconazole pharmacokinetics
4. Received ketoconazole, itraconazole, fluconazole or antiviral drugs within 1 month prior to the study.
5. Known history of azole antifungal agents or antiviral agent hypersensitivity.
6. Unwilling to give informed consent.

3.3.5.2 Study design

The study was one-sequence and three-periods without washout period pharmacokinetic interaction study.

Patients were hospitalized for 2 day at Songklanagarind hospital for blood sampling during each phase of the study.

Phase 1, Patients served as itraconazole control group, 200 mg of itraconazole was administered with 200 ml water in day 1 and continue thoughout the course of treatment. On day 14 of the study, Blood samples (approximately 5-7 ml) were obtained from an indwelling venous catheter before itraconazole administration (0 h) and at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12 and 24 h after administration of itraconazole. Each sample was added to the heparinized tube and centrifuged at 12,470 g for 15 min, and plasma was harvested and stored at -80°C until the time of analysis.

Phase 2, Patients served as efavirenz control group, 600 mg of efavirenz once daily was administered with 200 ml water in day 15 and continue thoughout the course of treatment. The drug was taken at bedtime (9.00 PM) to attenuate possible central nervous system adverse effect (Adkins, 1998). Efavirenz was combined with 300 mg of lamivudine and 40 mg (or 30 mg when B.W. was lower than 60 kgs.) of stavudine twice a day. On day 28 of the study, Blood samples (approximately 5 ml) were obtained from an

indwelling venous catheter before efavirenz administration (0 h) and at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12 and 24 h after administration of efavirenz. Each sample was added to the heparinized tube and centrifuged at 12,470 g for 15 min, and plasma was harvested and stored at -70°C until the time of analysis.

Phase 3, Patients served as study group, 600 mg of efavirenz was combined with 200 mg of itraconazole once daily in day 29 and continue thoughout the course of treatment. On day 42 of the study, Blood samples (approximately 5-7 ml) were obtained from an indwelling venous catheter before efavirenz and itraconazole administration (0 h) and at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12 and 24 h after administration of efavirenz and itraconazole. Each sample was added to the heparinized tube and centrifuged at 12,470 g for 15 min, and plasma was harvested and stored at -70°C until the time of analysis.

3.3.5.3. Pharmacokinetic analysis

The pharmacokinetic parameters (C_{max}, C_{min}, T_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $T_{1/2}$ and Cl) were analyzed by non-compartment model, with the use of WinNonlin Professional Software Version 1.1 (Pharsight, Mountain View, CA).

3.3.5.4. Statistical Analysis

All pharmacokinetics parameters were expressed as mean ± SD. The data shown normal distribution, therefore, parametric statistic test was used for data assessment. Paired *t*-test was treated for pair wise comparisons. The significance level was set at *p*-value of less than 0.05.

3.3.6 Protocol Approval

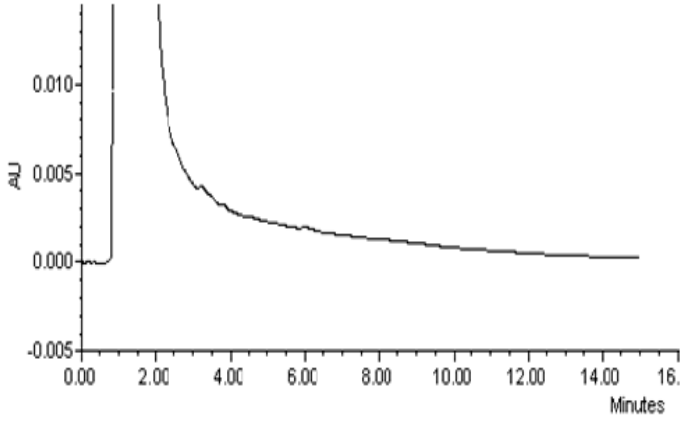
The study protocol was approved by the ethic committee of the Faculty of Medicine and Faculty of Science, Prince of Songkla University.

CHAPTER 4

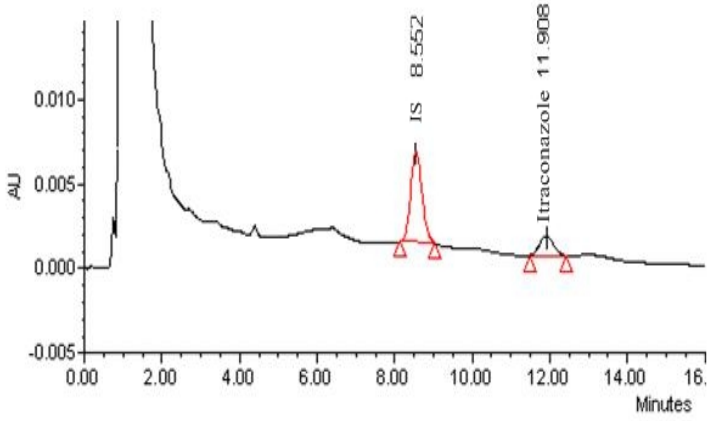
RESULTS

4.1. Analysis of Itraconazole in Plasma

Chromatograms of drug free plasma (a) and itraconazole 800 ng/mL with internal standard in plasma (b) are shown in figure 14. Itraconazole was eluted at 11.91 minutes and internal standard was eluted at 8.55 minutes as sharp and symmetrical peak.



(a)



(b)

Figure 14 HPLC chromatograms of drug free plasma (a) and itraconazole 800 ng/ml with internal standard in plasma (b)

4.1.1. Linearity

Calibration curves for plasma analysis were constructed for itraconazole in drug-free plasma to achieve the final concentrations of 50, 100, 200, 400, 800, 1,600 and 3,200 ng/ml. Then, calibration curves were plotted between the peak areas of itraconazole versus plasma itraconazole concentration (ng/ml), as shown in figure 15. Using the least-square linear regression analysis, the correlation coefficient (R-square) was 1 and the linear regression equation was:

$$Y= 0.0023X - 0.0208$$

Where:

X = plasma itraconazole concentration (ng/ml)

Y = the peak area of itraconazole

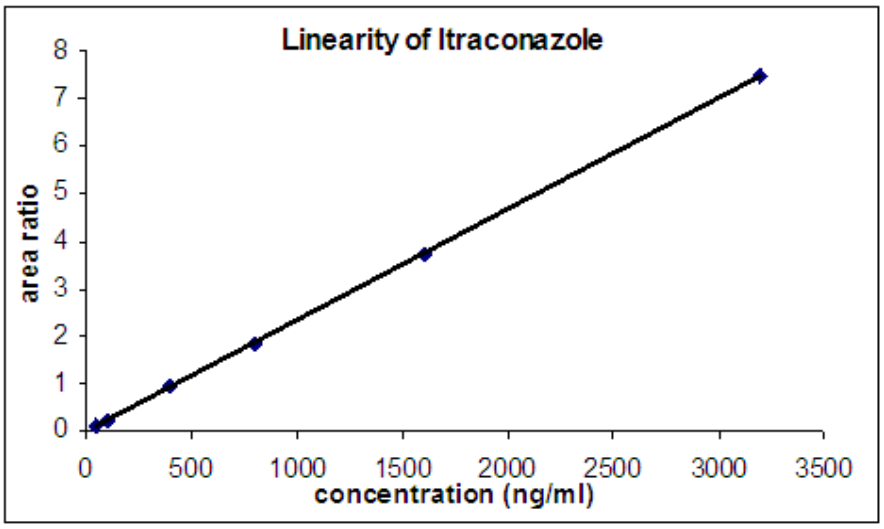


Figure 15 Calibration curves of itraconazole in plasma

($Y= 0.0023X - 0.0208$, $r^2 = 0.999$)

4.1.2. Recovery

Efficacy of deproteinization procedure was assessed from the percentage recovery, as shown in table 5. The mean percentage recoveries of itraconazole at the concentration of 50, 800 and 1,600 ng/ml were 106.42, 102.92 and 109.40%, respectively. These results had shown a good efficacy of deproteinization procedure owing to the high percentage of recovery with percentage CV less than 5%.

Table 5 Recovery of itraconazole

Plasma concentration (ng/ml)	Recovery (%) (n=5)	
	Mean	Percentage CV
50	106.42	4.96
800	102.92	2.91
1600	109.40	4.28

4.1.3. Lower limit of quantitation

The lower limit of quantitation (LLOQ) was obtained in this chromatographic condition and was found to be 50 ng/ml and the limit of detection (LOD) was found to be 25 ng/ml

4.1.4. Precision

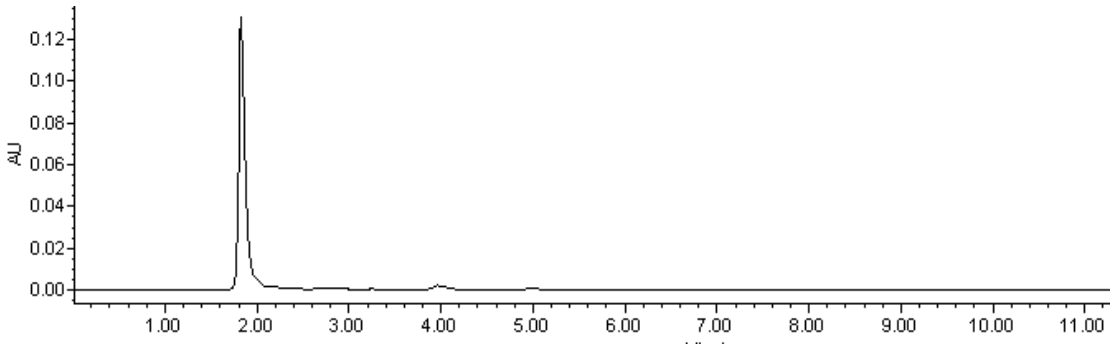
The precision of assay procedure was assessed from percentage CV of area under the curve itraconazole and retention times from intraday and interday results, as shown in table 6. Percentage CV of intraday precision for area under the peak of itraconazole was found in range of 1.11-8.07% and percentage CV of interday precision for area under the peak of itraconazole was found in range of 10.35-10.55%.

Table 6 Precision of the analytical method, intraday and interday precision

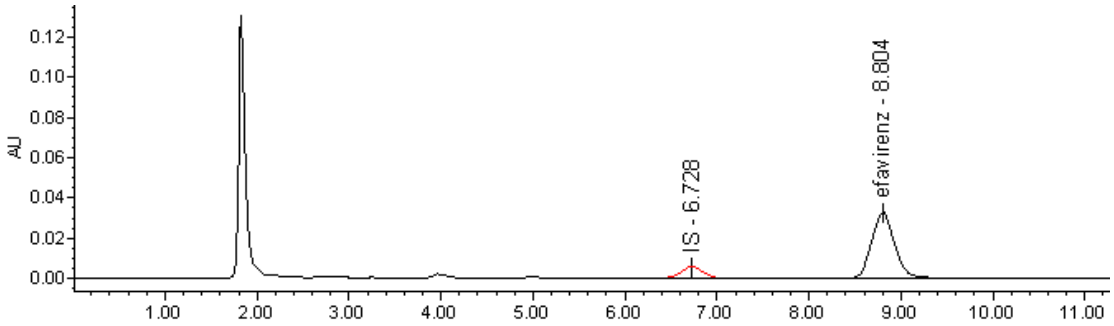
Concentration (ng/ml)	Mean concentration± SD (ng/ml)	CV (%)	Accuracy (%)
Intraday (n=5)			
50	55.17±2.76	5.01	110.34
800	725.14±58.52	8.07	90.64
1,600	1,392±15.39	1.11	87.02
Interday (n=5)			
50	50.97±5.28	10.35	101.94
800	770.52±81.23	10.54	96.31
1,600	1,581±166.84	10.55	98.85

4.2. Analysis of Efavirenz in Plasma

Chromatograms of drug free plasma (a) and efavirenz 0.8 µg/ml with internal standard in plasma (b) are shown in figure 16. Efavirenz was eluted at 8.80 minutes and internal standard was eluted at 6.73 minutes as sharp and symmetrical peak.



