INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a pandemic disease affecting several millions of adults and children in the developed and developing countries. Although this disease still remains a major social problem in several countries and a definitive cure is not currently available, in the last ten years impressive progress has been made in the treatment of AIDS infected patients [Gazzard *et al.*, 1998; US/DHHS, 2006]. The causative agent of AIDS is the human immunodeficiency virus (HIV), a retrovirus of the lentivirus family that produces a progressive immunosuppression by destruction of CD_4^+ T lymphocytes and macrophages, resulting in opportunistic infections, neurological and neoplastic diseases, and death. According to the AIDS Epidemic Update 2005 by UNAIDS/WHO, released on December 2005, 40.3 million people were living with the Human Immunodeficiency Virus (HIV) and 3.1 million have died from HIV/AIDS (UNAIDS/WHO, 2005). An estimated number of 1,054,684 were infected in Thailand at the end of 2003 and 450,742 have died from the infection (Phanuphak *et al.*, 2004).

During the past few years, significant advances have been made 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 the increased of CD_4^+ lymphocytes counts, causing on improvement of immunity and a decrease in the incidence of opportunistic infection (Powderly *et al.*, 1998; Sepkowitz, 1998).

To date, most clinical use of combination therapy in treatment-naïve individuals has been based on three different types of combination regimens, namely: NNRTI-based (1 NNRTI + 2 NRTI), PI (protease inhibitors)-based (1-2 PI+2NRTI), and triple NRTI-based regimens. NNRTI-based regimens are commonly prescribed as initial therapy for treatment-naïve patients. In general, these regimens have the advantage of lower pill burden as compared to most of the PI-based regimens. Use of NNRTI-based regimens can preserve the PIs for later use, reducing or delaying patient exposure to some of the adverse effects which are more commonly associated with PIs. The major

disadvantage of currently available NNRTIs is their low genetic barrier for development of resistance (DHHS, 2006).

There are two main categories of NNRTI-based antiretroviral drugs (ARV) currently under recommendation. The first type, NRTI, inhibits reverse transcriptase (RTase) by binding the enzyme's active site and adding to the growing DNA chain. The second category are the NNRTIs (namely, delavirdine, efavirenz and nevirapine) , bind RTase at a site that is distal from the active site, inducing a detrimental conformational change within the enzyme. These drugs show high antiviral activity and low toxicity *in vitro*, but are very specific. Single mutations have been shown to reduce or eliminate efficiency. For this reason, drugs of this type are usually coadministered with other drugs (Lythgo, 2004; Weller *et al.*, 2001).

To date, recommendations are designated as the initial regimen in treatment naïve patients, who have CD_4^+ falls below 350 cells/mm^3 in all asymptomatic individuals. The preferred regimen of NNRTI-based has been based on a combination of efavirenz plus 2NRTIs as shown in Appendix B (DHHS, 2006; WHO, 2005). The government of Thailand launched the programme aims to provide GPO-Vir, a generic fixed dose combination of stavudine, lamivudine and nevirapine, as a firstline HAART regimen to an additional 10,000 patients in 2003 (Phanuphak, 2004). Due to severe side effects, e.g. hepatotoxicity (Sanne *et al.*, 2005) and severe rash (Leth *et al.*, 2005) of nevirapine , and the results from several large cohort-studies have suggested that efavirenz is more effective and safer than nevirapine (Cozzi-Lepri *et al.*, 2002; Keiser *et al.*, 2002; Matthews *et al.*, 2002). In addition, the favorable side effect profile and diminished pill burden on patients due to once-a-day dosing have contributed to efavirenz's extensive use (Savini *et al.*, 2001). These factors make efavirenz an important component of NNRTI-based instead of nevirapine.

Several clinical studies have reported a reduction in the plasma levels of other cytochrome P450 (CYP)3A4 substrates when they are coadministered with efavirenz (Aarnoutse *et al.*, 2002; Clarke *et al.*, 2001, Falloon *et al.*, 2000). 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 (Berruet *et al.*, 2005; Mouly *et al.*, 2002).

Opportunistic infections (OIs) continue to cause morbidity and mortality in patients with human immunodeficiency virus (HIV)-1 infection throughout the world. Potent combination antiretroviral therapy has reduced the incidence of OIs for certain patients with access to care. However, certain patients in the developed and developing world do not have access to care and have OIs. Other patients do not have a sustained response to antiretroviral agents for multiple reasons, including poor adherence, drug toxicities, drug interactions, or initial acquisition of a drug-resistant strain of HIV-1. Therefore, OIs will continue to cause substantial morbidity and mortality in patients with HIV-1 infection. Fungal infection is one of the opportunistic diseases in HIV-infected patients. Oropharyngeal and esophageal candidiasis are common (Klein, 1988). On the other hand, infections with *Penicillium marneffei*, penicillosis, have been reported in patients infected with HIV, especially in Southeast Asia and South China (Clyti *et al.*, 2006; Supparatpinyo *et al.*, 1992; Tsang *et al.*, 1991; Tsui *et al.*, 1992). Penicillosis has been reported as the third most frequent opportunistic infection in Thailand. According to the Ministry of Public Health of Thailand, the cumulative number of AIDS patients during 1984—2004 with penicilliosis was 6,323 cases (Mootsikapun *et al.*, 2006).

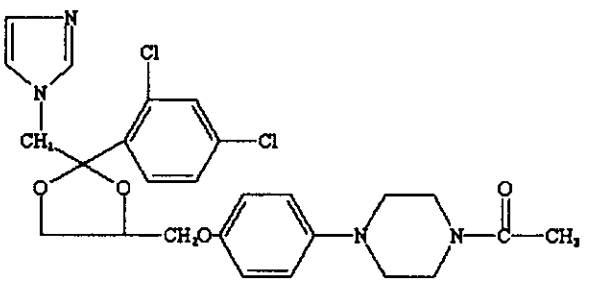
Ketoconazole, an imidazole piperazine antifungal agent, is also used for the threatment of penicillosis, which rapidly spreads among AIDS patients in Thailand (Hospenthal, 2000). The drug is also used for the treatment of candida infection, where oropharyngeal and esophageal candidiasis are the two most common fungal infection in HIV-infected patients (Glick *et al.*, 1994; Greenspan, 1990; Teanpaisan *et al.*, 1998).

Ketoconazole is extensively metabolized by hepatic microsomal enzymes (Rodriguez *et al.*, 1997). The major metabolic reactions in human are hydroxylation of imidazole ring and oxidative N-deacetylation of piperazine ring by CYP3A (Shannon *et al.*, 2005; Brown, 2001; Cupp *et al.*, 1998; Daneshmend *et al.*, 1988). The N-deacetyl ketoconazole (DAK) is a potent cytotoxicant than its parent

compound. It is directly toxic to liver cells in a distinct time- and dose-response relationship (Rodriguez *et al.*, 1997a).

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

Ketoconazole



MW = 531.44

Figure 1 : The chemical structure of ketoconaozle

Ketoconazole is a synthetic imidazole derivative antifungal agent. The structural formula of ketoconazole is shown in Figure 1, it has five-membered ring which contains two nitrogen atoms. It is a highly lipophilic compound. This property lead to high concentration of ketoconazole in fatty tissue and purulent exudates. Its oral absorption and solubility is optimal at acidic gastric pH (van der Meer *et al.*, 1980; Carlson *et al.*, 1983).

Mechanism of Action

As with all azole antifungal agents, ketoconazole has usually fungistatic action. The exact mechanism of action of the drug has not been fully determined. However, it has been suggested that ketoconazole may interfere with ergosterol synthesis, probably via inhibition of cytochrome P450

lanosterol 14-α-demethylase (Erg11p). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol. On the molecular level, one of the nitrogen atoms of the azole ring is thought to bind to the heam moiety of Erg11p (Lyman *et al.*, 1992; McEvoy ed., 2001; Sanglard *et al.*, 2002). The mechanism is shown in Figure 2.

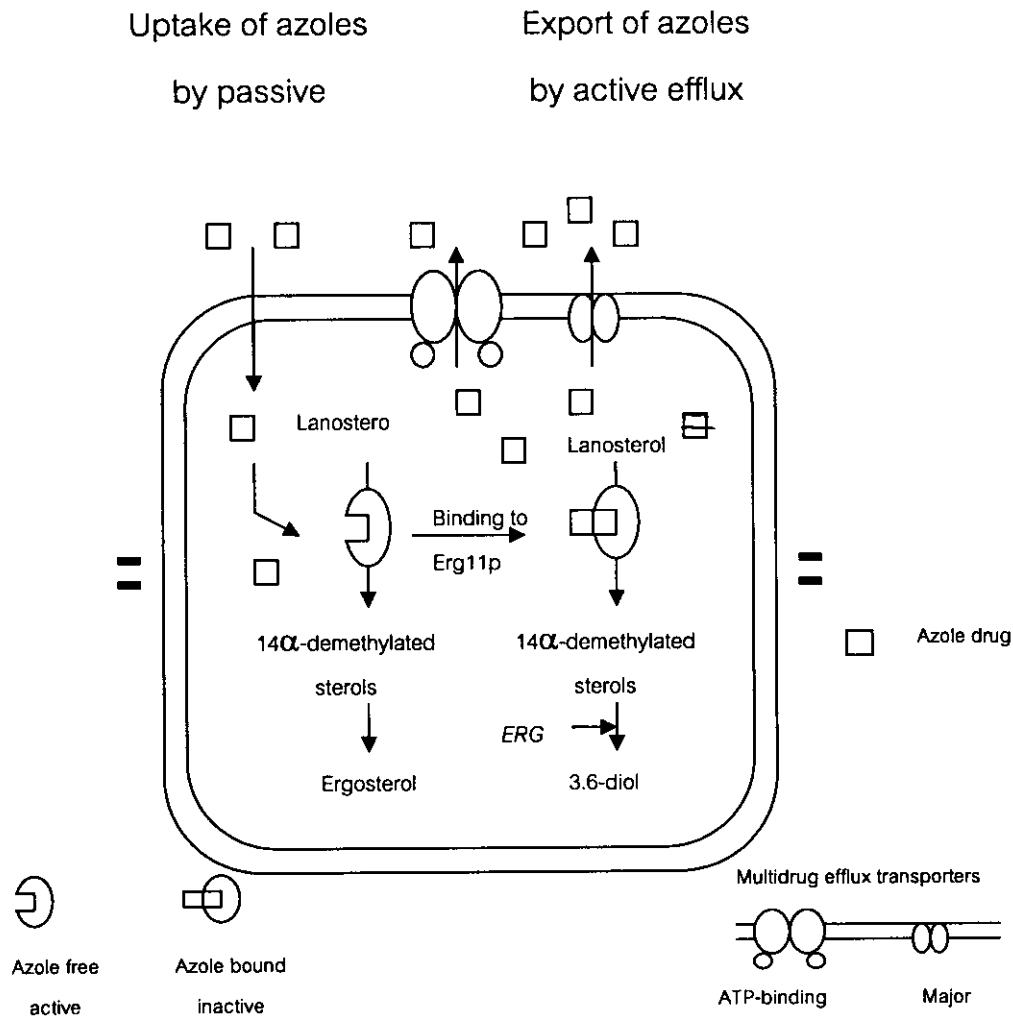


Figure 2 : Model for mechanism of action of ketoconazole (adapted from Sanglard *et al.*, 2002)

Pharmacokinetics

(1) Absorption

Ketoconazole is lipophilic drug (pK_a = 6.51, 2.94) and it should be absorbed across the gastrointestinal mucosa when it is in the solution (Carlson *et al.*, 1983). The mean maximum plasma concentration (C_{max}) of the single 200-mg ketoconazole tablet in healthy volunteers has been reported to be 4.2 μg/ml at 1.7 hours after oral administration (Huang, 1986). The bioavailability of oral ketoconazole depends on the pH of gastric contents in stomach. An increase in the pH results in decreased absorption of the drug (van der Meer *et al.*, 1980; Daneshmend, 1990; McEvoy ed., 2001).

(2) Distribution

Ketoconazole has been detected in urine, bile, saliva, sebum, cerumen, synovial fluid and cerebrospinal fluid following oral administration of a single 200-mg dose of the drug in adults (McEvoy ed., 2001). In blood, 84% of ketoconazole is bound to plasma proteins, primarily albumin, 15% is bound to erythrocytes, and 1% is free (Daneshmend *et al.*, 1988; Bennett, 1996).

The mean±SD of apparent oral clearance and the volume of distribution after 200-mg ketoconazole solution were 209.9±82.9 ml/min and 88.31±68.72 L, respectively (Huang *et al.*, 1986).

