5. DISCUSSION AND CONCLUSION

Ketoconazole is an azole antifungal agent with broad-spectrum antifungal activity. It has been shown to reduce the metabolism of other drugs sharing the CYP3A4 pathway such as alprazolam, triazolam, midazolam, nifedipine, nicardipine, amlodipine, felodipine, quinine, reboxine, oestrogen cyclosporine amprenavir cocaine and opioid (Venkatakrishnan, et al., 2000, Kosten, et al., 2001, Annas, et al., 2003, Abraham, et al., 2003). In addition, ketoconazole is a potent P-glycoprotein inhibitor. P-glycoprotein (P-gp) is the ATP-binding cassette multidrug transporter, exhibits a drug (substrate)- stimulatable ATPase activity. P-gp is tissue-specific, on the apical surface of enterocytes, the biliary canalicular membrane of hepatocytes, the apical surface of epithelial cells of kidney and placenta, and the apical surface of endothelial cell in brain capillaries. Owing to its intracellular localization, the P-gp transporter can limit cellular uptake of drug from the blood circulation into the brain and placenta, and from the gastrointestinal lumen into the enterocytes. On the other hand, this transporter can also enhance the elimination of drug out of hepatocytes, renal tubules, and intestinal epithelial cell into the adjacent luminal space. Thus, inhibition of P-gp transport of a drug by other drug could potentially and ATP binding site, or from the blockage of ATP hydrolysis process (Ramachandra, et al., 1998). P-gp inhibition results in an increase in the systemic exposure and tissue distribution of drug that is P-gp substrates, while P-gp induction lead to a decrease in the systemic exposure (Lin, 2003).

Mefloquine is an antimalarial drug, which is effective for the prophylaxis and treatment of *Plasmodium falciparum* and *Plasmodium vivax.* Mefloquine is structurally related to quinine, and CYP 3A4 is important for the 3-hydroxylation of quinine in humans *in vivo* (Mirghani, et al., 1999). In addition, the antifungal ketoconazole produced a strong concentration-dependent inhibition of mefloquine metabolism. Since the imidazole antifungals are known inhibitors of CYP3A4 *in vitro*

(Fontaine, et al., 2000). In the pharmacokinetic point of view, there is possibility of a pharmacokinetic interaction between mefloquine and ketoconazole in humans *in vivo.*

Our study design was based on the knowledge of the pharmacokinetics and pharmacodynamics of mefloquine and ketoconazole. The recommended dose of mefloquine for treatment of malaria in Thailand is 1,250 mg. In the present study, mefloquine was given to the healthy male volunteers at the dose of 500 mg in a single dose regimen because we want to minimize our subjects from toxicity and reduce side effect of mefloquine. Ketoconazole was given orally 400 mg for 10 days in healthy volunteers, because this dose is sufficient to inhibit hepatic microsome enzymes as described in previous studies (Backman, et al., 1998; Daneshmend, et al., 1983).

In the present study, the profile of plasma mefloquine concentrations and derived pharmacokinetic parameters in healthy subjects receiving a single oral dose of 500 mg mefloquine were similar to those previously reported (Mansor, et al., 1989; Sunbhanich, et al., 1997 and Ridtitid, et al., 2000). The mean peak plasma mefloquine concentration in each study depended on the oral dose of mefloquine. Nevertheless, the variations of these pharmacokinetic parameters may be influenced by inter-individual variation and environment factors (e.g., sex, races, diet, smoking, coffee and alcoholic drinking). In this study, there was considerable inter-individual variability in plasma concentration-time profiles, and as a consequence there were large variations in the derived pharmacokinetic parameters as previously reported by Crevoisier et al. (1997) and Ridtitid et al. (2000).

The plasma concentration–time profiles showed that the data were well described by one compartment model with first order kinetics for both absorption and elimination. The pharmacokinetic parameters such as the area under the time curve (AUC), the elimination rate constant (Ke), the elimination half life ($t_{1/2}$), the

maximum plasma concentration (C_{max}) and the apparent oral clearance (Cl/f) of the subjects receiving ketoconazole treatment were significant different from those receiving mefloquine alone. These findings suggest that ketoconazole alter the pharmacokinetics of mefloquine when the two drugs are concomitantly administered.

