Effect of Rifampicin on an Oral Single-Dose Mefloquine

Pharmacokinetics in Healthy Volunteers

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Master of Science Thesis in Pharmacology

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Thesis Title	Effect of Rifampicin on an Oral Single-Dose Mefloquine Pharmacokinetics in Healthy Volunteers
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Major Program	Pharmacology

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ชื่อวิทยานิพนธ์ ผลของไรแฟมปีซินต่อเภสัชจลนศาสตร์ของเมโฟลควินในอาสาสมัครสุขภาพปกติเมื่อให้โดยการรับประทานครั้งเดียว

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บทคัดย่อ

เมโฟลควินเป็นยาที่พัฒนาขึ้นโดยมีสูตรโครงสร้างคล้ายยาควินิน ยานี้มีประสิทธิภาพสูงในการรักษามาลาเรียที่เกิดจากเชื้อ *Plasmodium falciparum* ที่ดื้อต่อยาหลายชนิดซึ่งใช้ได้ทั้งในการรักษาและการป้องกันมาลาเรีย เป็นยาในอุดมคติสำหรับใช้ในการป้องกันมาลาเรียเพราะสามารถให้โดยการรับประทานครั้งเดียวได้และมีอาการข้างเคียงน้อยกว่าควินิน ส่วนไรแฟมปีซินเป็นยารักษาวัณโรคซึ่งมีฤทธิ์เหนี่ยวนำเอนไซม์ที่แรงมากในคนโดยเฉพาะอย่างยิ่งเอนไซม์ CYP 3A4 ซึ่งจะทำให้ประสิทธิภาพของยาหลายชนิดลดลงได้เมื่อใช้ร่วมกับไรแฟมปีซิน ดังนั้นในกรณีที่มีการใช้ยาไรแฟมปีซินและเมโฟลควินร่วมกัน ไรแฟมปีซินอาจมีผลเปลี่ยนแปลงเภสัชจลนศาสตร์ของเมโฟลควิน ซึ่งอาจส่งผลต่อประสิทธิภาพในการรักษาของยาเมโฟลควินได้ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของไรแฟมปีซินต่อเภสัชจลนศาสตร์ของเมโฟลควินในกรณีที่ให้ยาทั้ง 2 ชนิดนี้ร่วมกัน ซึ่งได้ทำการศึกษาในอาสาสมัครชายไทยสุขภาพปกติจำนวน 7 คน โดยให้ได้รับยาเมโฟลควินขนาด 500 มิลลิกรัมใน 2 กรณีคือ (ก) ได้รับยาเมโฟลควินอย่างเดียว (ข) หลังจากรับประทานยาไรแฟมปีซินขนาด 600 มิลลิกรัมวันละครั้ง เป็นเวลา 7 วันก่อนรับประทานยาเมโฟลควินและยังคงรับประทานยาไรแฟมปีซินขนาด 600 มิลลิกรัมวันละครั้ง ต่อไปอีก

เป็นเวลา 7 วันและหลังจากนั้นรับประทานยาไรแฟมปีซินต่อไปอีกสัปดาห์ละ 2 ครั้งไปจนครบ 56 วัน ความเข้มข้นของยาเมโฟลควินและสารแปรรูปในพลาสมาวัดโดย HPLC และนำข้อมูลมาเปรียบเทียบค่าพารามิเตอร์ทางด้านเภสัชจลนศาสตร์ทดสอบทางสถิติโดยใช้ Student's t-test พบว่าการให้ไรแฟมปีซินร่วมกับเมโฟลควินทำให้เมโฟลควินถูกกำจัดออกเร็วขึ้น (Cl) 4 เท่า (0.0214 ± 0.0038 vs 0.08 ± 0.03 l/hr/kg) ค่าครึ่งชีวิตของการกำจัดยา ($t_{1/2}$) เร็วขึ้น 2.5 เท่า (305.31 ± 47.15 vs 113.43 ± 49.71 hr) ความเข้มข้นสูงสุดของเมโฟลควินในพลาสมา (C_{max}) ลดลงอย่างมีนัยสำคัญ ($P< 0.05$) (855.63 ± 168.00 vs 695.67 ± 56.63 ng/ml) และพื้นที่ใต้กราฟของเมโฟลควิน (AUC) ลดลงประมาณ 3 เท่า (373.73 ± 57.47 vs 119.77 ± 54.94 mg/l.hr) นอกจากนี้ยังมีผลทำให้ค่าครึ่งชีวิตของการกำจัดยาของสารแปรรูปเมโฟลควิน ($t_{1/2}$) ลดลง 1.5 เท่า (506.66 ± 127.64 vs 307.45 ± 56.90 hr) เวลาที่ให้ความเข้มข้นสูงสุด (T_{max}) ลดลง 4 เท่า (220.62 ± 69.75 vs 52.48 ± 28.81 hr) นั่นคือไรแฟมปีซินมีผลทำให้ เมโฟลควินถูกแปรรูปได้เร็วขึ้นและถูกกำจัดออกจากร่างกายเร็วขึ้น โดยอาจจะผ่านเอนไซม์ CYP 3A4 ซึ่งจะทำให้ประสิทธิภาพในการรักษาด้วยยาเมโฟลควินลดลง ดังนั้นในระหว่างการใช้ยาไรแฟมปีซินจึงควรหลีกเลี่ยงการใช้ยาเมโฟลควิน เพื่อให้การใช้ยาได้ผลเกิดประสิทธิภาพสูงสุดและลดปัจจัยเสี่ยงต่อการเกิดปัญหาการดื้อยาของยาเมโฟลควิน

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ABSTRACT

Mefloquine, a structural analog of quinine, is effective single dose therapy for multidrug resistant *Plasmodium falciparum* malaria. It is used both in prophylaxis and treatment. It has ideal properties for prophylactic use because it has the advantage of a single day regimen and produces fewer adverse effects than quinine. Rifampicin, an antituberculosis drug, is a potent inducer of hepatic cytochrome P450 enzyme system (mainly CYP 3A4) resulting in numerous clinically significant drug interactions. Therefore, if mefloquine and rifampicin are coadministered during treatment, rifampicin may alter the pharmacokinetic of mefloquine resulting in changing the efficacy of mefloquine in malarial treatment. The objective of this study is to examine the effect of coadministration of rifampicin and mefloquine on the pharmacokinetics of mefloquine in healthy volunteers. The pharmacokinetic parameters of mefloquine were determined in 7 healthy male volunteers after receiving mefloquine 500 mg as an oral single-dose in 2 occasions : (a) mefloquine alone, (b) after pretreatment with rifampicin 600 mg given orally once daily for 7 days prior to mefloquine administration and continued

rifampicin 600 mg orally once daily from days 1 to 7 after rifampicin pretreatment, then 600 mg twice weekly from days 8 to 56. On study days 7, 500 mg mefloquine was orally administered 2 hours before rifampicin. The plasma mefloquine and mefloquine metabolite concentrations during 56 days were measured using High Performance Liquid Chromatography (HPLC). Statistical analysis using Student's t-test indicated that when mefloquine and rifampicin were coadministered, the oral clearance of mefloquine increased by about 4-fold (0.0214 $\pm$ 0.0038 vs 0.08 $\pm$ 0.03 l/hr/kg), the $t_{1/2}$ was shorter about 2.5-fold(305.31 $\pm$ 47.15 vs 113.43 $\pm$ 49.71 hr), the C_{max} decreased significantly difference (855.63 $\pm$ 168.00 vs 695.67 $\pm$ 56.63 ng/ml) and the AUC decreased about 3-fold (373.73 $\pm$ 57.47 vs 119.77 $\pm$ 54.94 mg/l.hr). In addition, the $t_{1/2}$ of mefloquine metabolite decreased about 1.5-fold (506.66 $\pm$ 127.64 vs 307.45 $\pm$ 56.90 hr) and T_{max} decreased about 4-fold (220.62 $\pm$ 69.75 vs 52.48 $\pm$ 28.81 hr). The alteration in mefloquine pharmacokinetic parameters may be mainly due to the induction of CYP 3A4 isozyme by rifampicin and may lead to reduce efficacy of mefloquine in malarial treatment. Therefore, mefloquine and rifampicin should not be coadministered in order to maximise therapeutic efficacy and prevent a risk of resistant of *P. falciparum.*

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LIST OF ABBREVIATIONS

ng	=	nanogram
µg	=	microgram
mg	=	milligram
g	=	gram
kg	=	kilogram
µl	=	microlitre
ml	=	millilitre
l	=	litre
nm	=	nanometer
µm	=	micrometer
mm	=	millimeter
min	=	minute
hr	=	hour
d	=	day
wk	=	week
yr	=	year
C_{max}	=	maximal plasma concentration
T_{max}	=	time to maximal plasma concentration
Ka	=	absorption rate constant
Ke	=	elimination rate constant
$t_{1/2}$ (abs)	=	absorption half-life
$t_{1/2}$	=	elimination half-life

LIST OF ABBREVIATIONS (Continued)

Cl/f	=	apparent oral clearance
Clo	=	oral clearance
Clu/F	=	unbound clearance
AUC	=	area under the concentration-time curve
Vd	=	volume of distribution
Vd/f	=	apparent volume of distribution
MIC	=	minimum inhibitory concentration
$\bar{x}$	=	mean
S.D.	=	standard deviation
C.V.	=	coefficient of variation
r	=	correlation coefficient
P	=	P value
%	=	percent
vs	=	versus
C.I.	=	confidence interval
^{o}C	=	degree Celcius
®	=	trade name
mV.F.S.	=	millivolt full scale
UV	=	ultraviolet
iv.	=	intravenous
eg.	=	exampli gratia
etc.	=	et cetera

LIST OF ABBREVIATIONS (Continued)

i.e.	=	id est
mmol	=	millimole
M	=	molar
M.W.	=	molecular weight
vol/vol/vol	=	volume by volume by volume

CHAPTER 1

INTRODUCTION

Half of the World 's population lives in malaria-endemic areas and about 20-30 million people, mainly Europeans and North Americans, either travel or stay in malarious areas. Morbidity and mortality from this disease is considerably high with approximately 200 million incident cases and two million deaths annually (Foster, 1994). In such an environment, prophylaxis and treatment of the disease are of prime public health importance.

Malaria is a deadly disease which constitutes one of the most serious public health problems in Thailand. Approximately 60% of malaria cases are caused by *Plasmodium falciparum*, almost all are chroroquine resistant, over 80% and 30% are sulfadoxine-pyrimethamine and quinine resistant, respectively. The use of mefloquine has been shown to be effective against multi-drug resistant *falciparum malaria* (Harinasuta et al., 1983 ; Karbwang and White, 1990). This drug seems to be the only available drug for prophylaxis in Thailand (Karbwang et al., 1991).

Mefloquine is a 4-quinoline methanol, structurally related to quinine and selected for development by the Walter Reed Army Institute of Research in the US from over 30,000 screened compounds. Although it was only marketed in 1985, mefloquine is effective single dose therapy for all species of malaria infecting humans, including multi-drug-resistant *Plasmodium falciparum*. It is used both in prophylaxis and treatment of the disease (Karbwang and White,

1990). Mefloquine is relatively well tolerated and has the advantage of a single daily dose regimen. It has ideal properties for prophylactic use (Nosten and Price, 1995). Mefloquine is a reasonably well tolerated drug for both curative therapy and prophylaxis of malaria, with minor side effects (Crevoisier et al., 1997). Because it produces fewer adverse effects than quinine, the drug has an important advance in the treatment of falciparum malaria (Goldsmith, 1992).