(3) Elimination

Ketoconazole is extensively metabolized in the liver. The major metabolic reactions in human are hydroxylation of the imidazole ring and oxidative N-deacetylation of piperazine ring. Ketoconazole itself appears to be oxidized by cytochrome P450 3A (Shannon *et al.*, 2005; Daneshmend *et al.*,

1988). Plasma concentration of the drug appeared to decline in biphasic manner, with a mean±SD half-life ($t_{½}$) of 1.7±0.6 h during the first 8 to 12 h and a mean±SD $t_{½}$ of 7.9±3.8 h after the administration of 200-mg of ketoconazole tablet (Huang *et al.*, 1986). The major route of elimination of ketoconazole and its metabolites appears to be excretion into feces via bile (Graybill *et al.*, 1980; McEvoy ed.,2001). There may be enterohepatic circulation because the double peaks plasma concentrations was seen at higher doses of ketoconazole (Brass *et al.*, 1982). Renal excretion of the drug is 13%, as unchanged form 2% to 4% (Graybill *et al.*, 1980). Renal insufficiency does not affect the plasma concentration or half-life, but the half-life is prolonged in patients with hepatic insufficiency (Brass *et al.*, 1982).

Clinical use

Oral ketoconazole is used for treatment of susceptible fungal infections, including candidal infection (i.e. oropharyngeal candidiasis and/or esophageal candidiasis, vulvovaginal candidiasis, candiduria, chronic mucocutaneous candidiasis), dimosphic infection (i.e. histoplasmosis, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, penicilliosis) and certain recalcitrant dermotophytosis (Como *et al.*, 1994; McEvoy ed.,2001).

Contraindication

Ketoconazole is contraindicated in patients with known hypersensitivity to the drug and do not use for treatment of fungal meningitis because it penetrates poorly into the CSF. Concomitant administration of ketoconazole and terfenadine, astemizole, or cisapride is contraindicated due

to the risk of potentially fatal cardiac arrest (McEvoy ed., 2001; Micromedex, 2004).

Adverse Drug Reaction

The major drawbacks of ketoconazole therapy are from the occasionally seen adverse reaction.

(1) Gastrointestinal (GI) effects

The most common adverse reactions of ketoconazole are nausea and/or vomiting (3% to 10%) (Como *et al.*,1994; Dismukes *et al.*, 1983). Other GI effects include abdominal pain, constipation, flatulence, GI bleeding and diarrhea (less than 1%). Adverse GI effect appear to be dose related. Administration of ketoconazole with food minimized adverse GI effect, which usually subside with continued therapy (McEvoy ed., 2001; Micromedex, 2004).

(2) Hepatic effects

Several cases of hepatotoxicity, hepatitis and transient elevation in liver enzymes (SGOT, SGPT and alkaline phosphatase), have been reported with ketoconazole therapy (Lewis *et al.*, 1984; Walsh *et al.*, 1991). Onset of the symptom has ranged from short-term (1 to 3 weeks) to long-term (12 to 15 months after initiation of therapy). Accompanying symptom include nausea, backache, fever and weakness. Hepatotoxicity has been observed in patients receiving 200 to 800 mg daily. Symptoms may progress to jaundice, anorexia, malaise and potential death. Ketoconazole should be discontinued, when signs or symptoms of hepatotoxicity occur (McEvoy ed., 2001; Micromedex, 2004). The severity of ketoconazole-induced hepatotoxicity was closely related

to the exposure level (AUC) of the drug (Ma *et al.*, 2003). Ketoconazole have been reported in the literature of which the deacetylated metabolite, N-deacetyl ketoconazole (DAK), is the major metabolite which undergoes further metabolism by the flavin-containing monooxygenases (FMO) to form a potentially toxic dialdehyde (Rodriguez *et al.*, 1997a).

(3) Endocrine effects

Bilateral gynecomastia with breast tenderness has occurred in some men during the therapy. A possible mechanism of ketoconazole-induced gynecomastia is inhibition of sterol synthesis through its direct inhibitory effect on adrenal steroidogenesis with a blunting of the cortisol response to adrenocorticotropin hormone. These would indicate that in some patients receiving ketoconazole, there may be a decrease in adrenal reserve. The steroid blockade usually persists for 4 to 16 hours following a daily dose and should not be of major significance. Although available data indicate that ketoconazole must be given in higher dose for certain resistant fungal diseases and more frequently (2 to 3 times daily), the patients may be at higher risk of developing a state of hypoadrenalism. The incidence of gynecomastia was 21%. Endocrinologic toxicity was dose related and increased at doses greater than 800 mg (O'Connor *et al.*, 2002; Thompson *et al.*, 1993; McEvoy ed., 2001; Micromedex, 2004).

(4) Other adverse effects

About 2% of patients receiving ketoconazole have experienced pruritus and less than 1% experienced rash, dermatitis and urticaria. Anaphylactic reactions occurring after the first dose of the drug has rarely been reported.

Headache, dizziness, somnolence, lethargy, asthenia, nervousness, insomnia, abnormal dreams, photophobia and paresthesia occurred in less than 1% of patients receiving ketoconazole (McEvoy ed., 2001; Micromedex, 2004).

Drug interactions

Since gastric acidity is necessary for the dissolution of ketoconazole, concomitant administration of drugs with decrease gastric acid output or increase gastric pH, such as, antacids, cimetidine, ranitidine, omeprazole, antimuscarinics, may decrease absorption of ketoconazole (McEvoy ed., 2001; Micromedex, 2004).

Amprenavir

Twelve healthy male volunteers received amprenavir 1200 mg, ketoconazole 400 mg, and amprenavir 1200 mg plus ketoconazole 400 mg. Each treatment was separated by 14 days. Coadministration of the drugs, the maximum concentration (C_{max}) and the area under the concentration-time curve (AUC) of ketoconazole were increased by an average of 19% and 44%, respectively. Ketoconazole decreased the amprenavir C_{max} by 16%, but the AUC was increased by 31% (Polk *et al.*, 1999).

Didanosine

Twenty-four healthy volunteers were randomized to received 200 mg of ketoconazole, single dose, or 200 mg of ketoconazole plus 400 mg capsule of didanosine, as an encapsulated enteric bead formulation.

Concomitant administration of didanosine 400-mg and ketoconazole 200-mg, indicated a lack of interaction (Damle *et al.*, 2002).

Phenytoin

Concomitant administration of phynytoin 300 mg/d and ketoconazole 600 mg/d, the area under the steady-state concentration of ketoconazole were 0.16 μg/ml during concurrent and 4.6 μg/ml after discontinue phenytoin. Patient failed to respond during concurrent therapy. The coadministration of phenytoin with ketoconazole leads to a profound reduction in serum ketoconazole concentrations. This decrease has an effect on the clinical response to therapy that appears to correlate with *in vitro* susceptibility results for the relevant fungal pathogen (Tucker *et al.*, 1992).

Rifampicin and isoniazid

Eleven tuberculous patients who were given rifampicin 10 mg/kg and ketoconazole 200 mg concurrently, plasma concentration of ketoconazole decreased 85% at 2 h ($p<0.025$) and 98% at 5 h ($p<0.025$). Another studies, eight male tuberculous patients were given isoniazid 5 mg/kg and ketoconazole 200 mg. Plasma concentrations were measured at 0, 2 and 5 hs after taking the drugs. When both drugs were given simultaneously ketoconazole plasma concentration decreased 75% at 2 hs ($p<0.025$) and 85% at 5 hs ($p<0.05$) (Pilheu *et al.*, 1989).

As in another pharmacokinetic study, concomitant administration of rifampicin 600-mg, isoniazid 300-mg and ketoconazole 200-mg, the AUC of ketoconazole after 5 months was decreased by 88.3% (Brass *et al.*, 1982).

Nevirapine

Twenty six HIV-infected patients with CD_{4+} count ≥ 100 cell/mm^3 were administered 400-mg of ketoconazole, q.d., for 5 days. Nevirapine was add on day 5 (200 mg/d for 14 days, than 400 mg/d for 14 days). Coadministration resulted in a significant 62.8% (p<0.05) and 39.5%(p<0.05) reduction in the AUC and C_{max} of ketoconazole, respectively. Ketoconazole had a slight inhibitory effect on nevirapine metabolism by 15 to 20% increase in C_{max} and minimum concentration (C_{min}) of nevirapine, although the inhibitory interaction may have been muted by the induction effect by nevirapine on ketoconazole (Lamson *et al.*, 1998).

2.2. Efavirenz

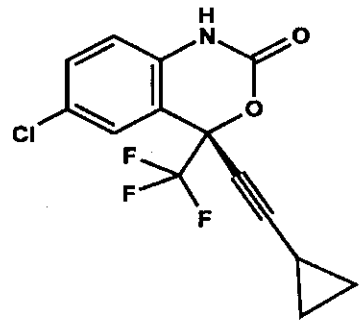


Figure 3 : The chemical structure of efavirenz

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which shows good inhibitory activity against HIV-1. In the treatment of HIV infection, efavirenz is used only in combination regimens (Adkins *et al.*, 1998; Micromedex, 2004). The structure formula of efavirenz is shown in Figure 3.

Mechanism of Action

The reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) is a heterodimer that consists of a 66 kDa subunit (p66) and a 51 kDa subunit (p51). Only the p66 polymerase domain has a DNA-binding cleft, a functional polymerase active site, and a site for binding non-nucleoside reverse transcriptase inhibitors (NNRTIs). The inhibition mechanism of HIV-1 RT by NNRTI, efavirenz, has been suggested that there are significant conformational differences in the p66 polymerase domain when efavirenz is thought to bind to the amino-acid residues of NNRTI-binding pocket in RT (Hsian *et al.*, 1996).

Pharmacokinetics

(1) Absorption

Efavirenz plasma concentrations reached steady-state level within 6 to 10 days during administration of the drug at 200, 400 or 600 mg/day to HIV-1-infected patients. The trough plasma concentration (C_{min}), maximum plasma concentration (C_{max}) and AUC at steady state of the single 600-mg once daily of efavirenz tablet in HIV-infected individuals have been reported to be 1.8 μg/ml, 4.1 μg/ml and 58.1 μg/ml.h, respectively (Adkins, 1998; Micromedex, 2004). The bioavailability of oral efavirenz was 42 % in animal studies after 2-mg/kg doses (Balani *et al.*, 1996; Young *et al.*, 1995). Treatment failure has been discussed as being associated with low efavirenz levels (<1μg/ml), and central nervous system (CNS) toxicity may be associated with high efavirenz levels (>4 μg/ml)(Marzolini *et al.*, 2001).

Efavirenz has been detected in urine, bile and cerebrospinal fluid following oral administration of a single 600-mg dose of the drug in adults (Micromedex, 2004). 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. In blood, 99.5% to 99.75% of efavirenz is bound to plasma proteins, mainly albumin (Adkins *et al.*, 1998).

(2) Elimination

Efavirenz is extensively metabolized in the liver, predominantly by the CYP3A4 and 2B6 isoenzymes (Chen *et al.*, 2003; Deeks, 1998). Hydroxylated metabolites are produced which have negligible antiviral activity. Appoximately 14 to 34% of radiolabelled dose of efavirenz 400 mg was excreted in urine in the form of metabolites and 16 to 61% was excreted in the faeces as unchanged drug. Less than 1% of an administered dose of efavirenz is excreted unchanged in the urine. The terminal plasma elimination half-life ($t_{½}$) of efavirenz was 52 to 76 and 40 to 55 hours, respectively, after single- and multiple- dose oral administration (Micromedex, 2004; Adkins, 1998). Several *in vivo* studies have reported a reduction in the plasma levels of other CYP3A4 substrates when they are co-administered with efavirenz 600-mg per day (Aarnoutse *et al.*, 2002; Clarke *et al.*, 2001, Falloon *et al.*, 2000). 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 (Berruet *et al.*, 2005; Mouly *et al.*, 2002).

Clinical use

Efavirenz in combination with other anitiretroviral agents is indicated for the treatment of HIV-1 infection (Micromedex, 2004).

Contraindication

Efavirenz is contraindicated in patients with known hypersensitivity to the drug. Concomitant administration of efavirenz and midazolam or triazolam is contraindicated due to the risk of excessive sedation and confusion. Concomitant administration of the drug and astemizole, or cisapride is contraindicated due to the risk of potentially fatal cardiac arrest (Micromedex, 2004).

Adverse Drug Reaction

The major drawbacks of efavirenz therapy are from the occasionally seen adverse reactions.

(1) Central nervous system

The most common adverse reactions of efavirenz on central nervous system symptom occur in 53% of patients on efavirenz and 25% of patients on control regimen. The adverse reactions which have been reported were dizziness 28%, insomnia 16%, somnolence 7%, abnormal dreams 6% and hallucinations 1.2%, with 2% of patients rating these symptoms severe enough to require discontinuation. Onset is rapid, within the first or second day of treatment, but may resolved spontaneously over 2 to 4 weeks of treatment, if tolerable. These symptoms are more tolerable if the drug is taken just prior to bedtime (Deeks, 1998; Micromedex, 2004).

