CHAPTER 5

DISCUSSION AND CONCLUSION

Ketoconazole and itraconazole are azole antifungal agents with broad spectrum antifungal activity. All the azoles are inhibitors of CYP3A4. Ketoconazole is the most potent CYP3A inhibitor, followed by itraconazole, and then by fluconazole. Ketoconazole and itraconazole are thus equally potent clinically important inhibitors of the clearance of CYP3A4 substrates such as cyclosporin, tacrolimus, triazolam, midazolam and terfenadine (Venkatakrishnan *et al.*, 2000). In addition, ketoconazole is a potent P-glycoprotein inhibitor (Lin, 2003). Although itraconazole may be a P-glycoprotein substrate, as shown by its increased brain accumulation in mdr1a(-/-) mice deficient in P-glycoprotein (Venkatakrishnan *et al.*, 2000). In contrast with the observation of Zhang and Benet (2001), itraconazole is a P-glycoprotein inhibitor. For these reasons it leads us to study the effect of ketoconazole and itraconazole on the pharmacokinetics of a single oral doses of praziquantel in Thai healthy male volunteers.

Our study design was based on the knowledge of the phamacokinetics and pharmacodynamics of praziquantel, ketoconazole and itraconazole. The recommended doses of praziquantel for opisthorchiasis in Thailand is a single oral dose of 40 to 50 mg/kg, with the cure rate of more than 90% (Bunnag and Harinasuta, 1983; Supanvanich *et al.*, 1982; Vivatanasesth *et al.*, 1982) or the three doses of 25 mg/kg taken 4 to 8 hours apart on the same day also result in high cure for infections with the liver flukes (Tracy and Webster, 2001). In the present study, praziquantel was given to the healthy males volunteers at the dose of 20 mg/kg in a single dose regimen because we want to protect subject from toxicity and reduced side effect of praziquantel. Ketoconazole and itraconazole were given orally 400 and 200 mg respectively for 5 days in healthy volunteers, because these doses were sufficient to inhibit hepatic microsomal enzymes as described in previous studies (Backman *et al.*,1998; Daneshmend *et al.*,1983). Our results showed that the plasma concentration-time

data of praziquantel were fitted to noncompartment model because the wide interindividual variations of subject which was similar to the previous study of Na-Bangchang *et al.* (1993). In another study, the serum concentration of praziquantel in healthy subjects after receiving a single oral dose of 20 mg/kg praziquantel were 0.80 mg/L (Sotelo and Jung, 1998) and 0.1-1 mg/L (Karbwang *et al.*,1993) while in the present study was 0.19 mg/L. The mean plasma concentration of praziquantel depended on the oral doses used in each study. The drug levels obtained of praziquantel showed wide individual variations that seem to be due to individual differences in pharmacokinetics (Jung *et al.*, 1990).

In this study, results revealed that when a single oral dose of praziquantel was coadministered with ketoconazole for 5 days, ketoconazole increased the mean C_{max}, *K*a, AUC_{0-12} and $AUC_{0-\infty}$ by 102.48% (2-fold), 119.96% (1.19-fold) 96.60% (1.97-fold) and 85.55% (1.86-fold respectively. But Cl/f and $V_{z/f}$ were significantly decreased by 58.45% (2.4-fold) and 66.39%(2.98-fold), respectively when compared with control. In the phase III, itraconazole significantly increased C_{max} when compared with phase I by 70.58% (1.7-fold).

The increasing of mean AUC, *K*a and C_{max} of a single oral dose praziquantel after pretreatment with ketoconazole for 5 days may be due to (a) inhibition of hepatic CYP3A4, (b) inhibition of gut wall first pass metabolism or inhibition of intestinal CYP3A4 and, (c) saturated first pass metabolism of PZQ.