In this study, it is revealed that when a single oral dose of mefloquine was co-administration with ketoconazole for 10 days, ketoconazole increased the mean of mefloquine $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, $t_{1/2}$, and C_{max} when compared to mefloquine alone (phase 1) by 1.8 fold (179.16%), 1.8 fold (175.66%), 1.4 fold (138.96%) and 1.7 fold (164.48%), respectively, whereas Ke, V_d/f and Cl/f were significantly decreased by 1.4 fold (72.72%), 1.4 fold (71.42%) and 2.1 fold (48.38%), respectively. In addition, the $AUC_{0\text{-last}}$, and C_{max} of mefloquine metabolite were decreased by 1.4 fold (71.54%) and 1.4 fold (69.23%), respectively, whereas V_d/f was significantly increased by 1.4 fold (141.59%) when compared to mefloquine alone. Cl/f was not significantly increased when compared to mefloquine alone.

The increasing of mean $AUC_{0\text{-last}}$, $AUC_{0\text{-}\alpha}$, C_{max} and $t_{1/2}$ of a single oraldose mefloquine after pretreatment with ketoconazole for 10 days may be due to inhibition of the hepatic mixed function oxidase system by ketoconazole, which were similar to those occurring with quinine (Mirghani, et al., 2003) since mefloquine has a chemical structure related to quinine. Significant quantities of CYP 3A4 were found in small bowel enterocytes and liver (Villikka, et al., 1997). CYP3A4 is the most abundantly expressed CYP and accounts for approximately 30 to 40% of the total CYP contents in human adult liver and small intestine (Wildt, et al., 1999). Since ketoconazole is a potent inhibitor of CYP3A4 in both liver and small bowel enterocytes, the obvious explanation of the strongly significant interaction observed between ketoconazole and mefloquine in this study was based on CYP3A4 activity inhibited by ketoconazole. The increase in mefloquine concentrations as represented by C_{max} and AUC after a ketoconazole co-

administration was due to inhibition of metabolic transformation of mefloquine in the liver rather than in gut wall, as t_{max} of mefloquine was not significantly affected by ketoconazole co-administration. Moreover, $t_{1/2}$ of mefloquine was significant increase by ketoconazole co-administration. Accordingly, the increase in bioavailability of mefloquine after ketoconazole co-administration is the result of a decrease in metabolism in the liver rather than in the small bowel enterocytes. In addition, ketoconazole also inhibits of P-gp, thereby decreasing P- gp-mediated drug transport and metabolism. As shown in the above study, co-administration of ketoconazole (50 mg/kg, i.v.) caused a 8-fold increase in brain level of nelfinavir and a 3.5-fold increase in plasma concentration in mice (Choo, et al., 2000). Both CYP3A4 and P-gp have broad substrate specificity. Therefore, there is striking overlap of substrate between CYP3A4 and P-gp. Because of overlapping substrate specificity, and because of coexpression of CYP3A enzymes and P-gp in the intestine, kidney, and liver, it is conceivable that P-gp may play an important role in drug absorption by limiting drug transport from the intestinal lumen, and metabolism (Lin, 2003). Since, mefloquine is a P-glycoprotein substrate, the possibility that inhibition of P-glycoprotein by ketoconazole contributed to the observed interaction cannot be excluded. The lower clearance in subjects with ketoconazole pre-treatment indicated a decreased hepatic metabolism of mefloquine. This suggests that ketoconazole inhibits the hepatic metabolism of mefloquine. In addition, CYP3A4 has been found in renal tissue, therefore, ketoconazole inhibition of renal tissue CYP3A4 causes the decrease of mean Cl/f of single oral doe of mefloquine after pre-treatment with ketoconazole for 10 days.

In phase II of this study, it was found that Vd/f of mefloquine was significant decreased after pretreatment with ketoconazole for 10 days. The decreasing in Vd/f of mefloquine may be caused by (a) circular changes and (b) competitive bound with P-gp.