(2) Gastrointestinal (GI) effects

The GI adverse reactions of efavirenz are diarrhea, nausea and/or vomiting and have been reported in 5% to 25% of adult patients and 12% to 39% of pediatric patients who received the drug (Micromedex, 2004).

(3) Hepatic effects

Elevation of liver enzymes (SGOT, SGPT and alkaline phosphatase) to 5-times of the upper limits of normal range has been observed in approximately 3% of 1,008 patients treated with efavirenz combinations. Up to 8% of patients with a prior history of hepatitis B or C may develop elevations of these enzymes (Micromedex, 2004).

(4) Other adverse effects

New onset rash was reported in 26% efavirenz-treated patients compare with 17% of patients in control group. Onset of rash was a median of 11 day. Antihistamines and/or corticosteroids may improve the tolerability and hasten the resolution of the rash (Micromedex, 2004).

Teratogenic effect, anencephaly, anophthalmia and cleft palate, have been occurred in some animal studies as monkeys, but have not been observed in rats and rabbits following standard dose of efavirenz. Efavirenz therapy should be avoided in the first trimester in pregnant women infected with HIV-1 (Deeks, 1998; Micromedex, 2004).

Drug interactions

Amprenavir

Concomitant administration of amprenavir 700-mg twice daily and efavirenz 600-mg once daily for 14 and 21 days, the area under the concentration-time curve (AUC) of amprenavir were decreased an average of 46% and 61%, respectively (Morse *et al.*, 2005). As in other pharmacokinetic studies involving fosamprenavir and efavirenz, AUC and C_{min} of fosamprenavir were decreased by 13% and 36%, respectively after coadministration with fosamprenavir 1400-mg and efavirenz 600-mg daily for 2 weeks (Micromedex, 2004).

Atorvastatin/Simvastatin/Pravastatin

Concomitant administration of efavirenz 600-mg once daily and atorvastatin 10-mg, simvastatin 40-mg or pravastatin 40-mg daily for 3 days decreased the area under the concentration-time curve (AUC) of atorvastatin, simvastatin and pravastatin by 43%, 58% and 40%, respectively (Gerber *et al.*, 2005).

Clarithromycin

In healthy volunteers given efavirenz 400-mg once daily and clarithromycin 500-mg twice daily for 7 day, the AUC of clarithromycin was decreased by 39% and the AUC of its hydroxyl-metabolite was decreased by 34% (Benedek *et al.*, 1998).

Ethinyl estradiol

The AUC of a single 50 μg dose of ethinyl estradiol was significantly decreased by 37% in healthy female volunteers who received concomitant efavirenz 400-mg once daily for 10 day (Adkins *et al.*, 1998; Joshi *et al.*, 1998).

Indinavir

Concomitant administration of efavirenz 200-mg once daily and indinavir 800-mg 3 times daily for 14 days decreased the maximum concentration (C_{max}) and the area under the concentration-time curve (AUC) of indinavir by 16% and 31%, respectively. An increase in the dosage of indinavir from 800 to 1000-mg 3 times daily is recommended in patients receiving concomitant efavirenz therapy (Adkins *et al.*, 1998; Micromedex, 2004). As in other pharmacokinetic studies involving indinavir, ritonavir and efavirenz, 18 healthy male volunteers received a combination of 800-mg indinavir and 100-mg ritonavir twice daily plus 600-mg efavirenz once daily for 14 days. Efavirenaz resulted in significant reductions in AUC, C_{max}, and C_{min} of indinavir by 25%, 50% and 17%, respectively. The significant decrease in the AUC, C_{max}, and C_{min} were 36%, 34% and 39%, respectively (Aarnoutse *et al.*, 2002).

Lopinavir/ritonavir

45 HIV-infected patients received 533.3/133.3-mg of lopinavir/ritonavir twice daily plus efavirenz 600 mg once daily. Efavirenz increased the mean steady state elimination rate constant of lopinavir by 36% (Dailly *et al.*, 2005).

Methadone

Eleven HIV-infected patients attending on stable methadone maintenance therapy. When combined with 600-mg once daily of efavirenz for 7 days there was marked decreased in the AUC_{0-24} and C_{max} of methadone by 52%(p=0.007) and 45%(p=0.012), respectively (Clarke *et al.*, 2001).

Nelfinavir

Concomitant of 750-mg nelfinavir 3 times daily plus 600-mg efavirenz once daily for 7 day decreased the mean clearance of nelfinavir metabolite (M8) by 43% (Labbe *et al.*, 2005).

Ritonavir

24 normal volunteers received 1875 mg of nelfinavir plus ritonavir 200 mg q.d. with a 300-kcal snack for 10 days. During days 11-20 efavirenz 600 mg q.d. 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

Administration of efavirenz 600-mg once daily for 10 days greatly decreased the C_{max} and AUC by 50% and 60%, respectively, of coadministered saquinavir 1200-mg 3 times daily as soft gelatin capsule in healthy volunteers. Use of efavirenz in combination with saquinavir as the sole protease inhibitor is not recommended (Adkins *et al.*, 1998; Micromedex, 2004).

Voriconazole

34 healthy male subjects received 400-mg once daily of efavirenz for 19 days and voriconazole 200-mg 2 times daily for 9 days after 10 days of efavorenz alone. Efavirenz decreased the mean steady state AUC and C_{max} of voriconazole by 77% and 61%, respectively. While voriconazole had a inhibitory effect on efavirenz metabolism by 38% and 44% increase in C_{max} and AUC of efavirenz, respectively (Liu *et al.*, 2005).

2.3. Cytochrome P450 System

The liver is frequently the target organ of toxic chemicals. It receives a dual supply of blood via the hepatic artery, arising from the aorta (25%), and via the portal vein (75%), which is a conglomerate of venous returns from the intestinal, spleen, and mesenteries. By lying between the portal and systemic circulation, the liver will receive drugs entering via portal system during oral absoption. Each liver cell carries out all chemical reactions associate with metabolism.

Drugs are usually lipophilic substances so they can pass plasma membranes and reach the site of action. Drug metabolism is basically a process that introduces hydrophilic functionalities onto the drug molecule to facilitate excretion. This biotransformation is an essential part of self-protection against toxic effects of drug. Two important detoxification mechanism in normally functioning livers are Phase I and Phase II metabolic reaction. Phase I reactions convert the parent hydrophobic drug to a more polar metabolite by oxidation, reduction, or hydrolysis. These reactions expose or introduce a functional group (-OH, $-NH_2$, -SH, or $-CO_2H$), and usually result in only a small increase in the hydrophilicity of the drug. Some examples of Phase I reactions

are presented in Figure 4. The bioactivation of drug candidates metabolites that sometimes can be even more toxic than the original drug. These Phase I metabolites are detoxified by Phase II conjugation reactions with cellular macromolecules such as glucuronide, sulfate, acetate, or an amino acid (as shown in Figure 5). Some examples of Phase II reactions are presented in Figure 6. Phase II preceding Phase I reaction, although less common, can also occur. These reactions, either alone or in concert, are responsible for the generation of readily excretable metabolites (Yan *et al.*,2001)

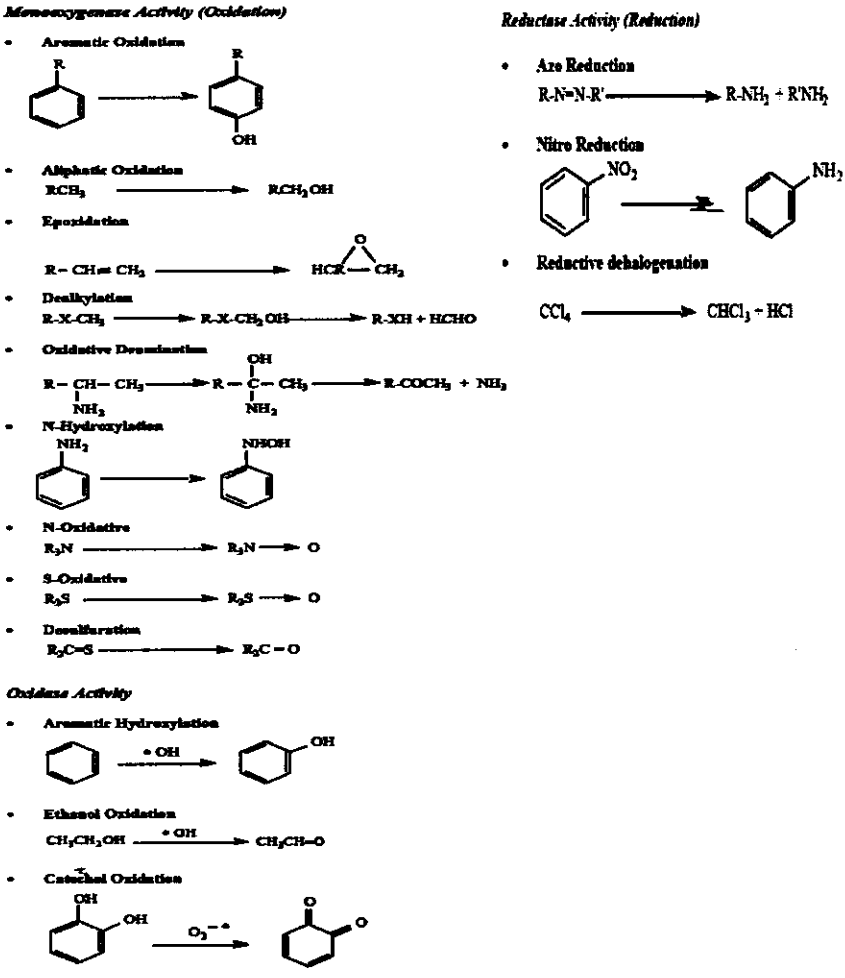


Figure 4 : Examples of Phase I Reactions (Yen *et al.*, 2001)

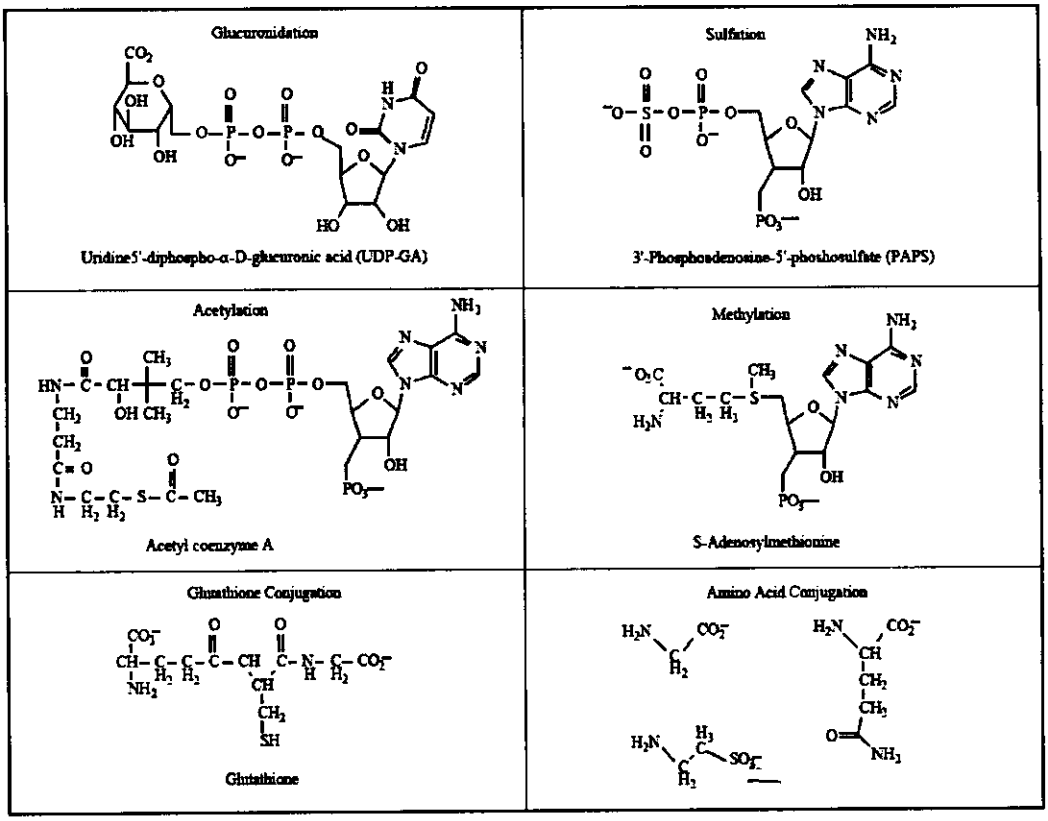


Figure 5 : Structures of cofactors for phase II biotransformation (Yen *et al.*, 2001).