Tuberculosis (TB) continues to be a very major problem throughout the world : in the early 1990s as many as 16 million cases of tuberculosis have been reported with, each year, 8 million new cases (one-half of which are infectious) and 3 million deaths due to the disease (Enarson, D.A. and Rouillon, A., 1994). In 1997, new cases of TB totaled an estimated 7.96 million. An estimated 1.87 million people died of TB (Dye, C. et al., 1999). These estimates are likely to be high; a more reasonable estimate of the total number of new cases each year is 5.5 million (Enarson, D.A. and Rouillon, A., 1994)

Rifampicin is a semisynthetic derivative of rifamicin B, a complex macrocyclic antibiotics. It is an antituberculosis drug which is usually administered for 4 to 12 months with other antituberculosis drugs. A potential for drug interactions often exists because rifampicin is a potent inducer of hepatic drug metabolism, as evidenced by a proliferation of smooth endoplasmic reticulum and an increase in the cytochrome P450 content in the liver. The induction is a highly selective process and not every drug metabolised via oxidation is affected (Venkatesan, 1992). The enzyme induction caused by rifampicin affects the metabolism of many other drugs,

induction caused by rifampicin affects the metabolism of many other drugs, increasing their metabolism and reducing their effects. The drugs affected include anticonvulsants, oral antidiabetic drugs, digoxin, anticoagulants, sex hormones including the contraceptive pills, steroids, vitamin D, ketoconazole, theophylline, protease inhibitors, zidovudine, delavirdine, itraconazole, nifedipine, midazolam, triazolam, nortriptyline, doxycycline and quinine (Mcnicol, et al., 1995 ; Strayhorn et al., 1997). Recently, Wanwimolruk, et al. (1995) reported that rifampicin pretreatment caused a marked increase in the clearance of quinine, possibly due to enzyme induction.

Mefloquine has a structure chemically related to quinine. Since quinine is extensively metabolized by CYP450 3A4 (Mirghani, et al., 1999); therefore, rifampicin would theoretically altered the metabolism of mefloquine similar to its effect on quinine pharmacokinetics. Very low plasma concentrations of mefloquine are associated with an increased risk of prophylaxis, treatment failure and spreading drug resistance.

With the increased number of immunosuppressed patients i.e. HIV infection, TB is now appearing with greater frequency. Therefore, the chance of using rifampicin in TB treatment is increased. Due to the increase in prescribing rifampicin, the possibility of rifampicin and mefloquine coadministration tends to have a chance to occur in clinical practice, and may lead to cause rifampicin-mefloquine drug interaction. The purposes of this investigation is to study the effect of rifampicin on an oral single-dose mefloquine pharmacokinetics in healthy volunteers. Due to the lack of interaction data between rifampicin and mefloquine, the present study may be

the guidance and useful data for decision making in case of coadministration of mefloquine and rifampicin.

CHAPTER 2

LITERATURE REVIEW

Mefloquine

Mefloquine[*dl*-erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol] is a quinoline methanol which structurally related to quinine (Figure 1) and selected for development by the Walter Reed Army Institute of Research in the US from over 30,000 screened compounds. Although it was only marketed in 1985, it is probably the most frequently studied of all antimalarials (Nosten, et al., 1995)

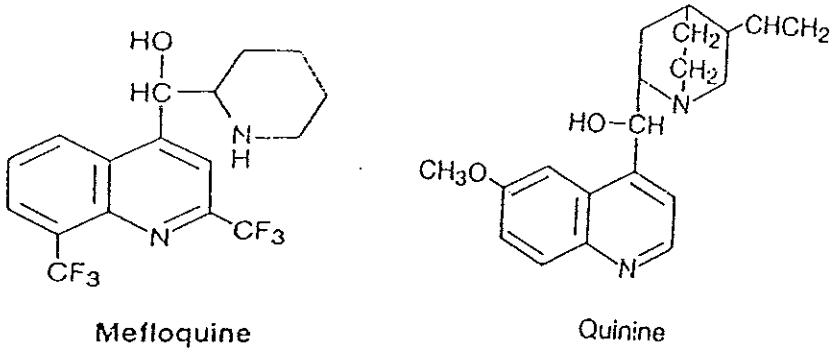

Figure 1 Structural Formula of Mefloquine and Quinine

Mefloquine has a molecular weight of 414.79 and is a very lipid-soluble. It is insoluble in water. Mefloquine is a basic, highly lipophilic drug (partition coefficient n-octanol/buffer pH 7.4= 630), with low aqueous

solubility at physiological pH (60 mg/100 ml at pH 1.2 and 30 mg/100 ml at pH 7.4) (Crevoisier, et al., 1997)

1. Pharmacodynamic properties

1.1 Mechanism of Action

The mechanism of action of mefloquine is unknown. In many respects, mefloquine behaves like quinine, but it does not intercalate with DNA. Mefloquine and quinine produce similar morphological changes in early ring stages of *Plasmodium falciparum* and *Plasmodium vivax*, the major ultrastructural abnormality produced by mefloquine in *P. falciparum* is swelling of secondary lysosomes. Like chloroquine, low extracellular concentrations of mefloquine raise the intravesicular pH of plasmodia in excess of that predicted from the passive distribution of a weak base. This suggests that there is a mechanism for concentration of mefloquine that has to be characterized. Mefloquine probably affects membranes of malarial parasites. Like quinine, mefloquine competes for accumulation of chloroquine in infected erythrocytes and inhibits chloroquine-induced clumping of pigment in the parasite (Webstar, 1991)

1.2 Pharmacologic Effects

Like quinine, mefloquine can slow cardiac conduction. Studies in animals have shown antifibrillary action and an increase in the pulse rate interval. The effect of mefloquine on the compromised cardiovascular system in humans has not been evaluated (Goldsmith, 1992).

1.3 Antimalarial Action

Mefloquine has strong blood schizonticidal activity against *P. falciparum* and *P. vivax*. Insufficient information is available to document effectiveness against *P. malariae* or *P. ovale*, but theoretically the drug should be effective against circulating schizonts of these species (Goldsmith, 1998)

1.4 Resistance

Sporadic and low levels of resistance to mefloquine have been reported from Southeast Asia and Africa. Resistance to the drug can emerge rapidly, and resistant strains have been found in areas where the drug has never been used (Goldsmith, 1998)

2. Pharmacokinetic properties

Mefloquine hydrochloride is a synthetic 4-quinoline methanol derivative chemically related to quinine. It can only be given orally because intense local irritation occurs with parenteral use. It is well absorbed, and peak plasma conccentrations are reached in 7-24 hours (Goldsmith, 1998). The bioavailability of commercial preparations exceeds 85%. Absorption is enchanced by food and impaired by vomiting within the first hour of ingestion and diarrhea in the following days (Nosten, et al., 1995). A single oral dose of 250 mg of the salt results in a plasma concentration of 290-340 ng/ml whereas continuation of this dose daily results in mean steady state plasma concentrations of 560-1,250 ng/ml. Plasma levels of 200-300 ng/ml may be necessary to achieve chemosuppression in *P. falciparum* infections (Goldsmith, 1998). The drug is highly bound to plasma proteins (98%) (Palmer, et al.,

1993 ; Webstar, 1991). Estimate of apparent volume of distribution is 20 L/Kg (Palmer, et al., 1993). The drug concentrated in red blood cells, and extensively distributed to the tissues, including the central nervous system (Goldsmith, 1998). Mefloquine is cleared in the liver, it under goes extensively to biliary and gastric secretion followed by reabsorption (Strickland, et al., 1991). Its acid metabolites are slowly excreted, mainly in the feces and only very small amounts of drug appear in the urine. Its elimination half-life, which varies from 13 days to 33 days, tends to be shortened in patients with acute malaria. The drug can be detected in the blood for months after dosing ceases. These findings are consistent with the large volume of distribution and low clearance drug (Goldsmith, 1998 ; Webstar, 1991). In rats, the gastrointestinal system serves as an important compartment for the drug as it undergoes a continuous enterohepatic and enterogastric circulation (Webstar, 1991). Clearance is increased in young children and late pregnancy (Nosten, et al., 1995). Pharmacokinetic parameters between Asian and Caucasian are different. Although the reason for this ethnic variation has not been investigated, it has been suggested that a smaller volume of distribution, secondary to a relatively lower body fat content, or differences in the enterohepatic circulation of mefloquine could be accounted for higher blood drug concentrations observed in Asians (Palmer, et al., 1993).

2.1 Studies in Healthy Volunteers

2.1.1 Absorption

The lack of an acceptable intravenous formulation has precluded determination of the absolute oral bioavailability of mefloquine. However, the

bioavailability of an early tablet formulation was determined from a comparison of the respective area under the plasma concentration-time curve (AUC) values of the tablet formulation relative to an aqueous suspension of mefloquine hydrochloride, and was estimated to be 65% in healthy Caucasian volunteers. A subsequent tablet formulation showed improved bioavailability, estimated at 89% in healthy Caucasian volunteers and 87% in Thai patients with malaria using a stable isotope technique (Palmer, et al., 1993).

The presence of food affects the pharmacokinetic properties of mefloquine, significantly enhancing the rate and extent of absorption, as evidenced by an increase in C_{max} and reduction in time taken to reach C_{max} (t_{max}) and a 40% increase in bioavailability (Crevoisier, et al., 1997). These data suggest that mefloquine should be taken at a standard time in relation to meals to minimise variation in blood concentrations (Palmer, et al., 1993).

2.1.2 Distribution and Elimination

The low systemic clearance and large apparent volume of distribution of mefloquine are consistent with the long elimination half-life of 14 to 28 days. Although the large volume of distribution suggests extensive tissue binding, in agreement with the high lipid solubility of the drug, extensive binding to plasma proteins in volunteers (98.2%) and patients (98.4%) (Palmer, et al., 1993). This phenomena may account for the rather low total plasma clearance of mefloquine.

2.2 Studies in Patients with Malaria

2.2.1 Absorption

Malaria does not appear to impair the absorption of mefloquine, indeed plasma C_{max} were higher in patients with acute uncomplicated falciparum malaria compared with uninfected individuals. Although ethnic differences may have contributed to the higher plasma C_{max}, recent studies directly comparing mefloquine kinetics in infected and uninfected Thai patients have demonstrated similar or higher plasma C_{max} values in patients with acute uncomplicated falciparum malaria (Boudreau, et al., 1990 ; Palmer, et al., 1993).

2.2.2 Distribution and Elimination

A comparison of overall means derived from pharmacokinetic investigations in patients with falciparum malaria and healthy volunteers suggests that the terminal elimination half-life and apparent volume of distribution of mefloquine are reduced in malarial infection (Palmer, et al., 1993). Karbwang, et al. (1988a) proposed that enterohepatic recirculation may be augmented during malarial infection. Palmer, et al., (1993) suggested that the fever and parasitaemia associated with severe infection affected the distribution of mefloquine. Indeed, malarial parasites are known to concentrate antimalarial drugs and this accumulation probably contributes to the low apparent volume of distribution during the first 3 days of treatment (Palmer, et al., 1993)

2.3 Studies in Children and Pregnant Woman

A more recent study demonstrated that following mefloquine 25 mg/kg, C_{max}, t_{max}, elimination half-life, volume of distribution and AUC in children aged 6 to 24 months were similar to those values observed in children aged 5 to 12 years. These values are similar to those in adults with falciparum malaria treated with a comparable dose (750 mg) suggesting that there are no age-related differences in mefloquine disposition (Palmer, et al., 1993).

