CHAPTER 5

DISCUSSION AND CONCLUSION

Quinine, the cinchona alkaloid, has been used for malarial treatment more than 350 years. It is recommended for treatment of chloroquine-resistant *P. falciparum* malaria, an important drug of choice for treatment of complicated and/or cerebral malaria (Tracy and Webster, 1996; WHO, 1990), and is also used for relief nocturnal leg cramps (Mackie and Davidson, 1995). Quinine has a narrow therapeutic window and its toxicity is serious, especially cardiotoxicity. It is mainly metabolized to 3-hydroxyquinine by cytochrome P4503A4 and its metabolism is inhibited by many drugs such as ketoconazole both *in vitro* (Zhao and Ishizaki, 19970) and *in vivo* (Mirghani *et al.*, 1999), etoposide (Zhao *et al.*, 1997), and cimetidine (Wanwimolruk *et al.*, 1986).

Ketoconazole and itraconazole are azole antifungal agents with broad spectrum antifungal activity. Nevertheless, they are potent inhibitor of CYP3A4. Previous studies reported that they interfered drug metabolism both in small intestine and liver. The CYP3A4 inhibition of these drugs resulted in some clinically important drug interactions. A variety of drug clearance decreased by ketoconazole and itraconazole such as chlordiazepoxide (Brown *et al.*, 1985), tirilazad (Fleishaker *et al.*, 1996), nisoldipine (Heining *et al.*, 1999), triazolam (Varhe *et al.*, 1994), midazolam (Tsunoda *et al.*, 1999 ; Olkkola *et al.*, 1994), quinidine (Damkier *et al.*, 1999), quinine (Mirghani *et al.*, 1999), reboxetine (Herman *et al.*, 1999), amprenavir (Polk *et al.*, 1999),

lovastatin and lovastatin acid (Neuvonen and Jalava, 1996), buspirone (Kivisto *et al.*, 1997), felodipine (Jalava *et al.*, 1997), terfinadine (Crane and Shih, 1993), clarithromycin (Auchair *et al.*, 1999) and bupivacaine (Palkama *et al.*, 1999).

For these reasons it leads us to study the effect of ketoconazole and itraconazole on the relationships between pharmacokinetics and pharmacodynamics of quinine in Thai male healthy volunteers.

Our study design was based on the knowledge of the pharmacokinetics and pharmacodynamics of quinine, ketoconazole and itraconazole. When quinine was given by intravenous infusion, it was shown that the electrocardiogram was changed in healthy volunteers with quinine concentration around 5 mg/l after receiving 10 mg/kg quinine (Karbwang *et al.*, 1993b). Ketoconazole and itraconazole were given orally 400 and 200 mg, respectively for 4 days in healthy volunteers, because these normal therapeutic doses were sufficient to inhibit CYP3A4 as described in previous studies (Olkkola *et al.*, 1994 ; Olkkola and Neuvonen, 1994 ; Kaukonen *et al.*, 1997 ; Jalava, Olkkola and Neuvonen, 1997).

Our results showed that the semi-logarithmic of quinine concentration-time profile fitted to one compartment open model and first-order kinetics for both absorption and elimination, which was similar to the previous studies of Alvan *et al.* (1991) ; Dyer *et al.* (1994) and Supanaranond *et al.* (1991) (Figure 10).

In the present study, the pharmacokinetic parameters of quinine in healthy subjects after receiving a single oral dose of 300 mg quinine sulphate

were comparable to other published data (Table 1) (Wanwimolruk, 1995 ; Auprayoon, 1995 ; Suwanmi and Salako, 1996 ; Ridtitid *et al.*, 1998). The mean plasma concentration of quinine depended on the oral doses used in each study. The variation of these parameters is also influenced by inter-individual variation of CYP3A4 activity in each subject, ketoconazole, itraconazole concentration and the environmental factors.

The hepatic CYP3A4 content has been shown to vary at least 20-fold, and the activity of CYP3A4 in small bowel which is found in the apical enterocytes and its content varies 11-fold among individuals (Dresser *et al.*, 2000). Moreover, the environmental factors (e.g., sex, diet, smoking, coffee, tea, alcoholic drinking and disease status) influence the activity of the enzymes. The plasma ketoconazole concentrations reached to peak about 3 hours after oral administration and are proportional to doses (Daneshmend *et al.*, 1981). Jame-Daw *et al.* (1997) and Varhe *et al.* (1994) showed that the C_{max} of ketoconazole might vary up to 10 times after oral administration. Ketoconazole would be expected to loose its metabolic inhibitory effect within 24 to 48 hours after the last dose (Venkatakrishnan *et al.*, 2000). However, in our study the doses and time for ketoconazole administration was sufficient to produce CYP3A4 inhibition.