(a)



(b)

Figure 16 HPLC chromatograms of drug free plasma (a) and efavirenz 0.8 µg/ml with internal standard in plasma (b)

4.2.1. Linearity

Calibration curves for plasma analysis were constructed for efavirenz in drug-free plasma to achieve the final concentrations of 250, 500, 1,000, 2,000, 4,000 and 8,000 ng/ml. Then, calibration curves were plotted between the peak area of efavirenz versus plasma efavirenz concentration (ng/ml), as shown in figure 17. Using the least-square linear regression analysis, the correlation coefficient (R-square) was 0.9989 and the linear regression equation was:

$$Y = 0.0004X + 0.0275$$

Where:

X = plasma efavirenz concentration (ng/ml)

Y = the peak area of efavirenz

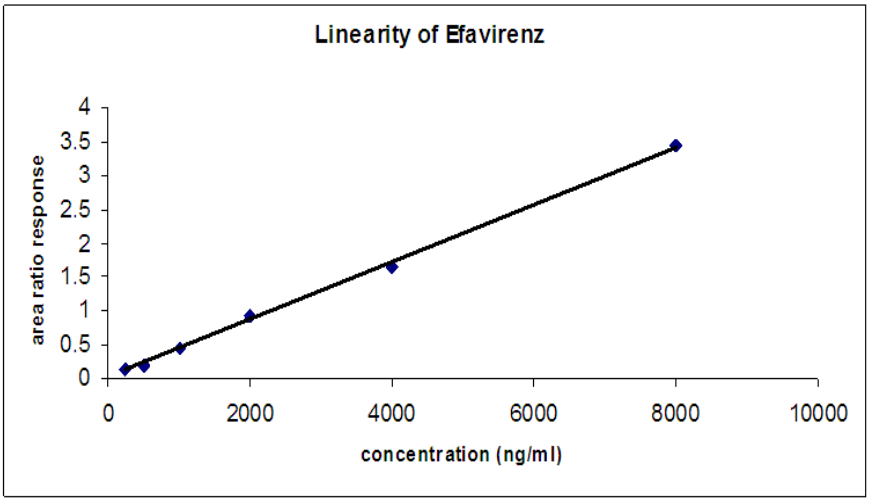


Figure 17 Calibration curves of efavirenz in plasma

($Y = 0.0004X + 0.0275$, $r^2 = 0.999$)

4.2.2. Recovery

Efficacy of deproteinization procedure was assessed from the percentage recovery, as shown in table 7. The mean percentage recoveries of efavirenz at the concentration of 250, 3,000 and 6,000 ng/ml were 71.69, 97.95 and 91.40 %, respectively. These results had shown a good efficacy of deproteinization procedure owing to the high percentage of recovery with percentage CV less than 5%.

Table 7 Recovery of efavirenz

Plasma concentration (ng/ml)	Recovery (%) (n=5)	
	Mean	Percentage CV
250	71.69	4.99
3,000	97.95	3.56
6,000	91.40	4.70

4.2.3. Lower limit of quantitation

The lower limit of quantitation (LLOQ) was obtained in this chromatographic condition and was found to be 250 ng/ml and the limit of detection (LOD) was found to be 125 ng/ml

4.2.4. Precision

The precision of assay procedure was assessed from percentage CV of area under the curve efavirenz and retention times from intraday and interday results, as shown in table 8. Percentage CV of intraday precision for area under the peak of efavirenz was found in range of 6.75-12.68% and percentage CV of interday precision for area under the peak of efavirenz was found in range of 6.59-11.08%.

Table 8 Precision of the analytical method, intraday and interday precision

Concentration (ng/ml)	Mean concentration± SD (ng/ml)	CV (%)	Accuracy (%)
Intraday (n=5)			
250	225.42±28.34	12.58	90.51
3,000	2,976±200.76	6.75	99.21
6,000	6,072.23±769.98	12.86	101.20
Interday (n=5)			
250	234.25±25.96	11.08	93.70
3,000	2,885.65±249.42	8.64	96.19
6,000	5,887.65±387.80	6.59	98.13

4.4. Plasma itraconazole concentrations

Four patients were enrolled in this study. One patient was withdrawn from the study due to adverse effect of efavirenz, therefore only three patients completed the study without serious adverse effect. Plasma itraconazole concentration from phase 1 (itraconazole alone) are presented in table 9 and those of phase 3 (concomitant itraconazole and efavirenz) are presented in table 10. The pharmacokinetic parameters of itraconazole in phase 1 and phase 3 were shown in table 11 and table 12, respectively. The mean±SD of C_{max}, C_{min}, T_{max}, AUC_{0-24}, $AUC_{0-\infty}$, $T_{1/2}$ and Cl of phase 1 (itraconazole alone) were 569.84±196.81 ng/ml, 256.54±208.82 ng/ml, 8.67±3.06 h, 9,240.30±5,707.57 ng·h/ml, 16,144±12,624.56 ng·h/mL, 14.20±2.60 h and 0.32±0.24 l/h, respectively. For phase 3 (concomitant itraconazole and efavirenz), the mean±SD of C_{max}, C_{min} and AUC_{0-24} were 42.91±18.12 ng/ml, 25.00±0.00 ng/ml and 716.69±143.98 ng·h/ml, respectively. All parameters were shown in table 9-13. The mean plasma itraconazole concentration-time data of the two phases are depicted in figure 18, and individual patient profile are shown in appendix D.

Table 9 Plasma itraconazole concentration for each patient during phase 1 (itraconazole alone)

Patient No.	Plasma itraconazole concentration(ng/ml) at each time of blood drawn (h)												
	0	1	2	2.5	3	3.5	4	4.5	5	6	8	12	24
1	99.99	50.76	83.37	91.62	72.28	64.49	78.43	130.42	173.57	345.64	127.08	134.02	30.20
2	603.00	386.47	381.34	420.41	404.33	397.01	358.35	454.83	407.49	401.92	714.10	663.69	297.75
3	529.05	443.53	441.69	471.46	522.50	490.64	543.78	537.75	523.49	475.96	516.69	649.78	456.71
Mean	410.68	293.59	302.13	327.83	333.04	317.38	326.85	374.33	368.18	407.84	452.62	482.50	261.55
SD	271.59	212.22	191.84	206.15	233.43	223.96	234.27	215.26	178.24	65.36	298.71	301.87	215.55

Table 10 Plasma itraconazole concentration for each patient during phase 3 (concomitant itraconazole and efavirenz)

Patient No.	Plasma itraconazole concentration(ng/ml) at each time of blood drawn(h)												
	0	1	2	2.5	3	3.5	4	4.5	5	6	8	12	24
1	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	41.45	42.51	25.00*	25.00*
2	25.00*	28.51	25.00*	25.00*	25.00*	25.00*	25.00*	31.12	39.53	53.01	61.24	38.82	25.00*
3	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*	25.00*
Mean	25.00	26.17	25.00	25.00	25.00	25.00	25.00	27.04	29.84	39.82	42.92	29.61	25.00
SD	0	2.03	0	0	0	0	0	3.53	8.39	14.08	18.12	7.98	0

* Calculated from LOD of itraconazole

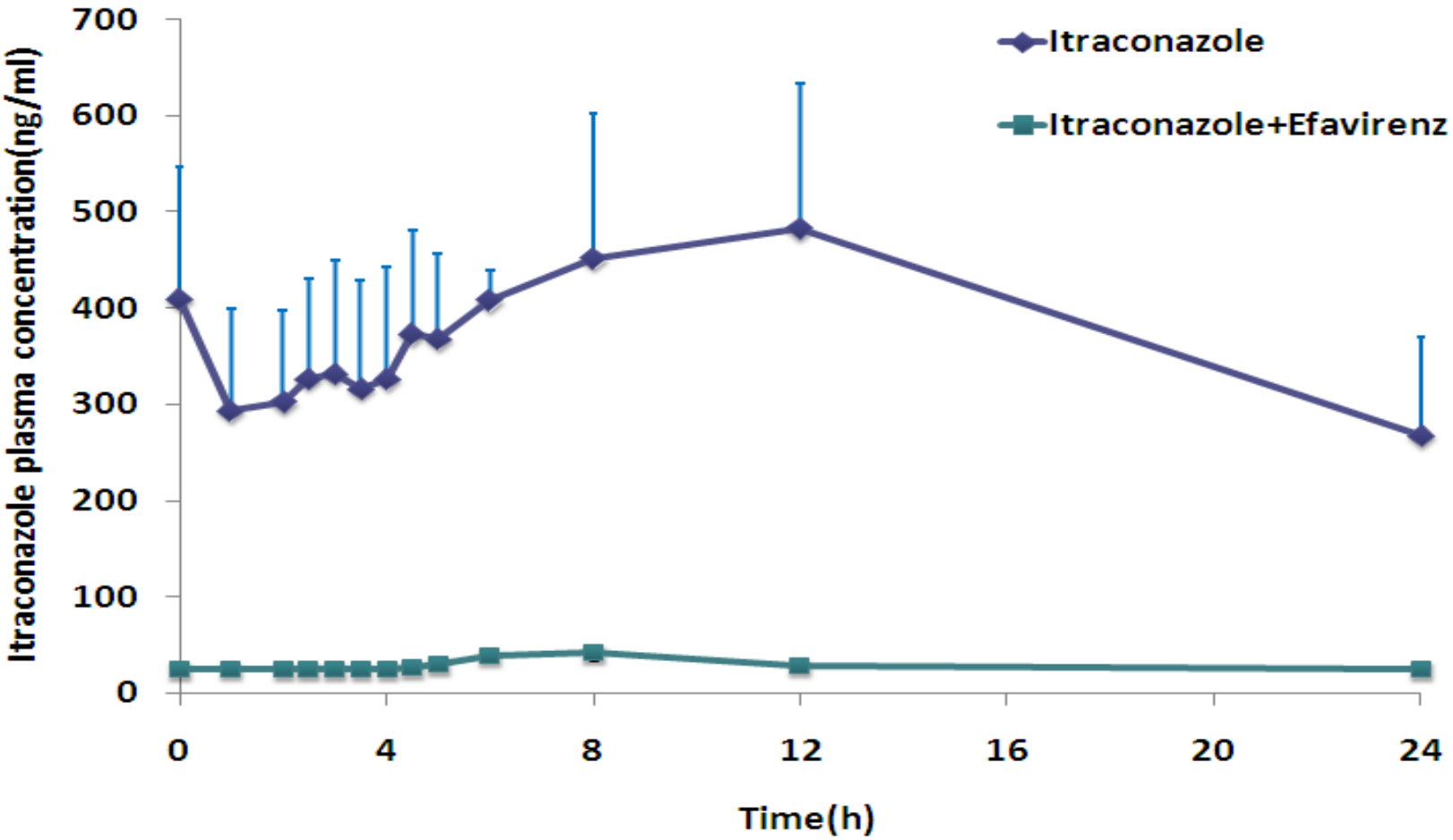


Figure 18 Mean plasma concentration-time curves of itraconazole after multiple oral doses of itraconazole alone (14 days) and in combination with efavirenz once daily for 14 days. (◆): multiple oral 200 mg itraconazole alone once daily for 14 days; (■): multiple oral 200 mg itraconazole in combination with 600 mg efavirenz once daily for 14 days.

Table 11 Pharmacokinetic parameters of itraconazole in each of the three patients during phase 1 (itraconazole alone)

Parameters	Patient number (S1-S3)				
	1	2	3	Mean	SD
C_{max} (ng/ml)	345.64	714.10	649.78	569.84	196.81
C_{min} (ng/ml)	30.20	297.75	441.69	256.54	208.82
T_{max} (h)	6	8	12	8.67	3.06
AUC_{0-24} (ng·h/ml)	2,665.14	12,138.26	12,917.51	9,240.30	5,707.57
$AUC_{0-\infty}$ (ng·h/ml)	2,974.12	17,319.15	28,141.18	16,144.00	12,624.56
λ_z (h^{-1})	0.20	0.35	0.20	0.25	0.09
$t_{1/2,z}$ (h)	17.09	12.06	13.46	14.20	2.60
Cl/f (l/h)	0.10	0.57	0.30	0.32	0.24
V_z/f (l/kg)	2.05	0.6	0.61	1.09	0.83

Table 12 Pharmacokinetic parameters of itraconazole in each of the three patients during phase 3 (concomitant itraconazole & efavirenz)

Parameters	Patient number (S1-S3)				
	1	2	3	Mean	SD
C_{max} (ng/ml)	42.50	61.24	25.00*	42.91	18.12
C_{min} (ng/ml)	25.00*	25.00*	25.00*	25.00*	0
T_{max} (h)	8	8	N/A	N/A	N/A
AUC_{0-24} (ng·h/ml)	677.21	878.86	600.00	716.69	143.98
$AUC_{0-\infty}$ (ng·h/ml)	865.57	1,363.86	N/A	N/A	N/A
λ_z (h^{-1})	0.13	0.15	N/A	N/A	N/A
$t_{1/2,z}$ (h)	5.28	3.49	N/A	N/A	N/A
Cl/f (l/h)	0.23	0.66	N/A	N/A	N/A
V_z/f (l/kg)	1.74	2.45	N/A	N/A	N/A

* Calculated from LOD of itraconazole
N/A: not available

Table 13 Effect of efavirenz on itraconazole pharmacokinetics in each of three HIV-infected patients

Patient No.	C_{max} (ng/ml)		C_{min} (ng/ml)		T_{max} (h)		AUC_{0-24} (ng·h/ml)		$AUC_{0-\infty}$ (ng·h/ml)	
	Phase		Phase		Phase		Phase		Phase	
	ITR	ITR+EFV	ITR	ITR+EFV	ITR	ITR+EFV	ITR	ITR+EFV	ITR	ITR+EFV
1	345.64	42.50	30.20	25.00*	6	8	2,665.14	677.21	2,974.12	N/A
2	714.10	61.24	297.75	25.00*	8	8	12,138.26	878.86	17,319.15	N/A
3	649.78	25.00*	441.69	25.00*	12	N/A	12,917.51	600.00*	28,141.18	N/A
Mean	569.84	49.21	256.54	25.00*	8.67	N/A	9,240.30	716.69	16,144.00	N/A
SD	196.81	18.12	208.82	0	3.06	N/A	5,707.57	143.98	12,624.56	N/A
p-value	0.04		0.19		N/A		0.12		N/A	