Pharmacokinetic studies of mefloquine have been performed in pregnant woman, assuming bioavailability was not reduced, systemic clearance was increased in late pregnancy compared with that observed in healthy nonpregnant adults. The quantity of mefloquine excreted in human breast milk after a maternal dose of 250 mg is unlikely to be harmful to a breasted infant (Palmer, et al., 1993)

2.4 Metabolism

Data on the metabolism of mefloquine in humans are limited. Animal studies indicated that mefloquine is metabolised in the liver, being excreted predominantly in the bile and faeces. In human volunteers the plasma concentration of a primary human mefloquine metabolite, the 4-carboxylic acid derivative (Ro21-5104), exceeds that of the parent compound after single dose, divided dose and multiple dose administration (Karbwang and White, 1990 ; Palmer, et al., 1993). In rodents, parenterally administered [^{14}C]-labelled drug was excreted primarily in the faeces ; 30% appeared as unchanged drug in the mouse, but virtually no parent drug was excreted in the rat. In these studies, mefloquine was well absorbed, extensively distributed throughout the body,

and concentrated in the bile and gastric juice. Five metabolites have been isolated from the faeces of rats, two of which have been identified as 2,8 bis-trifluoromethyl 4-hydroxymethyl quinoline and a carboxylic metabolite. This suggests that biotransformation occurs in the piperidine ring but not in the aromatic portion of the mefloquine molecule. The structurally defined components account for about 40% of the administered dose.

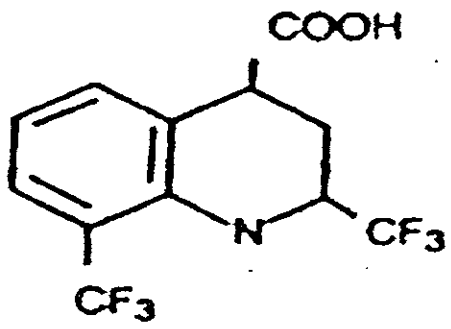

Figure 2 Structural Formula of a carboxylic metabolite

These workers concluded that the relatively high plasma concentrations of the mefloquine metabolite observed after oral administration in humans are explained by its smaller apparent volume of distribution (Vd/F) (Karbwang and White, 1990)

Mefloquine is largely biotransformed into 2,8-bis(trifluoromethyl)-4-quinoline carboxylic acid (Figure 2). Schwartz, et al. (1980) studies the disposition of the carboxylic acid metabolite in human subjects given 1 g mefloquine base in the form of its hydrochloride orally. The metabolite appeared in the blood 2 to 4 hours after oral administration of mefloquine. Its concentration rose steadily to maximum values of 1.1 to 1.4 μg/ml within 1 or

2 weeks of administration and remained practically constant for about 2 to 3 weeks to decline thereafter at a rate similar to that of mefloquine. The authors compared the kinetic studies of 250 mg mefloquine and 204 mg of its metabolite in dogs, using i.v. administration. In these experiments, both compounds showed virtually the same elimination half-life but the initial plasma levels of mefloquine metabolite was about 30 times higher than those of mefloquine. The volume of distribution of this compound was less than that of mefloquine. On comparing the AUC of metabolite formed after i.v. administration of 250 mg mefloquine with that measured after i.v. administration of 204 mg of metabolite, it was found that only 26% of mefloquine had been metabolically converted to mefloquine metabolite. These workers concluded that the relatively high plasma concentrations of the mefloquine metabolite observed after oral administration in humans are explained by its smaller apparent volume of distribution (Vd/F) (Karbwang and White, 1990). Schwartz, et al. (1982) studied the oral single dose kinetics of mefloquine in 16 male volunteers. Unchanged mefloquine and one of its metabolites were measured in plasma. The levels of its metabolite surpassed those of mefloquine, resulting in a 2.4-5.1 larger AUC. Franssen, et al. (1989) reported that the concentration of the metabolite in whole blood was lower than that in plasma but also exceeded the whole blood concentration of mefloquine. Mimica, et al. (1983) reported steady-state plasma concentration of mefloquine and carboxylic acid metabolite in 5 volunteers receiving 250 mg weekly for 21 weeks. The mean plasma levels of the metabolite ranged between 1.47 and

5.55 μg/ml, while the mean metabolite to mefloquine ratio measured at steady state was found to have an interindividual range of between 2.3 and 8.6.

3.Toxicology

UNDP/World Bank/WHO Update(1983) reported the toxicity of mefloquine in the rats, dogs, monkeys and mice. The results of acute, subacute and chronic toxicity showed that the drug did not cause mutagenic nor teratologic changes. The toxic effects on the development of the offspring of rats were produced during the postnatal period only in those nursed by dams given very high doses of the drug. There was no evidence of toxic effects in dogs given doses ranging from 5 to 150 mg/kg/week for 1 year, other than a reduction in the rate of weight gain in those receiving 25 mg/kg or more of drug per week. When the drug was given for 28 consecutive days, no adverse effects were noted at doses of 5 mg/kg/day, and the only abnormality detected at doses of 30 mg/kg/day was slight lymphocytopenia. However, doses of 150 mg/kg/day were lethal within 2 weeks. In male rats daily doses of more than 20 mg/kg for 13 weeks induced degenarative lesions in the epididymis. Teratological studies in female rats showed an increased incidence of externally visible soft tissue and skeletal anomalies when the drug was given orally in very large doses (100 mg/kg) from day 6 to day 15 of gestation, but no abnormalities were seen at doses of 10 or 20 mg/kg. Similar doses in mice reduced fetal growth and produced cleft palate in some offsprings, A 2-year study in rats showed that mefloquine is not carcinogenic at doses of 30

mg/kg/day or below. Thus, it possesses a relatively satisfactory overall profile of animal toxicity (Karbwang and White, 1990).

4. Adverse Reactions

Mefloquine, given orally in single doses up to 1,500 mg or in 500 mg doses each week for 1 year, it generally well tolerated (Webstar, 1991). The frequency and intensity of reactions are dose-related. Most of the symptoms, however, can also occur as a result of malaria itself.

4.1 Prophylactic Doses : Minor and transient adverse effects include gastrointestinal disturbances (nausea, vomiting, epigastric pain, diarrhea), headache, dizziness, syncope and extrasystoles, with an incidence not much higher than for placebo or other antimalarials. Transient leukocytosis, thrombocytopenia, and aminotransferase elevations have been reported. Transient neuropsychiatric events (convulsions, depression, psychoses) have been reported rarely.

4.2 Treatment Doses : Particularly with therapeutic doses over 1,000 mg, gastrointestinal symptoms and fatique are more likely and the incidence of neuropsychiatric symptoms (dizziness, headache, visual disturbances, tinnitus, insomnia, restlessness, anxiety, depression, confusion, acute psychosis, or seizure) may be as high as 1%. Pruritus, skin rash, alopecia and myalgia are rare. Reactions may occur up to 2-3 weeks after the last dose of mefloquine.

4.3 Animal Toxicity : Ocular lesions (lens opacity, retinal degeneration) have been reported in animals but not in humans

4.4 Mutagenicity, Carcinogenicity, and Teratogenicity : Mutagenicity and carcinogenicity studies have been negative in a variety of assay systems. At high doses, the drug is teratogenic and embryotoxic in experimental animals. Adequate controlled studies in human pregnancy are not available (Goldsmith, 1998).

5. Contraindication & Cautions

Mefloquine is contraindicated if there is a history of epilepsy, psychiatric disorders, arrhythmia, cardiac conduction defect, or sensitivity to quinidine or related drugs. The drug should not be used for children under 15 kg or under 2 years of age. In these groups, the drug appears to be poorly tolerated, and efficacy has not been established. Also contraindicated is concurrent administration of mefloquine with quinine, quinidine, or halofantrine. If these agents precede the use of mefloquine, 12 hours should elapse before mefloquine is started. Because of the long half-life of mefloquine, extreme caution is required if quinine or quinidine is used to treat malaria after mefloquine has been taken.

The drug should preferably not be used in the first trimester of pregnancy, and woman of childbearing potential who take mefloquine for malarial prophylaxis should use reliable precautions against conception for 3 months after the last dose.

The development of neuropsychiatric symptoms (anxiety, depression, restlessness, confusion) during prophylaxis is an indication to stop using mefloquine. The drug should not be taken by persons whose work requires

fine coordination and spatial discrimination (eg, airline pilots). Patients taking anticonvulsant drugs (particularly valproic acid and divalproex sodium) may have break through seizures ; concurrent administration of mefloquine and chloroquine or quinine increases the risk of convulsions.

The safety of mefloquine usage beyond 1 year has not been established. Therefore, if the drug is used for prolonged periods, periodic evaluations, including liver function tests and complete ophthalmologic examinations, are recommended (Goldsmith, 1998)

6. Clinical Uses

6.1 Prophylaxis of Chloroquine-Resistant Strains of *P. falciparum* :

Dose : One 250 mg tablet salt (228 mg base). Give a single dose of mefloquine weekly starting 1 week before entering the endemic area, while there and for 4 weeks after leaving.

Mefloquine is effective in prophylaxis against most strains of chloroquine-resistant or pyrimethamine- sulfadoxine-resistant *P. falciparum* and is curative when taken weekly for 4 weeks after leaving an endemic area. When used for this purpose, the drug also provides prophylaxis against *P. vivax* and probably against *P. ovale* and *P. malariae*. Eradication of *P. vivax* and *P. ovale*, however, requires a course of primaquine against their hepatic stages (as used with chloroquine in eradicating *P. vivax* infections).

Although the drug is effective against chloroquine-sensitive *P. falciparum*, it should be reserved for use in malarial areas in which chloroquine is not effective.

6.2 Treatment of Chloroquine-Resistant *P. falciparum* Infection :

Dose : Mefloquine, 1,250 mg (salt) once or 750 mg followed after 6-8 hours by 500 mg. Mefloquine is indicated in oral treatment of mild to moderate mefloquine-susceptible *P. falciparum* infections. Since mefloquine apparently does not act as quickly as quinine or quinidine and cannot be given parenterally, it should not supplant these drugs in management of severely ill patients (Goldsmith, 1998)

7. Drug Interactions

Sulfadoxine-Pyrimethamine

Mefloquine is marketed in Thailand in combination with both sulfadoxine and pyrimethamine (Fansimef®). In a recent study comparing the effects of mefloquine alone with those of mefloquine plus sulfadoxine-pyrimethamine, both regimens are equally effective in producing cure (Pinichpongse, et al., 1987). There was significant difference in the half-life for patients who received mefloquine alone and for those receiving mefloquine-sulfadoxine-pyrimethamine. The devived mean half-life of mefloquine was longer following ingestion of "Fansimef" compared to that following mefloquine alone. This suggested that sulfadoxine and/or pyrimethamine may influence mefloquine elimination (Karbwang and White, 1990)

Quinine

Quinine should not be used concurrently with mefloquine. It can potentiate the dose-related adverse effects of mefloquine. If this compound is to be used in the initial treatment of severe malaria, mefloquine should not be administered within 12 hours of the last dose of quinine (Stern (letter), 1988)

Chantavanich, et al. (1985) showed that mefloquine concentrations rose abruptly when quinine administration ceased. They suggested that competition for plasma and red cell binding sites might explain this finding. The combined effects of quinine and mefloquine on the cardiovascular system warrant urgent investigation so that appropriate treatment recommendation can be made

Primaquine

A study was conducted to examine the pharmacokinetics of mefloquine in healthy Thai men who received either mefloquine alone or mefloquine and primaquine. The results suggested that primaquine did not alter the pharmacokinetics of mefloquine in Thai volunteers (Karbwang, et al. (letter), 1992)

Metoclopramide

A study of the pharmacokinetics of 750 mg mefloquine when administered concomitantly with metoclopramide showed that metoclopramide increased maximum concentration of mefloquine and AUC. This suggested that metoclopramide increased gastrointestinal motility. Since accelerated gastric emtying can lead to an increase in the rate of drug absorption from the small intestine (Na Bangchang, et al., 1991)

Oral Contraceptive Steroids

The effects of oral contraceptive steroids on the pharmacokinetics of a single oral dose mefloquine (750 mg) were studied in six healthy Thai women volunteers who regularly used oral contracceptive steroids (OCS) and in twelve Thai woman patients with falciparum malaria, six of whom were also using OCS. The pharmacokinetic parameters of mefloquine were not significantly different in the two patient groups. The study suggested that the oral contraceptive steroids had no effect on the pharmacokinetics of mefloquine (Karbwang, et al., 1988b)

Ampicillin

Karbwang, et al. (1991) examined the pharmacokinetics of a single oral dose of mefloquine in healthy Thai volunteers who took either mefloquine alone or in combination with ampicillin. The results showed a significantly higher maximum whole blood mefloquine concentration after coadministration with ampicillin, significantly reduced terminal half-life and increased in mean residence time and increased volume of distribution at steady state. The rationale for this study was to examine the posible role of enterohepatic recycling (EHC) in the prolonged half-life of mefloquine. Although in the ampicillin phase a small decrease in half-life was seen, this was secondary to a decrease in the apparent volume of distribution and the clearance was unchanged. The most likely explanation of this finding is altered tissue binding: although it should be noted that with an intact EHC the gastrointestinal tract will be part of the volume of distribotion, but if EHC is interrupted this will no longer be so.

