CHAPTER 5

DISCUSSION AND CONCLUSION

Praziquantel, a pyrazinoisoquinoline derivative, is a broad-spectrum anthelmintic which is widely used in trematode infections. Praziquantel is highly metabolized in the liver with a high extraction ratio (Na-Bangchang *et al.*, 1993). It is metabolized by a set of cytochrome P450 isozymes induced by phenobarbital (Masimirembwa *et al.*, 1993) producing monohydroxylated derivatives in which *tran*-4-hydroxypraziquantel is the main metabolite in human (Schepmann and Blaschke, 2001). The hydroxylated metabolites are excreted in the urine as conjugates with glucuronic acid and/or sulphuric acid (Meier and Blaschke, 2000). The results from Masimirembwa and Hasler (1994(b)) study suggested that CYP1A2, CYP2C9, CYP2C10, CYP2D6 and CYP2E1 are not involved in the hydroxylation of praziquantel to its major metabolite 4-hydroxypraziquantel in rat, and it is likely that CYP2B1 and CYP3A, which are inducible by phenobarbital, are predominantly responsible for the formation of 4-hydroxypraziquantel. In another report, Zhang and Guan (1997) indicated that CYP3A is involved in the monohydroxylation of praziquantel in rat. Its metabolism in human is induced by many drugs such as dexamethasone (Vazquez *et al.*, 1987), phenytoin, phenobarbital (Na-Bangchang *et al.*, 1995), and carbamazepine (Bittencourt *et al.*, 1992), but the exact cytochrome P450 isozyme is still unidentified.

Rifampicin is the most potent inducer of the CYP450 enzyme system. Backmann and Juregui (1993) found that rifampicin induced several cytochrome P-450 isoenzymes, not only CYP3A4 but also CYP1A and CYP2C. These findings are consistent with the highly significant interactions reported in the literature for drugs metabolized by these isoenzymes (Strayhorn *et al.*, 1997). For example, glyburide and glipizide are metabolized by CYP2C9 (Neimi *et al.*, 2001), and theophylline by CYP1A2, and each of these drugs have interactions of major importance with rifampicin. Zhou *et al.* (1997) recently reported the induction of CYP2C19 by rifampicin. Clinical studies in healthy volunteers demonstrated a reduction in the plasma concentrations and half-life of ondansetron following treatment with rifampicin, and concluded that the interaction is most likely the result of induction of the CYP3A4-mediated metabolism of ondansetron (Villkka *et al.*, 1999). Another clinically important drug interaction with rifampicin was concomitant administration with oral contraceptives, and it was found that a 4-fold increase in the rate of hydroxylation of estradiol and ethinylestradiol in patients treated with rifampicin was associated with an increases of CYP content in liver biopsies (Lin and Lu, 1998).

In addition, rifampicin also induces some isoforms of the UDP-glucuronosyltransferases (UGT) enzyme system (Dilger *et al.*, 2000). For example, zidovudine (Gallicano *et al.*, 1999), morphine (Fromm *et al.*, 1997) and lamotrigine were metabolized by UDP- glucuronosyltransferases. Ebert *et al.* (2000) showed that rifampicin was able to reduce the AUC and $t_{1/2}$ of lamotrigine and to increased both the CL/F and the amount of lamotrigine in urine excreted as glucuronide. Rifampicin altered pharmacokinetics of

lamotrigine due to induction of the hepatic enzymes responsible for glucuronidation.

For these reasons it leads us to study the effect of rifampicin on the pharmacokinetics of single and multiple oral doses of praziquantel in Thai healthy male volunteers.

Our study design was mainly based on the knowledge of the phamacokinetics of praziquantel and rifampicin. The recommended dose 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 infection with the liver flukes (Tracy and Webster, 2001). In the present study, praziquantel was given to the healthy male volunteers at the dose of 40 mg/kg in a single dose regimen or the three doses of 25 mg/kg in a multiple dose regimen. Rifampicin was given orally 600 mg for 5 days in healthy volunteers, because these doses were sufficient to induce hepatic microsomal enzymes as described in previous studies (Miguet *et al.*, 1977; Borcherding, *et al.*, 1992; Freitag *et al.*, 1999; Tracy and Webster, 2001).