* Calculated from LOD of itraconazole
N/A: not available

4.5. Plasma efavirenz concentrations

Four patients were enrolled in this study. One patient was withdrawn from the study due to adverse effect of efavirenz, therefore only three patients completed the study without serious adverse effect. Plasma efavirenz concentration from phase 2 (efavirernz alone) are presented in table 14 and those of phase 3 (concomitant efavirenz and itraconazole) are presented in table 15. The pharmacokinetic parameters of efavirenz in phase 2 (efavirernz alone) and phase 3 (concomitant efavirenz and itraconazole) are shown in table 16 and table 17, respectively. The mean±SD of C_{max}, C_{min}, T_{max}, AUC_{0-24}, AUC $_{0-\infty}$, $T_{1/2}$ and Cl of phase 2 (efavirenz alone) were 502.13±300.15 ng/ml, 185.47±104.75 ng/ml, 6.67±1.15 h, 7,745.27±5,155.6 ng·h/ml, 9,471.89±5,305.49 ng·h/ml, 24.66±14.18 h and 0.08±0.02 l/h, respectively. For phase 3 (concomitant efavirenz and itraconazole), the mean±SD of C_{max}, C_{min}, T_{max}, AUC_{0-24}, AUC $_{0-\infty}$, $T_{1/2}$ and Cl were 592.44±374.55 ng/ml, 164.86±69.04 ng/ml, 4.67±1.26 h, 8,039.31±3,281.64 ng·h/ml, 13,473.22±5,731.12 ng·h/ml, 26.44±16.80 h and 0.05±003 l/h, respectively. All parameters shown in table 14-18. The mean plasma efavirenz concentration-time data of two phases are depicted in figure 19, and individual patient profile are shown in appendix D.

Table 14 Plasma efavirenz concentration for each patient during phase 2 (efavirenz alone)

Patient No.	Plasma efavirenz concentration(ng/ml) at each time of blood drawn (h)												
	0	1	2	2.5	3	3.5	4	4.5	5	6	8	12	24
1	338.25	306.41	326.46	377.28	497.96	352.89	559.46	574.31	653.01	848.70	677.28	548.45	601.39
2	131.81	125.00*	125.00*	125.00*	152.48	155.76	193.48	203.38	255.84	326.30	272.02	186.14	221.43
3	125.00*	125.00*	125.00*	137.09	135.85	176.65	208.57	200.82	133.53	241.55	331.40	201.46	125.00*
Mean	198.35	185.47	192.15	213.12	262.10	228.43	320.50	326.17	347.46	472.18	426.90	312.02	315.94
SD	121.20	104.74	116.31	149.29	204.43	108.29	207.08	214.90	371.59	328.81	218.86	204.90	251.86

* Calculated from LOD of efavirenz

Table 15 Plasma efavirenz concentration for each patient during phase 3 (concomitant efavirenz and itraconazole)

Patient No.	Plasma efavirenz concentration(ng/ml) at each time of blood drawn (h)												
	0	1	2	2.5	3	3.5	4	4.5	5	6	8	12	24
1	311.02	301.42	881.90	972.23	994.12	1,024.91	813.08	764.96	727.91	605.90	483.20	421.62	244.58
2	125.00*	125.00*	134.36	131.49	170.11	195.27	318.63	372.80	257.42	290.12	200.36	125.00*	125.00*
3	125.00*	125.00*	125.00*	125.00*	125.00*	125.00*	147.03	365.60	326.85	379.60	255.24	194.13	125.00*
Mean	187.01	183.81	380.42	409.57	429.74	448.39	426.25	501.12	437.39	425.21	312.93	246.92	164.86
SD	107.40	101.86	434.32	487.29	489.28	500.51	345.82	228.52	253.98	162.76	149.99	155.20	69.04

* Calculated from LOD of efavirenz

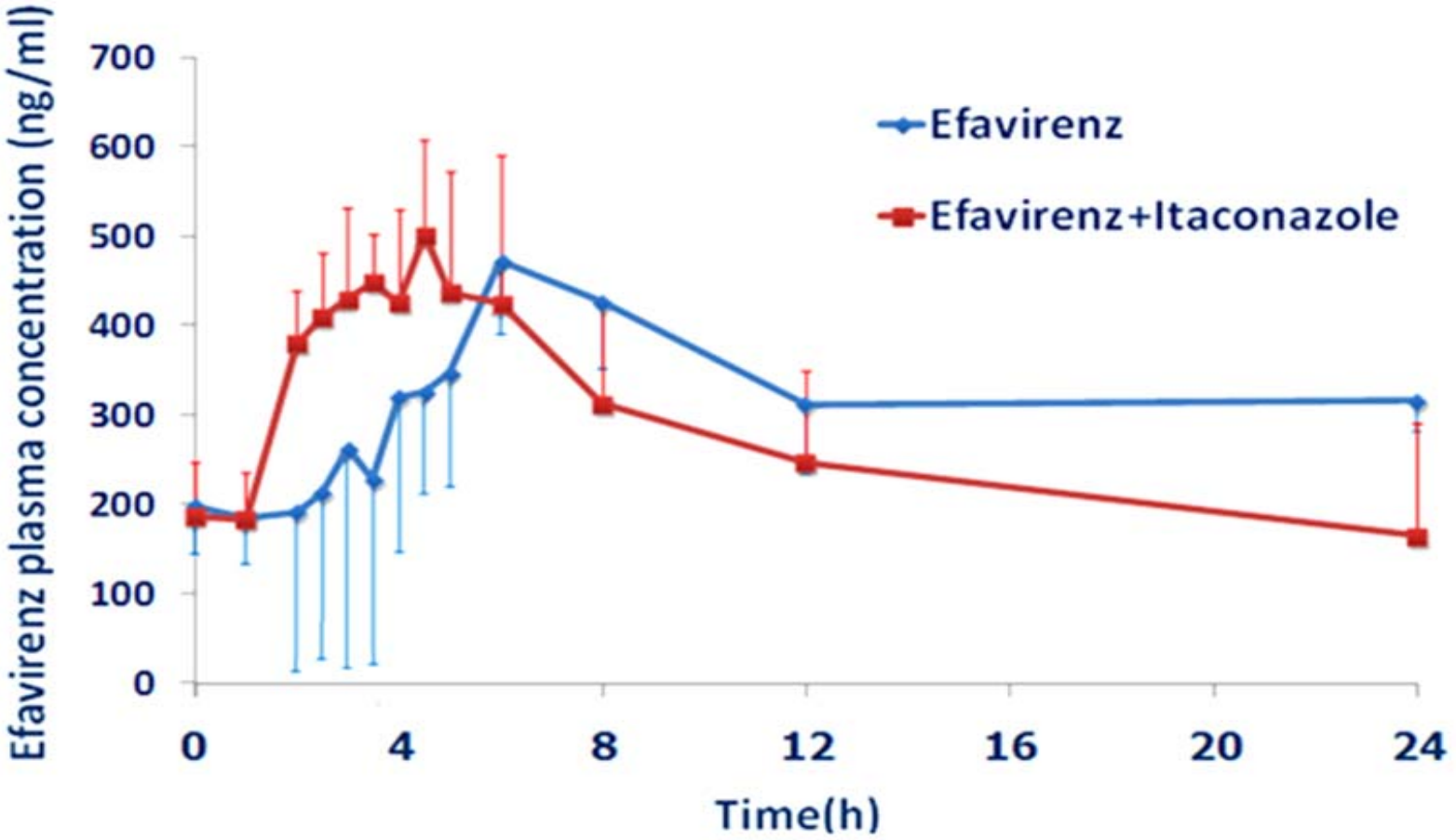


Figure 19 Mean plasma concentration-time curves of efavirenz after multiple oral doses of efavirenz alone (14 days) and in combination with itraconazole once daily for 14 days. ( ): multiple oral 600 mg efavirenz alone once daily for 14 days; ( ): multiple oral 600 mg efavirenz in combination with 200 mg itraconazole once daily for 14 days.

Table 16 Pharmacokinetic parameters of efavirenz in each of the three patients during phase 2 (efavirenz alone)

Parameters	Patient number (S1-S3)				
	1	2	3	Mean	SD
C_{max} (ng/ml)	848.70	326.30	331.40	502.13	300.15
C_{min} (ng/ml)	306.41	125.00*	125.00*	185.47	104.75
T_{max} (h)	6	6	8	6.67	1.15
AUC_{0-24} (ng·h/ml)	13,691.92	5,014.79	4,529.09	7,745.27	5,155.68
$AUC_{0-\infty}$ (ng·h/ml)	15,504.99	7,377.03	5,533.64	9,471.89	5,305.49
λ_z (h^{-1})	0.03	0.09	0.12	0.08	0.05
$t_{1/2,z}$ (h)	41.00	17.40	15.58	24.66	14.18
Cl/f (l/h)	0.06	0.08	0.10	0.08	0.02
V_z/f (l/kg)	3.53	2.86	1.87	2.75	2.84

* Calculated from LOD of efavirenz

Table 17 Pharmacokinetics parameters of efavirenz in each of the three patients during phase 3 (concomitant efavirenz & itraconazole)

Parameters	Patient number (S1-S3)				
	1	2	3	Mean	SD
C_{max} (ng/ml)	1,024.91	372.80	379.60	592.44	374.55
C_{min} (ng/ml)	244.58	125.00*	125.00*	164.86	69.04
T_{max} (h)	3.5	4.5	6	4.67	1.26
AUC_{0-24} (ng·h/ml)	11,147.83	8,361.75	4,608.36	8,039.31	3,281.64
$AUC_{0-\infty}$ (ng·h/ml)	17,398.48	16,124.69	6,896.48	13,473.22	5,731.12
λ_z (h^{-1})	0.04	0.02	0.06	0.04	0.02
$t_{1/2,z}$ (h)	45.81	17.87	15.66	26.44	16.80
Cl/f (l/h)	0.04	0.04	0.08	0.05	0.03
V_z/f (l/kg)	3.88	3.65	1.59	30.4	1.26

* Calculated from LOD of efavirenz

Table 18 Effect of itraconazole on efavirenz pharmacokinetics in each of three HIV-infected patients

Patient No.	C_{max} (ng/ml)		C_{min} (ng/ml)		T_{max} (h)		AUC_{0-24} (ng·h/ml)		$AUC_{0-\infty}$ (ng·h/ml)		$t_{1/2}$ (h)		Cl(l/h)	
	Phase		Phase		Phase		Phase		Phase		Phase		Phase	
	EFV	**EFV+ITR**	**EFV**	**EFV+ITR**	**EFV**	**EFV+ITR**	**EFV**	**EFV+ITR**	**EFV**	**EFV+ITR**	**EFV**	**EFV+ITR**	**EFV**	**EFV+ITR**
1	848.70	1,024.91	306.41	244.58	6	3.5	13,691.92	11,147.83	15,504.99	17,398.48	41.00	45.81	0.06	0.04
2	326.30	372.80	125.00	125.00	6	4.5	5,014.79	8,361.75	7,377.03	16,124.69	17.40	17.87	0.08	0.04
3	331.40	379.60	125.00	125.00	8	6	4,529.09	4,608.36	5,533.64	6,896.48	15.58	15.66	0.10	0.08
Mean	502.13	592.44	185.47	164.86	6.67	4.67	7,745.27	8,039.31	9,471.89	13,473.22	24.66	26.44	0.08	0.05
SD	300.15	374.55	104.75	69.04	1.15	1.26	5,155.68	3,281.64	5,305.49	5,731.12	14.18	16.80	0.02	0.03
p-value	0.17		0.42		0.02		0.88		0.23		0.86		0.06	

CHAPTER 5

DISCUSSION AND CONCLUSION

Administration of the multiple and long-term antiretroviral therapy in HIV-infected patients is not only for treatment of AIDS, but also for strengthening the immune system and therefore helps protect against opportunistic infections (Fauci, 2004). Opportunistic infections have been defined as infections that are more frequent or more severe because of immunosuppression (CD_4^+ cell counts have dropped to less than 200 cells/mm^3) in patients with HIV disease. As treatments for some common opportunistic pathogens and management of AIDS patients permits longer survivals. The spectrum of opportunistic infections range from fungal infection, bacterial and mycobacterial infections, parasitic infection and viral infection. Cryptococcosis, pneumocystis carinii pneumonia and candidiasis are common opportunistic infections in HIV-infected patients. Itraconazole, an orally absorbed triazole derivative, is an effective and well tolerated systemic drug used for treatment of fungal infections. The treatment of HIV is defined as treatment with at least three active antiretroviral medications typically two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) plus non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs). The issue of drug-drug interactions arises as one of the major problems associated the current therapy.