Cimetidine

Sunbhanich, et al. (1997) investigated the inhibition of the metabolism of mefloquine by cimetidine. Pretreatment with cimetidine 400 mg twice a day after breakfast and at bedtime for 7 days caused markedly significant reduction ($P<0.05$) in the apparent oral clearance (Clo) (0.051 ± 0.026 versus 0.031 ± 0.015 l/h/kg) and a significant increase ($P<0.05$) in the elimination half-life ($t_{1/2}$) after cimetidine treatment (9.6 versus 14.4 days, respectively). Therefore, the present data suggest that cimetidine reduces the Clo and prolongs the elimination $t_{1/2}$ of mefloquine in a manner similar to quinine. These results may reflect the decrease in the metabolism of mefloquine by cimetidine. These effects of cimetidine on the mefloquine pharmacokinetics may be mediated by inhibiting the hepatic-mixed function oxidase system, being similar to those occuring in quinine

Valproic acid

When used prophylactically at a dosage of 250 mg mefloquine has been reported to reduce the half-life of valproic acid, possibly by increasing metabolism, resulting in a decrease in seizure control in several epileptic patients. Therefore, concomitant use of these 2 agents is not advised (Palmer, et al., 1993)

Rifampicin

Rifampicin (Figure 3) was first introduced in 1963 (Mcnicol, et al.,1995). The rifamycins are a group of structurally similar, complex macrocyclic antibiotics produced by *Streptomyces mediterranei* ; rifampicin is a semisynthetic derivative of one of these rifamycin B (Mandell and Sandle, 1991).

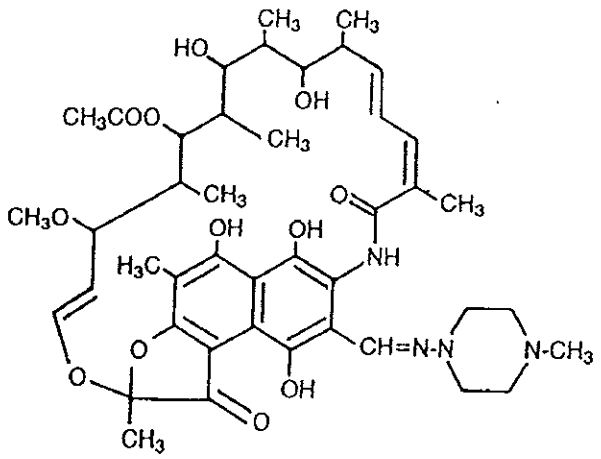

Figure 3 Structural Formula of Rifampicin

Rifampicin has a large molecular weight (MW 823) and is soluble in organic solvents and in water at acidic pH (Mandell and Sandle, 1991 ; Chambers and Jawetz, 1998)

1. Pharmacodynamic properties

1.1 Mechanism of Action

Rifampicin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms, leading to suppression of initiation of chain

formation (but not chain elongation) in RNA synthesis. More specifically, the β-subunit of this complex enzyme is the site of action of the drug, although rifampicin binds only to the holoenzyme. Nuclear RNA polymerase from a variety of eukaryotic cells does not bind rifampicin, and RNA synthesis is correspondingly unaffected. While rifampicin can inhibit RNA synthesis in mamalian mitochondria, considerably higher concentrations of the drug are required than for the inhibition of the bacterial enzyme. Rifampicin is bactericidal for both intracellular and extracellular microorganisms (Mandell and Sandle, 1991).

1.2 Antibacterial Activity

Rifampicin inhibits the growth of most gram-positive bacteria, as well as many gram-negative microorganisms such as *Escherichia coli, Pseudomonas, indole-positive* and *indole-negative Proteus,* and *Klebsiella.* Rifampicin is very active against *Staphylococcus aureus* and *coagulase-negative staphylococci* ; bactericidal concentrations range from 3 to 12 ng/ml. The drug is also highly active against *Neisseria meningitidis* and *Haemophilus influenzae* ; minimal inhibitory concentrations range from 0.1 to 0.8 μg/ml. Rifampicin is very inhibitory to *Legionella* species in cell culture and in animal models (Mandell and Sandle, 1991).

Rifampicin in concentrations of 0.005 to 0.2 μg/ml inhibits the growth of *M. tuberculosis* in vitro. Among nontuberculous mycobacteria, *M. kansasii* is inhibited by 0.25 to 1 μg/ml. The majority of strains of *M.scrofulaceum, M. intracellulare,* and *M. avium* are supressed by concentrations of 4 μg/ml, but certain strains may be resistant to 16 μg/ml. *M. fortuitum* is highly resistant to

the drug. Rifampicin increases the *in vitro* activity of streptomycin and isoniazid, but not that of ethambutol, against *M. tuberculosis* (Mandell and Sandle, 1991).

1.3 Resistance

Microorganisms, including mycobacteria, may develop resistance to rifampicin rapidly in vitro as a one-step process, and one of every 10^7 to 10^8 tubercle bacilli is resistant to the drug. This also appears to be the case in vivo, and therefore the antibiotic most not be used alone in the chemotherapy of tuberculosis. Microbial resistance to rifampicin is due to an alteration of the target of this drug, DNA-dependent RNA polymerase. Certain rifampicin-resistant bacterial mutants have decreased virulence. Tuberculosis caused by rifampicin-resistant mycobacteria has been described in patients who had not received prior chemotherapy, but this is very rare (usually less than 1%) (Mandell and Sandle, 1991).

2. Pharmacokinetic properties

2.1 Absorption

The oral administration of rifampicin produces peak concentrations in plasma in 2 to 4 hours ; after ingestion of 600 mg this value is about 7 μg/ml, but there is considerable variability. If taken after food, absorption is delayed and peak levels are lower and later. Aminosalicylic acid may delay the absorption of rifampicin, and adequate plasma concentrations may not be reached. If these agents are used concurrently, they should be given separately

at an interval of 8 to 12 hours (Mandell and Sandle, 1991 ; Boman, et al., 1971 ; Mcnicol, et al., 1995 ; Siegler, et al., 1974).

Food has been demonstrated to cause a 1 to 2 hours delay in the T_{max} and a reduction in the C_{max} and AUC of rifampicin. In studies in which rifampicin was given in combination with other antituberculosis drugs, some notable effects upon absorption and bioavailability have been reported. Rifampicin does not influence the serum concentrations of isoniazid but conflicting reports have been published on the effect of isoniazid on the former. On the other hand, para-aminosalicylic acid was found to delay the T_{max} of rifampicin from 2 to 4 hours, reduce its C_{max} from 8.0 to 3.8 μg/ml, and decrease the AUC by approximately 50% (Boman, et al, 1974). This effect was thought to be due to impairment of gastrointestinal absorption of rifampicin by either alteration of the physicochemical properties of the mucosa by para-aminosalicylic acid, or a decrease in gastric emptying rate with an increased intestinal transit time. In other studies, simultaneous administration of para-aminosalicylic acid and rifampicin has also been shown to decrease the C_{max} of rifampicin due to the absorption of the latter onto the bentonite content of the para-aminosalicylic acid granules, thereby leading to decreased intestinal absorption of rifampicin (Acocella and Conti, 1980 ; Boman, et al., 1971). Excipients such as talcum and kaolin have also been suspected to reduce gastrointestinal absorption of rifampicin. In addition, food may decrease its absorption (Seigler, et al, 1974), and decreased serum concentrations of rifampicin have been observed after prolonged administration (Holdiness, 1984)

2.2 Distribution

Rifampicin is widely distributed with good penetration of all normal tissues including bone and serous fluid. It does not penetrate the normal blood brain barrier, but penetrates well when there is inflammation. The tissue levels attained are about 100 times higher than the MIC for sensitive strains (Mcnicol, et al., 1995). At physiological pH, only 25% of the compound of rifampicin is ionised, but the molecule as a whole is lipid soluble (Holdiness, 1984). Rifampicin is relatively highly protein-bound. In normal subjects protein binding is approximately 80% suggested that the γ-globulins may be the main serum binding proteins (Holdiness, 1984 ; Chambers and Jawetz, 1998).

Rifampicin is distributed throughout the body and is present in effective concentrations in many organs and body fluids, including the CSF. This is perhaps best exemplified by the fact that the drug may impart an orange-red color to the urine, feces, saliva, sputum, tears and sweat ; patients should be so warned (Mandell and Sandle, 1991)

2.3 Metabolism

Rifampicin is slowly acetylated in the liver and the unchanged drug together with acetylrifampicin is excreted in the bile. There is a significant enterohepatic circulation of rifampicin and very high concentrations are achieved in bile. Desacetylrifampicin is not reabsorbed and most of the drug is excreted in the faeces in this form. It is also excreted in the urine where very high concentrations are attained. The pathway of rifampicin metabolism, and metabolic derivatives are illustrated in Figure 4.