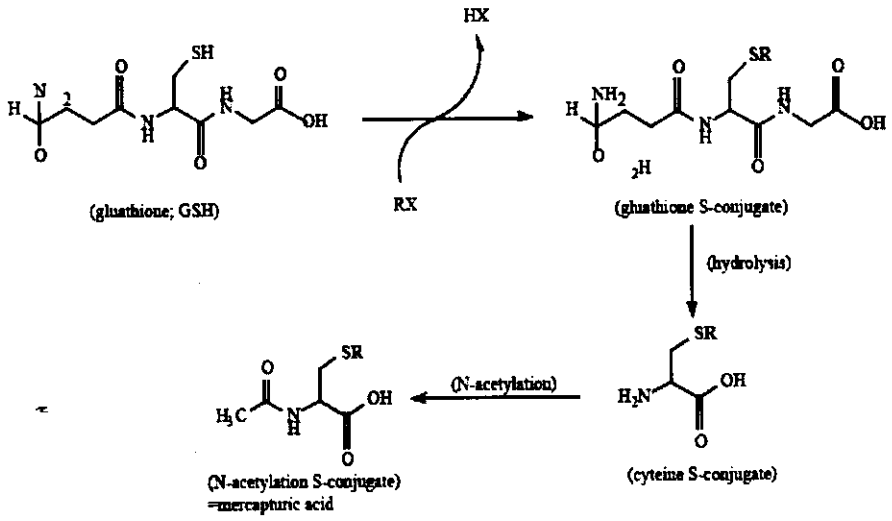


Figure 6 : Reaction of electrophilic metabolites with GSH (Yen *et al.*, 2001)

Cytochrome P450 (CYP), 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). It refers to a family of over 100 enzymes in human body that modulate various physiologic functions (Preskron *et al.*, 1995). The CYP450 enzyme system contains two large subgroups: steroidogenic and xenobiotic enzymes. The steroidogenic group is involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. The xenobiotic group includes four major enzyme families, CYP1, CYP2, CYP3, and CYP4. These enzymes perform a number of physiologic functions, they are vital formation of arachidonic acid metabolites, but their primary role involves the Phase I metabolism of drugs (Gonzalez,1992; Guengerich *et al.*,1993; Nebert, 1991; Nelson *et al.*, 1993). Most of the metabolism enzymes are located in the endoplasmic reticulum (ER) (Yan *et al.*, 2001).

Approximately 70% of human liver CYP is accounted for by CYP1A2, CYP2A6, CYP2B, CYP2C, CYP2D6, CYP2E1 and CYP3A enzymes. Among these, CYP3A and CYP2C are the most abundant subfamilies, accounting for 30% and 20%, respectively (Lin *et al.*, 1998).

In general, significant drug-drug interaction occurs only when two or more drugs compete for the same enzyme and when the metabolic reaction catalysed by this enzyme is a major elimination pathway. Drug-drug interactions can also occur when the CYP responsible for the metabolism of a drug is induced by long term treatment with an other drug.

The human CYP3A4 is responsible for approximately 60% of P450-mediated metabolism of drugs in therapeutic use today implicating this enzyme as important with respect to the action, duration, and disposition of drugs and their metabolites (Gibson *et al.*, 2002). Because of the considerable role that CYP3A4 plays in drug metabolism, hepatic and intestinal expression of this P450 can mediate the therapeutic outcome of many agents. Wide variation in tissue concentrations of this enzyme has been found among individuals that ultimately affects drug disposition often making disposition difficult to predict (Wrighton *et al.*, 2000). Variability in CYP3A4 expression can result from a variety of factors and is partially explained by the ability of various xenobiotics to increase the expression of this P450. Of these xenobiotics, many are therapeutic agents that enhance hepatic and/or intestinal CYP3A4 expression (Guengerich, 1999). At least five categories of agents are considered CYP3A inducers: steroid hormones having either glucocorticoid or anti-glucocorticoid activities, Phenobarbital (PB) and PB-like agents such as PCBs and organochlorine pesticides, macrolide antibiotics, imidazole antifungal agents, and receptor and enzyme antagonists (e.g., nifedipine, troglitazone, lovastatin) (Quattrochi *et al.*, 2001). The inducibility of CYP3A4 gene expression, coupled with the remarkable versatility of CYP3A catalytic activities, creates the potential for drug-drug interactions.

Mechanisms of inhibition of CYP

The catalytic cycle of CYP consist of at least 7 discrete steps :

(i) binding of the substrate to the ferric form of the enzyme

(ii) reduction of heam group from the ferric to the ferrous state by an electron provided by NADPH via CYP reductase

(iii) binding of molecular oxygen

(iv) transfer of a second electron from CYP reductase and/or cytochrome b5

(v) cleavage of the O-O bond

(vi) substrate oxygenation

(vii) product release (Figure 7).

Although impairment of any one of steps can lead to inhibition of CYP enzyme activity, step (i), (iii) and (vi) are particularly vulnerable to inhibition.

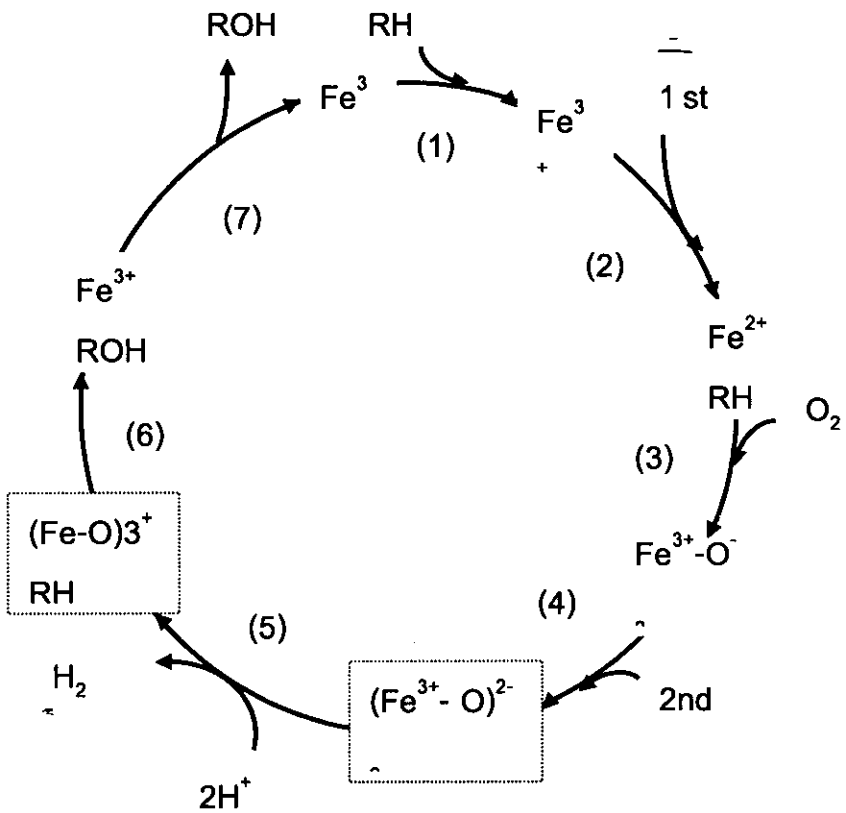


Fig 7 : The catalytic cycle of cytochrome P450 (Siroka *et al)*

The mechanisms of CYP inhibition can be divided into three categories

1) Reversible inhibition

Reversible inhibition is probably the most common mechanism responsible for documented drug interactions. In mechanistic terms, reversible interactions arise as a result of competition at the CYP active site and probably involve only the first step of the CYP catalytic cycle. Many of the potent reversible CYP inhibitor are nitrogen-containing drugs, including imidazole, pyridines and quinolines. These compounds can not only bind to the prosthetic heam iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone-electron pair and the prosthetic heam iron (Lin *et al.*, 1998).

2) Quasi-irreversible inhibition via metabolic intermediate complexation

A large number of drug, including methylenedioxybenzenes, alkylamines, macrolide and hydrazines, undergo metabolic activation by CYP enzyme to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic heam of CYP, called metabolic intermediate (MI) complex, so that the CYP is sequestered in the functionally inactive state. MI 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. Dissociation or displacement of MI complex results in the reactivation of CYP functional activity. However, in *in vivo* situations, the MI 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 MI complexation is, therefore, considered to be quasi-irreversible (Lin *et al.*, 1998).

3) Irreversible inactivation of CYP

Drug containing certain functional groups can be oxidized by CYP to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site. The mechanism-based inactivation of CYP may result from irreversible alteration of heam or protein, or a combination of both. In general, modification of the heam 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.

Drug containing terminal double-bond (olefins) or triple-bond (acetylenes) can be oxidized by CYP to radical intermediates that alkylates the prosthetic heam group and inactivate the enzyme. The evidence for heam alkylation includes the demonstration of equimolar loss of enzyme and heam, as well as the isolation and structural characterization of the heam adducts. Heam akylation is initiated by the addition of activated oxygen to the internal carbon of the double or triple bond and is terminated by binding to heam pyrrole nitrogen.

The best known example of inactivation of CYP through protein modification by suicide inactivator is that of chloramphenicol. The dichloroacetamido group is oxidised to an oxamyl moiety that acylates a lysine residue in the CYP active centre. This acylation event interferes with the

transfer of electrons from CYP reductase to the heam group of the CYP and thereby prevents catalytic turnover of the enzyme (Lin *et al.*, 1998).

Mechanisms of induction of CYP

A central part of this defense is the adaptive increase of *CYP* gene expression, induction, which leads to enhanced metabolism and termination of the pharmacological action of drugs (Okey, 1990). The induction mechanisms for major drug metabolising *CYP* genes have been studied intensively, and recent findings indicate that a common general pathway is utilized : exposure to drugs activates specific members of the nuclear receptor (NR) superfamily which in turn bind to their cognate DNA elements and stimulate the *CYP* target gene transcription (Johnson *et al.*, 1996; Kliewer *et al.*, 1999; Honkakoski *et al.*, 2000). This leads to increased synthesis of CYP enzymes and enhanced metabolism and clearance of the drugs.

Several clinical studies have reported a reduction in the plasma levels of other cytochrome P450 (CYP) 3A4 substrates when they are coadministered with efavirenz (600 mg/d) (Aarnoutse *et al.*, 2002; Falloon *et al.*, 2000; Clarke *et al.*,2001). Furthermore, the mechanism may be efavirenz increase CYP3A4 activity. Recent studies suggest that the human pregnane X receptor (hPXR), an orphan nuclear receptor, serves as a key regulator 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 (Honkakoski *et al.*, 2000). Efavirenz is an efficacious activator of hPXR (Hariparsad *et al.*, 2004).

2.4. The HIV life cycle (CATIE, 2003; Lythgo, 2004)

Like all retroviruses, HIV cannot multiply by itself. It must get inside a cell in order to make copies of itself. When HIV infects a cell, it takes over the cell's control centre. From there, the virus starts to make new copies of itself (it reproduces or replicates). These newly minted viruses then go on to infect other cells. Without treatment, experts estimate that up to 10 billion copies of HIV may be made every day.

The HIV virus is part of the lentivirus family, and is a sexually transmitted pathogenic retrovirus which can be divided into two types. HIV-1 is currently widespread among humans, and begins showing symptoms within 5 years of infection. HIV-2 is localized in Africa, and it takes longer for symptoms to appear. HIV-1 is classified into three sub-groups based on the sequences of *gag* and *env* genes: Group O (outliers), Group M (majority), and Group N (non-M/O).

The HIV Virus

Human immunodeficiency virus (HIV) is made up of two strands of genetic material called RNA. Along with the RNA, HIV contains three key enzymes:

- reverse transcriptase
- integrase
- protease

These enzymes are chemicals that help the virus make copies of itself. The outer surface of the virus is covered with glycoproteins called gp120 and gp41 (as shown in Figure 8).

