CHAPTER 5

DISCUSSION AND CONCLUSION

In this study, the pharmacokinetic parameters of DEC were analyzed by one-compartment model. They were obtained from the treatment of DEC alone, DEC after pretreatment with rifampicin, and DEC after pretreatment with ketoconazole. This results showed that neither rifampicin nor ketoconazole significantly alter the pharmacokinetic parameters of DEC (P>0.05). The urine pH did not affect the excretion of DEC throughout this study because there was not significantly difference among the mean urine pH of 3 phases (P>0.05). This study indicated that DEC may not be mainly metabolized by CYP and P-gp may not bee involved in DEC metabolism.

Diethylcarbamazine (DEC), a piperazine derivative, is a drug of choice for control and treatment of lymphatic filariasis, and therapy of tropical pulmonary eosinophilia (TPE) caused by *W. bancrofti* and *B. malayi* (Ottensen and Ramachandran, 1995). DEC is rapidly and extensively metabolized in liver (Micromedex Healthcare Series, 2004). Over 50% of oral dose appears in acidic urine as the unchanged drug, but this value is decreased in alkaline urine (Tracy and Webster, 2001). A major metabolite of DEC in human is DEC-*N*-oxide which is eliminated mainly in urine. About 10% was excreted as DEC-*N*-oxide; between 4 to 5% was recovered in the faeces (Edward *et al*., 1988). Ilondu *et al*. (2000) reported that DEC was found in saliva. Metabolic pathway of DEC was not clearly indicated. However, Tracy and Webster (2001) indicated that DEC metabolism is occurred in liver. Generally, cytochrome P450 (CYP) has been previously viewed as major contributors to drug metabolism. Rifampicin and ketoconazole are potent CYP3A4 inducer and inhibitor, respectively. Thus, we have investigated the effects of rifampicin and ketoconazole on the pharmacokinetics of DEC in healthy volunteers.

Rifampicin is the most potent inducer of the CYP enzymes. Backman and Juregui (1993) found that rifampicin induced several CYP isoenzymes, not only CYP3A4 but also CYP1A, CYP2C and CYP2D. 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 (Niemi *et al*., 2001), and theophylline by CYP1A2. 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 (Villikka *et al*., 1999). Hoogerwerf and Pasricha (2006) reported that ondansetron is extensively metabolized in the liver by CYP1A2, CYP2D6, and CYP3A4, followed by glucuronide or sulfate conjugation. 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 increase of CYP content in liver biopsies (Lin and Lu, 1998). Rifampicin is also a potent inducer P-gp (Dürr *et al*., 2000; Finch *et al*., 2002; Lilja *et al*., 2004; Magnarin *et al*., 2004; Granzotto *et al*., 2004). To date, rifampicin was found to be able to induce CYP3A4 and strongly correlated with P-gp levels. So far, the mechanisms by which rifampicin induces multi-drug resistant1 (MDR1) expressions are poorly understood. Recent studies demonstrate that the nuclear receptor pregnane X receptor (PXR) is involved in xenobiotic induction of CYP3A4. Interestingly, CYP3A4 and MDR1 are often co-induced (Geick *et al*., 2001).

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, amprenavir, cocaine, and opioid (Venkatakrishnan *et al*., 2000, Kosten *et al*., 2002, Annas *et al*., 2003, Abraham *et al*., 2003). In addition, ketoconazole is a potent P-gp inhibitor. 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 and the apical surface of endothelial cells 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, 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 leads to a decrease in the systemic exposure (Lin, 2003).

In the present study, pharmacokinetic drug interaction was studied on the basis of

the knowledge of the pharmacokinetics and pharmacodynamics of DEC, rifampicin and ketoconazole. The recommended dose of DEC for treatment of lymphatic filariasis is 6 mg/kg (Tracy and Webster, 2001). These doses are sufficient to induce or inhibit hepatic microsomal enzymes as described in previous studies (Varhe *et al*., 1994; Villikka *et al*., 1997; Lamberg *et al*., 1998; Herman *et al*., 1999; Niemi *et al*., 2000; 2004; Seidegård, 2000; Backman *et al*., 2005; Lucksiri *et al*., 2005).