(a) Circular changes such as those associated with bed rest and ambulating may also affect distribution volume but in our studies, each of which lasted 24 hours, subjects maintained routine daily activity. Thus systemic circulatory is not likely to have significant effect.

(b) Competitive by bound with P-gp resulted that ketoconazole might limit accession of mefloquine to certain body compartment i.e.CSF,liver. Co-administration of ketoconazole with some substrate of P-gp demonstrates that ketoconazole inhibit P-gp leading to the accumulation of those P-gp substrates in various tissues. In addition, mefloquine also competitive inhibitor of P-gp,it may competitive with mefloquine bind to P-gp result in a larger fraction of mefloquine would remain in plasma or tissue and Vd/f would appear to decrease likely decreased in Vd of quinine by ketoconazole (Ridtitid, et al.,2001).

The mean Cl/f of a single oral dose of mefloquine after pretreatment with ketoconazole 10 days was decreased may be caused by (a) decreased hepatic clearance and (b) inhibition of renal tissue CYP3A4.

(a) Hepatic 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 plasma drug concentration data can also be obtained from the relationship; hepatic clearance = (rate of elimination by liver)/(plasma drug concentration). Accordingly, ketoconazole decreased hepatic clearance of mefloquine because ketoconazole is selective and highly potent inhibitor of human liver microsomal CYP3A activity and mefloquine undergoes extensive metabolism by the set of cytocrome P450 enzymes. All in all, co-administration of ketoconazole with mefloquine causes the decreasing Cl/f of mefloquine.

(b) Ketoconazole is selective and highly potent inhibitor of CYP3A activity and mefloquine undergoes extensive metabolism by the set of cytocrome

P450 isozymes. In addition, CYP3A4 found in renal tissue. Therefore, ketoconazole inhibition of renal tissue CYP3A4 cause the mean Cl/f of single oral dose mefloquine after pretreatment with ketoconazole for 10 days to be decreased.

In phase II of this study, the significant decreased in the $AUC_{0\text{-last}}$ and C_{max} of mefloquine metabolite (carboxylic acid metabolite) on co-administration of ketoconazole may be due to inhibition of the hepatic mixed function oxidase system by ketoconazole. In addition, CYP3A4 is involved in the formation of this metabolite since the drug is a potent inhibitor of this enzyme.

The mean $t_{1/2}$ and Cl/f of mefloquine metabolite of a single oral dose of mefloquine after pre-treatment with ketoconazole for 10 days was not significantly different from mefloquine alone. This result may be caused by inter-individual variation. Differences in drug metabolism and their determinants in the human organism have been intensively investigated over years. In general, genetic factor (polymorphism) are more important than environmental ones. Hesselink, et al. (2003) found polymorphism of CYP3A4 in metabolism of tacrolimus, as a group, patients with the CYP3A4*1B carriers require more tacrolimus to reach target trough concentrations compared with CYP3A4*1A homozygotes. However, among the latter, age, nutrition, disease and drug interaction are common factors that alter drug metabolism. Finally, renal elimination of either drug or metabolite is subject to several factors such as urine pH, changes in renal blood flow. While, focusing on pharmacokinetic variability, it should be appreciated that the determination of drug response provides an ever present challenge to clinical pharmacology. It is evident that there may be considerable inter-individual differences in receptors (number, affinity of drugs) and enzymes (Wernsdorfer and Karbwang, 2001).

The mean t_{max} values of mefloquine and mefloquine metabolite after pretreatment with ketoconazole were not significant different from the respective values of the control. Therefore, result in this study indicates that ketoconazole does

not effect on the rate of mefloquine absorption. Our results were similar to the study of interaction between cimetidine and mefloquine in human (Sunbhanich, et al., 1997)

The limitation of experimental unit in our study comprise: (a) sample size (b) the interval between each phase and (c) variability of subjects.