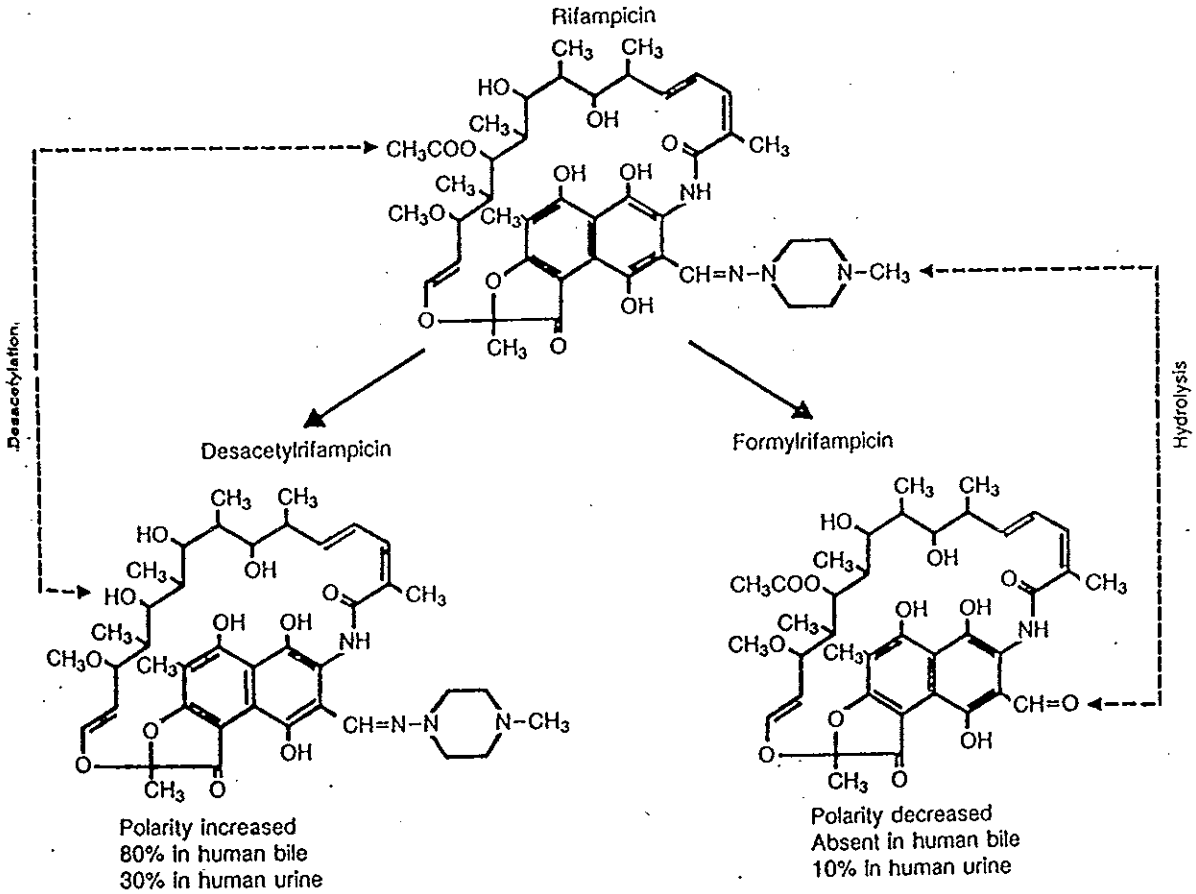

Figure 4 Principal metabolic derivatives of rifampicin in man, polarity and percentage recovery in bile and urine (Holdiness, 1984 : 523).

In figure 4, it presents the metabolic routes of rifampicin in man, the major pathway being desacetylation at the C-25 position, most probably in the liver. This results in a more water-soluble compound with an increased capacity for biliary excretion. In a separate metabolism pathway, hydrolysis yields a 3-formyl-rifampicin derivative. The desacetylated form accounts for approximately 80% of the microbiological activity in human bile and its rate of transfer into bile is 10 to 20 times greater than that of the parent compound. The desacetylating enzymes are thought to be located in the smooth endoplasmic reticulum of the hepatocytes as evidenced by the proliferation smooth endoplasmic reticulum with continued administration of rifampicin (demonstrated by electron microscopic examination). Along with these ultrastructure changes are concomitant increases in hepatic cytochrome P-450, β-glucuronidase, paranitrophenolglucuronyltransferase, β-N-acetylglucuronidase, β-N-acetylglucosaminidase, and corticosteroid hydroxylase.

It has also been demonstrated recently that 15 to 20% of desacetylrifampicin is converted to a glucuronide, this process being commonly catalysed by smooth endoplasmic reticulum enzymes (Holdiness, 1984).

In the first few days of rifampicin administration serum levels tend to fall progressively as a result of the induction of hepatic enzymes concerned with its own metabolism (Mcnicol, et al., 1995)

2.4 Excretion

The half-life of rifampicin varies from 1.5 to 5 hours and is increased in the presence of hepatic dysfunction ; it may be decreased in patients receiving isoniazid concurrently who are slow inactivators of this drug. The half-life of rifampicin is progressively shortened by about 40% during the first 14 days of treatment, owing to induction of hepatic microsomal enzymes with acceleration of deacetylation of the drug. Up to 30% of a dose of the drug is excreted in the urine ; less than half of this may be unaltered antibiotic. Adjustment of dosage is not necessary in patients with impaired renal function (Mandell and Sandle, 1991).

3. Pharmacokinetics in Various Pathophysiological States

3.1 Renal Disease

Rifampicin can also be administratered in full therapeutic doses to patients with severely impaired renal function without toxic effects. Studies in the early 1970s with 300 mg rifampicin found little difference between normal patients and those with severe renal insufficiency. However, when the dose was increased to 600 mg 34 to 40% increases in AUC and $t_{1/2}$ were noted probably due to oversaturation of the hepatic metabolising capacity as well as decreased renal clearance.

Reduced renal clearance of rifampicin seems to be compensated by enhanced biliary elimination, and a dose of 600 mg/day does not appear to need reduction in patients with limited renal function. Studies of peritoneal

and haemodialysis patients receiving rifampicin also indicate that the antibiotic is dialysable without difficulty (Holdiness, 1984).

3.2 Liver Disease

Numerous studies have demonstrated increased serum concentrations of rifampicin and an increased elimination half-life in patients with liver diseases given oral doses of 300 to 900 mg rifampicin. Following a lower dose of 450 mg, serum half-lives of 2.5, 6.0 and 6.5 hours have been observed in normal, cirrhotic and hepatitis subjects, respectively. In one study slight increases in the AUC of rifampicin were observed in patients with chronic liver disease compared with normal subjects, the AUC_{0-12} values in the 2 groups after 7 days of treatment with 600 mg rifampicin daily being 55.1 μg/ml h (normals) and 105.2 μg/ml h (liver disease). Associated with this was a small increase in serum $t_{1/2}$ in the chronic liver disease patients (3.2 h vs 1.8 h in the normal subjects on the seventh day of treatment). Despite the longer serum $t_{1/2}$ values, no marked differences in total urinary excretion were noted in the above studies, suggesting that patients with various liver diseases have the same ability to metabolise rifampicin as normal individuals. However, the increased serum concentrations of rifampicin could give rise to increased bilirubin levels as a result of competition for common biliary excretion mechanisms. Thus in the presence of severe hepatic disease, there may be a need to reduce the dosage of rifampicin and monitor serum concentrations in order to avoid the occurrence of hyperbilirubinaemia (Holdiness, 1984).

3.3 Infants and Children

Reduced serum concentrations of rifampicin in comparison with adults have been noted in neonates and children up to 18 months of age given equivalent doses in terms of mg/kg bodyweight. Mean peak serum concentrations of 3.5 to 4.2, 7.1 and 9.6 to 12.0 μg/ml after preprandial administration of single rifampicin doses of 10, 15 and 20 mg/kg bodyweight, respectively, have been recorded in children. Peak serum concentrations of rifampicin in children were found to be approximately one-third to one-tenth those of adults given a similar dose based on bodyweight ; this difference is possibly due to the large total body compartments in infants (Holdiness, 1984).

In newborns less than 3 days of age, peak serum concentrations after a 10 mg/kg dose were reached up to 8 hours after dosing and were followed by comparatively slow elimination. This is possibly due to the undeveloped hepatic capacity for the drug, and a high gastric pH. Following repeated administration of doses of 10 mg/kg in newborns, accumulation of the drug has been noted. A study of the urinary excretion of rifampicin in newborns (aged less than 3 days) and young children (aged 4 to 18 months) given doses of 10 mg/kg showed that 37% of the dose was recovered in urine in the first 12 hours in the newborns compared with 2.5% of the dose in the older children (Holdiness, 1984).

3.4 Elderly Patients

Recently, the pharmacokinetic of rifampicin have been studied in 6 elderly individuals (aged 78 to 95 years) after single oral doses of 10 mg/kg. The C_{max} and $t_{1/2}$ of rifampicin were 8.83 ± 1.72 μg/ml and 4.09 ± 2.59 hours,

respectively, which are comparable to those reported in younger adults. The same also applies to the C_{max} (1.93 ± 0.53 μg/ml) and the $t_{1/2}$ (4.65 ± 2.61 h) values of desacetylrifampicin. However, the renal clearance of rifampicin (7.5 ± 3.6 ml/min) and desacetylrifampicin (14.6 ± 2.7 ml/min) during a 24-hour period were lower than those observed in younger individuals (rifampicin, 30 ± 7.6 ml/min ; desacetylrifampicin, 22.5 ± 10 ml/min). The authors suggested that since the drug is eliminated via the liver to such an extent that serum concentrations are the same as in younger adults, for therapeutic purposes the metabolism of rifampicin may be globally considered as unaltered in elderly patients (Holdiness, 1984).

3.5 Pregnancy

There is no convincing evidence of teratogenicity in the human but high doses are teratogenic in rats and mice. It was formerly recommened that rifampicin should be avoided during the first 3 months of pregnancy, but in the absence of firm evidence of teratogenicity in humans this recommendation has been abandoned. Particularly when disease is extensive or the patient is ill, it is generally agreed that active tuberculosis is a much more serious threat to the mother and fetus than the use of rifampicin which is an important component of the most effective antituberculosis regimens. Rifampicin is excreted in breast milk. This does not appear to be associated with clinically important problems and is not a contraindication to breast feeding (Mcnicol, et al., 1995).

4. Adverse Reactions

Rifampicin is generally well tolerated. When given in usual doses, less than 4% of patients with tuberculosis have significant adverse reactions ; the most common are rash (0.8%), fever (0.5%) and nausea and vomiting (1.5%). The most notable problem is the development of jaundice. Sixteen deaths associated with this reaction have been recorded in 500,000 treated patients. Hepatitis from rifampicin rarely occurs in patients with normal hepatic function ; likewise, the combination of isoniazid and rifampicin appears generally safe in such patients. However, chronic liver disease, alcoholism and old age appear to increase the incidence of severe hepatic problems when rifampicin is given alone or concurrently with isoniazid (Mandell and Sandle, 1991) .

Administration of rifampicin on an intermittent schedule (less than twice weekly) and/or daily doses of 1,200 mg or greater is associated with frequent side effects and the drug should not be used in this manner. A flu-like syndrome with fever, chills and myalgias develops in 20% of patients so treated. The syndrome may also include eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia and shock (Mandell and Sandle, 1991).

Since rifampicin is a potent inducer of hepatic microsomal enzymes, its administration results in a decreased half-life for a number of compounds, including prednisone, digitoxin, quinidine, ketoconazole, propranolol, metoprolol, clofibrate and the sulfonylureas. There is a similar and significant interaction between rifampicin and oral anticoagulants of the coumarin type,

which leads to a decrease in efficacy of the latter agents. This effect appears about 5 to 8 days after rifampicin administration is started and persists for 5 to 7 days after it is stopped. Rifampicin also appears to enhance the catabolism of a variety of steroids ; for this reason it decreases the effectiveness of oral contraceptives. Methadone metabolism is also increased, and the precipitation of withdrawal syndromes has been reported. Rifampicin may reduce biliary excretion of contrast media used for visualization of the gallbladder (Mandell and Sandle, 1991).

5. Clinical Uses

5.1 Mycobacterial Infections : Rifampicin, usually 600 mg/day (10 mg/kg/day) orally, is administered together with INH, ethambutal, or another antituberculous drug in order to prevent emergence of drug-resistant mycobacteria. In some short-course therapies, 600 mg of rifampicin is given twice weekly. Rifampicin also is effective in some atypical mycobacterial infections and in leprosy when used together with a sulfone. Rifampicin is an alternative to INH prophylaxis for patients who are unable to take INH or who have had close contact with a case of active tuberculosis caused by an INH-resistant, rifampicin-susceptible strain.

5.2 Other Indications : Rifampicin is used in a variety of other clinical situations. An oral dosage of 600 mg twice daily for 2 days can eliminate meningococcal carriage. Rifampicin, 20 mg/kg/day for 4 days, is used as prophylaxis in contacts of children with *Haemophilus influenzae* type b disease. Rifampicin combined with a second agent is used to eradicate

staphylococcal carriage. Rifampicin combination therapy is also indicated for treatment of serious staphylococcal infections such as osteomyelitis and prosthetic valve endocarditis. Rifampicin has been recommened also for use in combination with ceftriaxone or vancomycin in treatment of meningitis caused by highly penicillin-resistant strains of pneumococci (Chambers and Jawetz, 1998).