Our results showed that the plasma concentration-time data of praziquantel were fitted to noncompartment model because, the wide interindividual variations of the subject and the different drug administration schedual (single and multiple dose regimen), which was similar to the previous study of Na-Bangchang *et al.* (1993).

In the present study, the pharmacokinetic parameters of praziquantel in healthy subjects after receiving a single oral dose of 40 mg/kg praziquantel

could be comparable to other published data (Table 15) (Castro *et al.*, 2000; Mandour *et al.*, 1990; Masimirembwa *et al.*, 1994; Homeida *et al.*, 1994). The mean plasma concentration of praziquantel depended on the oral doses used in each study. The drug levels obtained for praziquantel showed wide individual variations that seem to be due to individual differences in pharmacokinetics (Jung *et al.*, 1990).

The present results revealed that when a single oral dose of praziquantel was administered with rifampicin for 5 days, plasma concentrations of praziquantel could only be detected in 3 out of 10 subjects. In 3 subjects with measurable concentrations, the mean AUC_{0-12}, $AUC_{0-\infty}$, C_{max} and $t_{1/2}$ reduced by 85% (6.7-fold), 83% (6-fold), 81% (5.2-fold) and 45% (1.8- fold), respectively, while the mean λ_z, Cl/f and V_z/f increased by 112% (2.1-fold), 684% (7.8-fold) and 248% (3.5-fold), respectively, compared with those values when praziquantel was administered alone. The mean AUC_{0-12} and C_{max} of praziquantel in subjects with undetectable concentrations after rifampicin pretreatment compared to those values after praziquantel alone reduced by 94% (15.9-fold) and 99% (93.5-fold), respectively.

For a multiple oral dose of praziquantel was administered with rifampicin for 5 days, plasma concentrations of praziquantel could only be detected in 5 of 10 subjects. In 5 subjects with measurable concentrations, the mean AUC_{0-12}, $AUC_{0-\infty}$, C_{max} and $t_{1/2}$reduced by 80% (5-fold), 78% (4.5-fold), 74% (3.8-fold) and 43% (1.7-fold), respectively, while the mean λ_z, Cl/f and V_z/f increased by 65% (1.6-fold), 375% (4.7-fold) and 173% (2.7-fold), respectively, compared with those values when praziquantel was administered alone. The mean AUC_{0-12} and C_{max} of praziquantel in subjects with

undetectable concentrations after rifampicin pretreatment compared to those values after praziquantel alone reduced by 89% (9-fold) and 98% (64.7-fold), respectively.

The mean λ_z, $t_{1/2}$ and Cl/f of a multiple oral dose praziquantel after pretreatment with rifampicin in 5 subjects whose praziquantel plasma concentrations could be measured were significantly different from praziquantel alone. In single dose these parameters were different between subjects receiving praziquantel alone compared with rifampicin pretreatment in 3 subjects with measurable concentrations but there were no statistical significance. The latter results may be due to wide inter-individual variations in metabolism of praziquantel since the number of subjects was rather small (3 subjects), and these results were similar to the study of Bittencourt *et al.* (1992).

Pretreatment with rifampicin for 5 days prior to praziquantel resulted in the increase in oral clearance of praziquantel. These changes led to corresponding largely decreases in C_{max} and AUC of praziquantel both a single and multiple oral doses phase, suggesting that the biotransformation of praziquantel was increased. In theory, the half-life and elimination rate constant (Ke or λ_z) are known as dependent parameters because their values depend on the clearance and volume of distribution of the agent: $t_{1/2}$ = (0.693 x V/Cl, Ke = Cl/V. The half-life and elimination rate constant for a drug can change either because of a change in clearance or a change in the volume of distribution. In the present study the $t_{1/2}$ and λ_z of praziquantel was moderately changed by rifampicin indicated that Cl/f and V_z/f of praziquantel were increased.