In this study, plasma DEC concentrations profile and derived pharmacokinetic parameters were similar to those previously reported (Shenoy *et al*., 2000). The mean peak plasma DEC concentration in each study depended on the oral dose of DEC. Nevertheless, the variation of these pharmacokinetic parameters may be influenced by inter-individual variations and environmental factors (e.g. sex, races, diet, smoking, coffee and alcoholic drinking). The amount of DEC excretion by renal depends on the urine pH. The mean urine pH values were not significantly different when compared among 3 phase (Table 5). Therefore, the urine pH of each subject in this investigation did not influence the plasma concentrations of DEC. Generally, the t_{max} of DEC was 1-2 h (Edward *et al*., 1988; Tracy and Webster, 2001). Our study showed that t_{max} is 1-3 h as similar to the study of Shenoy *et al*. (2000) and Bolla *et al*. (2002).

Joseph *et al*. (1984) suggested that CYP was at least partly involved in the oxidation pathway of DEC. Li *et al*. (2003) studied in vitro system using rat liver microsomes (RLM), human liver microsomes (HLM), and recombinant CYP, classified as low clearance drugs (<30% of liver blood flow). DEC was difficult to estimate the relative contributions of CYPs to their elimination in HLM because of the immeasurably long $t_{1/2}$ in rCYPs even at high enzyme concentration of 20 pmol per incubation.

CYPs is the major contributor to oxidative xenobiotic metabolism (Kruger and Williams, 2005). Flavin-containing monooxygenases (FMOs) have been previously viewed as minor contributors to drug metabolism. However, the advantages associated with using FMOs to diversity the metabolism of a drug are now being recognized (Cashman, 2004). FMOs oxidizes the nucleophilic nitrogen, sulfur and phosphorus-containing xenobiotics (Parkinson, 1996). Currently, five forms of the FMO genes are known, but FMO3 is the major form in adult human liver that is likely responsible for a majority of FMO-mediated metabolism (Cashman and Zhang, 2002). Of the five functional human FMOs known, FMO3 appear to be the most important FMO present in adult human liver. Based on immunoreactivity investigations, FMO3 is expressed at

levels approaching 60% of the expression levels of the major CYPs present in adult liver (CYP3A4) (Cashman, 2004). Role of FMOs involved with metabolism of *N*-containing xenobiotics. For example, the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is readily N-oxygenated by FMO to the N-oxide. Another example is N-deacetyl ketoconazole which is *N*-hydroxylated by a number of FMOs (Krueger and William, 2005). Thus, there is a possibility that the FMOs may partly contribute to the *N*-oxidation of DEC. However, FMOs-mediated DEC metabolism has not yet been studied and it needed for further investigation. Mushiroda *et al*. (2000) indicated that ketoconazole was not FMOs inhibitor because it could not decrease a primary metabolite of itopride. Other FMOs inhibitors (methimazole and thiourea) could inhibit itopride *N*-oxide formation. The study showed that itopride metabolism involved with FMOs but not CYP3A4. However, in present study, we could not exclude that FMOs did not involve or affect plasma DEC concentrations. Thus, we have suggested that further investigation for DEC metabolism pathway correlated with FMOs *in vivo* and *in vitro* should be considered.

The involvement of P-gp pathway has not been study with DEC metabolism, in the present study using rifampicin and ketoconazole, which are P-gp inducer and inhibitor, respectively. However, the results in this study showed that all of pharmacokinetic parameters of DEC are not altered by rifampicin or ketoconazole pretreatment. These results could imply and suggest that P-gp does not play an important role in DEC metabolism and excretion.

While the normal human urine pH range is 5-6.5, the present study showed that the mean urine pH values were 5.93 ± 0.44, 5.77 ± 0.44 and 5.91 ± 0.50 in subjects who received DEC alone and after pretreatment with rifampicin or ketoconazole, respectively. When compared the mean urine pH values among the 3 phase-study, there is no statistically significant difference. The renal excretion of DEC depends on the urine pH. Awadzi *et al*. (1986) studied the effect of moderate alkalinisation on low dose DEC therapy in patients with onchocerciasis. The plasma elimination half-life and the total urinary excretion of DEC were significantly less in patients receiving sodium bicarbonate than in control group. Another study, patients received DEC at 6 mg/kg or 3 mg/kg with or without $NaHCO_3$ at 75 mg/kg body weight. There was a significant difference between the $t_{1/2,}\lambda_z$ for the patients receiving DEC at 3 mg/kg plus $NaHCO_3$ at 75 mg/kg body weight, while the general tendency $t_{1/2},\lambda_z$ and $AUC_{0-\infty}$ increased in patients treated with DEC at 6 mg/kg plus $NaHCO_3$ at 75 mg/kg body weight (Njenga *et al*., 1997). In this study, the

results showed that after a single oral dose of DEC was coadministered with rifampicin or ketoconazole for 5 days, the mean of urine pH among the 3 phases of the study was not significantly different. Thus, urine pH in each specific time points in the present study did not affect plasma concentrations of DEC throughout the study.