(a) Ketoconazole is a selective and highly potent inhibitor of human liver microsomal CYP3A activity and praziquantel undergoes extensive metabolism by the set of cytochrome P450 isozymes. So coadministration of ketoconazole with praziquantel increased AUC and C_{max} of praziquantel

(b) Ketoconazole inhibit gut wall first pass metabolism. CYP3A4 is the dominant P450 isozyme of the mucosal enterocytes of human small intestine. In a study examining the relative expression of the various P450s in 60 human livers, CYP3A4 was found to account for, on average, 29% of

the total P450 present. As much as 60% of the P450 present in microsomes from an exceptional human liver was found to be CYP3A4. Furthermore, the levels of CYP3A4 determined immunochemically in a bank of human liver samples exhibited a 20-fold range. CYP3A4 is also the dominant microsomal P450 in the mucosal epithelial barrier of the small intestine. Its expression is higher in the proximal (duodenum-jejunum) small bowel, in comparison to the distal (ileum) bowel. The mean level of duodenal mucosal microsomal CYP3A in a bank of human small intestines was found to be approximately 44% of that in the human liver, but like the liver, individual levels varied more than 20-fold. Whereas some of the intestinal variability may be the result of *ex vivo* enzyme degradation, much of it is likely to result from the effects of endogenous and exogenous factors. There are several mechanisms that may account for widely varying expression of hepatic CYP3A4. The levels of CYP3A4 are induced or increased by exposure of the patient to a number of drugs. The levels of CYP3A4 are also decreased by a number of mechanism-based or suicidal inhibitors. In addition, basal expression of the enzyme may be under the control of endogenous circulating hormones. If first pass gut wall metabolism is extensive, inhibitors of CYP3A4 could affect the systemic AUC of substrate through the inhibition of first pass gut wall metabolism as well as hepatic metabolism. Ketoconazole inhibits intestinal CYP3A4. It has also been suggested, but not proven, that the profound and preferential inhibition of intestinal metabolism by high-affinity reversible CYP3A4 inhibitors, such as ketoconazole, persists well beyond the time window of inhibitor absorption. Inhibition of CYP3A4 activity in the small intestine may occur through the formation of a slowly reversible metabolite-heme complex as discussed earlier (part CYP). By this mechanism, inhibition of first pass metabolism with drug could occur at

systemic concentration well below the reversible inhibition constant (Ki), because it depends on the fraction of the cumulative dose that is converted to the proximal inhibitory metabolite. If dissociation of complex is slow relative to its rate of formation, it will accumulate over time (Thummel *et al.*, 2000). Although the level of CYP3A in the intestine are generally 10 to 50% lower than those found in the liver, CYP3A concentrations equaling or exceeding those in the liver have been observed in some individual. Indeed, despite the relatively low amount of CYP in intestine compare with liver, intestinal CYP3A can play a major role in drug metabolism for two reason: (i) its strategic placement at the tip of the villus; and (ii) its positioning in relation to p-glycoprotein. Hence, the intestine has the opportunity to metabolize drug as it is being absorbed. Although there is an apparent structural similarity in CYP enzyme in both the liver and intestine, no correlation in activity exists between the two location, thereby indicating that CYP3A4 in the intestine and liver are independently regulated (Doherty and Charman, 2002). As previous describe, we conclude that increased *K*a.

(c) Saturated first pass metabolism of praziquantel. Praziquantel appears that hepatic first pass metabolism might reach satulation at serum concentrations within the rang of 200-1000 μg/ml (Sotelo and Jung, 1998). In the study reported here, ketoconazole increase the bioavailability of praziquantel and increase maximum concentration to 371.31 ± 141.10 ng/ml. Therefore the level of praziquantel in plasma reach to satulation first pass metabolism of praziquantel causes the increasing of absorption phase.