6. Drug Interactions

Rifampicin is used clinically in the treatment of tuberculosis, usually being administered for 4 to 12 months together with other antituberculosis agents or additional medications for an accompanying disease. It is a potent inducer of drug metabolism in humans and has been shown to produce a proliferation of smooth endoplasmic reticulum and to increase the cytochrome P450 content of human liver. There is a remarkable selectivity in the enzyme induction by rifampicin and not every drug metabolised by oxidation will be affected (Venkatesan, 1992).

6.1 Effects of Rifampicin on Drugs

6.1.1 Oral Anticoagulants

One of the first reported rifampicin interaction was with oral anticoagulants. Several groups of workers noticed that patients on long term anticoagulants require an increase in the daily dose when rifampicin is coadministered.

O' Reilly (1974), who measured prothrombin time and plasma warfarin concentrations in 10 male volunteers after single oral and intravenous dose of

warfarin 1.5 mg/kg both before and during rifampicin treatment, observed a highly significant decrease in the mean areas under the prothrombin time-time curve and a corresponding decrease in plasma warfarin concentrations. That there was no significant alteration in the absorption of the anticoagulant by rifampicin suggests an induction of warfarin metabolising enzymes.

In a subsequent study (1975), the same author administered warfarin 7.5 to 10 mg together with rifampicin 600 mg daily to 8 volunteers for 21 days, and noted a highly significant decrease of hypoprothrombinaemic effect and plasma warfarin concentrations associated with increased excretion of warfarin metabolites in urine and stool for the last 10 days of the study. Rifampicin withdrawal decreased the warfarin requirements by 50 to 60% (Venkatesan, 1992).

Heimark, et al. (1987) have shown that the reduction in hypoprothrombinaemic response of warfarin by rifampicin is due to increased clearance of both warfarin enantiomers, thereby ruling out any regioselectivity or stereoselectivity of warfarin hydroxylating microsomal enzymes induced by rifampicin. A need for increased doses of acenocoumarol and phenprocoumon during concomitant administration of rifampicin has also been reported.

In cases of simultaneous rifampicin and anticoagulant therapy, the dosage of the latter should be adjusted on the basis of prothrombin time, which must be monitored, especially when oral anticoagulant therapy is either initiated or terminated. In any case, the treatment of patients with anticoagulants is a highly individualised matter (Venkatesan, 1992).

6.1.2 Cardioactive Agent

Digoxin and Digitoxin

Since the elimination of digoxin is primarily dependent on renal function. The impact of rifampicin on digoxin pharmacokinetics should be most notable in patients with severely compromised renal function. This interaction was reported by Novi, et al. (1980) in a patient receiving dialysis, in whom initiation of concomitant rifampicin therapy led to a development of symptoms of congestive heart failure and also decreased plasma digoxin concentrations. Subtherapeutic concentrations persisted despite increased digoxin administration, until rifampicin was substituted by ethambutol (1,000 mg/day).

Gault et al. (1984) described 2 dialysis-dependent patients who required substantial increases (34 to 100%) in digoxin doses to maintain therapeutic digoxin concentrations after the commencement of rifampicin 300 to 600 g/day. When rifampicin therapy was stopped, therapeutic concentrations of digoxin were obtained with about 50% of the dose required previously.

The serum digoxin concentration of a patient with atrial fibrillation decreased from 2.9 to 1.7 μg/L 4 days after initiation of rifampicin 600 mg/day. Further reductions occurred despite increasing the digoxin dose, while stopping rifampicin therapy (and reducing of the digoxin dose) resulted in serum digoxin concentrations of 1.6 and 2.6 μg/L at 8 and 15 days after rifampicin therapy (Bussey, et al., 1984).

Serum drug concentration data from another patient on concomitant digoxin, quinidine and rifampicin suggest that rifampicin may directly increase

the metabolism of quinidine and thereby negate the influence of the latter to produce a doubling or tripling in the serum digoxin concentration (Bussey, et al., 1984).

Because digitoxin elimination is primarily dependent on the hepatic metabolism, a reduction in the serum digitoxin concentration during concomitant rifampicin therapy should not be surprising. There are, in fact, reports describing decreases of approximately 30 to >50% (Venkatesan, 1992).

Quinidine

A patient who responded well to quinidine 200 mg orally for syncope and palpitations associated with ventricular dysrhythmia had a relapse within 1 week of initiation of therapy with rifampicin 600 mg/day concurrently with ethambutol for coexistent tuberculosis. Despite increased quinidine dosage the peak plasma quinidine concentration decreased from 5 to 1 mg/L. The patient had therapeutic quinidine concentrations 1 week after rifampicin was replaced with isoniazid and the quinidine dose was increased from 1,200 to 1,600 mg/day. The AUC for quinidine was reduced by 4 to 6 fold, while its $t_{1/2}$ was enhanced 3 to 6 fold with concurrent oral administration of rifampicin to 8 volunteers receiving quinidine sulphate 6 mg/kg either intravenously or orally (Venkatesan, 1992).

In view of rifampicin-quinidine interactions, the quinidine dose must be increased to control arrhythmia. Serum concentrations of quinidine should be monitored. Alternative agents such as procainamide should be tried if proper control of arrhythmia is not possible with manipulation of quinidine dosage (Venkatesan, 1992).

Metoprolol and Propranolol

Bennett, et al. (1982) conducted a controlled study on 12 healthy volunteers receiving rifampicin for 15 days concomitantly with metoprolol. The AUC of metoprolol was found to be reduced by 33%. In another controlled study of 6 healthy volunteers, (Herman et al., 1983) coadministration of rifampicin 600 mg/day for 3 weeks reduced the plasma concentration of propranolol via a 2 to 3 fold increase in its oral clearance. Both studies failed to show any significant change in elimination half-lives ($t_{1/2}$) of metoprolol and propranolol. The effect of rifampicin persisted for several days after its discontinuation.

Patients receiving rifampicin concurrently with either propranolol or metoprolol need frequent monitoring of therapeutic response and may require an increase in the β-blocker dosage (Venkatesan, 1992).

Verapamil

A rifampicin-verapamil interaction has been reported in a 67-year-old patient with pulmonary tuberculosis treated with rifampicin and supraventricular arrhythmia uncontrolled with verapamil. A 4-fold increase in serum verapamil concentration after rifampicin was stopped, appeared to control the supraventricular arrhythmia (Barbarash, 1985). In a subsequent single-dose, crossover study of the pharmacokinetics of intravenous and oral verapamil in 6 healthy volunteers before and after rifampicin therapy, Barbarash et al. (1987) observed a significant reduction (92%) in the oral bioavailability of a single dose of verapamil after 15 days of rifampicin

therapy. There was an 18% reduction in the AUC of verapamil and a 24% increase in its clearance, even with intravenous administration.

Lorcainide

Mauro, et al. (1987) reported a patient with ventricular tachycardia [suppressed with intravenous lidocaine (lignocaine) but nonresponsive to quinidine, flecainide, procainamide and tocainide] who received rifampicin 600 mg/day for 5 months previously for pulmonary tuberculosis. The patient required a daily dose of lorcainide 800 to 900 mg to suppress his arrhythmias and achieve a therepeutic concentration of 0.29 mg/L. This dose was 3 times the normal oral maintenance dose in the absence of rifampicin. Until more is known about this interaction (which may result from rifampicin-induced lorcainide metabolising enzyme activity) caution is advisable and serum concentrations should be monitored.

6.1.3 Oral Contraceptives

Oral contraceptive steroids continue to be the most popular form of revesible contraception. Their efficacy is impaired with rifampicin coadministration, leading to breakthrough bleeding and occasionally pregnancy.

Bolt, et al. (1975, 1977) showed that rifampicin enhances the metabolism of both estrogenic and progesterogenic components of the oral contraceptive steroids in humans. A 4-fold increase in the rate of hydroxylation of estradiol and ethinylestradiol in patients treated for 6 to 10 days with rifampicin 600 mg/day was paralleled by an increase of cytochrome P450 content in liver biopsies. In a further study in volunteers, a rifampicin-induced

60% increase in the rate of elimination of ^{3}H - ethinylestradiol from plasma and a 2-fold increase in the hydroxylation rate of ethinylestradiol occurred (Venkatesan, 1992).

A molecular basis has recently been reported for the rifampicin-oral contraceptive steroids interactions. Guengerich (1988) and Combalbert, et al. (1989) showed that rifampicin induces a human liver cytochrome P450 which is a product of the P450 3A gene subfamily. The isozyme (P450 Nf, P450 3A3) is one of the major forms involved in the 2-hydroxylation of ethinylestradiol.

It is imperative to counsel women who will receive oral contraceptives and rifampicin concurrently. As rifampicin clearly affects both estrogen and progesterone components of combined contraceptive steroids, no oral preparation is free from interaction. Since there are wide interindividual variations in response to rifampicin and it is usually given for a relatively short time, oral contraceptive steroids should not be given to woman taking rifampicin and alternative contraceptive measures should be tried (Venkatesan, 1992).

6.1.4 Glucocorticoids

Prolonged administration of rifampicin increases the metabolism of many steroids, including cortisol and prednisolone. An increase in cortisone acetate replacement therapy from 50 to 100 mg/day was required when rifampicin 450 to 600 mg/day was given simultaneously to patients with Addison's disease. Patients with tuberculosis pericarditis, nephrotic syndrome

or rejection of renal allografts also require increased glucocorticoid doses during concurrent administration of rifampicin (Venkatesan, 1992).

Several workers have studied the pharmacokinetics of prednisolone in patients treated with rifampicin. There was a reduction of prednisolone AUC by 45 to 66% and the total body clearance increased by 45 to 91%, while the $t_{1/2}$ for prednisolone decreased by 45 ± 1.8% (Venkatesan, 1992)

Rather than Switching to other chemotherapy the dose of prednisolone should be at least doubled if rifampicin is to be administered concurrently (Venkatesan, 1992).

6.1.5 Hypoglycaemics

Tolbutamide

Syvalahti, et al. (1974) observed a decrease in serum tolbutamide concentrations in tubercular patients who were receiving oral tolbutamide in conjunction with rifampicin. In a subsequent study by the same group (Syvalahti, et al., 1975), 9 patients with tuberculosis showed altered pharmacokinetics of the antidiabetic agent in response to intravenous dose of tolbutamide 1g 4 weeks after starting therapy with rifampicin 450 to 600 mg/day. The $t_{1/2}$ of tolbutamide declined by 43 and 41% at 180 and 360 min, respectively, after concomitant rifampicin therapy.

Chlorpropamide

Diabetic control is poorer in patients on chlorpropamide during rifampicin coadministration. A 62-year-old patient with diabetes required an increase in his daily dosage from chlorpropamide 250 to 400 mg when

rifampicin 600 mg/day was introduced as part of a tuberculosis regimen (Self and Morris, 1980).

The serum chlorpropamide concentration decreased during rifampicin therapy and rose dramatically, with a concomitant decrease in blood glucose levels, when that drug was stopped. Eventhough blood glucose levels had declined, no adjustment in the dosage of chlorpropamide appeared to be necessary as the patient continued to do well clinically.

Other Hypoglycaemics

A decrease in the mean $t_{1/2}$ of glymidine (Glycodiazine) from 230 to 156 min has been reported in 6 patients treated with a combination of rifampicin, isoniazid and ethambutol. An interaction between rifampicin alone and glibenclamide was also observed (Venkatesan, 1992).