Itraconazole is a broad-spectrum triazole antifungal agent which is a primarily fungistatics at clinically achievable serum concentrations and acts by impairing the synthesis of ergosterol, an essential component of the fungal cell membrane. A prospective, randomized trial in HIV positive and AIDS patients with oropharyngeal candidosis using oral solution 200 mg/day of itraconazole has found that the clinical response rates were 97% with few adverse effects of drug. Steady-state of itraconazole concentrations are reached after 14 days of oral administration in humans (Thompson *et al.,* 2009). Itraconazole is extensively metabolized by various pathways, mainly oxidation, into a large number of metabolites. Hydroxyitraconazole is a major metabolite in human. There have been

few studies of the pharmacokinetics interaction between itraconazole and antiretroviral drugs. There is one case report showed interaction between itraconazole and nonnucleoside reverse-transcriptase inhibitors (efavirenz) in patient with AIDS that had been receiving antiretroviral therapy consisting of efavirenz, lamivudine and stavudine, while receiving itraconazole capsules (200 mg once daily) for treatment of disseminated histoplasmosis. Over one year follow up, it's found the patient's urine *Histoplasma* antigen level was not decreased and plasma itraconazole concentration could not be detected (<0.05 μg/ml) (Koo *et al.,* 2007). Retrospective cohort study in ten HIV-infected patients treated with antiretroviral therapy (NNRTIs-based regimen) and itraconazole for disseminated histoplasmosis between January 2003 and December 2006, clinical data of HIV-infected patients demonstrated that coadministration of an NNRTIs and itraconazole resulted in significant decrease in itraconazole blood concentrations likely by induction the CYP3A4 enzyme system (Andrade *et al.,* 2009). Efavirenz is an antiretroviral drug that belongs to the class of drugs called non-nucleoside reverse transcriptase inhibitors (NNRTIs) used as part of highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV) (Adkins *et al.,* 1998; Maggiolo, 2009; DHHS, 2012). In our study, all patients received an oral triple antiretroviral therapy that included efvirenz and two NRTIs, lamivudine and stavudine. There is no evidence that any of this NRTIs drugs interacted with CYP3A4 (Berry *et al.,* 1999). Efavirenz is an enzyme inducer of hepatic drug metabolism in human and rat, especially CYP3A4, but does not modify intestinal absorption if coadministered with substrates of P-glycoprotein (Berruet *et al.,* 2005; Mouly *et al.,* 2002). In *in vitro* studies efavirenz inhibited the isozyme CYP2C9, CYP2C19 and CYP3A4 (Adkins *et al.,* 1998; VON *et al.,* 2001). It is known to interact with a number of drugs such as amprenavir, atazanavir, atorvastatin, simvastatin, pravastatin, clarithromycin, ethinyl estradiol, fluconazole, indinavir, ketoconazole, lopinavir, ritonavir, methadone, nelfinavir, rifabutin, rifampin, saquinavir and voriconazole (Adkins *et al.,* 1998; Benedek *et al.,* 1998; Joshi *et al.,* 1998; Gerzenstein *et al.,* 2000; Falloon *et al.,* 2000; Kuper *et al.,* 2000; Wintergerst *et al.,* 2000; Xavier *et al.,* 2000; Clarke *et al.,* 2001; Veldkamp *et al.,* 2001; Aarnoutse *et al.,* 2002; Spradling *et al.,* 2002; Bergshoeff *et al.,* 2003; Boyd *et al.,* 2003; Cvetkovic *et al.,* 2003; DiCenzo *et al.,* 2003; Eap *et al.,* 2004;

Edelstein *et al.,* 2004; Lee *et al.,* 2004; Micromedex, 2004; Porte *et al.,* 2004; Wire *et al.,* 2004; Dailly *et al.,* 2005; Gerber *et al.,* 2005; Hsu *et al.,* 2005; Kappelhoff *et al.,* 2005; Labbe *et al.,* 2005; Le Tiec *et al.,* 2005; Liu *et al.,* 2005; Weiner *et al.,* 2005; Bruce *et al.,* 2006; Dailly *et al.,* 2006; Sriwiriyajan *et al.,* 2007; Jamois *et al.,* 2009; Sustiva, 2011). In the clinical point of view, there is the possibility of a pharmacokinetic interaction between itraconazole and efavirenz in humans.

For the above reason, this study was designed to examine drug interaction between itraconazole and efavirenz in HIV infected patients. The aim of this study is to examine the pharmacokinetic drug interactions between itraconazole and efavirenz in HIV infected patients, in order to compare the pharmacokinetic parameters of itraconazole when administered alone and when combined with efavirenz in HIV infected patients and to compare the pharmacokinetics parameter of efavirenz when administered alone and when combined with itraconazole in HIV infected patients.

Our study design was mainly based on the knowledge of the pharmacokinetics and pharmacodynamics of itraconazole and efavirenz. The results of the present study revealed that efavirenz increased the metabolism of itraconazole when they were administered concurrently for 14 days which was due most likely to induction of CYP3A4. Moreover, itraconazole increased the absorption of efavirenz, probably due to the inhibition of P-glycoprotein but the half-life and clearance did not change when compared to efavirenz alone. Firstly, in order to compare the pharmacokinetic parameters of itraconazole when administered alone and in combination with efavirenz in HIV-infected patients, recommended doses of itraconazole for treatment of systemic and superficial fungal infection are ranging between 200 to 600 mg/day (Grant and Clissold, 1989). In the present study, itraconazole was given to the HIV-infected patients at the dose 200 mg once a day (2 capsules of 100 mg itraconazole) for 14 day, while efavirenz was given orally 600 mg once a day for 14 day at bedtime to attenuate possible central nervous system adverse effect of this drug (Deeks, 1998; Micromedex, 2004). The results of the present study showed that the pharmacokinetic parameters of itraconazole and efavirenz were calculated base on noncompartment model because the variation of these pharmacokinetic parameters may be influenced by inter-individual variations of

patients (high value of standard deviation) and environmental factors such as sex, races, diet, smoking, coffee and alcohol. The possible explanation of this variation might be due to difference in absorption as evidence by the different in bioavailability of itraconazole between oral solution and capsules in HIV-infected patients which was reduced by approximately 20% and 50% compared to normal volunteers (Smith *et al.,* 1991).

Efavirenz has been reported to be a potent inducer of CYP3A4, and to a lesser degree CYP2B6 in several *in vitro* and *in vivo* studies and also a weak substrate of P-glycoprotein (Hariparsad *et al.,* 2004; Chandler *et al.,* 2006). Certainly, it has been well documented that itraconazole is a potent inhibitor of CYP3A4 and also a substrate of CYP3A4 (Grant and Clissold, 1989). In pharmacokinetics point of views, itraconazole and ketoconazole have been reported to have a variety of drug interactions with several co-administered drugs of which their metabolism is mediated through CYP3A4. When multiple oral doses of efavirenz and NRTIs (lamivudine and stavudine) were co-administered with itraconazole for 14 days (Phase 3), the means of C_{max} of itraconazole was significantly decreased by 92.47% but the C_{min} and AUC_{0-24} of itraconazole were not significantly decreased, although C_{min} and AUC_{0-24} were decreased by 10 and 13 fold, respectively when compared to itraconazole alone (Phase 1). Enzyme induction can lead to an increased (in case of the use of a prodrug) and a decreased drug effect. The decreasing of the C_{max}, C_{min} and AUC_{0-24} of itraconazole when compared to itraconazole alone (phase 1), suggesting that the metabolism of itraconazole was increased. The T_{max} was not changed indicating that efavirenz did not affect the rate of itraconazole absorption. However, individual results had shown that the AUC_{0-24} of itraconazole were decreased by more than 10-fold after pretreatment with efavirenz. In theoretical section, increased in enzyme activity, in effect an increase in total clearance, reflected by increased hepatic clearance of drugs metabolized by the induced enzyme. Pharmacokinetically, the drugs affected by enzyme induction generally demonstrate reduced half-life ($T_{1/2}$) as a reflection of increased total clearance (Cl). But in this study evaluate the half-life ($T_{1/2}$) and total clearance (Cl) of itraconazole only 2 of 3 patients because in phase concomitant itraconazole and efavirenz of patient No.3 did not detect itraconazole level. The half-life ($T_{1/2}$) and total clearance (Cl) of itraconazole in individual patients

(P1 and P2) shown the half-life ($T_{1/2}$) shorter than itraconazole alone (Phase 1) and total clearance (Cl) were increased when compared to itraconazole alone (phase 1). The mechanism of the pharmacokinetic interaction between itraconazole and efavirenz is most to be due to induction of CYP3A4 of itraconazole by efavirenz. Efavirenz is a well known inducer of CYP3A4 (Adkins, 1998; Moyle, 1999). It has been shown that efavirenz caused a concentration-dependent CYP3A4 induction and activation of human pragnane X receptor (hPXR), a key transcriptional regular of CYP3A4, *in vitro*. In the presence of an activating ligand, PXR form a heterodimer with the retinoid X receptor (RXRα). This heterodimer binds to the xenobiotic response element (XBE) in the promoter sequence of CYP3A4, leading to increased in gene transcription (Waxman, 1999; Moore *et al.,* 2000 Hariparsad *et al.,* 2004). Although, itraconazole is a P-glycoprotein substrates (Shon *et al.,* 2005) but efavirenz did not appear to induce intestinal CYP3A4 or intestinal p-glycoprotein (Mouly *et al.,* 2002; Berruet *et al.,* 2005), hence it might has no effected on itraconazole bioavailability.

One of the objectives of this study was to compare the pharmacokinetic parameters of efavirenz when administered alone and in combination with itraconazole in HIV infected patients. The results of the present study have shown that when multiple oral doses of efavirenz were co-administered with itraconazole for 14 days,the T_{max} of efavirenz was significantly decreased from 6.67±1.15 h to 4.67±1.26 h but the means of C_{max}, C_{min}, AUC_{0-24}, $AUC_{0-\infty}$, $T_{1/2}$ and Cl of efavirenz were not significantly altered by itraconazole co-administration. (502.13±300.15 ng/ml vs 592.44±374.55 ng/ml, 85.47±104.74 ng/ml vs 164.86±69.04 ng/ml, 7,745.27±5,155.68 ng.h/ml vs 8,039.31±3,281.64 ng.h/ml, 9,471.89±5,305.49 ng.h/ml vs 13,473.22±5731.12 ng.h/ml, 24.66±14.18 h vs 26.44±16.80 h and 0.08±0.02 l/h vs 0.06±0.03, respectively) when compare to efavirenz alone (phase 2). The results suggest that when itraconazole was concomitantly administered with efavirenz the absorption of efavirenz was increased. As previous study has shown that itraconazole is an inhibitor of P-glycoprotein (Venkatakrishnan *et al.,* 2000) and efavirenz is a weak substrate of P-glycoprotein (Chandler *et al.,* 2006). Itraconazole moderately increased plasma concentrations of oral morphine, probably by enhancing its absorption by inhibiting P-glycoprotein. (Heiskanen *et al.,* 2008) and itraconazole

markedly increased the plasma concentrations of aliskiren, probably mainly explained by inhibition of the P-glycoprotein-mediated efflux of aliskiren in the small intestine (Tapaninen *et al.,* 2011). Thus, itraconazole increase absorption of efavirenz, probably due to the inhibition of P-glycoprotein. However, the half-life ($T_{1/2}$) and clearance (Cl) of efavirenz did not change when compared to efavirenz alone. These results were in contrast to the results of the study of Liu *et al*. (2007) which has shown that concomittant administration of 400 mg voriconazole with efavirenz 400 mg once daily in healthy male subject leads to significant increased in the mean AUC_{0-24} and C_{max} of efavirenz by 43% and 37%, respectively, probably due to the inhibition of CYP3A4 by voriconazole. In our experimental design, there was a limitation in duration of blood sample collection i.e.between 0 to 24 h although the half life of efavirenz is very long i.e.40-55 h. This limitation was due to ethical problem as efavirenz must be continuously given every day and we could not stop giving the drug during the course of the antiretroviral therapy. Therefore, the pharmacokinetic parameters in the elimination phase could not be calculated and inhibitory effect of CYP3A4 by itraconaconazole could not be observed.

In conclusion, in a long term efavirenz treatment it has been shown that efavirenz markedly decrease the plasma concentration of itraconazole and significantly decreased in C_{max} of itraconazole by 92.47% and decreased C_{min} and AUC_{0-24} of itraconazole by 10 and 13 folds, respectively. These effects may be mainly due to inducition of CYP3A4 isozyme by efavirenz. However, itraconazole slightly increased the absorption of efavirenz probably by inhibition of P-glycoprotein but its inhibitory activity on metabolism of efavirenz could not be demonstrated in this study due to limitation in blood sample collection. Therefore, the dosage of itraconazole should be increased and its therapeutic outcome be closely monitored when these two agents must be concomitantly administered, especially in patients who are on a long-term therapy in order to get the optimum result of therapy or considered alternative antifungal agents such as fluconazole. A change in antiretroviral therapy to a protease inhibitor-based regimen is an alternative option when itraconazole is necessary to treat HIV-infected patients.