In phase II of this study, it was found that V_z/f of praziquantel was significantly decreased after pretreatment with ketoconazole 5 days. The decreased in V_z/f of praziquantel may be caused by (a) increase in plasma protein binding or increase in

praziquantel tissue binding, (b) circulatory changes. Each factor could be explained as following :

(a) Ketoconazole increased praziquantel plasma protein binding or decrease praziquantel tissue binding. Praziquantel and ketoconazole are extensively bound to plasma protein (80% and more than 99%, respectively), the major protein was albumin for both praziquantel and ketoconazole. Less is known about praziquantel tissue binding or how ketoconazole affects it but Brown *et al.* (1985) showed that ketoconazole decreased the volume of distribution of chlordiazepoxide.

(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. Thus systemic circulatory is not likely to have a significant effect.

The mean Cl/f of a single oral dose of praziquantel after pretreatment with ketoconazole for 5 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 praziquantel because ketoconazole is a selective and highly potent inhibitor of human liver microsomal CYP3A activity and praziquantel undergoes extensive metabolism by the set of cytochrome P450 isozymes. All in all, coadministration of ketoconazole with praziquantel cause the decreasing Cl/f of praziquantel.

(b) Ketoconazole is a selective and highly potent inhibitor of CYP3A activity and praziquantel 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 a single oral dose praziquantel after pretreatment with ketoconazole for 5 days to be decreased.

In phase II, the mean $t_{1/2}$ of a single oral dose of praziquantel after pretreatment with ketoconazole for 5 days was not significantly different from praziqunatel alone. This result may be caused by (a) inter-individual variability, (b) others.

(a) Pharmacokinetic parameters differ not only between individuals, but also in the same individual at different occasions. Both rate and extent of drug absorption are important determinants of drug response as is the extent of any first-pass effect (where the drug is metabolized in the gut wall and/or liver on its initial passage from the gastrointestinal tract) and the degree of enterohepatic circulation. Differences in drug distribution are largely determined by physico-chemical properties such as lipophilicity and binding to plasma protein (albumin, α_1-acid glycoprotein) or to tissue proteins. Factors altering protein binding include hypoalbuminaemia, renal failure and displacement by other drugs. 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. However, among the latter, age, nutrition, disease and drug interaction are common factors that alter drug metabolism. Finally, renal elimination of either drug or metabolites is subject to several factors such as urine pH, changes in renal blood flow. While focussing 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)

(b) Others: Since the C_{max} and AUC of praziquantel were markedly increased by ketoconazole, with no significant change in the mean $t_{1/2}$, it is likely that this interaction is mainly explained by inhibition of the CYP-mediated first-pass metabolism of praziquantel in the intestine and the liver. Because praziquantel is a high clearance drug, its clearance is not sensitive to change in hepatic enzyme activity, which probably explains the lack of effect on the mean $t_{1/2}$ of praziquantel. Our results were similar to the studies of interaction between grapefruit juice and praziquantel in human (Castro *et al.*, 2002), bioavailability of praziquantel increases with concomitant administration of food (Castro *et al.*, 2000) and pharmacokinetic study of praziquantel administered alone and in combination with cimetidine in a single-day therapeutic regimen (Jung *et al.*, 1997).

In phase III of this study, The mean C_{max} and *Ka* of a single oral dose praziquantel after pretreatment with itraconazole for 5 days was increased. This result may be caused by inhibition of gut wall first pass metabolism or inhibition of intestinal CYP3A4; (see phase II). However the mean AUC increased but did not significantly different from the control value. This effect may be caused by (a) It has been suggested that itraconazole is a selective inhibitor of fungal cytochromes more than its effects on the mammalian CYP system (Venkatakrishnan *et al.*, 2000); (b) Praziquantel metabolized by a set of cytochrome P450 isozyme not only CYP3A4; (c) Bioavailability of traconazole influence by food intake (Poirier and Cheymol, 1998).