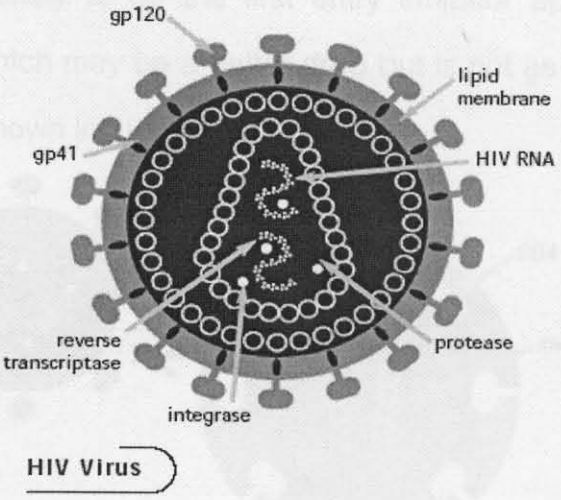


Figure 8 : The structure of HIV (CATIE, 2003)

2.4.1. HIV enters a cell

HIV has an affinity for CD4+ T-cells and monocytes. HIV uses the glycoproteins that mediate entry are transmembrane protein gp41 and gp120, gp120 is non-covalently linked to gp41, and recognizes the CD4 ligand on host cells. Upon binding, a conformational change within gp120 is induced which exposes co-receptor binding sites in gp120. The coreceptors bind a host chemokine receptor – either CXCR4 or CCR5 depending on the type of HIV particle. Once HIV is attached to the receptors, the virus can fuse with the cell into the cytoplasm.Then the contents of the virus are inserted into the cell. Not all of the cells in your body have CD4 receptors; the most important cells that do are called *CD4+ T cells* or *T4 cells*.

Drugs known as *entry inhibitors* are being developed to prevent HIV from getting inside cells. Some of these experimental drugs are designed to block the co-receptors while others prevent the virus from fusing with the cell. Although none of these drugs are approved for use yet, there are several currently being studied in clinical trials. Included are T-20, also known as

pentafuside, which is likely to be the first entry inhibitor approved, and a similar drug, T-1249, which may be a better drug but is not as far along in the approval process (as shown in Figure 9).

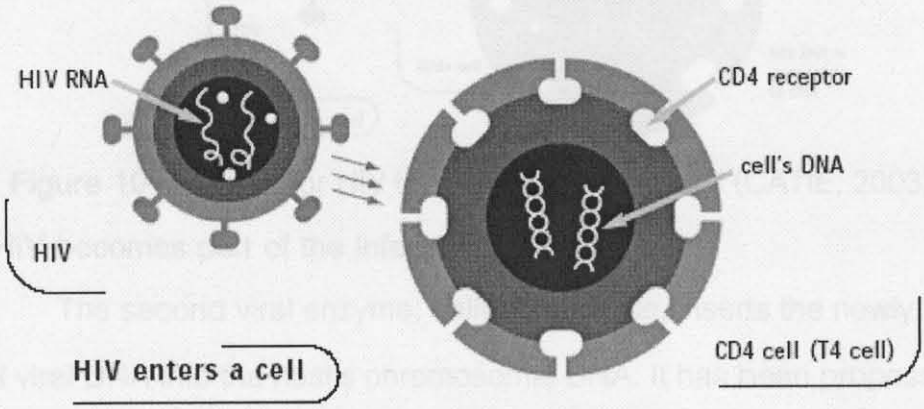


Figure 9 : Model for HIV enters a cell (CATIE, 2003)

2.4.2. HIV takes control of the cell

Inside the cell, the viral lipid envelope is left behind in the host's lipid bilayer, and the viral capsid is released into the cell. Immediately after entry, viral reverse transcriptase (RTase) transcribes the viral genome into cDNA, and the cDNA travels to the nucleus. Now the genetic material of the virus matches the genetic material of the cell (as shown in Figure 10). Drugs called *reverse transcriptase inhibitors* slow down or stop the action of the RT enzyme. The three types of these drugs are:

2.4.2.1 .nucleoside analogue reverse transcriptase inhibitors (NRTIs)

2.4.2.2. non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs)

2.4.2.3. nucleotide analogue reverse transcriptase inhibitors (nucleotide RTIs)

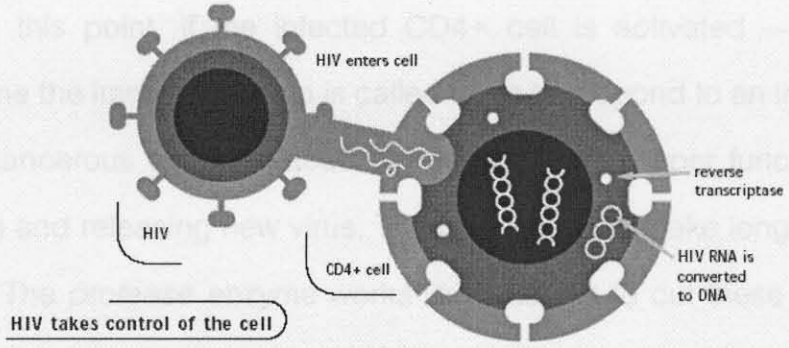


Figure 10 : Model for HIV takes control of the cell (CATIE, 2003)

2.4.3. HIV becomes part of the infected cell

The second viral enzyme, called *integrase*, inserts the newly converted viral DNA into the host's chromosomal DNA. It has been proposed that distortion characteristics in the DNA (for example bound proteins) influence where the viral cDNA is inserted. With the viral DNA integrated into the DNA of the cell, the virus has become part of the cell.

This process has sometimes been compared to putting a "bug" in a computer software program. Researchers are working to develop drugs that will interfere with the action of integrase. Right now, there are no approved *integrase inhibitors* (as shown in Figure 11).

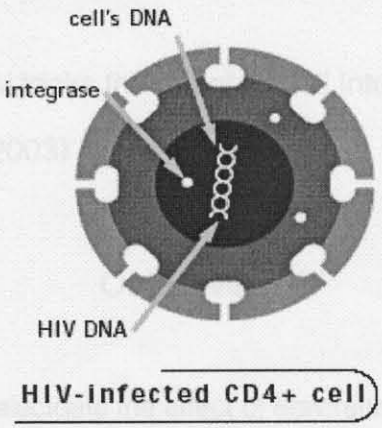


Figure 11 : Model for HIV becomes part of the infected cell (CATIE, 2003)

2.4.4. HIV tricks the infected cell into making copies of itself

At this point, if the infected CD4+ cell is activated — which happens any time the immune system is called upon to respond to an infection or allergen or cancerous cell — instead of performing its proper functions, it will start making and releasing new virus. The first step is to make long chains of viral protein. The *protease* enzyme works like scissors to cut these protein chains into the smaller pieces that make up HIV. The newly cut pieces are assembled into new virus particles, which then "bud" out from the host cell and can go on to infect other cells (as shown in Figure 12).

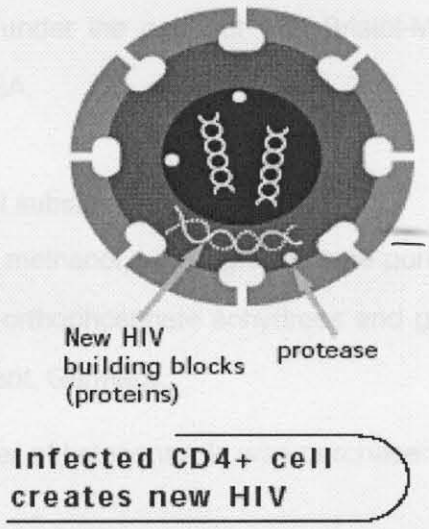


Figure 12 : Model for HIV tricks the infected cell into making copies of itself (CATIE, 2003)

Objectives

This study was conducted to elucidate the effect of efavirenz on ketoconazole pharmacokinetics in HIV-infected patients.

MATERIALS AND METHODS

Materials

Drugs

Ketoconazole used in this study was Nizoral® (Lot No.B420006) from OLIC(Thailand) limited, Ayudthaya, Thailand, under the contract with Janssen Pharmaceutica LTD.

Efavirenz (Storcrin®, Lot No.G0119) was purchased from B.L.H.Trading Co.,LTD., Bangkok, Thailand, under the contract with Bristol-Mayers Squibb Holding Pharma limited, Puerto Rico, USA.

Reagents and Chemical substances

Acetonitrile and methanol, HPLC grade, were purchased from J.T.Baker, NJ, USA. Disodium hydrogen orthophosphate anhydrous and glacial acetic acid were purchased from Merck Darmstadt, Germany.

Standard powder of ketoconazole was purchased from Sigma Chemical Co., St.Louis, MO, USA.

Water was purified for HPLC by the MiLLi Q Water Purification System, Millipore, Milford, USA.

Equipment

HPLC model

- Waters 2695 pump, autosampler (Waters Associates, Milford, USA.)
- Waters 2475 spectrofluorometer detector (Waters Associates, Milford, USA.)
- Empower software (Waters Associates, Milford, USA.)

- μ-Bondapak® C_{18} column : a reverse phase column C_{18}, 10 μm particle, 30 cm length x 3.9 mm internal diameter. (Waters Associates, Milford, USA.)
- μ-Bondapak® C_{18} guard column : packed with resolved C_{18} (Waters Associates, Milford, USA.)

Instruments

- Vortex mixer
- Centrifuge machine
- pH meter
- Micropipette (200 and 1,000 μl)
- Pipette tip
- Disposable needle (22G)
- Haparin 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

Methodology

Determination of ketoconazole in plasma

Sample preparation

The 250 μl of plasma sample was transferred into an appendorf microcentrifuge tube and equal volume of acetonitrile was added for deprotienizaton. The mixture was vortexed for 30 seconds using vortex mixer and centrifuge at 5,000 g for 15 minutes. The supernatant was transferred into a new vial and 50 μl was injected into the column.

Chromatographic conditions

Plasma ketoconazole concentrations were determined by high-performance liquid chromatography (HPLC). The assay was modified from Yeun and Peh study (1998) using the following parameter :

Column : reverse phase column (μ-Bondapak C_{18}, 10 μm particle, 300 mm length, 3.9 mm internal diameter)

Guard column : μ-Bondapak C_{18} pack with resolve C_{18}, 1.5 cm

Mobile phase : Mixture of Acetonitrile : 0.05 M disodium hydrogen orthophosphate (45:55 %v/v) and pH was adjusted to 6.0 with glacial acetic acid

Flow rate : 1.5 ml/min

Injection volume : 50 μl

Detector : Fluorescence, an excitation wavelength of 260 nm and an emission wavelength of 375 nm.

Temperature : room temperature (~25°C)

Mobile phase preparation

Mobile phase consisted of acetonitrile and 0.05 M disodium hydrogen orthophosphate anhydrous (45:55 % by volume). The mixture was adjusted to pH 6.0 with glacial acetic acid and filtered through PTFE filter, pore size 0.45 μm, and degassed by sonification for 15 minutes prior to using.

Standard curve

Plasma contained a known quantity of ketoconazole was run in parallel with the samples on each day of analysis. Standard curve was prepared by diluted stock solution (1,000 μg/ml) to serial concentrations 16, 8, 2, 0.4 and 0.1 μg/ml with drug-free plasma. All samples were proceeded following the procedures as described in 3.3.1.1. and 3.3.1.2. Standard calibration curves were conducted by the least-square linear regression of the ketoconazole concentration and peak area ketoconazole. Unknown concentrations of ketoconazole in patient's plasma were calculated from the standard curves by reverse prediction.

Recovery study

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

Lower limit of quantification

Lower limit of quantification was obtained by adding known amount of ketoconazole to drug-free plasma (0.01-16 μg/ml) and deproteinized as above. The peak areas of ketoconazole were calculated and plotted for the correlation between the concentration of ketoconazole and peak area. The lowest concentration of ketoconazole which was still linearly correlated was regarded as lower limit of quantification (LLOQ).

Precision of the assay procedure

Intraday (within-day) and interday (between-day) were established by adding a series of known amount of ketoconazole to drug-free plasma (0.4, 2, 8 μg/ml). The precision was calculated as percentage of coefficient of variation (%CV). It should be less than 5% for intraday and 10% for interday.

$$\%\ CV = [SD / \overline{X}] \times 100$$

Where : SD = standard deviation of the mean

$\overline{X}$ = mean value

Sample size calculation

The study was conducted to determine the influence of efavirenz on pharmacokinetics of ketoconazole in HIV-infected patients. However, there were no report on correlation of ketoconazole and efavirenz in HIV-infected patients. So, the study of Clarke et al. (2000) on the influence of efavirenz on the pharmacokinetics of methadone in 11 HIV-infected patients was used to calculate sample size. They found

that the AUC_{0-24} of methadone was also significantly reduced from 12,341 to 5,309 $ng.ml^{-1}.h$ in the presence of efavirenz, $p = 0.012$ (SD= 5,599 $ng.ml^{-1}.h$).

The different AUC of ketoconazole (d) = 12,341-5,309 = 7,032 $ng.ml^{-1}.h$

Type I error 5% (α=0.05), Z_α= 1.645, and Type II error 10% (β=0.10), Z_β= 1.282

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

$$= \frac{2(1.645+1.282)^2(5599)^2}{(7032)^2}$$

$$= 10.86$$

$$\approx 11$$

A total sample size of 11 patients should be enough to detect a significant pharmacokinetic difference in AUC for ketoconazole in the presence or absence of efavirenz. Hence, the number of sample size of the HIV-infected patients in this study was twelve.