The present results revealed that after pretreatment with 400 mg ketoconazole for 4 days, the mean AUC increased by 107% (2-fold), the mean Ka reduced by 49% (1.9-fold), the mean T_{max} in creased by 59% (1.6-fold), the mean C_{max} increased by 34% (1.3-fold), the mean Ke reduced by 38% (1.6-fold), the mean $t_{1/2}$ increased by 70% (1.7-fold), the mean Vd/f increased

by 22% (1.3-fold) and the mean CL/f reduced by 55% (2.2-fold). When compared with the study of Mirghani *et al.* (1999), which revealed that pretreatment with 100 mg ketoconazole given orally 12 and 1 hours before and at 12, 24, 36, 48, 60 and 72 hours after 500 mg quinine orally results in the mean AUC of quinine increased by 45% ($P < 0.001$), the mean apparent oral clearance of quinine decreased by 31% ($P < 0.001$) and the mean elimination half-life increased by 16% ($P < 0.01$). Thus, these results suggest that the inhibition of CYP3A4 by ketoconazole related to doses and the time of ketoconazole administration.

Since the elimination half-life of quinine was increased, and quinine is rapidly absorbed with the bioavailability was 88% (Salako and Sowanmi, 1992), therefore, the effect of CYP3A4 in small intestine in prehepatic metabolism may not much concerned (Ho *et al.*, 1999). Our present data in this study support the inhibition of CYP3A4 in the liver by ketoconazole.

The T_{max} was prolonged after pretreatment with ketoconazole. In general, the mechanism altered drug absorption is depending on, (a) interfere pH in gastrointestinal tract, (b) gastric emptying rate (c) intestinal motility (Birkett *et al.*, 1991) and (d) increase only amounts of absorption but not the rate. Although ketoconazole prolong T_{max} of many drug such as midazolam, amprenavir and nisoldipine, but less is known about the mechanism (Olkkola *et al.*, 1994 ; Polk *et al.*, 1999 ; Heinig *et al.*, 1999).

There was significantly decreased in Vd/f in ketoconazole pretreated group. The alteration in Vd/f of quinine may caused by (a) increase plasma protein binding or decrease quinine tissue binding, (b) circulatory changes, (c)

liver blood flow and (d) competitive bound with P-glycoprotein, which is function as an efflux pump of some substrate of P-glycoprotein such as saquinavir, itraconazole, rhodamine and doxorubicin (Eagling et al., 1999 ; Miyama *et al.*, 1998 ; Yamamoto *et al.*, 1999 ; Smit *et al.*, 1998)

(a) Ketoconazole increased quinine plasma protein binding or decrease tissue binding. Quinine and ketoconazole are extensively bound to plasma protein (69-92% and more than 99%, respectively), the major protein was α_1-acid glycoprotein for quinine and albumin for ketoconazole, respectively. Less is known about quinine tissue binding or how ketoconazole affects it but Brown *et al.* (1985) showed ketoconazole had no effect on chlordiazepoxide (the major binding of albumin) binding to plasma protein.

(b) Circulatory changes such as those associated with bed rest and ambulating may also affect distribution volume but in our studies, each of which lasted 48 hours, subjects maintained routine daily activity.

(c) It is not known if ketoconazole affects liver blood flow, but quinine is a low clearance drug (CL = 0.072-0.24 l/hr/kg) and low presytemic clearance (bioavalibility 88%) (Birkett, 1991) thus liver blood flow is not likely to have a significant effect.

(d) Competitive bound with P-glycoprotein result in ketoconazole might limit accession of quinine to certain body compartments i.e. CSF, liver. Coadministration of ketoconazole with some substrate of P-glycoprotein as in the outline that ketoconazole inhibit P-glycoprotein

leading to the accumulate of those P-glycoprotein substrate in various tissue. In addition, quinine also competitive inhibitor of P-glycoprotein, it may competitive with quinine bind to P-glycoprotein resulted in a larger fraction of quinine would remain in plasma or tissue and Vd would appear to decrease likely decreased in Vd of chlordiazepoxide by ketoconazole competitive for transport mechanism for organic base distribution (Brown *et al.*, 1985).

