

DISCUSSION AND CONCLUSION

From the present data of method validation tests which included the linearity of the standard calibration curve, precision, accuracy, limit of quantitation were suitable for analysis of mefloquine and mefloquine carboxylic metabolite in plasma samples since CVs of intra- and inter-day assay for mefloquine and mefloquine carboxylic metabolite analysis in plasma samples was less than or very close to 10% and the recovery of mefloquine and mefloquine carboxylic metabolite in plasma were 83.19-99.48%.

Ketoconazole is azole antifungal agents 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 (Venkatakrisnan, 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 faciparum* and *Plasmodium vivax*. Mefloquine is structurally related to quinine, and cytochrome P450 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 malaria in Thailand is 1250 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 protect subject 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 Ridditid, et al., 2000). The mean peak plasma mefloquine concentration in each study was 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 Riditid et al. (2000).

The plasma concentration–time profiles show that the data are 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 (K_e), 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 if the two drugs are concomitantly administered.

In this study, results 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-last} , $AUC_{0-\infty}$, $t_{1/2}$, 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 K_e , 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-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-last} , $AUC_{0-\infty}$, C_{max} and $t_{1/2}$ of a single oral dose 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 are 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 strong 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-glycoprotein, thereby decreasing P-glycoprotein-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). Although it is not known whether 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 dose 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 significantly decreased after pretreatment with ketoconazole 10 days. The decreasing in Vd/f of mefloquine may be caused by circulatory 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.

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 cytochrome 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 cytochrome 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-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 Vd/f of mefloquine metabolite of a single oral dose of mefloquine after pretreatment with ketoconazole 10 days was increased may be caused by increase in plasma protein binding of parent drug. Mefloquine are extensively bound to plasma protein about 98%, the major protein albumin for both mefloquine and mefloquine metabolite. Since, decreasing of AUC_{0-last} and C_{max} of major metabolite (carboxylic acid metabolite). Hence, mefloquine metabolite cannot competitive to bind plasma binding protein with parent drug. Another possibility of decreasing C_{max} of major metabolite may be due to

a more distribution of metabolite from central to peripheral compartment (metabolite is more soluble than parent compound, mefloquine).

Although 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*1 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 has 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 be 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 1 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 (Venkatakrisnan, et al., 2000).

Clinically, mefloquine is an antimalarial drug, which is effective for the prophylaxis and treatment of *Plasmodium falciparum* and *Plasmodium vivax*. Plasma concentrations of 200-300 ng/ml may be necessary to achieve chemosuppression in *Plasmodium falciparum* 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 falciparum* 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 for prevent adverse effects.

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 faciparum* (Crevoisier, et al., 1997). 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.

In conclusion, oral pre-treatment and co-administration of ketoconazole with mefloquine for 10 days did not affect the absorption of orally co-administered mefloquine, while distribution and elimination of mefloquine were significantly affected. These findings suggested that hepatic metaolism of mefloquine was decreased. One of the possibility of a dercrease in hepatic mefloquine metabolism may be due to the inhibition of CYP3A4 isozyme by ketoconazole. 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 faciparum*. 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 a drug of malaria while ketoconazole is widely prescribed to patients with fungal infections therapy. Thus, clinicians should consider reducing the dose of mefloquine to prevent the drug pharmacokinetic drug interaction and toxicity, and should adjusted the dose of mefloquine to maximize the therapeutic efficacy and to reduce the cost of therapy.

. 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.