Interestingly, the organic anion transporter (OAT) plays a critical role in the renal excretion and detoxification of a wide variety of compounds including drugs, toxins, hormones, and neurotransmitter metabolites. Every OAT identified thus far is expressed in the kidney where their function is a major determinant of toxicity and the therapeutic action of drugs. In addition to the kidney, active OAT is also an important function of other barrier epithelia including liver, placenta, brain capillaries, and choroids plexus. For organic anions, active transepithelial transport across the renal proximal tubule followed by elimination via the urine is a major pathway in this detoxication process. Accordingly, a large number of organic anion transport proteins belonging to several different gene families have been identified and found to be expressed in the proximal tubule. The function of these transporters, in combination with the high volume of renal blood flow, predisposes the kidney to increased toxic susceptibility (Sweet, 2005). van Aubel *et al*. (2000) focused on the molecular aspects of renal organic anion transporters. The OAT substrates were drugs (e.g., antibiotics, chemotherapeutics, diuretics, nonsteroidal anti-inflammatory drugs, radiocontrast agents, cytostatics), drug metabolites (especially conjugations products with glutathione, glucuronide, glycine, sulfate, acetate), and toxicants and their metabolites (e.g., mycotoxins, herbicides, plasticizers, glutathione S-conjugates of polyhaloalkanes, polyhaloalkanes, hydroquinones, aminophenols).

Organic cations (OCs) constitute a diverse array of compounds of physiological, pharmacological, and toxicological importance. Renal secretion of these compounds occurs principally along the proximal portion of the nephron (Wright, 2005). The majority of drugs for therapeutic use including many antihistaminines, antacids, antiarrhythmics, antihypertensives and anticholinergics are organic cations or weak bases, i.e. molecules with a transient or permanent positive net charge (Müller *et al*., 2005). OCs are positively charged at physiological pH (Ciarimboli and Schlatter, 2005). It is very well known, however, that membrane transporters play an important role in drug absorption across the gastrointestinal tract. One of the best known examples is the cephalosporin and prodrug transport via the intestinal proton-coupled peptide transporter PEPT1. Main candidates as membrane transporters for cationic drugs are the organic

cation transporters (OCT) of the SLC22 family; OCT1, OCT2 and OCT3 also called extraneuronal monoamine transporter (EMT: dopamine, epinephrine, norepinephrine, serotonin, histamine). In human, OCT1 is expressed primarily in the liver, with some expression in heart, intestine, and skeletal muscle, whereas OCT2 is expressed in abundance in human kidney. Human OCT3 is expressed in liver, kidney, intestine, and placenta (Giacomini and Sugiyama,2006).

DEC, a piperazine derivative, is a basic compound (Lee *et al*., 1997; Tracy and Webster, 2001). Müller *et al*. (2005) indicated that OCT substrates (e,g., antihistamines, antacids, antiarrhythmics, antihypertensives and anticholinergics) are organic cations or weak bases. As previously mentioned, the elimination of DEC in both patients and healthy subjects is by renal and extrarenal routes. Thus, there is a possibility that DEC excreted by OCT might be occurred.

Theorically, DEC is a drug of choice of lymphatic filariasis used for prophylaxis and treatment of *W. bancrofti* and *B. malayi*. Therapeutic range is 800 to 1,000 ng/ml (Shenoy *et al*., 2000). Oral administration of DEC 6 mg/kg gives enough plasma concentration for antifilarial activity because peak plasma concentration is above the therapeutic level. The present study showed that administration of DEC alone, DEC plus rifampicin, and DEC plus ketoconazole did not alter the plasma DEC concentration, and in each phase studied the C_{max} was higher than the therapeutics concentration range. Therefore, DEC co-administration with rifampicin or ketoconazole may not alter the efficacy of prophylaxis and treatment of lymphatic filariasis.

In conclusion, this study showed that rifampicin or ketoconazole considerably unchanged plasma DEC concentrations by CYPs induction, P-gp induction, CYPs inhibition, or P-gp inhibition. The precise metabolic pathway of DEC is unknown. However, the results in this study suggested that CYPs may not be involved in DEC metabolism. DEC is still widely used in the developing countries including Thailand, since lymphatic filariasis, tuberculosis, and fungal infections are still the important public health problems. However, co-administration of these two drugs with DEC does not alter pharmacokinetics of DEC. Further studies are needed to clarify the DEC metabolic pathways. FMOs and OCTs, the two interested factors for DEC metabolic pathway, should be considered for further investigation.