(a) All the azole antifungal agents are inhibitors of CYP3A4, albeit with different potencies. Ketoconazole is selective and highly potent inhibitor of human liver microsomal CYP3A activity and causes clinically significant drug interactions with CYP3A substrates such as cyclosporin and tacrolimus. The Ki values for ketoconazole to wards CYP3A index substrates are usually in the nanomolar range, whereas the potency of itraconazole is at

least an order of magnitude lower. Nevertheless, intrahepatic concentrations of itraconazole are much higher than Ki values toward CYP3A substrate, resulting in significant drug interactions upon coadministration. It has been suggested that itraconazole is a selective inhibitor of fungal cytochromes and its effects on the mammalian CYP system are negligible (Venkatakrishnan *et al.*, 2000).

(b) There are fewer reports of praziquantel metabolized. In the study of Masimirembwa (1994), praziquantel is metabolized by the set of cytochrome P450 isozyme but not indicate specific CYP. If praziquantel is metabolized by many CYP and itraconazole has lower potency of CYP3A4 inhibition than ketoconazole, so AUC of praziquantel after pretreated with itraconazole did not increased significantly different from the control.

(c) Bioavailability of traconazole influence by food intake. Absorption of lipophilic weak base itraconazole seemed to be promoted by low stomach pH and high-fat meal. If volunteers had a meal raising the pH of the gastrointestinal tract, the bioavailability of itraconazole will be decreased. Daneshmend (1984) reported that food has not reduced ketoconazole absorption. In addition, itraconazole is the potent reversible CYP inhibitor (see Chapter 2). All of above factor, due to itraconazole can not increased mean AUC significantly different from the control value.

The mean t_{max} values of praziquantel after pretreatment with ketoconazole or itraconazole were not significantly different from the respective values of the control. Therefore, results in this study indicated that ketoconazole or itraconazole has no effect on the rate of praziquantel absorption. Our results were similar to the study of interaction between grapefruit juice and praziquantel in human (Castro *et al.*, 2002).

The limitation of the 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 the quantity of subjects (Farongsak, 2003).

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

n = number of subject

z = z-parameter ($1 - \alpha / 2$)

s = standard deviation

h = margin of sampling error

From table in appendix-6 depicts that 6-10 subjects are acceptable to do the experiment.

(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 everyday in order to relieve the variance that cause by circadian rhythm (Farongsak, 2003). In our experiment, we designed the wash-out period equal 2 weeks as compare the time period with "Plasma concentrations and effects of oral methylprednisolone are condederably increased by itraconazole" (Varis *et al.*, 1998) which the study design has defined the interval between the phased equal 4 weeks. Consequently, we can conclude that our designed wash-out interval and the $t_{1/2}$ of ketoconazole and itraconazole made credible in our experiment design.

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

It is well established that praziquantel undergo extensive first-pass metabolism by the liver. So the mean C_{max} and AUC_{0-24} of praziquantel in phase II study were markedly significant increased in subjects after ketoconazole pretreatment, thus it could suggest that the presystemic metabolism of praziquantel was markedly decreased. These

results were in good agreement with those of other studies of the effect of keoconazole 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 decreased by ketoconazole inhibiting the hepatic and intestinal first pass metabolism of orally administered cyclosporin((Venkatakrishnan *et al.*,2000). There are fewer reports of interaction between itraconazole and cyclosporin. Addition of itraconazole 200 mg/day to renal and heart transplant recipients on cyclosporin immunosuppression causes 2 to 3-fold increase in trough cyclosporin concentration accompanied by nephrotoxicity.

Praziquantel is highly metabolized in the liver with a high extraction ratio (Na-Bangchang *et al.*, 1993), producing 4- hydroxypraziquantel which is the main metabolite in human (Schepmann and Blaschke, 2001). In rat, Zhang and Guan (1997) indicated that CYP3A is involved in the hydroxylation of the ring A of praziquantel. The hydroxylated metabolites are also excreted in the urine as conjugated with glucuronic acid and/or sulphuric acid (Meier and Blaschke, 2000).