These effects may be increased the hepatic metabolism by rifampicin. To combat this clinically important interaction between rifampicin and 16 hypoglycaemics, the dose of the latter should be increased until blood glucose level is controlled.

6.1.6 Narcotics and Analgesics

Garfield, et al. (1975) reported the occurrence of narcotic withdrawal symptoms in a group of patients on a methadone maintenance regimen who were also given rifampicin for coexistent tuberculosis. The serum concentrations of methadone were significantly lower than those seen when rifampicin was stopped.

Kreek, et al. (1976) observed narcotic withdrawal symptoms in 70% of patients on methadone maintenance given rifampicin 600 mg/day for

tuberculosis. In an extended study by those authors on 6 of these patients with severe withdrawal symptoms, plasma methadone concentration were found to be lowered by 33 to 68% during rifampicin therapy ; however, the plasma methadone $t_{1/2}$ did not change.

6.1.7 Cyclosporin

Cyclosporin is a cyclic endecapeptide with potent immunosuppres-sive properties, metabolised in the liver by the cytochrome P450 system. It is therefore not surprising that rifampicin increases the metabolism of this drug by inducing cytochrome P450 activity, thus diminishing the blood drug concentrations. Langhoff and Madson (1983) reported low blood concentrations of cyclosporin in a patient with cadaveric kidney transplant despite increasing doses during rifampicin administration. Additional pharmacokinetic study in 1 patient showed decreases in cyclosporin AUC of approximately 75 and 63% at 6 and 8 h, respectively, after starting rifampicin therapy.

Cyclosporin has a narrow therapeutic range and the various toxic effects are mostly concentration dependent. Close surveillance of serum cyclosporin is necessary in transplant recipients receiving concomitant rifampicin to ensure continuous and efficacious immunosuppression and to prevent acute rejection. It may be necessary to modify both the cyclosporin dose and the frequency of administration. If possible, rifampicin should be replaced by an alternative antimicrobial agent (Venkatesan, 1992).

6.1.8 Antifungal Agents

Ketoconazole

Brass, et al. (1982) observed a 47% decrease in the AUC of the antimycotic agent ketoconazole 200 mg/day after a single dose of rifampicin 600 mg, and a decrease of 88% after 5 months of concurrent therapy with isoniazid 300 mg/day and rifampicin 100 mg/day.

Fluconazole

Fluconazole is a triazole antifungal agent which is likely to be used extensively for the treatment of concomitant fungal infections in tuberculosis patients receiving rifampicin. Unlike ketoconazole, fluconazole is excreted mainly unchanged in the urine and so its elimination is not as markedly induced by rifampicin. Nevertheless, Apseloff, et al. (1991) have found a 19% decrease in the half - life of fluconazole, with no change in AUC, in 16 healthy male volunteers who received fluconazole on days 1 and 22 and rifampicin on days 8 to 27. The same workers have also suggested an increased dosage of fluconazole for the treatment of concomitant fungal infections in tuberculosis patients receiving rifampicin.

6.1.9 Theophylline

Six to 14 days ' treatment with rifampicin 600 mg/day in 6 to 10 healthy men has been shown to elevate the clearance of oral theophylline by 20 to 82% and intravenous theophylline by 38 to 80% (Venkatesan, 1992). Hauser's and Powell-Jackson's groups found 32 and 17% increases, respectively, in the volume of distribution (Vd) of theophylline from single intravenous doses.

6.1.10 Phenytoin

In epileptic patients receiving phenytoin and rifampicin concurrently the $t_{1/2}$ of phenytoin is reported to decrease ; the magnitude of the reduction was similar in patients taking rifampicin alone or with isoniazid and ethambutol (Kay, et al., 1985). It has also been reported that no further changes were noted in the pharmacokinetics of phenytoin after a 3-month period of antituberculosis treatment with all 3 drugs.

The addition of rifampicin to phenytoin might decrease the serum phenytoin concentration to subtherapeutic levels, resulting in seizures . Conversely, stopping rifampicin therapy in a patient receiving phenytoin might increase the serum phenytoin concentrations to toxic levels if the dose is not decreased proportionately. Serum phenytoin concentrations should, therefore, be measured periodically, particularly on starting and withdrawing rifampicin therapy. Further studies are needed (Venkatesan, 1992).

6.1.11 Vitamins and Hormones

Vitamin D

Rifampicin reduce circulating levels of 25-hydroxy-cholecalciferol, with no significant effect on the plasma levels of 1,25-dihydroxy-cholecalciferol, immunoassayable parathyroid hormone or calcitonin (Venkatesan, 1992).

Thyroxine

Rifampicin therapy causes a significant decrease in plasma concentrations of thyroxine and an increase in those of triiodothyroxine . However, the clinical significance of the change with respect to possible

alteration of thyroid function needs to be evaluated by additional studies (Venkatesan, 1992).

6.1.12 Antituberculosis Drugs

Administration of rifampicin with para-aminosalicylic acid (PASA) has resulted in conflicting data from no effect of either drug on the other to a decrease in the AUC of rifampicin which has been shown to be due to absorption of that drug on to an excipient component (bentonite) of the PASA preparation (Boman, et al, 1975 ; Holdiness, 1984).

Rifampicin has no effect on either the metabolism or excretion of pyrazinamide. The pharmacokinetic properties of both drugs were not altered significantly when rifampicin and isoniazid were administered in conjunction. Although some clinical studies and case reports suggest that the combination of rifampicin and isoniazid may be more hepatotoxic than either drug alone, Holdiness (1984) has emphatically reported that the vast majority of individuals receiving both drugs together do not develop clinically evident synergistic hepatotoxicity.

6.1.13 Antileprosy Drugs

Simultaneous administration of rifampicin is reported to decrease the $t_{1/2}$ of dapsone by a factor of about 2 and to lower its plasma, skin and nerve concentrations considerably and the dapsone-lowering effect of rifampicin did not vary among various dapsone acetylator pheno-types. The interactions are not clinically significant, as the serum dapsone concentration is still higher than the minimum inhibitory concentration (MIC) for the drug (Venkatesan, 1992).

6.1.14 Drug for Human Immunodeficiency Virus (HIV) Infection

Zidovudine

Zidovudine and rifampicin are commonly used together in HIV-infected patients. Burger, et al. (1993) evaluated 4 patients receiving coadministration of rifampicin, 600 mg/d, and zidovudine (doses ranging from 100 to 300 mg/d). In all patients, clearance of zidovudine was significantly higher and AUC lower vs 69 patients receiving zidovudine monotherapy. In 1 patient, zidovudine AUC doubled following discontinuation of rifampicin use. The interaction between these 2 agents is most likely attributable to rifampicin induction of a glucuronide metabolite of zidovudine.

Protease Inhibitors

Saquinavir is extensively metabolized by cytochrome P-450 enzymes, primarily CYP 3A4. In a premarketing clinical study of 12 healthy volunteers, a dose of saquinavir, 600 mg, was given 3 times daily concurrently with rifampicin, 600 mg/d. At steady state concentrations, a dramatic decrease of about 80% in both AUC and maximum plasma concentration (C_{max}) of saquinavir was observed. Based on the significance of this study, concomitant administration of saquinavir with rifampicin should be avoided. Studies of this interaction with a new formulation of saquinavir, which has enhanced bioavailability, are being conducted (Strayhorn, et al., 1997)

Since ritonavir and indinavir are both primarily metabolized by CYP 3A4, induction of metabolism by rifampicin is probable. Healthy volunteers were given either concomitant rifampicin, 600 mg/d, and indinavir, 800 mg every 8 hours, or indinavir alone. Rifampicin use resulted in a drastic 92%

decrease in indinavir AUC. Rifampicin induction of ritonavir metabolism appears to be less significant. Administration of ritonavir, 500 mg twice daily, with rifampicin 600 or 300 mg/d, resulted in a decrease in ritonavir AUC by 35% and Cmax by 25%. A newer protease inhibitor, nelfinavir, is also primarily metabolized by CYP 3A4. Rifampicin administration has resulted in a 3- to 11-fold increase in nelfinavir oral clearance in 12 healthy volunteers (Strayhorn, 1997).

Interactions between protease inhibitors and rifampicin are of obvious importance because many patients with HIV infection require concurrent treatment for tuberculosis (TB). The Centers for Disease Control and Prevention has addressed this issue and provided current guidelines for treating these patients. In patients diagnosed as having drug-susceptible TB and HIV infection but who are not currently receiving protease inhibitor therapy, the suggested approach is to complete TB therapy with rifampicin as part of an appropriate regimen before initiating protease inhibitor treatment. In patients who are already receiving protease inhibitor therapy at the time of diagnosis of TB, the Centers for Disease Control and Prevention offers other options to be applied on a case-by-case basis pending further research (Strayhorn, et al., 1997)

Delavirdine

Borin, et al. (1997) studied the effect of rifampicin on delavirdine pharmacokinetics. Twelve patients received delavirdine, 400 mg, every 8 hours for 30 days ; 7 of the patients also received rifampicin, 600 mg/d, on days 16 to 30 of delavirdine therapy. In the rifampicin group, oral clearance of

delavirdine increased nearly 27-fold, and plasma through concentrations of delavirdine were almost negligible after 2 weeks of concomitant therapy. Based on the results of this study, concurrent administration of delavirdine with rifampicin should be avoided.

6.1.15 Psychotropic Agents

Nortriptyline

Rifampicin use is documented in 2 case reports to decrease nortriptyline levels, which consequently leads to increased dosage requirements of nortriptyline. Bebchuk and Stewart (1991) described a 51-year-old man receiving rifampicin, 600 mg/d, as part of anti-TB therapy concurrently with nortriptyline, 175 mg/d. On discontinuation of rifampicin use, the nortriptyline dosage required a reduction to 75 mg for serum concentrations to remain within the therapeutic range. Self, et al. (1996) described a 43-year-old woman receiving concurrent rifampicin, 600 mg/d, with nortriptyline, 75 mg/d. In this patient, nortriptyline levels were undetectable until 2 weeks after discontinuation of rifampicin use. This time frame is consistent with the expected enzyme induction effect of rifampicin, which often begins to wane 1 week after discontinuation of therapy but lasts 2 weeks or longer. The authors of these reports suggest using caution when administering nortriptyline or other tricyclic antidepressants with rifampicin (Borcherding, et al., 1992).

Benzodiazepines

Reports of rifampicin interacting with benzodiazepine have previously been reviewed (Borcherding, et al, 1992) and a new study involving midazolam has been reported. Backman, et al. (1996) studied 10 patients

receiving rifampicin, 600 mg/d, or a placebo for 5 days. Midazolam, 15 mg/d orally, was introduced on day 6. Midazolam values for C_{max} decreased by 94%, and for AUC by 96% ; the elimination half-life was 40% compared with the control value. The mechanism of this interaction is postulated to be due to enzymatic induction in the gut wall, since AUC decreased much more than the half-life.

Rifampicin also has a marked pharmacokinetic and pharmacodynamic effect on triazolam. In a double-blind, randomized, cross-over study of 10 healthy volunteers, use of rifampicin, 600 mg/d, for 5 days reduced triazolam AUC by 95% and C_{max} by 88%. Consistent with the drastic reduction in serum concentrations, the patients experienced virtually no pharmacologic effect from the single 0.5-mg oral dose of triazolam as tested by psychomotor performance (Villikka, et al., 1997).