Pharmacokinetic study

Patient selection

Inclusion criteria :

- Twelve HIV-infected patients, who had CD_4 T-lymphocyte absolute cell count less than 350 cell/mm^3 (obtained within the proceeding 2 months) and the age were over 18 years, participated in the 1-sequence, 2-period pharmacokinetics interaction study. Written informed consent was obtained from all patients, and the study protocol was approved by the ethic committee, Songklanagarind Hospital.

Exclusion criteria:

Patients were excluded from the study in the following cases :

- renal or hepatic impairment
- diarrhea or vomiting during the study period
- currently received the agents known to influence on ketoconazole pharmacokinetics.
- received ketoconazole, itraconazole, fluconazole or antiretroviral drugs within 1 month prior to the study
- known history of azole antifungal agents or antiretroviral agents hypersensitivity

Study design

The study was a 1-sequence and 2-periods without washout period pharmacokinetic interaction study.

Patients were hospitalized for 2 days at Songklanagarind hospital for blood sampling during each phase of the study. They were fasted from 12 PM on the night prior to the study and until 4 hours after the ketoconazole administration.

Phase 1, Patients served as control group, 400 mg of ketoconazole was administered with 200 ml water under supervision on day 1.

Blood samples (approximately 5 ml) were obtained from an indwelling venous catheter before ketoconazole administration and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12 and 24 h after administration of ketoconazole. Each sample was added to the heparinized tube and centrifuged at 1000g for 10 min, and plasma was harvested and stored at –80°C until the time of analysis.

Phase 2, Patients served as study group, each same patient from phase 1 took 600 mg of efavirenz once daily at bedtime (9.00 PM) to attenuate possible central nervous system effect of this drug (Adkins, 1998). The patient also received 150 mg of

lamivudine and 40 mg (or 30 mg when B.W. was lower than 60 kgs.) of stavudine twice daily on day 2 to 16, they were instructed to ingest the drugs in the morning and evening at 12-h interval. There is no evidence that any of this NRTI drugs interact with CYP3A4 (Berry, 1999). Lamivudine, stavudine and efavirenz were taken in day 2 and continue throughout the course of treatment. On day 16 of the study, patient received 400 mg of ketoconazole with 200 ml water. Blood samples were collected as previously mentioned.

Pharmacokinetic analysis

Plasma ketoconazole concentration-time data were fitted using WinNonlin Version 4.1 (Pharsight, Mountain View, CA) by non-compartment model with first-order absorption and the first-order elimination. The maximun plasma concentration (C_{max}) and the time for occurrence of C_{max} (T_{max}) were determined by visual inspection of individual plasma concentration-time profiles. The area under the concentration-time curve between 0 and 24 h (AUC_{0-24}) was estimated by using the linear trapezoidal rule.

Statistical analysis

All results were expressed as mean $\pm$ standard deviation (SD). The data show normal distribution, therefore, parametric statistic test was used for data assessment. Paired t-test was treated for pairwise comparisons. The priority value of significance was set at *p*-value of less than 0.05.

RESULTS

Analysis of Ketoconazole in Plasma

Chromatograms of drug free plasma (a); ketoconazole 8 μg/ml in plasma (b); and ketoconazole 8 μg/ml in methanol (c) are shown in Figure 13. Ketoconazole was eluted at 11 minutes as sharp and symmetrical peak.

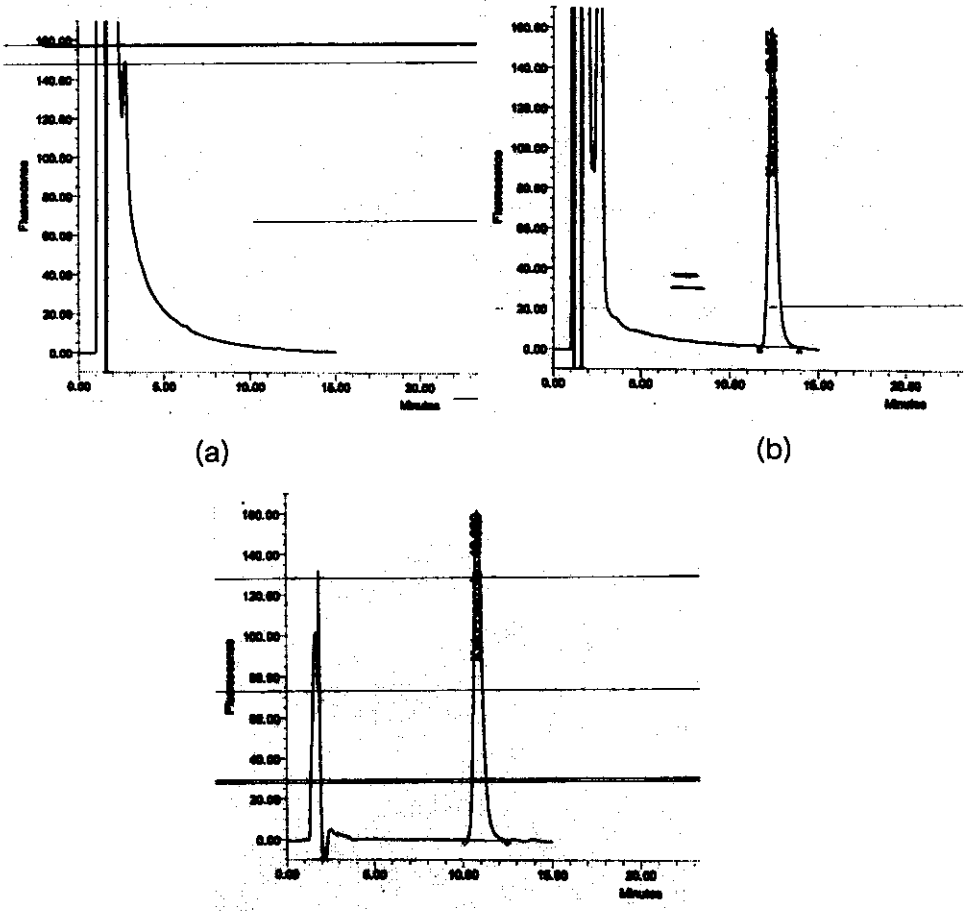


Figure 13 Representative HPLC chromatograms of drug free plasma (a); ketoconazole 8 μg/ml in plasma (b); and ketoconazole 8 μg/ml in methanol (c). Chromatogram of Ketoconazole was clearly separeated from plasma.

Linearity

Calibration curves for plasma analysis were constructed for ketoconazole in drug-free plasma to achieve the final concentrations of 0.01, 0.1, 0.4, 2, 8 and 16 μg/ml. Then, calibration curves were plotted between the peak area of ketoconazole versus plasma ketoconazole concentration (μg/ml), as shown in Figure 14. Using the least-square linear regression analysis, the correlation coefficient (R-square) was 0.9999 and the linear regression equation was :

$$Y = 534041\ X + 12823$$

Where :

X = plasma ketoconazole concentration (μg/ml)

Y = the peak area of ketoconazole

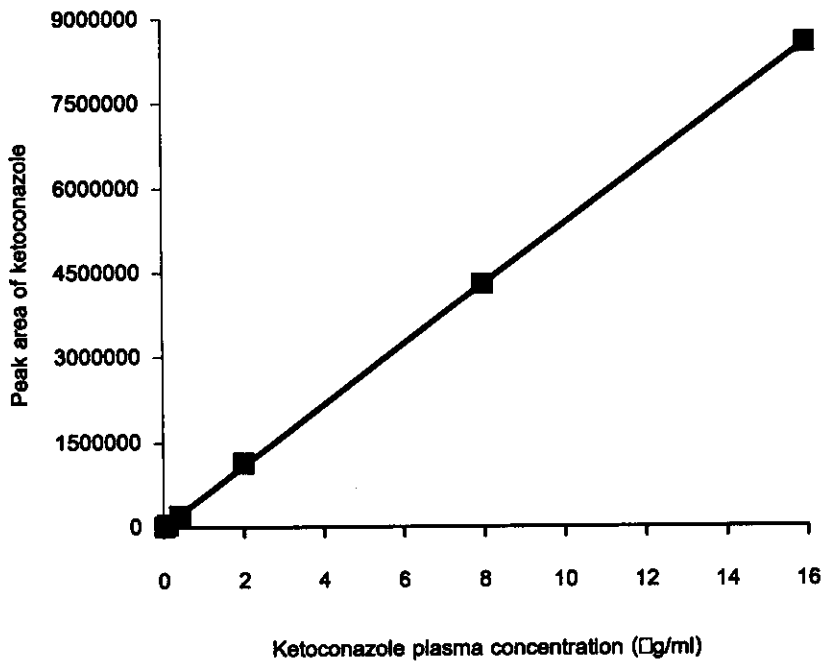


Figure 14 Calibration curve of ketoconazole in plasma

($Y = 534041\ X + 12823$, $r^2 = 0.9999$)

Recovery

Efficacy of deproteinization procedure was assessed from the percentage recovery, as shown in Table 1. The mean percentage recovery of ketoconazole at the concentration of 0.4, 2 and 8 μg/ml were 101.43, 102.59 and 100.11%, respectively. These results had shown a good efficiency of deproteinization procedure owing to the high percentages of recovery with %CV less than 5%.

Table 1 Recovery of ketoconazole

Plasma concentration (μg/ml)	Recovery (%) (n=5)	
	Mean	%CV
0.4	101.43	3.16
2	102.59	3.57
8	100.11	1.98

4.1.3. Lower limit of quantitation

The lower limit of quantitation (LLOQ) was obtained in this chromatographic condition and was found to be 0.01 μg/ml.

4.1.4. Precision

The precision of the assay procedure was assessed from %CV of area under the ketoconazole curve and retention times from intraday and interday results, as shown in Table 2. %CV of intraday precision for area under the peak of ketoconazole was found in the range of 1.00 to 2.67 % and retention time was found in the range of 0.02 to 0.09 %. %CV of interday precision for area under the peak of ketoconazole was found in the range of 3.58 to 4.17 % and retention time was found in the range of 1.00 to 1.87 %. Although internal standard was not used in this study, the results demonstrated a good precision of the assay method.

Table 2 Precision of the analytical method, intraday and interday precision

Concentration (μg/ml)	Mean ± SD of area under ketoconazole	%CV	Mean ± SD of Retention time	%CV peak
Intraday (n=5)				
0.4	221736 ± 5911	2.67	11.25 ± 0.007	0.06
2	1144402 ± 11408	1.00	11.23 ± 0.002	0.02
8	4476478±76330	1.71	11.20 ± 0.010	0.09
Interday (n=10)				
0.4	217243 ± 8853	4.08	11.21 ± 0.112	1.00
2	1098212 ± 45863	4.17	11.22 ± 0.210	1.87
8	4312581 ± 154645	3.58	11.21 ± 0.189	1.69

Table 3 Demographic data of the patients in the study

Patient No.	Sex	Age (years)	BMI (kg/m^2)	CD_4 ($cell/mm^3$)	WBC	Hb	Hct	Lymph	Mono	BUN mg%	Cr mg%	SGOT U/L	SGPT U/L	Alb g%	Preg. test
1	F	41	20.75	336	6,300	11.9	36	31	5	14.0	0.95	20	25	4.0	Neg
2	F	22	20.99	345	7,100	15.4	43	18	7	10.2	1.12	30	34	4.1	Neg
3	F	55	28.04	296	6,500	11.5	33	41	7	10.8	1.07	33	31	4.2	Neg
4	F	26	20.81	252	7,100	11.9	38	35	5	13.7	1.04	34	35	4.3	Neg
5	F	28	21.02	57	4,700	11.1	34	28	10	12.8	1.15	24	14	4.0	Neg
6	M	34	22.19	272	3,600	11.9	32	5	5	9.2	1.20	33	37	4.1	NA
7	F	26	26.56	298	2,900	12.3	37	45	6	14.1	0.70	18	8	4.0	Neg
8	M	34	16.07	46	4,400	10.1	32	12	6	16.7	1.06	35	29	3.6	NA
9	F	27	26.71	250	5,600	13.6	42	29	10	9.7	0.65	21	5	4.6	Neg
10	F	36	25.51	343	4,400	12.9	38	39	9	13.1	0.66	27	11	4.4	Neg
11	M	37	20.34	239	5,400	15.6	45	27	11	15.6	1.04	35	34	4.4	NA
12	F	39	21.72	90	5,240	12.3	37	52	10	9.2	0.66	34	32	3.8	Neg
Mean	-	33.75	22.56	235.33	5,270.0	12.5	37.3	30.17	7.58	12.43	0.94	28.67	24.58	4.13	-
SD	-	8.97	3.45	109.54	1,336.3	1.6	4.3	13.63	2.27	2.55	0.21	6.39	11.72	0.28	-