The interaction of ketoconazole or itraconazole with praziquantel is probably caused by inhibition of CYP, which is involved in the praziquantel metabolism including CYP3A isoform (Zhang and Guan, 1997). The CYP3A4 is the most abundant isoform, accounting for about 30% of total CYP in the liver (Pea and Frlanut, 2001). Thus, in our study the CYP3A4 is most likely involved in the metabolism of praziquantel in the liver. Besides, this interaction may be caused by inhibition of other CYP450 isoforms, however, there were no evidences to support. In addition, ketoconazole or itraconazole also inhibition of P-glycoprotein, thereby decreasing P-glycoprotein-mediated drug elimination. Although it is not known whether praziquantel is a P-glycoprotein substrate,

the possibility that inhibition of P-glycoprotein by ketoconazole or itraconazole contributed to the observed interaction cannot be excluded.

Clinically, praziquantel is a broad spectrum anthelmintic drug with activity against all species of schistosomes pathogenic to human as well as against a wide variety of trematodes and cestodes, including cysticercosis and used either as a single oral dose of 40 mg/kg or multiple oral doses of 25 mg/kg (Goldsmith, 2001 ; Tracy and Webster, 2001). Peak serum concentrations of 200-2000 ng/ml of the unchanged drug are reach 1-3 hours after a therapeutic dose (Goldsmith, 2001). In man, the threshold plasma concentration of praziquantel for therapeutic effect is about 1.0 μM (approximately 300 ng/ml) and this has to prevail for about 6 hours in order to affect schistosomes lethally (Andrew, 1988). Furthermore, plasma concentrations of praziquantel increase when the drug is orally coadministered with a high-lipid diet and a high-carbohydrate diet in healthy volunteers (Castro *et al.*, 2000). Our results have shown that the mean C_{max} of praziquantel after ingestion of the single doses of 20 mg/kg praziquantel alone in 10 subjects was 183.38 $\pm$ 138.82 ng/ml, whereas the mean C_{max} of praziquantel after pretreated with ketoconazole 400 mg/kg and itraconazole 200 mg/kg for 5 days was 371.31 $\pm$ 141.10 ng/ml and 312.81 $\pm$ 204.98 ng/ml, respectively. Therefore the therapeutic doses of praziquantel given alone in this study showed levels of mean peak plasma praziquantel concentration which is not sufficient to produce an efficacy for anthelmintic activity because it was obviously smaller than that of the minimum effective concentration of praziquantel (300 ng/ml, 6 hours) for anthelmintic activity. In case of coadministration of praziquantel with ketoconazole or itraconazole, mean peak plasma concentrations of praziquantel were increased 2-fold and 1.7-fold, respectively. Thus, coadministration of ketoconazole 400 mg/day or itraconazole 200 mg/day with praziquantel could increase praziquantel concentrations in medical practice if the same conditions are assumed to be as in patients. Thus it could be an advantage for prescribing ketoconazole with the reduced dose of praziquantel, however, the clinical relevant would be justified.

In conclusion, our result indicated that ketoconazole (400 mg/kg for 5 days) caused a great raised in plasma concentrations of praziquantel, which will lead to the toxicity if receive therapeutic dose of praziquantel. 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, liver flukes (especially *Opisthorchis viverrini*) and fungal infections are still the important problems especially in the northeastern and northern region of the country. Praziquantel is a drug of choice and widely used in mass chemotherapy of opisthorchiasis while ketoconazole is widely prescribed to patients with fungal infections therapy. Thus, clinicians should consider reducing the dose of praziquantel to prevent the pharmacokinetic drug interaction and toxicity. The CYP450s play a major role in the metabolism of praziquantel. The possibility of other mechanism are inhibition of other CYP450 isoforms that involved in praziquantel biotransformation or inhibition of P-glycoprotein. Further studies are needed to clarify the mechanism of drugs interaction between ketoconazole or itraconazole and praziquantel.