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APPENDIX A

NNRTIs – Based Regimens (1-NNRTIs + 2-NRTIs/2-PIs)

The Panel's Recommendations

- *Preferred NNRTIs – Based Regimens :*
    - Efavirenz + Tenofovir + Emtricitabine (should not be used during the first trimester of pregnancy or in women of childbearing potential who are trying to conceive or not using effective and consistent contraception or in patients with renal insufficiency)
- *Alternative NNRTIs – Based Regimens :*
    - Efavirenz + Abacavir + Lamivudine
    - Rilpivirine + Tenofovir + Emtricitabine
    - Rilpivirine + Abacavir + + Lamivudine
- *Acceptable NNRTIs – Based Regimens :*
    - Efavirenz + Zidovudine + Lamivudine
    - Nevirapine + Tenofovir/Emtricitabine or Zidovudine/Lamivudine)
    - Nevirapine + Abacavir + + Lamivudine
    - Rilpivirine + Zidovudine + Lamivudine

The Panel does not recommend the following *NNRTIs* as initial therapy

- Nevirapine should not be used in patients with moderate to severe hepatic impairment.
- Nevirapine should not be used in women with pre-antiretroviral therapy CD_4^+ count >250 cells/mm^3 or in men with pre-antiretroviral therapy CD_4^+ count >400 cells/mm^3.
- Selection of a regimen should be individualized on the basis of virologic efficacy, toxicity, pill burden, dosing frequency, drug-drug interaction potential, resistance testing results, and comorbid conditions.

Referance: DHHS. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. 27 March, 2012: pp F3-F4.

APPENDIX B

SUB.EC 51-031-19-2-1

คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์
ตำบลคอหงส์ อำเภอหาดใหญ่
จังหวัดสงขลา 90110

หนังสือรับรองนี้ให้ไว้เพื่อแสดงว่า

โครงการวิจัยเรื่อง : ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาอีฟาวิเร็นซ์กับไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวี

หัวหน้าโครงการ : ผู้ช่วยศาสตราจารย์ นายแพทย์วีรวัฒน์ มหัทธนตระกูล
ภาควิชา/คณะ : ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านกระบวนการพิจารณารับรองจากคณะอนุกรรมการพิจารณาจริยธรรมการวิจัยในคนจากเวชระเบียนและสิ่งส่งตรวจจากร่างกายมนุษย์ ของคณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 1 กันยายน 2551

.....ประธานอนุกรรมการ
(รองศาสตราจารย์นายแพทย์วีระพล จันทร์ดียิ่ง)
รองคณบดีฝ่ายวิจัย

SUB.EC 51-031-19-2-1

Documentary Proof of Ethical Clearance

The Ethics Committee, Faculty of Medicine, Prince of Songkla University

The Project Entitled : Pharmacokinetic Interaction between Efavirenz and Itraconazole in HIV-infected Patients

Principal Investigator : Werawath Mahatthanatrakul, M.D.

Approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University.

Date of Approval : September 1, 2008

Verapol Chandeying

(Assoc.Prof. Verapol Chandeying, M.D.)

Associate Dean for Research Affairs

วท-จธ/51/12-1

ที่ ศธ 0521.1.09/ 784

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90112

หนังสือรับรองโครงการวิจัย

การศึกษาวิจัยที่ทำการทดลองในมนุษย์เรื่อง : ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาอีฟาวิเร็นซ์ กับไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวี

หัวหน้าโครงการวิจัย : ผู้ช่วยศาสตราจารย์ นายแพทย์วีรวัฒน์ มหัทธนตระกูล
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณาและเห็นชอบจาก คณะกรรมการจริยธรรมการวิจัยที่ทดลองในมนุษย์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 8 กันยายน 2551

(รองศาสตราจารย์ ดร.วิไลวรรณ โชติเกียรติ)
รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา ปฏิบัติราชการแทน
คณบดีคณะวิทยาศาสตร์

ใบยินยอมเข้าร่วมโครงการ

ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาอีฟาวิเร็นซ์กับไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวี

ก่อนที่จะลงนามในใบยินยอมในการทำวิจัยนี้ ข้าพเจ้า (นาย, นาง, นางสาว).....
นามสกุล.....ได้รับคำอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมถึงประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว ข้าพเจ้ายังได้รับสำเนาของหนังสือฉบับนี้ รวมทั้งเอกสารแนบท้ายเชิญชวนร่วมโครงการ 1 ชุด และอ่านข้อความข้างต้นทั้งหมดแล้ว

ผู้วิจัยขอรับรองว่าจะตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดปังซ่อนเร้น จนข้าพเจ้าพอใจ

ในระหว่างร่วมโครงการ หากการกระทำและคำชี้แจงนี้ของผู้วิจัยยังไม่เป็นที่เข้าใจ ข้าพเจ้าจะมีสิทธิ์แจ้งต่อประธานอนุกรรมการจริยธรรมของคณะวิทยาศาสตร์ได้ (รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา โทร.(074)446660) และหากข้าพเจ้าไม่พอใจในการเข้าร่วมโครงการ ข้าพเจ้ามีสิทธิ์ปฏิเสธการเข้าร่วมในโครงการได้ทันที โดยไม่เสียสิทธิ์ในการรับการรักษาในโรงพยาบาลสงขลานครินทร์ต่อไป

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

.....	.....
(ลายเซ็นของผู้ยินยอม)	วัน เดือน ปี
.....	.....
(ลายเซ็นของพยาน)	วัน เดือน ปี
.....	.....
(ลายเซ็นของแพทย์)	วัน เดือน ปี

เอกสารเชิญชวนเข้าร่วมในโครงการ

ชื่อเรื่อง ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาอีฟาวิเร็นซ์กับไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวี

เรียน ท่านผู้อ่านที่นับถือ

ท่านกำลังได้รับการเชิญชวนให้เข้าร่วมในโครงการวิจัยเรื่องดังกล่าวข้างต้น เพราะท่านได้รับการวินิจฉัยว่ามีการติดเชื้อไวรัสเอชไอวี ที่ทำให้ภูมิคุ้มกันของท่านลดลง เอกสารนี้จะให้ข้อมูลเพื่อช่วยให้ท่านตัดสินใจว่าจะเข้าร่วมโครงการนี้หรือไม่ ท่านควรจะได้เข้าใจรายละเอียดที่เกี่ยวข้องทั้งหมดก่อนที่ท่านจะตกลงเข้าร่วมการศึกษานี้ หากท่านยังมีคำถามที่ยังไม่ได้รับการอธิบายโดยละเอียดในเอกสารนี้ โปรดสอบถามแพทย์ที่ร่วมทำการศึกษานี้ได้ ท่านไม่ควรตกลงเข้าร่วมโครงการหากยังไม่เข้าใจวิธีการที่เกี่ยวข้องอย่างละเอียดดี

ลักษณะและวัตถุประสงค์ของการศึกษา

โครงการนี้เป็นการศึกษาเปรียบเทียบทางด้านเภสัชจลนศาสตร์เพื่อศึกษาถึงอิทธิพลต่อกันทางเภสัชจลนศาสตร์ของยาอีฟาวิเร็นซ์และยาไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวี ซึ่งยาทั้งสองนี้จะบริหารร่วมกันในผู้ป่วยติดเชื้อเอชไอวี แพทย์ผู้ทำการศึกษาวิจัยจะขอให้ท่านพิจารณาเข้าร่วมในโครงการนี้ โดยที่การศึกษานี้จะแสดงให้เห็นถึงอิทธิพลต่อกันทางเภสัชจลนศาสตร์ของยาอีฟาวิเร็นซ์และยาไอทราโคนาโซลในผู้ป่วยติดเชื้อเอชไอวีว่ามีมากน้อยเพียงใด ซึ่งจะมีผลต่อการกำหนดเป็นแนวทางในการบริหารยาทั้งสองร่วมกัน

วิธีการ

การศึกษานี้จะเริ่มด้วยการคัดเลือกผู้ป่วยเข้าร่วมในโครงการศึกษา โดยแพทย์ผู้ทำการศึกษาจะซักประวัติทางการแพทย์ ตรวจร่างกาย ตรวจทางห้องปฏิบัติการ ได้แก่ การตรวจนับเม็ดเลือดสมบูรณ์ การตรวจนับเม็ดเลือดขาวซีดีโฟร์ หน้าที่การทำงานของตับและไต การฉายภาพรังสีทรวงอก และตรวจการตั้งครรภ์ ซึ่งเป็นการตรวจปกติที่แพทย์จะต้องทำต่อผู้ป่วยในกรณีดังกล่าวอยู่แล้ว

ผู้ป่วยที่ได้รับการคัดเลือกทุกคนจะต้องเข้าร่วมโครงการทั้งหมด 42 วัน

ในการทดลองนี้ใช้ผู้ป่วยจะได้รับยาไอทราโคนาโซล ขนาด 200 มิลลิกรัม (ครั้งละ 2 แคปซูล, แคปซูลละ 100 มิลลิกรัม) ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 20.00 น. นาน 14 วัน โดยวันที่ 14 ของการทดลอง จะให้ผู้ป่วยเข้านอนในโรงพยาบาล ในเย็นวันที่ 14 หลังได้รับยา

ในระหว่างทำการทดลองจะมีการเฝ้าติดตามอาการและตรวจร่างกาย รวมทั้งการตรวจทางห้องปฏิบัติการที่จำเป็น เพื่อเฝ้าระวังผลข้างเคียงที่อาจเกิดขึ้นได้ ถ้าเกิดผลข้างเคียงของยาที่รุนแรงจะหยุดการทดลองทันที และผู้ป่วยจะได้รับการดูแลโดยแพทย์ในโครงการอย่างใกล้ชิด

<u>การทดลองนี้มีความเสี่ยงอะไรบ้าง</u>

การเจาะเลือดจะใช้วิธีเก็บจากสายที่คาไว้ในหลอดเลือดดำ ดังนั้น ท่านจะเจ็บครั้งแรกตอนเจาะเลือดเพื่อคาสายไว้ในหลอดเลือดดำเท่านั้น หลังจากนั้นเวลาเก็บเลือดแต่ละครั้งจะไม่เจ็บ การคาสายไว้ในหลอดเลือดดำจะเกิดผลแทรกซ้อนน้อยมาก ได้แก่ การอักเสบของหลอดเลือดดำ เป็นต้น

ส่วนยาที่ท่านได้รับเป็นยาที่ใช้ในการรักษาตามมาตรฐาน โดยยาอีฟาวิเร็นซ์เป็นยาที่อาจเกิดผลข้างเคียงบ้าง ได้แก่ ปวดศีรษะ มึนงง ผื่นผิวหนัง ฝันประหลาด เป็นต้น ส่วนยาไอทราโคนาโซลอาจทำให้เกิดผลข้างเคียง ได้แก่ คลื่นไส้ อาเจียน ปวดศีรษะ เป็นต้น

ประโยชน์ที่คาดว่าจะได้รับ

การเข้าร่วมในโครงการศึกษานี้ ท่านจะไม่ต้องเสียค่าใช้จ่ายค่ายาในระหว่างทำการศึกษา ท่านจะได้รับการดูแลเป็นอย่างดีโดยแพทย์ผู้มีประสบการณ์ด้านนี้โดยเฉพาะ และประการสุดท้ายที่สำคัญคือข้อมูลที่ได้จากการเข้าร่วมการทดลองของท่านจะเป็นประโยชน์อย่างมหาศาลต่อการกำหนดแนวทางในการบริหารยาอีฟาวิเร็นซ์และไอทราโคนาโซลร่วมกัน

นอกจากนี้ท่านจะได้รับค่าตอบแทนในการเข้าร่วมโครงการวิจัยนี้เป็นเงิน 3,000 บาท (สามพันบาทถ้วน) เมื่อเข้าร่วมโครงการจนครบตามระยะเวลาที่กำหนด

ผู้ที่ท่านติดต่อ

หากท่านมีคำถามเพิ่มเติมเกี่ยวกับการศึกษาวิจัยนี้ หรือต้องการสอบถามเกี่ยวกับสิทธิในฐานะผู้เข้าร่วมในการศึกษา ท่านสามารถติดต่อกับ นายแพทย์วีรวัฒน์ มหัทธนตระกูล ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์(074)2888174 หรือนายแพทย์สุเทพ จารุรัตนศิริกุล ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์ (074)451485

การเก็บข้อมูลเป็นความลับ

ข้อมูลทุกอย่างที่ได้จากผลการทดลอง อันเนื่องมาจากการเข้าร่วมการทดลองครั้งนี้จะถูกเก็บเป็นความลับอย่างเคร่งครัด ท่านจะได้รับแจ้งข้อมูลที่มีความสำคัญต่อสุขภาพของท่าน แต่จะไม่มีการเปิดเผยข้อมูลดังกล่าวต่อบุคคลที่ 3 ผลจากการศึกษาทั้งหมดจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย

อาสาสมัคร

เกณฑ์การคัดเลือกอาสาสมัครเข้าร่วมโครงการ (Inclusion criteria)

- อาสาสมัครอายุมากกว่าหรือเท่ากับ 18 ปี
- ติดเชื้อ HIV ที่มี CD^{+}_{4} น้อยกว่าหรือเท่ากับ 350 เซลล์/ลบ.มม.
- ผ่านการตรวจสัญญาณชีพ (Vital signs) เป็นปกติ
- ยินยอมเข้าร่วมการศึกษาด้วยความเต็มใจ และลงนามในหนังสือแสดงความยินยอมแล้ว

เกณฑ์การคัดเลือกอาสาสมัครออกจากการศึกษา (Exclusion criteria)

- ไตวาย โดยมีค่า Creatinine (Cr) มากกว่าหรือเท่ากับ 1.4
- ค่า SGOT และ SGPT มากกว่า 3 เท่าของค่าปกติ
- อยู่ในภาวะช๊อค
- มีประวัติการแพ้ยา Itraconazole , Efavirenz และยาอื่นที่มีสูตรโครงสร้างทางเคมีคล้ายกัน
- อาเจียน
- ท้องเสีย
- หญิงตั้งครรภ์และ/หรือให้นมบุตร
- มีประวัติการดื่มสุราเป็นประจำ และมีการใช้สารเสพติด
- มีประวัติการสูบบุหรี่เป็นประจำ (มากกว่า 10 มวนต่อวัน) หรือมีการสูบบุหรี่ปานกลาง(น้อยกว่า 10 มวนต่อวัน) และไม่สามารถอดการสูบบุหรี่ได้ก่อนเริ่มการศึกษา
- ได้รับยาต่อไปนี้ในระยะ 6 เดือนก่อนเริ่มทำการทดลอง ได้แก่ Ketoconazole, Itraconazole, Efavirenz, Terfenadine, Astemizole, Loratadine, Erythromycin, Clarithromycin, Nevirapine, Rifampicin และ Protease inhibitors เป็นต้น รวมทั้งยาอื่นๆที่มีผลยับยั้งหรือกระตุ้นการทำงานของ Enzyme CYP450 โดยเฉพาะ 3A4 (ซึ่งต้องมีการพิจารณาเป็นรายๆไป)
- เคยเข้าร่วมการทดลองการศึกษาทางคลินิกอื่นๆภายใน 1 เดือนก่อนการศึกษา

ภายหลังจากการซักประวัติและตรวจร่างกายเพื่อคัดเลือกผู้ป่วยร่วมในโครงการแล้วผู้ป่วยเหล่านี้จะถูกบันทึกข้อมูลพื้นฐาน ได้แก่ อายุ เพศ น้ำหนัก ผลการตรวจทางห้องปฏิบัติการ ได้แก่ (CBC, CD4, Blood glucose, BUN, creatinine, liver function test และ pregnancy test)

เกณฑ์การถอนตัวออกจากการศึกษา (Subject withdrawal criteria)

- อาสาสมัครเกิดอาการไม่พึงประสงค์ที่แพทย์เห็นควรให้ออกจากการศึกษา
- อาสาสมัครไม่ปฏิบัติตนตามข้อกำหนดของการศึกษา
- อาสาสมัครต้องการถอนตัวออกจากการศึกษา

ยาที่ใช้ในโครงการ

- Efavirenz ได้รับบริจาคจาก M&D ประเทศไทย
- Itraconazole ซื้อจากบริษัท Janssen ประเทศไทย

วิธีการบริหารยา

อาสาสมัครที่ได้รับการคัดเลือกทุกคนจะต้องเข้าร่วมโครงการทั้งหมด 3 ระยะๆละ 14 วัน

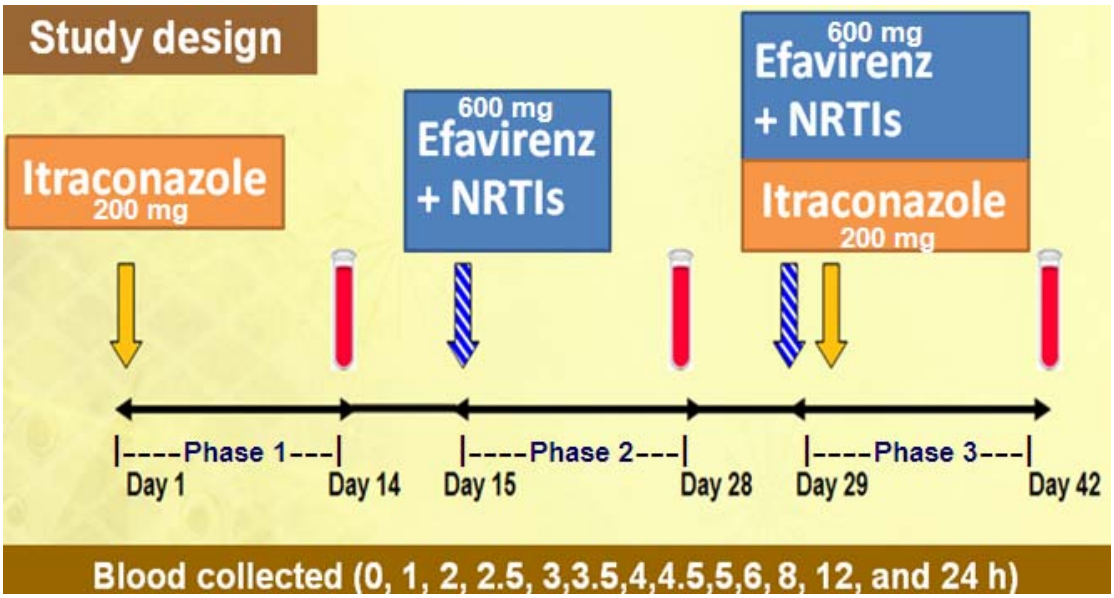
- **ระยะที่ 1** ผู้ป่วยจะได้รับยา itraconazole ขนาด 200 มิลลิกรัม (100 มิลลิกรัม 2 แคปซูล) ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 20.00 นาฬิกา นาน 14 วัน โดยในเช้าวันที่ 14 ของการทดลอง จะให้ผู้ป่วยเข้าพัก (admit) ในโรงพยาบาล ในเย็นวันที่ 14 หลังได้รับยา itraconazole ขนาด 200 มิลลิกรัม เพียงอย่างเดียวพร้อมกับน้ำดื่ม 200 มิลลิลิตร หลังอาหารเวลา 20.00 นาฬิกา อาสาสมัครจะได้รับการคาสายพลาสติกไว้ในหลอดเลือดดำที่แขน (คา heparin lock) พร้อมทั้งรับประทานอาหารและเครื่องดื่มที่ผู้วิจัยจัดเตรียมให้ และจะต้องได้รับการเก็บตัวอย่างเลือดเพื่อนำไปวิเคราะห์ต่อไป โดยผู้ป่วยจะกลับบ้านได้หลังจากเจาะเลือดที่เวลา 24 ชั่วโมงหลังรับประทานยา

- **ระยะที่ 2** ผู้ป่วยจะได้รับยา efavirenz ขนาด 600 มิลลิกรัม ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 20.00 นาฬิกา นาน 14 วัน (day 15 ถึง day 28) ร่วมกับยาต้านไวรัสตัวอื่น (Lamivudine+Tenofovir) โดยในเช้าวันที่ 28 ของการทดลอง จะให้ผู้ป่วยเข้าพัก (admit) ในโรงพยาบาล ในเย็นวันที่ 28 หลังได้รับยา efavirenz ขนาด 600 มิลลิกรัม ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 20.00 นาฬิกา ร่วมกับยาต้านไวรัสตัวอื่น (Lamivudine+Tenofovir) อาสาสมัครจะได้รับการคาสายพลาสติกไว้ในหลอดเลือดดำที่แขน (คา heparin lock) พร้อมทั้งรับประทานอาหารและเครื่องดื่มที่ผู้วิจัยจัดเตรียมให้ และจะต้องได้รับการเก็บตัวอย่างเลือดเพื่อนำไปวิเคราะห์ต่อไป โดยผู้ป่วยจะกลับบ้านได้หลังจากเจาะเลือดที่เวลา 24 ชั่วโมงหลังรับประทานยา

- **ระยะที่ 3** ผู้ป่วยยังคงได้รับยา efavirenz ขนาด 600 มิลลิกรัม และยาต้านไวรัสตัวอื่น (Lamivudine+Tenofovir) ร่วมกับยา itraconazole ขนาด 200 มิลลิกรัม (100 มิลลิกรัม 2 แคปซูล) โดยรับประทานทั้งหมดเวลา 20.00 นาฬิกาตามด้วยน้ำ 200 มิลลิลิตร หลังอาหาร นาน 14 วัน (day 29 ถึง day 42) โดยในเช้าวันที่ 42 ของการทดลอง จะให้ผู้ป่วยเข้าพัก (admit) ในโรงพยาบาล ในเย็นวันที่ 42 หลังได้รับยา efavirenz ขนาด 600 มิลลิกรัมและยาต้านไวรัสตัวอื่น (Lamivudine+Tenofovir) ร่วมกับยา itraconazole ขนาด 200 มิลลิกรัม ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 20.00 นาฬิกา อาสาสมัครจะได้รับการคาสายพลาสติกไว้ในหลอดเลือดดำที่แขน (คา heparin lock) พร้อมทั้งรับประทานอาหารและเครื่องดื่มที่ผู้วิจัยจัดเตรียมให้ และจะต้องได้รับการเก็บตัวอย่างเลือดเพื่อนำไปวิเคราะห์ต่อไป โดยผู้ป่วยจะกลับบ้านได้หลังจากเจาะเลือดที่เวลา 24 ชั่วโมงหลังรับประทานยา

ในระหว่างที่อาสาสมัครรับประทานยาที่บ้าน ผู้ทำการทดลองได้ให้สมุดบันทึกการรับประทานยาไปด้วยเพื่อให้อาสาสมัครจดบันทึกวันและเวลาในการรับประทานยา เมื่ออาสาสมัคร

ในระหว่างทำการทดลองจะมีการเฝ้าติดตามอาการและตรวจร่างกายรวมทั้งการตรวจทางห้องปฏิบัติการที่จำเป็น เพื่อเฝ้าระวังผลข้างเคียงของยาที่อาจเกิดขึ้นได้ ถ้าเกิดผลข้างเคียงของยาที่รุนแรงจะหยุดการทดลองทันที และผู้ป่วยจะได้รับการดูแลโดยแพทย์ในโครงการอย่างเหมาะสม



การเก็บตัวอย่างเลือด

อาสาสมัครจะถูกเก็บเลือดปริมาตร 5-7 มิลลิลิตร ผ่านทาง heparin lock ที่คาไว้ในหลอดเลือดดำที่แขน ณ เวลา 0 (ก่อนบริหารยา), 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12 และ 24 ชั่วโมงหลังบริหารยาในวันที่ 14 ทั้งในระยะที่ 1, 2 และ 3 ใส่ในหลอดทดลองที่มี heparin 1: 5,000 จำนวน 20 µl นำเลือดที่ได้ไปปั่นแยกพลาสมาและเก็บไว้ที่อุณหภูมิ -70°C จนกว่าจะนำไปวัดระดับยาด้วยเครื่อง HPLC ตามวิธีที่เหมาะสมต่อไป

หมายเลขโทรศัพท์ติดต่อ

รศ.นพ.วีรวัฒน์ มหัทธนตระกูล 074-288174; 089-9749089

ศ.นพ.สุเทพ จารุรัตนศิริกุล 074-281485

ตารางบันทึกเวลาการรับประทานยา

ชื่อผู้ป่วย นาย/นาง/นางสาว_____

วันที่	เวลา	เวลาจริง	ลงชื่อ
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		
___/___/___	20.00 น.		

*** ยาวันที่ 14 ของการศึกษาให้มารับประทานก่อนการเจาะเลือด**

ข้อปฏิบัติ

1. ทานยา จำนวนตามที่แพทย์สั่ง วันละ 1 ครั้ง เวลา 2 ทุ่ม หากมีปัญหาไม่สามารถทานยาเวลา 2 ทุ่ม สามารถทานยาได้ในช่วงเวลา 19.30 – 20.30 น. หากลืมทานในเวลาที่กำหนด ให้ทานทันทีที่นึกได้ ก่อนนอนในวันนั้น

   *** หากนึกได้ตอนเช้าไม่ต้องทานแต่ให้ลงบันทึกว่า* "ลืมทานยา" *ที่ช่องลงเวลา*
2. ยาที่แพทย์สั่งเป็นยาที่ต้องทานอย่างต่อเนื่องอย่างเคร่งครัด อย่าลืมกินยาหรืออย่าหยุดยาเองเพื่อสุขภาพที่ดีของคุณ
3. หากพบว่าผลข้างเคียงของยารุนแรง ให้ติดต่อ นพ.สุเทพ จารุรัตนศิริกุล
   โทร.074-451452, 074-451485
4. เบอร์โทรนักศึกษาผู้ทำวิจัย 086-7051910 กฤษณา

นัดครั้งต่อไปวันที่.....เวลา.....น. สถานที่.....