F = Female , M = Male , Neg = Negative , ND = Not applicable

Table 4 Plasma ketoconazole concentration for each patient during phase 1

Patient	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
No.	0.5	1	1.5	2	2.5	3	3.5	4	6	8	12	24
1	6.25	6.33	5.60	4.69	4.44	3.78	3.37	2.94	1.11	0.51	0.12	0.05
2	0.97	8.60	11.20	12.20	12.71	10.01	10.50	7.53	4.90	2.94	0.57	0.08
3	4.78	8.79	8.74	8.30	7.93	7.43	7.17	6.43	3.08	2.51	1.41	0.33
4	0.10	2.03	4.18	5.25	3.11	0.90	0.34	0.19	0.11	0.04	0.03	0.02
5	4.11	9.19	16.45	16.02	14.98	15.25	14.65	14.06	6.89	6.71	3.66	0.54
6	3.78	6.41	7.45	7.36	6.24	5.89	5.55	4.64	1.65	0.77	0.13	0.06
7	0.61	7.58	9.97	13.34	12.26	11.14	11.00	10.21	5.84	3.80	1.38	0.03
8	4.99	8.34	9.62	12.46	16.47	16.08	14.79	15.88	12.89	11.14	8.49	1.90
9	1.94	6.33	10.72	10.33	8.82	7.73	7.48	6.98	3.01	1.77	0.54	0.02
10	0.32	1.62	3.27	6.99	10.11	15.87	13.96	13.33	6.99	4.83	2.17	0.12
11	8.29	8.15	7.69	6.88	6.06	5.76	5.54	4.87	3.02	2.21	1.17	0.35
12	2.57	5.79	5.13	4.64	3.63	3.27	2.93	2.63	0.89	0.42	0.16	0.03
Mean	3.23	6.60	8.34	9.04	8.90	8.59	8.11	7.47	4.20	3.14	1.65	0.29
SD	2.59	2.49	3.64	3.76	4.46	5.13	4.86	4.95	3.59	3.20	2.40	0.53

Table 5 Plasma ketoconazole concentration for each patient during phase 2

Patient	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
No.	0.5	1	1.5	2	2.5	3	3.5	4	6	8	12	24
1	0.24	2.50	4.60	6.23	7.12	5.66	3.87	2.82	0.34	0.07	0.02	0.01
2	1.66	6.99	10.02	11.26	6.78	4.83	3.64	2.36	0.23	0.10	0.04	ND
3	1.57	1.87	5.66	4.72	3.76	3.00	2.43	1.92	0.49	0.16	0.13	ND
4	0.42	0.93	0.64	0.49	0.58	0.56	0.61	0.40	0.06	ND	ND	ND
5	2.38	5.11	10.21	10.68	11.78	9.53	7.76	6.42	1.79	0.45	0.06	0.02
6	1.06	2.95	2.98	3.02	3.26	3.32	2.54	2.44	1.27	0.82	0.35	0.02
7	2.17	2.68	2.51	4.64	6.40	6.33	5.01	3.75	0.71	0.16	0.01	ND
8	0.02	0.13	2.46	2.12	1.72	1.45	1.49	2.03	0.98	0.36	0.10	ND
9	0.25	0.76	4.21	3.54	4.12	4.85	4.23	5.06	0.91	0.25	0.02	ND
10	0.12	1.22	2.93	2.18	3.08	4.66	6.47	6.18	1.57	0.36	0.06	0.02
11	4.43	6.66	6.70	5.43	3.95	2.67	2.03	1.34	0.10	0.03	0.01	ND
12	0.47	0.89	2.04	4.27	2.13	1.87	1.81	1.68	0.44	0.09	0.03	0.01
Mean	1.23	2.72	4.58	4.88	4.56	4.06	3.49	3.03	0.74	0.26	0.08	0.02
SD	1.30	2.33	3.06	3.25	3.04	2.46	2.12	1.92	0.57	0.23	0.10	0.01

ND = Not detectable, the limit of quantitation was 0.01 μg/ml.

Table 6 Pharmacokinetic parameters of ketoconazole in each of the twelve patients during phase 1

Parameters	Patient number (S1-S12)													
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
C_{max} (μg/ml)	6.33	12.71	8.79	5.25	16.45	7.45	13.34	16.47	10.72	15.87	8.29	5.79	10.62	4.23
AUC_{0-24} (μg/ml.h)	25.92	66.08	61.56	8.89	129.33	34.15	80.01	199.75	51.17	89.28	54.40	21.77	68.53	52.89
$AUC_{0-\infty}$ (μg/ml.h)	26.13	66.41	64.19	9.34	132.75	34.40	80.11	217.68	51.24	89.80	57.38	21.95	70.95	57.18
λ_z (h^{-1})	0.23	0.24	0.13	0.04	0.16	0.24	0.31	0.11	0.28	0.23	0.12	0.16	0.19	0.08
$t_{½,Z}$ (h)	2.97	2.87	5.54	16.85	4.38	2.88	2.26	6.54	2.48	2.99	5.90	4.35	5.00	3.99
T_{max} (h)	1.0	2.5	1.0	2.0	1.5	1.5	2.0	2.5	1.5	3.0	0.5	1.0	1.67	0.75
Cl/f (L/h)	15.31	6.02	6.23	42.65	3.01	11.63	4.99	1.84	7.81	4.45	6.97	18.22	10.76	11.17
V_z /f (L/kg)	65.50	24.92	49.85	1037.14	19.08	48.30	16.30	17.34	27.98	19.27	59.35	114.53	124.96	288.68

Table 7 Pharmacokinetic parameters of ketoconazole in each of the twelve patients during phase 2

Parameters	Patient number (S1-S12)													
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
C_{max} (μg/ml)	7.12	11.26	5.66	0.93	11.78	3.32	6.40	2.46	5.06	6.47	6.70	4.27	5.95	3.20
AUC_{0-24} (μg/ml.h)	19.75	26.38	15.62	2.68	42.28	20.54	21.48	10.47	19.92	22.88	17.92	10.29	19.18	9.76
$AUC_{0-\infty}$ (μg/ml.h)	19.78	26.52	15.94	2.74	42.34	20.62	21.49	10.74	19.95	22.95	17.93	10.37	19.28	9.74
λ_z (h^{-1})	0.32	0.28	0.41	0.93	0.32	0.23	0.71	0.38	0.64	0.28	0.68	0.13	0.44	0.24
$t_{½Z}$ (h)	2.14	2.45	1.69	0.74	2.16	2.99	0.98	1.82	1.09	2.44	1.01	5.44	2.08	1.27
T_{max} (h)	2.5	2.0	1.5	1.0	2.5	3.0	2.5	1.5	4.0	3.5	1.5	2	2.29	0.89
Cl/f (L/h)	20.23	15.08	25.09	146.02	9.46	19.39	18.61	37.32	20.05	17.43	22.30	38.58	32.46	36.73
V_z /f (L/kg)	62.51	53.37	61.49	156.41	29.46	83.82	26.29	98.06	31.55	61.32	32.62	304.32	83.44	78.68

Table 8 Effect of efavirenz on ketoconazole pharmacokinetics in each of twelve HIV-infected patients

Patient No.	C_{max} (μg/ml)		AUC_{0-24} (μg/ml.h)		$AUC_{0-\infty}$ (μg/ml.h)		T_{max} (h)		$t_{½,Z}$ (h)		Cl/f (L/h)	
	Phase		Phase		Phase		Phase		Phase		Phase	
	1	2	1	2	1	2	1	2	1	2	1	2
1	6.33	7.12	25.92	19.75	26.13	19.78	1.0	2.5	2.97	2.14	15.31	20.23
2	12.71	11.26	66.08	26.38	66.41	26.52	2.5	2.0	2.87	2.45	6.02	15.08
3	8.79	5.66	61.56	15.62	64.19	15.94	1.0	1.5	5.54	1.69	6.23	25.09
4	5.25	0.93	8.89	2.68	9.34	2.74	2.0	1.0	16.85	0.74	42.65	146.02
5	16.45	11.78	129.33	42.28	132.75	42.34	1.5	2.5	4.38	2.16	3.01	9.46
6	7.45	3.32	34.15	20.54	34.40	20.62	1.5	3.0	2.88	2.99	11.63	19.39
7	13.34	6.40	80.01	21.48	80.11	21.49	2.0	2.5	2.26	0.98	4.99	18.61
8	16.47	2.46	199.75	10.47	217.68	10.74	2.5	1.5	6.54	1.82	1.84	37.32
9	10.72	5.06	51.17	19.92	51.24	19.95	1.5	4.0	2.48	1.09	7.81	20.05
10	15.87	6.47	89.28	22.88	89.80	22.95	3.0	3.5	2.99	2.44	4.45	17.43
11	8.29	6.70	54.40	17.92	57.38	17.93	0.5	1.5	5.90	1.01	6.97	22.30
12	5.79	4.27	21.77	10.29	21.95	10.37	1.0	2.0	4.35	5.44	18.22	38.58
Mean	10.62	5.95	68.53	19.18	70.95	19.28	1.67	2.29	5.00	2.08	10.76	32.46
SD	4.23	3.20	52.89	9.76	57.18	9.74	0.75	0.89	3.99	1.27	11.17	36.73
p-value	0.002		0.006		0.008		0.063		0.049		0.018	

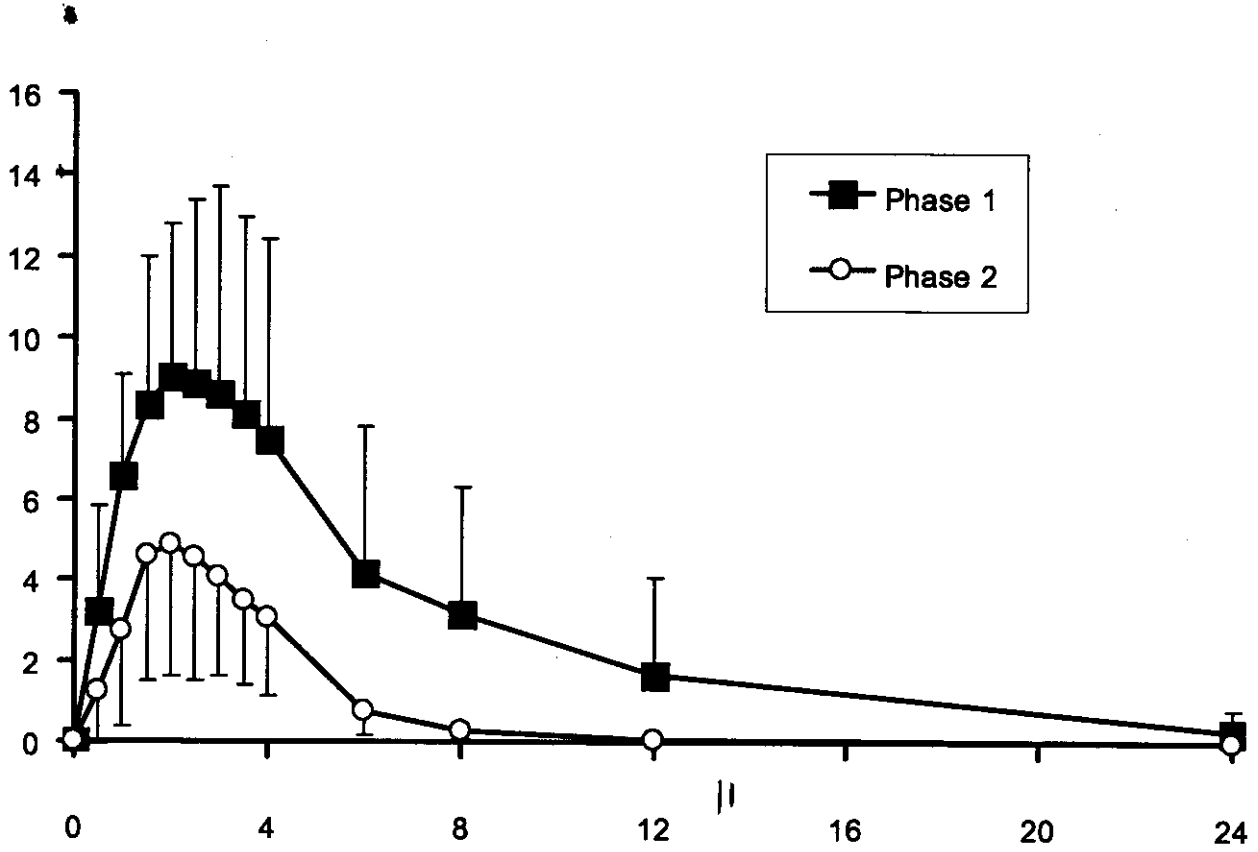


Figure 15 The mean plasma concentration-time profiles of ketoconazole in 12 HIV-infected patients after a singal dose of 400 mg ketoconazole alone, Phase 1 (■) and combination of 150 mg lamivudine and 30 or 40 mg stavudine twice daily, plus 600 mg efavirenz once daily, Phase 2 (O).