For the itraconazole-quinine interaction, the mean AUC of quinine after pretreatment with itraconazole was increased by 96% (2-fold), Ke decreased by 36% (1.6-fold), $t_{1/2}$ increased by 71% (1.7-fold) and CL/f decreased by 44% (1.8-fold). The plasma concentration time-curve of itraconazole in healthy volunteers was wide inter-individual variation (Hardin *et al.*, 1988). The mean AUC, Ke, $t_{1/2}$ and CL/f of quinine after pretreatment with itraconazole were significantly different from quinine alone. But there was no significant difference from those pretreated with ketoconazole. The mean AUC and C_{max} after pretreatment with itraconazole were slightly less than pretreatment with ketoconazole, which is similar to the study of Olkkola, *et al.* (1994). Itraconazole also increased $t_{1/2}$ and decreased quinine clearance like the previous reviewed (Chapter 2) by inhibiting CYP3A4 in the liver and Vd/f of quinine slightly decreased but not significant, it may competitively binding with P-glycoprotein to reuptake quinine like ketoconazole.

In the pharmacodynamic points of views, when compared control (before study) with quinine alone, there were no significant difference in blood pressure and QT_c but significantly decreased in pulse rate at 0.5 and 1.5 hours.

After pretreatment with ketoconazole, when compared with control, there was significant decrease in diastolic blood pressure at 2.5 hours and pulse rate at 0.5, 1, 1.5 and 24 hours. When compared with quinine alone, the pulse rate significantly reduced at 0.5, 1, 2.5, 4 and 24 hours.

After pretreatment with itraconazole, only the systolic blood pressure at 0.5 hours was significantly reduced from control.

The decrease in pharmacodynamic parameters were not related to the rise in plasma concentration of quinine, excepted only the diastolic blood pressure after pretreatment with ketoconazole at 2.5 hours which were altered nearly the time to peak of quinine. The QT_c interval was not altered in all phases.

The present results also suggested that no cardiovascular toxicities occurred as evidences by the cardiovascular parameters measured after pretreatment with ketoconazole or itraconazole were significantly altered when compared with control and quinine alone. However, there were significant changes in pulse rate at some interval times after pretreatment with ketoconazole or itraconazole compared with control and quinine alone. These results may be due to the increased in subjects activities since they were not related to plasma concentration of quinine, and do not have significantly changes in clinical as evidence by the no serious abnormal clinical symptom was found during the study.

Our present results found that mean peak plasma concentration of quinine in subjects administered a single oral dose of 300 mg quinine alone was 2.35 μg/ml, and after pretreatment with ketoconazole and itraconazole were 3.14 and 2.84 μg/ml, respectively. The intention of this study is to observe only

quinine pharmacokinetics without producing any side effects. For these reasons, we used a 300 mg quinine dose instead of 600 mg dose and expected that after pretreatment with ketoconazole or itraconazole the peak plasma concentration of quinine would not be high enough to produce toxicities, especially cardiotoxicity.

Generally, the effective plasma quinine concentration was 8-15 μg/ml and after 600 mg oral dose of quinine sulphate the plasma concentration may reach 15-20 μg/ml. Mild toxicity usually occurred at plasma quinine concentration above 10 μg/ml (Powell and McNamara, 1972) and cardiovascular toxicity was observed when the plasma concentrations was above 16 μg/ml. Karbwang *et al.* (1993) reported that the electrocardiogram may change in healthy volunteers with quinine concentrations around 5 μg/ml after 10 mg/kg intravenously. However, if we duplicate the dose of quinine to 600 mg dose, the peak plasma quinine concentration after pretreatment with ketoconazole and itraconazole would be 6 and 5.7 μg/ml, respectively, which seem likely to produce electrocardiogram changes.

However, in the clinical practice the toxicity produced by quinine after coadministration with ketoconazole or itraconazole may be occurred if high doses of quinine are administered i.e., loading dose, and longterm treatment with ketoconazole or itraconazole. Because, not only coinfection of malaria and HIV patients are found but also both malaria and fungal infection are serious problems in tropical zone, therefore these would be lead to increase coadministration of quinine and azole antifungal agents (ketoconazole or itraconazole) in clinical practice.

In conclusion, pretreatment with ketoconazole and itraconazole augmented the increase in plasma concentration of quinine mainly by inhibition of CYP3A4 in the liver. Moreover ketoconazole may compete quinine to bind with P-glycoprotein during the distribution phase. Drug monitoring of quinine was considered in patients who need coadministration of these drugs.