จำนวนยาที่ได้ไปวันนี้มีจำนวน.....เม็ด นัดครั้งต่อไปจะต้องเหลือยาจำนวน.....เม็ด

APPENDIX C

Table 19 Demographic data, laboratory results and concurrent drug used during the study data of the patients in the study

Data	Patient No.			
	1	2	3*	4
Sex	Male	Male	Female	Female
CD_4^+ cell count (cells/mm^3)	324	170	47	172
WBC (cells/mm^3)	7.77×10^3	6.52×10^3	4.47×10^3	10.84×10^3
RBC (cells/mm^3)	3.32×10^6	3.23×10^6	4.35×10^6	3.43×10^6
Platelet (cells/mm^3)	271×10^3	446×10^3	230×10^3	441×10^3
Hb (g/dl)	10.5	9.4	12.4	10.3
Hct (%)	30.7	29.2	38.3	31.5
BUN (mg%)	11.5	13.5	6.4	6.7
Creatinine (mg%)	0.71	1.00	0.85	0.82
Toatal bilirubin (mg%)	0.16	0.12	0.15	0.07
SGOT (U/L)	26	22	32	26
SGPT (U/L)	16	31	35	24
Concurrent drug used during the study	Aspirin Carvedilol Enalapril Furosemide Cilostazol Spinolactone	-	TA cream 0.1% Vitamin B complex Azithromycin Dapsone	Ferrous fumarate

* Patient was withdrawn from the study due to adverse effect reaction.

APPENDIX D

Profile of plasma itraconazole concentration at each time point of blood drawn from individual patient

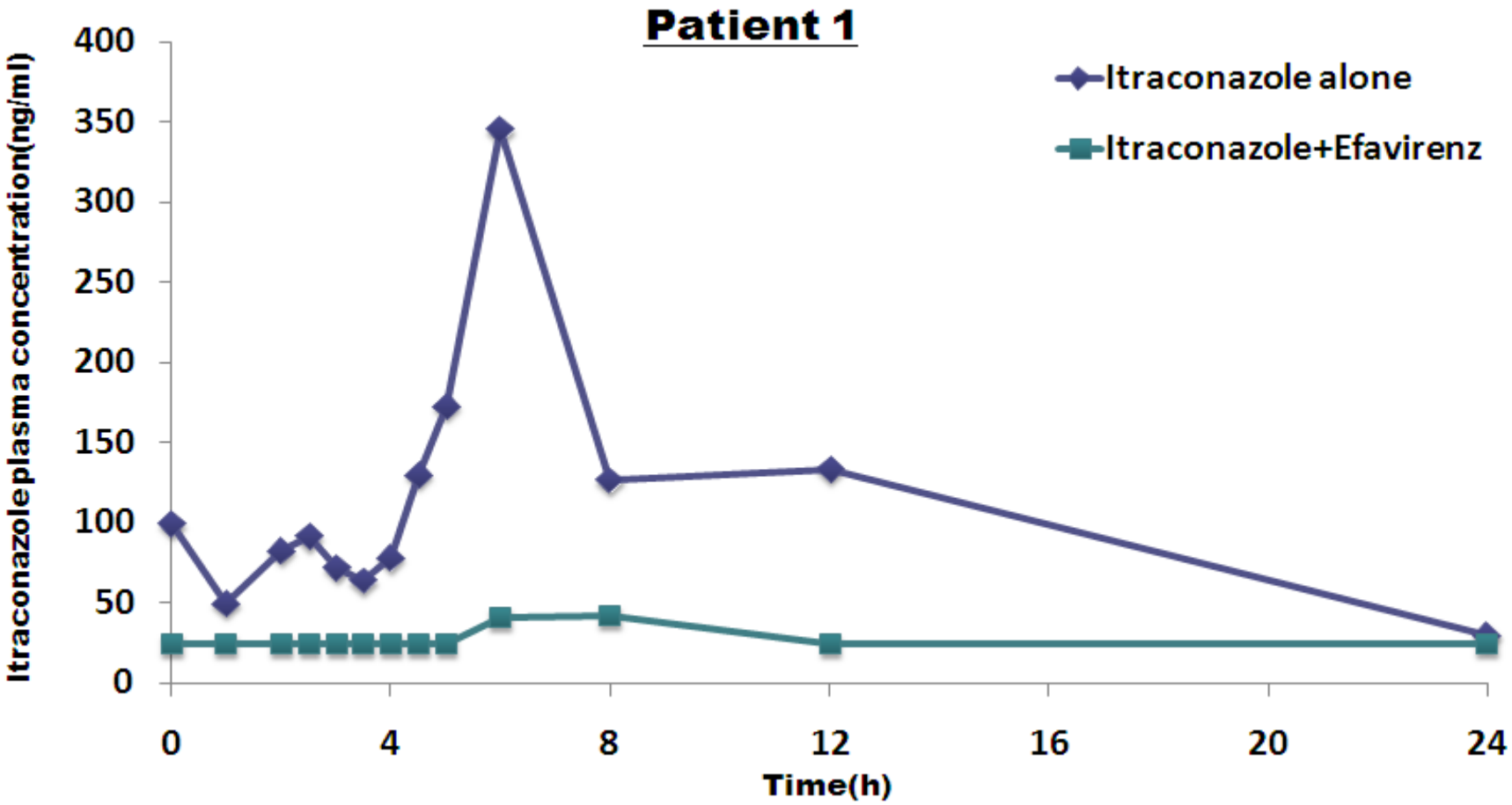


Figure 20 Plasma itraconazole concentration-time curve of patient No.1

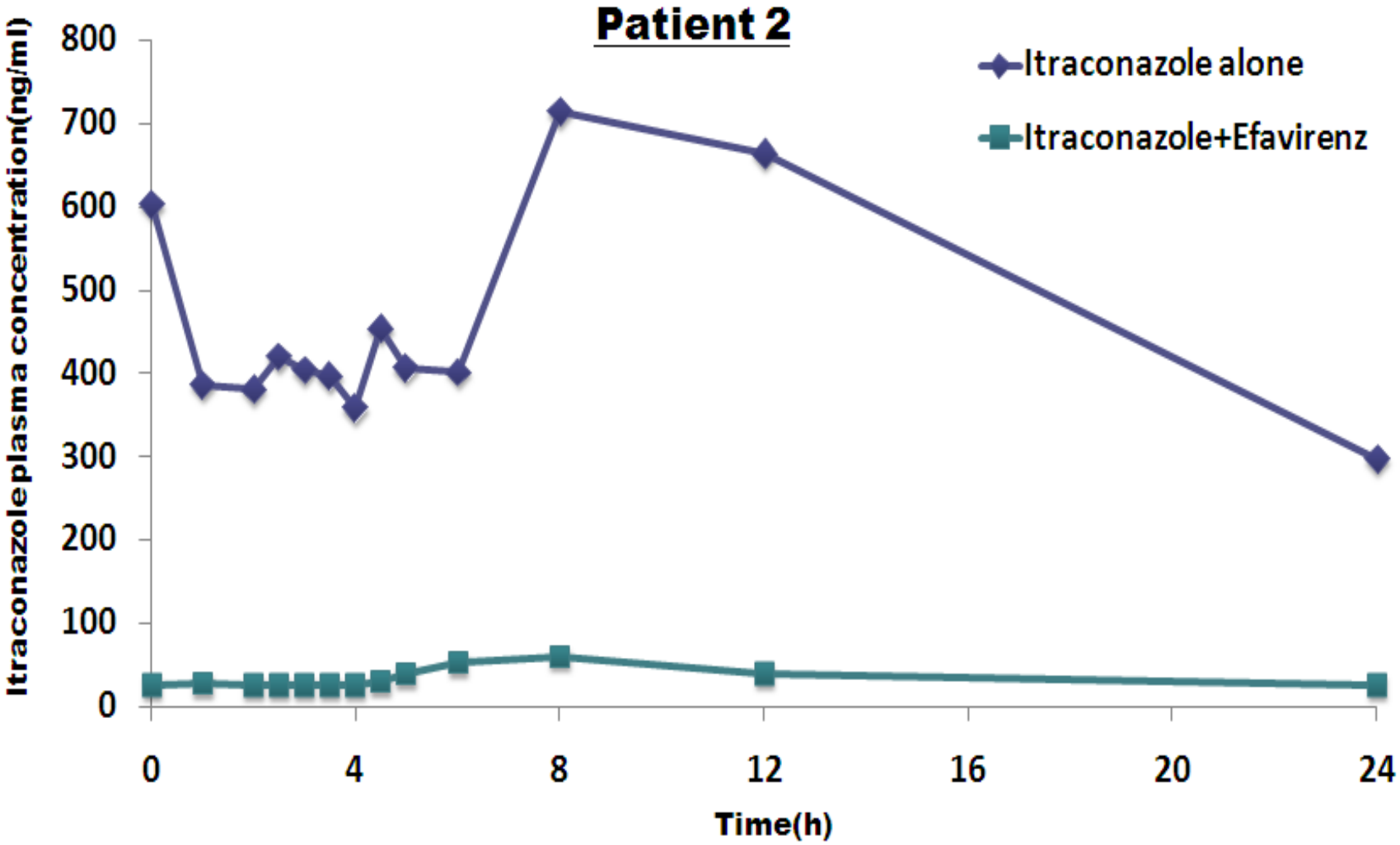


Figure 21 Plasma itraconazole concentration-time curve of patient No.2

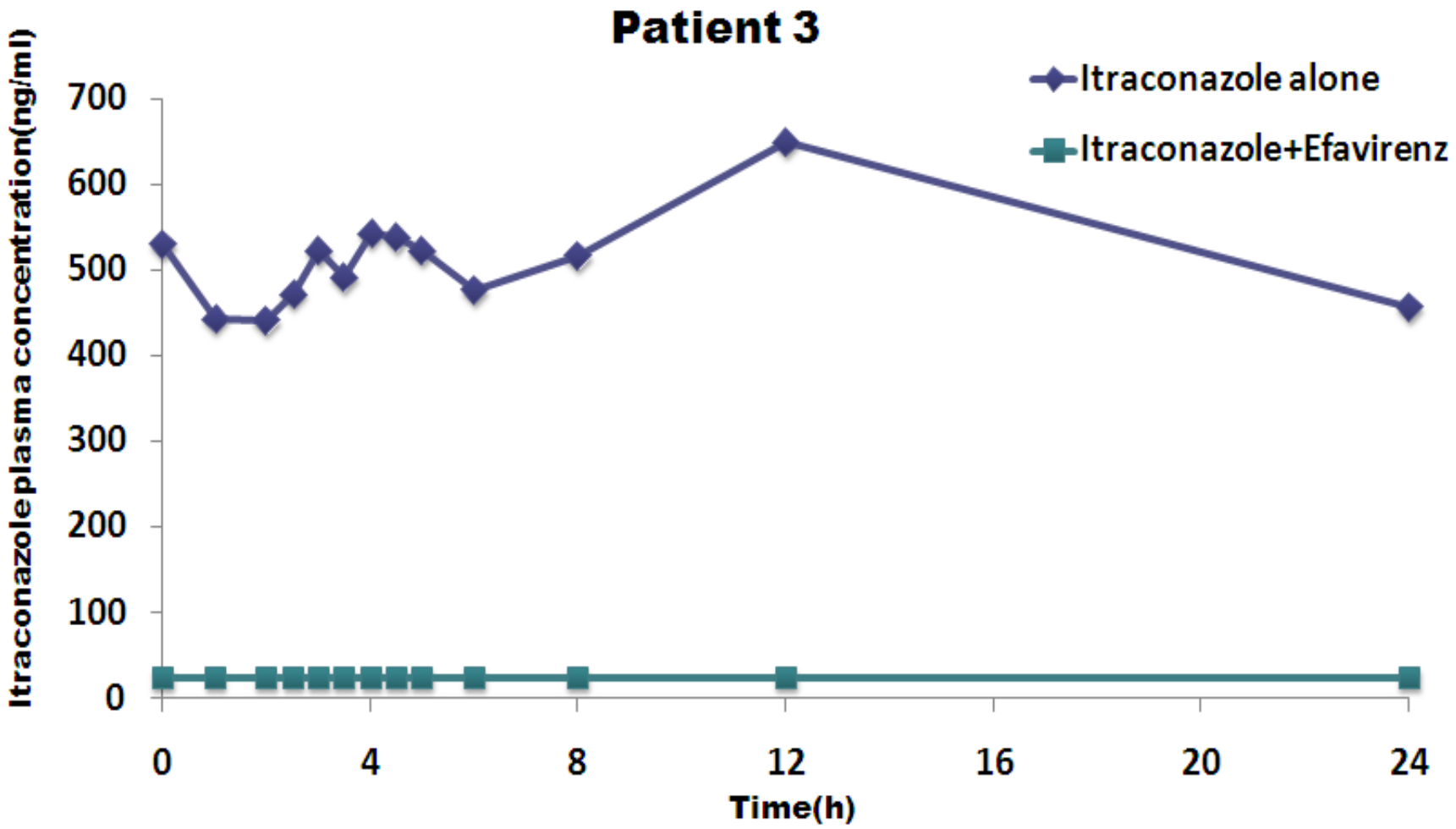


Figure 22 Plasma itraconazole concentration-time curve of patient No.3

Profile of plasma efavirenz concentration at each time point of blood drawn from individual patient

