CHAPTER 5

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.

For the above reasons, 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.