There was significantly increased in V_z/f of praziquantel both a single and multiple dose phase in rifampicin pretreated group. The alteration in V_z/f of praziquantel may be caused by (a) a decrease in plasma protein binding or increase in praziquantel tissue binding and (b) systemic circulatory changes. Each factor could be explained as following:

(a) Rifampicin decrease praziquantel plasma protein binding or increases praziquantel tissue binding. Praziquantel and rifampicin are extensively bound to plasma protein (80% and 89%, respectively), the major protein is albumin for both praziquantel and rifampicin. Less is known about praziquantel tissue binding or how rifampicin affects it but the lone paired electron of rifampicin is higher than praziquantel, therefore, rifampicin-albumin complex is also stronger than praziquantel-albumin complex. It is possible that praziquantel is displaced from its binding site on albumin by rifampicin, resulting in an increase in the free drug concentration of praziquantel causing increase in volume of distribution. Thus displacement of praziquantel from plasma protein binding is likely to have a significant effect on the increase in volume of distribution of praziquantel.

(b) Systemic circulatory changes such as those associated with bed rest and ambulating may also affect volume of distribution 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 t_{max} values of praziquantel in single or multiple doses after pretreatment with rifampicin were not significantly different from the respective values of the control. Therefore, the results from this study indicated that rifampicin has no effect on the rate of praziquantel absorption.

It is well established that praziquantel undergoes extensive first-pass metabolism by the liver. Since the mean C_{max} and AUC_{0-12} of praziquantel in this study were markedly decreased in both a single and multiple dose phase after rifampicin pretreatment, thus it could suggest that the presystemic metabolism of praziquantel was markedly increased. These results were in good agreement with other studies of the effect of rifampicin on the pharmacokinetics of CYP3A4 substrates that undergo extensive presystemic metabolism (Hebert *et al.*, 1992 ; Backman *et al.*, 1996 ; Villikka *et al.*, 1997 ; Kivisto *et al.*, 1998 ; Kyrklund *et al.*, 2000; Niemi *et al.*, 2000). For example, in the study of repaglinide-rifampicin interaction, the C_{max} values of repaglinide decreased by 31%, the $AUC_{0-\infty}$ by 57% and the $t_{1/2}$ by 21% with rifampicin treatment, suggesting that presystemic metabolism and elimination phases of repaglinide increased (Niemi *et al.*, 2000).

For instance, the same treatment with rifampicin decreased the AUC of midazolam, triazolam and simvastatin acid to 4%, 5% and 7% of the control, respectively (Backman *et al.*, 1996; Villikka *et al.*, 1997; Kyrklund *et al.*, 2000). This may be party explained by a large effect of rifampicin on the first-pass metabolism of these drugs, reflecting the amount of CYP3A4 in the small intestine (Zhang *et al.*, 1999). The increase in presystemic metabolism of praziquantel seemed to be largely due to induction of the cytochrome P450 enzymes in the liver. Another supportive evidence from the present study to

this assumption, which suggested that the metabolism of praziquantel was enhanced in the liver rather than the gut wall, as the t_{max} of praziquantel was unaffected by rifampicin (Ridtitid *et al.*, 2000). However, the present study did not show any evidence to exclude contribution of the intestine to increased praziquantel first-pass metabolism after induction of CYP3A4 with rifampicin. Rifampicin is a potent inducer of CYP3A4 not only in the liver but also in the intestine (Kolars *et al.*, 1992 ; Combalbert *et al.*, 1989), and several studies have shown drug interactions between rifampicin and other drugs (Venkatesan, 1992 ; Strayhorn *et al.*, 1997). Consequently, there were published reports the interaction between rifampicin and other CYP3A4-substrate drugs such as tamoxifen and toremifene, ondansetron, mefloquine and simvastatin acid, and indicated that CYP3A4 is most likely responsible for the enhanced metabolism in the liver (Kivisto *et al.*, 1998 ; Villikka *et al.*, 1999 ; Ridtitid *et al.*, 2000 ; Kyrklund *et al.*, 2000).

Rifampicin also induces some isoforms of the uridinediphosphate-glucuronosyl-transferases (UGT) enzyme system (Lin and Lu, 1998). For example, the studied of Dilger *et al.* (2000) suggested that bioavailability of propafenone decreased 87% after pretreatment with rifampicin. Rifampicin induced both phase 1 metabolism (*N*-desalkylation) and phase 2 metabolism (glucuronide) of oral propafenone.