(a) The confidence of our experimental depend on the quantity of used subjects, but due to the insufficient of experimental expenses, we must use the following equation to find quantity of subjects (Farongsank, 2003)

$$n = \frac{z^2 \ \sigma^2}{h^2}$$

n = number of subject

z = z- parameter (1-α/2)

σ = standard deviation

h = margin of sampling error

(b) The interval between each phase: in the research methodology, wash out period must be equal in each of subject and should not less than 10 times of the mean of elimination half life. Such long period must be regulated because the physiology of subjects may be varied. Treating of drugs for experiment should feed at a constant time of every day in order to relieve the variance that cause by circadian rhythm (Farongsank, 2003). In our experiment, although we designed the washout period equal 3 month but the plasma concentration time curve profile showed a valid degradation of mefloquine and metabolite as illustrated in figure 10. Consequently, we can conclude that our designed washout interval made credible in our experiment design.

(c) Variability of subjects: see " inter-individual variability" of phase II.

It is well established that mefloquine undergo extensive first pass metabolism by the liver. So the mean C_{max} and AUC of mefloquine in phase II

study were markedly significant increased in subjects after ketoconazole pretreatment, thus it could suggest that the presystemic metabolism of mefloquine was markedly decreased. These results were in good agreement with those of other studies of the effect of the ketoconazole on the pharmacokinetics of CYP3A4 substrates that undergo extensive presystemic metabolism. For example, in the study of cyclosporin-ketoconazole interaction, the AUC values of cyclosporin increased 3 fold produced by a single 400 mg oral dose of ketoconazole in renal allograft recipients on low level cyclosporin immunosuppression, suggesting that presystemic metabolism and elimination phases of cyclosporin decrease by ketoconazole inhibiting the hepatic and intestinal first pass metabolism of orally administered cyclosporin (Venkatakrishnan, et al., 2000).

Clinically, mefloquine is an antimalarial drug, which is effective for the prophylaxis and treatment of *Plasmodium faciparum* and *Plasmodium vivax.* Plasma concentrations of 200-300 ng/ml may be necessary to achieve chemosuppression in *Plasmodium faciparum* infections (Katzung, 1998). In this study, maximum plasma concentration of mefloquine after mefloquine administration alone and co-administration with ketoconazole were 345.10 ± 43.22 ng/ml and 567.65 ± 88.69 ng/ml, respectively which are adequate for a schizontocidal activity for *Plasmodium faciparum* malaria.

Food increases the rate and the extent of mefloquine absorption, administration of mefloquine after a high fat meal resulted in a 4% higher prophylactic efficacy compared with administration during a complete fasting (Crevoisier, et al., 1997). Therefore, if mefloquine and ketoconazole were co-administration, mefloquine should not be administered with high fat diet to prevent adverse effects. Our result indicated that ketoconazole (400 mg for 10 days) caused a great raised in plasma concentrations of mefloquine, which increased the therapeutic benefit if receive subtherapeutic dose of mefloquine.

It has been reported that mefloquine concentrations in blood samples from volunteers who experienced prophylaxis failure were all below 400 ng/ml, suggesting that higher mefloquine concentrations are necessary to suppress *Plasmodium falciparum* (Crevoisier, et al., 1997).

In conclusion, this study shows that ketoconazole considerably enhance plasma concentrations of mefloquine by inhibiting its metabolism. Enzyme inhibition, probably involved in CYP3A4-mediated metabolism, is a possible explanation for the interaction between ketoconazole and mefloquine in this study. Although the clinical significance of this interaction is not clear, it is reasonable to assume that ketoconazole and other potent inhibitors of CYP3A4 may enhance the therapeutic efficacy of mefloquine in the treatment of *Plasmodium falciparum*. In fact, the possibility of these two drugs prescribed by the physicians for the same patient is not frequent. However, in the developing countries such as Thailand, malaria and fungal infections are still the important problems. Mefloquine is an antimalarial drug while ketoconazole is widely prescribed to patients with fungal infections. Thus, clinicians should consider reducing the dose of mefloquine to prevent the pharmacokinetic drug interaction and toxicity. The CYP450s play a major role in the metabolism of mefloquine. The possibilities of other mechanism are inhibition of other CYP450s isoforms that involved in mefloquine metabolism or inhibition of P-glycoprotein. Further studies are needed to clarify the mechanism of drugs interaction between ketoconazole and mefloquine.