Patients

Fourteen HIV infected patients who had CD_4 T-lymphocyte absolute cell count less than 350 cell/mm^3 and met the inclusion criteria were enrolled into the study. Two patients dropped out from the study due to adverse effects, both patients reported rash and headache. Three patients were male and nine patients were female. The mean age was 33.7±8.9 years (ranging from 22 to 55 years) and the mean body mass index was 22.56±3.45 kg/m^2 (ranging from 20.34 to 28.04 kg/m^2). CD_4 T-lymphocyte counts were in the range from 46 to 345 cell/mm^3. Demographic data of the patients in the study are summarized in Table 3.

Plasma ketoconazole concentrations

Twelve patients completed the study without serious adverse effects. Plasma ketoconazole concentration were measured; all plasma drug concentration from Phase 1 are presented in Table 4 and those of Phase 2 are presented in Table 5. The pharmacokinetic parameters of ketoconazole in Phase 1 and 2 were showed in Table 6 and Table 7, respectively. The mean ± SD of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, Cl/f and $t_{½}$ of Phase 1 were 10.62±4.23 μg/ml, 68.53±52.89 μg/ml.h, 70.95±57.18 μg/ml.h, 10.76±11.17 L/h and 5.00±3.99 h, respectively. For Phase 2, the mean ± SD of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, Cl/f and $t_{½}$ were 5.95±3.20 μg/ml, 19.18±9.76 μg/ml.h, 19.28±9.74 μg/ml.h, 32.46±36.73 L/h and 2.08±1.27 h, respectively. All parameters showed statistically significant difference ($p<0.05$) as shown in Table 8. The mean plasma ketoconazole concentration-time data of two phases are depicted in Figure 15, and individual patient profile are shown in appendix E. Coadministration efavirenz resulted in a decrease in ketoconazole C_{max}, AUC_{0-24} and $t_{½}$ in all patients as shown in appendix F.

DISCUSSION AND CONCLUSION

Patients with HIV disease have to receive multiple and prolonged dosing regimens not only for the treatment of HIV disease, but also for the treatment and prophylaxis of opportunistic infections (Fogelman *et al.*, 1994). The standard of care for antiretroviral therapy involves using a triple combination of antiretrovirals, usually consisting of two nucleoside analogue reverse transcriptase inhibitors, and either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (DHHS, 2006). The issue of drug-drug interactions arises as one of the major problems associated with current therapy (Berry *et al.*, 1999).

Ketoconazole, an imidazole antifungal agent, is used for the treatment of superficial and deep fungal infection in HIV-infected patients. The drug has been reported to be primarily metabolized in the liver by oxidation of the imidazole ring, degradation of the oxidized imidazole, oxidative *O*-dealkylation, oxidative degradation of the piperazine ring, and aromatic hydroxylation to a large number of metabolites by hepatic microsomal enzymes (Heel *et al.*, 1982; Gascoigne *et al.*, 1981). It has been previously demonstrated that inducers of CYP450 may alter the metabolism of ketoconazole (Brass *et al.*, 1982; Lamson *et al.*, 1998). There have been few studies of the potential interactions between ketoconazole and antiretroviral drugs. It has been shown that nevirapine, an inducer primarily of CYP3A4, and ketoconazole should not be administered concomitantly because decreases in ketoconazole plasma concentrations may reduce the efficacy of the drug (Lamson *et al.*, 1998). In another studies (Brass *et al.*, 1982; Pilheu *et al.*, 1989), concomitant administration of ketoconazole and rifampicin, a potent inducer of CYP450 isozymes significantly reduce the ketoconazole plasma concentrations. Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which shows potent inhibitory activity against HIV-1. In the treatment of HIV infection, efavirenz is used in combination which is a preferred regimen of NNRTI-based (Adkins *et al.*, 1998; Micromedex, 2004; DHHS, 2006). In our study, all patients commenced an oral triple antiretroviral therapy that included efavirenz and two NRTIs,

lamivudine and stavudine. There is no evidence that any of this NRTI drugs interact 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 of coadministered substrates of p-glycoprotein (Berruet *et al.*, 2005; Mouly *et al.*, 2002). In *in vitro* studies efavirenz inhibited the isozymes CYP2C9 and 2C19 (Adkins *et al.*, 1998). It is known to interact with a number of drugs such as amprenavir, atorvastatin, simvastatin, clarithromycin, indinavir, ritonavir, lopinavir, methadone, nelfinavir, saquinavir (Adkins *et al.*, 1998; Benedek *et al.*, 1998; Joshi *et al.*, 1998; Clarke *et al.*, 2001; Aarnoutse *et al.*, 2002; Micromedex, 2004; Porte *et al.*, 2004; Dailly *et al.*, 2005; Gerber *et al.*, 2005; Labbe *et al.*, 2005). In the clinical point of view, there is the possibility of a pharmacokinetic interaction between ketoconazole and efavirenz in humans.

This study was designed to investigate the effect of efavirenz on the pharmacokinetics of a single oral dose of ketoconazole in HIV-infected patients.

Our study design was mainly based on the knowledge of the pharmacokinetics of ketoconazole and efavirenz. The recommended dose of ketoconazole for systemic infection is 200 to 400 mg once daily depending on the infection (Greenspan, 1994; Lambert *et al.*, 1992). In serious infections, the recommended dose is 400 mg/day. In the present study, ketoconazole was given to the patients at the dose of 400 mg in a single dose regimen. While efavirenz was given orally 600 mg once daily for 14 days at bedtime to attenuate possible central nervous system side effect of this drug (Adkins *et al.*, 1998). Because this dose was sufficient to induce hepatic CYP3A4 as described in previous study (Mouly *et al.*, 2002).

Our results showed that the plasma concentration-time data of ketoconazole were fitted to noncompartment model, because of the wide inter-individual variations of the patient (high value of SD). The possible explanation of this variation might be due to dissimilar absorption which might be affected by the increase of gastric pH and led to malabsorption of ketoconazole. Also gastric hypoacidity (gastric pH>3) in

AIDS patients is controversial in varied incidences ranging from 22 to 93%(Belistos *et al.*, 1992; Lake-Bakaar *et al.*, 1996).

It was revealed that when a single oral dose of ketoconazole was administered after pretreatment with efavirenz, lamivudine and stavudine for 14 days (Phase 2), The mean of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$ and $t_{½}$ of ketoconazole were significantly decreased by efavirenz by 43.97%, 72.01%, 72.83% and 58.40%, respectively when compared to ketoconazole alone (Phase 1). Although the result of almost all patients show significantly decreased of C_{max}, but there was one patient (patient No.1) who was increased in C_{max}. It could be explained as individual variation. On the contrary, the Cl/f was significantly increased by 201.67%, but T_{max} was not significantly increased when compared to ketoconazole alone. Therefore, results in our study indicated that efavirenz did not affect on the rate of ketoconazole absorption. Although a clinical correlation has not been demonstrated, it is assumed that successful treatment requires ketoconazole concentrations exceeding the MIC for the organism. Antifungal efficacy, like antibacterial efficacy, is theoretically dependent on the area under the concentration-time curve (AUC) of the antifungal agent above MIC and on the duration of time the serum antifungal concentrations remain above the MIC at the site of infection. Reduction of ketoconazole bioavailability will affect the treatment results in patients who has plasma concentration lower than minimum inhibitory concentration of ketoconazole on organism such as *Candida species* (MIC=0.25 mg/L) (Cartledge *et al.*, 1997). Since it may not adequate for the treatment in every and may cause drug resistance to ketoconazole.

Pretreatment with efavirenz for 14 days prior to ketoconazole resulted in increase in clearance of ketoconazole. These changes led to corresponding markedly decreases in C_{max} and AUC of ketoconazole, suggesting that the metabolism of ketoconazole was increased. In theory, the half-life ($t_{½}$) and elimination rate constant (λ_z) are known as dependent parameters because their values depend on the clearance (Cl/f) and volume of distribution (V_z/f) of the agent according to the equation: $t_{½} = 0.693 \times V_z/Cl$, $\lambda_z = Cl/V_z$. The half-life and elimination rate constant of a drug can

be changed either because of a change in clearance or a change in volume of distribution. In this study, the $t_{½}$ and λ_z of ketoconazole were moderately changed by efavirenz indicated that Cl/f was increased, but the V_z was decreased.

In phase 2 of this study, V_z/f of ketoconazole was increased in 9 patients, but decreased in 3 patients when compared to ketoconazole alone. The alteration in V_z/f of ketoconazole may be caused by plasma protein binding or ketoconazole tissue binding changes. It could be explained as efavirenz decrease ketoconazole plasma protein binding or increase ketoconazole tissue binding. Ketoconazole and efavirenz are extensively bound to plasma protein, especially albumin, 84% and 99.5%, respectively (Daneshmend *et al.*, 1988; Bennett, 1996; Adkins *et al.*, 1998). Knowledge about ketoconazole tissue binding or how efavirenz affects it is not well known. But the lone paired electron of efavirenz is higher than ketoconazole, therefore, efavirenz-albumin complex is also stronger than ketoconazole-albumin complex. It is possible that ketoconazole is displaced from its binding site on albumin by efavirenz, resulting in an increase in volume of distribution. Nevertheless, there were 3 patients (patient No.1, 4 and11) whose V_z/f were decreased in phase 2. One explanation of the difference of V_z/f might be due to individual variation.

The mean Cl/f of single oral dose of ketoconazole after pretreatment with efavirenz 14 days was significantly increased, which was thought to be due to increase in hepatic clearance. Indeed, the total clearance is defined by renal clearance and non-renal clearance, But previously study (Brass *et al*, 1982) found that C_{max} and $t_{½}$ of ketoconazole did not changed by renal impairment. Because ketoconazole is extensively metabolized by liver, and the elimination of unchanged drug and metabolites takes place primarily through the biliary tract. Only a small amount of unchanged drug (2 to 4%) is excreted via the kidney. In theory, if a drug is metabolized exclusively by liver, the total clearance (Cl/f) of the drug is equal to the hepatic clearance (Cl_H). Heptic clearance is defined as the volume of blood perfusing the liver that is cleared of drug per unit of time. Calculation of hepatic clearance based on total hepatic blood flow (Q_H), fraction unbound of drug in the blood (f_u) and the free intrinsic clearance (Cl_{int}): Cl_H =

$Q_H x\ f_u x\ Cl_{int} / Q_H + f_u x\ Cl_{int}$. The Q_H had no effect on ketoconazole clearance due to the same patients. In addition, f_u was increased as described in V_z/f that efavirenz could decrease ketoconazole plasma protein binding. While Cl_{int} was increased in phase 2 since a major part of this parameter is metabolism. It is important to note that the pharmacokinetic consequences of enzyme induction should always be a decrease in plasma concentrations, because cytochrome P450 induction will cause an increase in the hepatic metabolism and a decrease in bioavailability. Accordingly, efavirenz increased hepatic clearance of ketoconazole because efavirenz is an inducer of CYP3A4 and ketoconazole undergoes extensive metabolism by the set of cytochrome P450 enzymes.

With respect to the mechanism of the pharmacokinetic interaction between efavirenz and ketoconazole combination, it seems most likely that efavirenz caused induction of CYP3A4, the cytochrome P450 isozyme that play a role in the biotransformation of ketoconazole. Efavirenz is known to act as an inducer of CYP3A4 (Moyle, 1999; Adkins, 1998). It has been shown that efavirenz caused a concentration-dependent CYP3A4 induction and activation of the human pragnane X receptor (hPXR), a key transcriptional regulator of CYP3A4, *in vitro*. 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 (XBE) in the promoter sequence of CYP3A4, leading to increased in gene transcription (Hariparsad *et al.*, 2004). Although, ketoconazole is a p-glycoprotein substrate (Wang *et al.*, 2002) but efavirenz did not appear to induce intestinal CYP3A4 or intestinal p-glycoprotein (Berruet *et al.*, 2005; Mouly *et al.*, 2002), hence it might has no effected on ketoconazole bioavailability. Induction of metabolic enzymes is in accordance with data from this study showing increase in clearance of ketoconazole.

In conclusion, in a long term efavirenz treatment it has shown that efavirenz markedly decreases the plasma concentration of ketoconazole and significantly increased in clearance and decreased in half-life of ketoconazole. These effects may be mainly due to the induction of CYP3A4 isozyme by efavirenz. Therefore,

the dosage of ketoconazole should be monitored during co-administration with efavirenz and clinical outcome should be observed in order to minimize therapeutic failure.