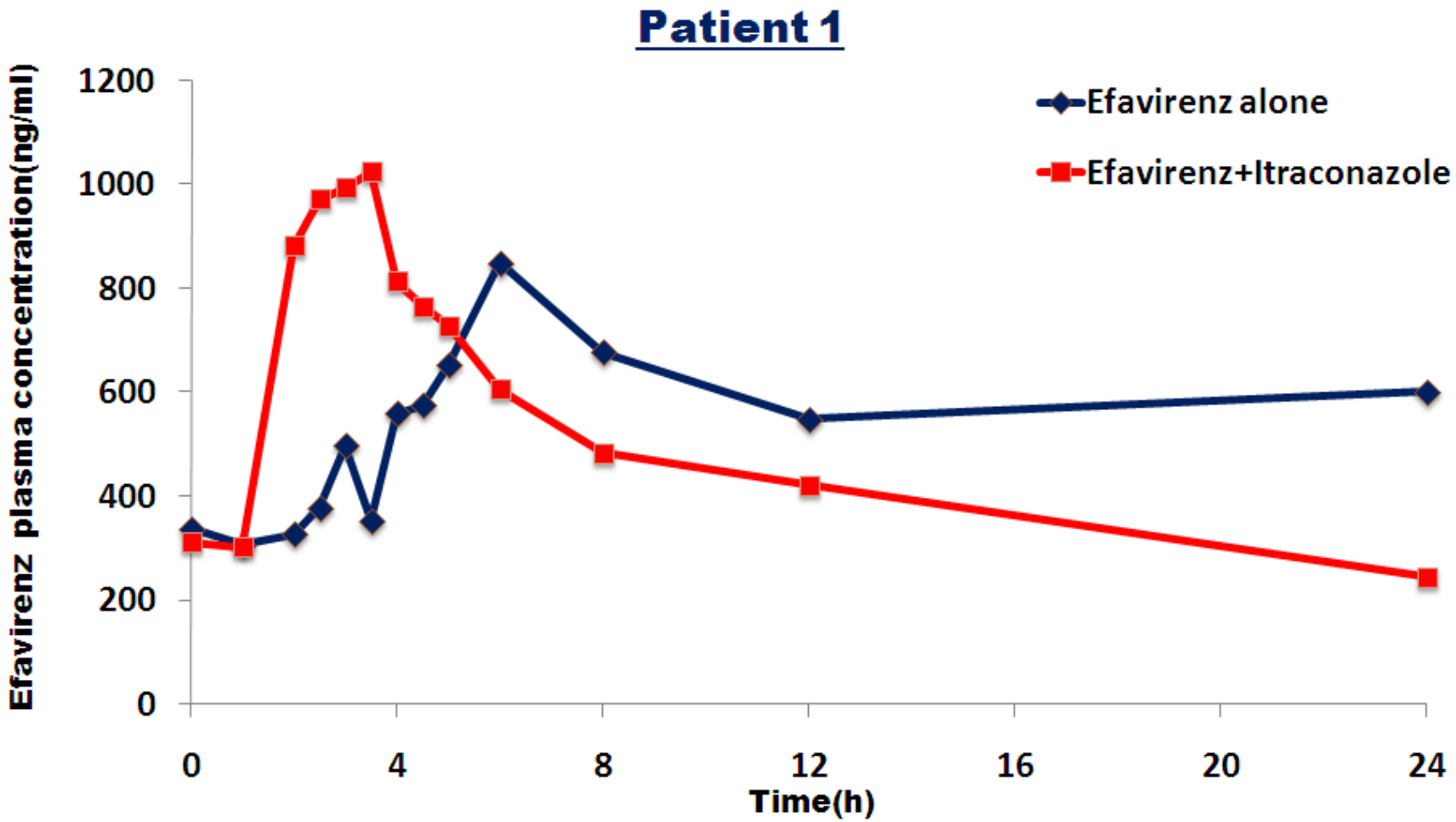


Figure 23 Plasma efavirenz concentration-time curve of patient No.1

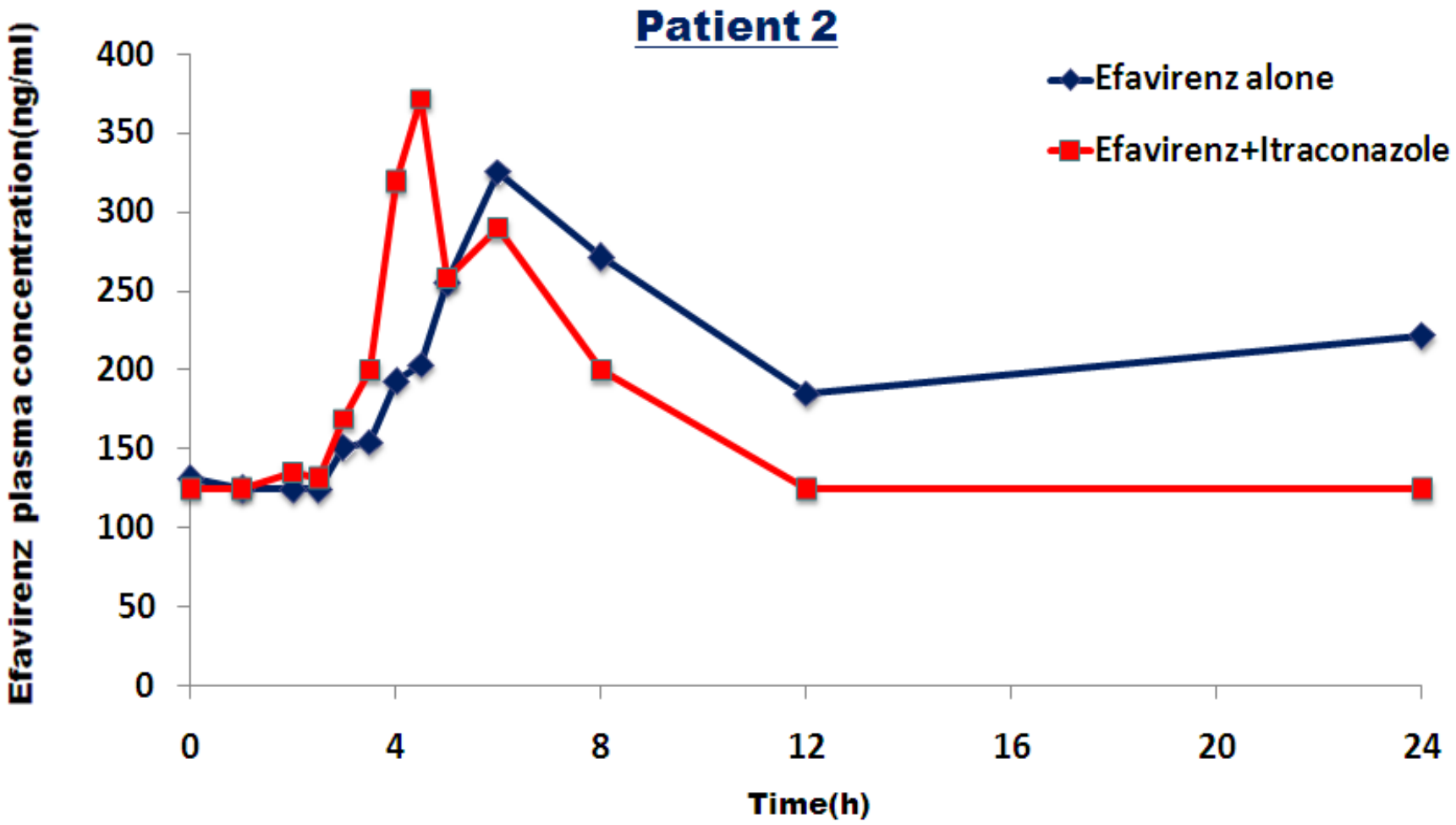


Figure 24 Plasma efavirenz concentration-time curve of patient No.2

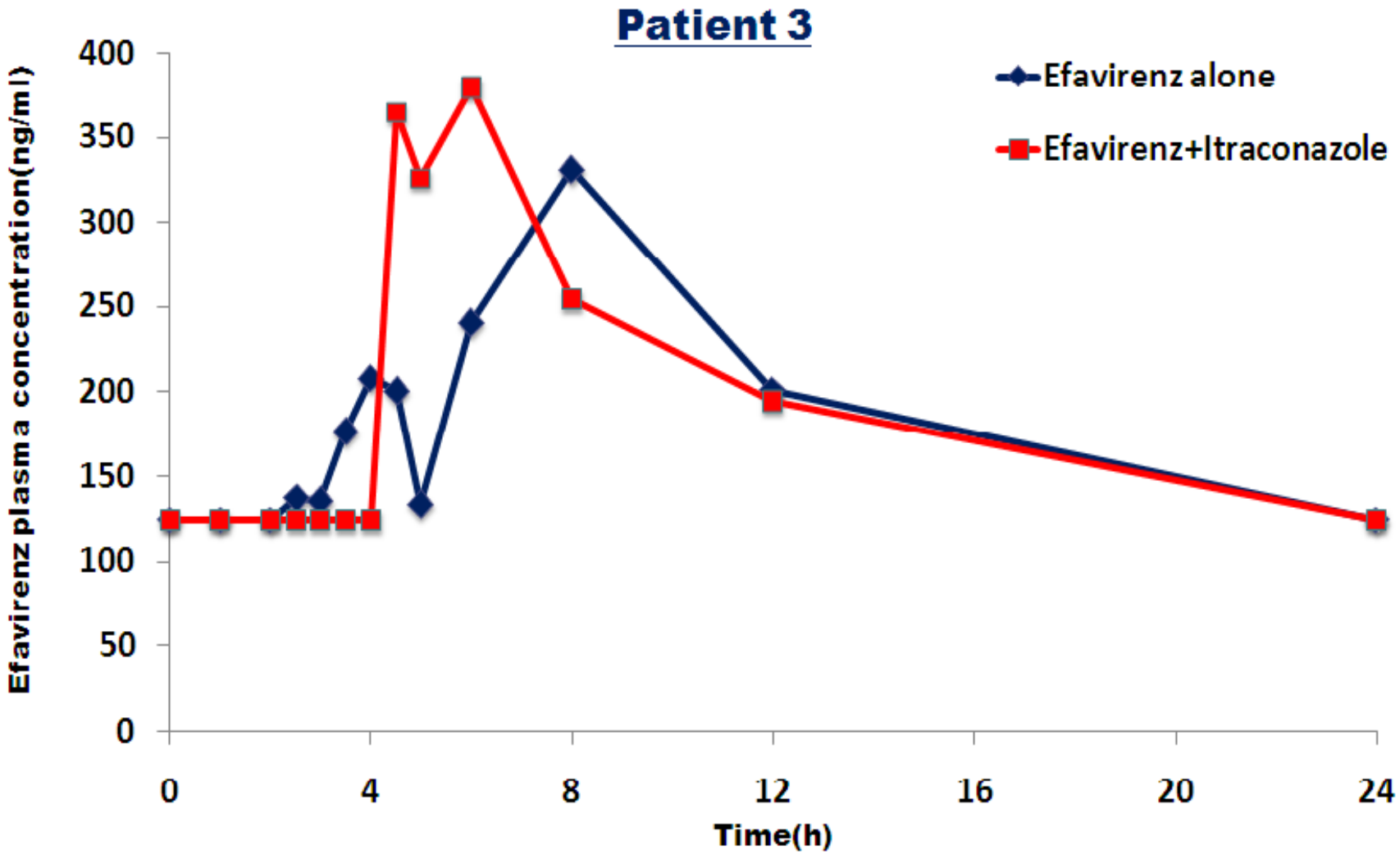


Figure 25 Plasma efavirenz concentration-time curve of patient No.3

VITAE

Name Miss Kritsana Kongklao

Student ID 5210220007

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Nurse	Prince of Songkla University	2008

Scholarship Awards during Enrolment

Research Assistant, Faculty of science, Prince of Songkla University

List of Publication and Proceeding

Kritsana Kongklao, Werawath Mahatthanatrakul, Sutep Jaruratanasirikul, Malinee Wongnawa and Somchai Sriwiriyajan. The preliminary study: Effect of efavirenz on the pharmacokinetics of itraconazole in HIV- infected patients. *The 22nd National Graduate Research Conference,* October 6th - 7 th, 2011, Bangkok, Thailand.