Apart from rifampicin, there are other drugs (eg dexamethasone, carbamazepine, phenytoin and phenobarbital) (Vazquez *et al.*, 1987; Bittencourt *et al.*, 1992; Na-Bangchang *et al.* 1995) which are potent inducers of CYP enzymes. These drugs produced an interaction with praziquantel. For example, Na-Bangchang *et al.* 1995 studied that pretreatment with phenytoin

or phenobarbital results in significantly increase clearance secondary to induction of extensive first-pass metabolism of praziquantel, and relatively low plasma/CSF availability of the drug consequently resulted. The effect of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetic of praziquantel were studied in rats. The phenobarbital pretreated rats showed a 6-fold decrease in AUC, 6-fold decrease in C_{max} and 8-fold increase in total Cl compared to the saline treated controls. The 3-methycholanthrene-pretreated rats and their olive oil treated control did not show any statistically significant differences in the above parameters. These results suggested that praziquantel is extensively metabolized by phenobarbital-inducible isoforms. These findings also suggested that the bioavailability of praziquantel could be altered to a significant extent in humans taking drugs that are phenobarbital-type induced (Masimirembwa *et al.*, 1993). It is likely that CYP2B1 and CYP3A, both inducible by phenobarbital, are predominantly responsible for the formation of 4-hydroxypraziquantel (Masimirembwa and Hasler, 1994(b)). Hoppa (1999) suggested that rifampicin and some anticonvulsants, such as phenobarbital, phenytoin and carbamazepine, belong to the clinically most-important inducers of CYP3A activity.

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, 2001).

The interaction of rifampicin with praziquantel is probably caused by induction of CYP450, 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 induction of other CYP450 isoforms, however, there were no evidences to support this conclusion. In addition, rifampicin also induces P-glycoprotein, thereby increasing P-glycoprotein-mediated drug elimination. Although it is not known whether praziquantel is a P-glycoprotein substrate, the possibility that the induction of P-glycoprotein by rifampicin contributed to the observed interaction cannot be excluded.

Clinically, praziquantel is a drug of choice in the treatment of schistosome infections of all species and most other trematode and cestode infections, 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 reached 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 40 mg/kg

praziquantel alone in 10 subjects was 1024 ± 417.48 ng/ml, whereas the mean C_{max} of praziquantel after ingestion of the multiple doses of 25 mg/kg alone was 763.50 ± 378.44 ng/ml. It was seen that the therapeutic doses of praziquantel given alone in this study either as single or multiple oral doses showed levels of peak plasma praziquantel concentrations, which are sufficient to produce an efficacy for anthelmintic activity. The C_{max} of praziquantel of subjects with measurable or undetectable concentrations in the single and multiple doses after rifampicin pretreatment varied from 12.25 to 294 ng/ml which was obviously smaller than that of the minimum effective concentration of praziquantel (300 ng/ml) for anthelmintic activity, therefore, subsequently leading to the treatment failure in medical practice if the same conditions are assumed to be as in these patients.

In conclusion, results of the present study show that 5-days pretreatment with 600 mg of oral rifampicin causes a great reduction in plasma concentrations of either a single oral dose (40 mg/kg) or multiple oral doses (25 mg/kg) of praziquantel, which will lead to the failure of treatment if these interactions occur in pateints. In fact, the possibility of these two drugs to be 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 *Mycobacterium tuberculosis* 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 rifampicin is widely prescribed to patients with tuberculosis in the short-course therapy. Thus, clinicians should consider increasing the dose of praziquantel in a patient who is taking rifampicin

especially if the patient does not respond to an initial treatment with praziquantel. Additionally, CYP3A4 is most likely to play a major role in the metabolism of praziquantel in the liver since CYP3A4 is the most abundant isoform, accounting for about 30% of total CYP in the liver. The possibility of other mechanisms may be due to the induction of other CYP450 isoforms that involved in praziquantel biotransformation, induction of UDP-glucuronosyltransferases (UGT) enzyme system, induction of P-glycoprotein or displacement from protein binding site. Further studies are needed to clarify the mechanism of this interaction.