6.1.16 Quinine

Wanwimolruk, et al. (1995a) studied the effect of rifampicin pretreatment on the pharmacokinetics of quinine after a single oral dose (600 mg quinine sulphate) was studied in nine healthy young Thai male volunteers. The mean clearance (CL/F) of quinine coadministered with rifampicin was significantly greater than that of quinine alone. The mean difference in clearance from the control treatment was 0.73 l/h/kg, with 95% confidence interval (C.I.) of 0.48 to 0.98. The unbound clearance (CLu/F) of quinine, which reflects the activity of the drug metabolizing enzymes was considerably greater (6.9-fold) in subjects when rifampicin was coadministered with quinine than that of quinine alone. The mean elimination half-life of quinine when

coadministered with rifampicin was significantly shorter than when quinine was given alone. These results indicate that rifampicin pretreatment caused a marked increase (6.2-fold) in the clearance of quinine, possibly due to enzyme induction. The extent to which the elimination of quinine is enhanced by the concomitant administration of rifampicin is likely to have important clinical consequences. Although the clinical significance of these findings is unknown, they indicate the need for caution in the administration of quinine to patients who are concurrently taking rifampicin as an anti-tuberculosis medication.

6.1.17 Others

Chloramphenicol

Prober (1985) reported decrease of 86.5 and 63.8% in serum concentrations of chloramphenicol in 2 children treated with intravenous chloramphenicol and rifampicin concomitantly. Kelly, et al (1988) also found chloramphenicol-rifampicin interactions in children. Although additional studies are required to validate this interaction, serum concentrations of chloramphenicol may be tested as a precautionary measure when this drug is administered concomitantly with rifampicin.

Trimethoprim

When rifampicin was given in conjunction with trimethoprim decreased blood concentrations and $t_{1/2}$ of both drugs occurred, and a significant decrease in the renal clearance of trimethorprim was seen, after 7 days' combined treatment (Venkatesan, 1992).

Barbiturates

Zilly, et al. (1975, 1977) and Breimer, et al. (1977) observed a decrease in $t_{1/2}$ and an increase in the metabolic clearance of hexobarbital in both patients with cirrhosis and volunteers receiving intravenous hexobarbital following rifampicin theapy.

6.2 Effect of Drugs on Rifampicin

Coadministered medications have also been reported to alter the pharmacokinetics of rifampicin by any one of the following mechanisms ; impaired absorption, decreased hepatocellular uptake through either competition or inhibition and altered hepatic metabolism. The strength of these interactions in altering the therapeutic efficacy of rifampicin remains to be evaluated by further studies (Venkatesan, 1992).

Cytochrome P-450

1. Introduction

In human, the metabolizing processes of foreign compounds require a large number of enzymes and almost all occurs in the liver. Most of the enzymes have been classified as belonging to phase I or phase II pathways of metabolism. Phase I enzymes include reductases, oxidases and hydrolases. Phase II enzymes are all transferases. These reactions will transform a hydrophobic compound into a form that is more water soluble and can be easily eliminated from the organism through urine or bile.

Among drug-metabolizing enzymes in phase I pathway, cytochrome P450 (P450s or CYPs) are the most active one. These enzymes are also principally responsible for activation of procarcinogens and promutagens. Most clinically used drugs are metabolized to some degree by P450s.

P450s represent a superfamily of enzymes. They are found in animals, plants, yeast and bacteria. In mammals, some P450s are involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. However, the vast majority of these enzymes, the foreign compound metabolizing P450s, appear to oxidise chemicals that are not normal constituents of the body. P450s are named with the root CYP followed by an arabic number and upper case letter designating the family and subfamily, respectively. Individual P450 forms are denoted by an arabic number that follows the subfamily letter (Gonzalez and Idle,1994).

The cytochrome P450 proteins are embedded in the lipid bilayer of the smooth endoplasmic reticulum. An important associated protein, NADPH-

cytochrome P450 oxidoreductase, is also attached to this lipid bilayer in a stoichiometry of about ten P450 molecules to one reductase (Benet, et al., 1991). A simplified scheme of the oxidative cycle is presented in Figure 5. Briefly, oxidized (Fe^{3+}) cytochrome P450 combines with a drug substrate to form a binary complex (step 1). NADPH donates an electron to the flavoprotein reductase, which in turn reduces the oxidized cytochrome P450-drug complex (step 2). A second electron is introduced from NADPH via the same flavoprotein reductase, which serves to reduce molecular oxygen and to form an "activated oxygen-cytochrome P450-substrate" complex (step 3). This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product (step 4) (Correia, 1998).

2. Human Hepatic Cytochrome P450s (P450s)

The P450s comprise a superfamily of haemoproteins which contain a single iron protoporphyrin IX prosthetic group. This superfamily is subdivided into families and subfamilies that are defined solely on the basis of amino acid sequence homology. To date, at least 14 CYP gene families have been identified in mammals (Nelson et al., 1996). The mammalian P450 families can be functionally subdivided into 2 major classes, those that involve the biosynthesis of steroids and bile acids and those that primarily metabolise xenobiotics. Three main P450 gene families, CYP1, CYP2 and CYP3 are responsible for most hepatic drug metabolism. Although the CYP1 and CYP3 gene families are relatively simple (i.e. CYP1A, CYP1B and CYP3A), the CYP2 gene family is

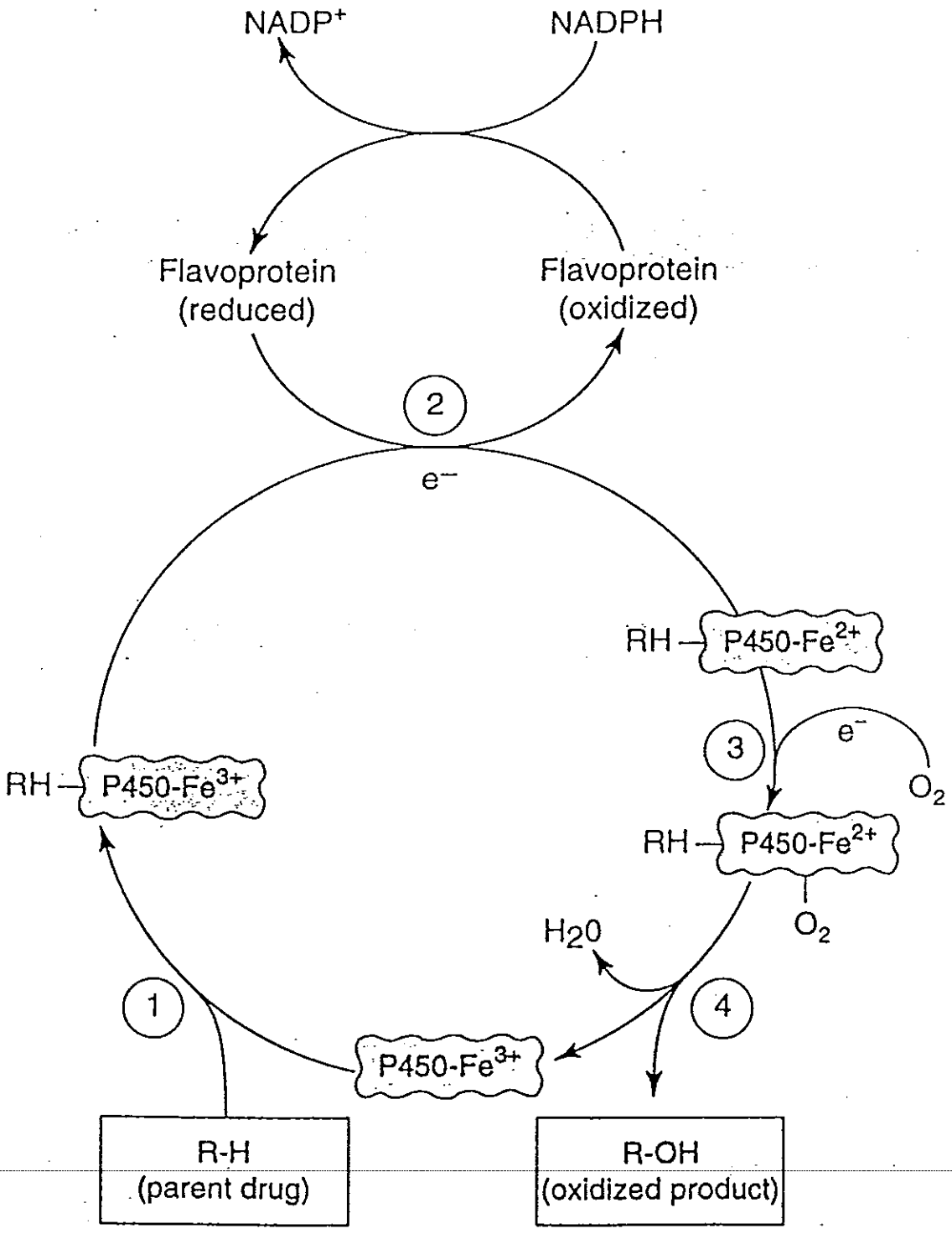

Figure 5 Cytochrome P-450 cycle in drug oxidations

(Correia, 1998 : 52)

comprised of many subfamilies (e.g., CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, etc). These isoforms have the same oxidising centre (the haem iron), but differ by their protein structures (Lin and Lu, 1998).

For different P450, specificity control is governed by the entry of the substrate into the active site and the direct interaction of amino acids in the active site with the substrate. Because the interaction of substrates and mammalian P450 generally lacks absolute complementarity, substrates often bind to the enzyme active site in several different configurations, resulting in multiple metabolites with regio- and stereospecificity unique to each isoform.

3. Mechanisms of Inhibition of P450

The catalytic cycle of P450 consists of at least 7 discrete steps :

(i) binding of the substrate to the ferric form of the enzyme

(ii) reduction of the haem group from the ferric to the ferrous state by an electron provided by NADPH via P450 reductase

(iii) binding of molecular oxygen

(iv) transfer of a second electron from P450 reductase and/or cytochrome b5

(v) cleavage of the O-O bond

(vi) substrate oxygenation

(vii) product release.

Although impairment of any one of these steps can lead to inhibition of P450 enzyme activity, steps(i), (iii) and (vi) are particularly vulnerable to inhibition.

The mechanisms of P450 inhibition can be divided grossly into 3 categories : reversible inhibition, quasi-irreversible inhibition and irreversible inhibition. Among these, reversible inhibition is probably the most common mechanism responsible for the documented drug interactions (Halpert , 1995).

3.1 Reversible Inhibition

Many of the potent reversible P450 inhibitors are nitrogen-containing drugs, including imidazoles, pyridines and quinolines. These compounds can not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron. For example, both ketoconazole and cimetidine are imidazole-containing compounds that interact with ferric P450 at its sixth axial ligand position to elicit a type II optical difference spectrum. The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the hexacoordinated haem by a strong ligand, gives rise to a "type II" binding spectrum. However, cimetidine is a relatively weak reversible inhibitor of P450, an apparent result of an intrinsic low binding affinity to microsomal P450. This latter property is most probably because of the low lipophilicity of cimeticine (logP=0.4). On the other hand, ketoconazole, a potent P450 inhibitor, has a high lipophilicity (logP=3.7). Similarly, fluconazole contains a triazole that binds to the prosthetic haem iron but is a weak reversible P450 inhibitor, again due mainly to its low lipophilicity.

The quinolines are another class of nitrogen heterocycles that exhibit potent P450 inhibition. Ellipticine is a quinoline-containing compound that interacts with both ferrous and ferric P450 forms

Many antimalarial agents (such as primaquine, chloroquine, amodiaquine and mefloquine) contain a quinoline ring and are potent reversible P450 inhibitors. However, the inhibition activity is not associated with the quinoline structure, since the pyridine nitrogen is sterically hindered. Instead, the amino group in substituents on the quinoline ring appears to be the primary determinant of the observed inhibition potency. The terminal amino group in the 8-substituent of primaquine is believed to be involved in the direct binding to the haem iron of the ferric P450 (Lin and Lu, 1998)

3.2 Quasi-Irreversible Inhibition via Metabolic Intermediate Complexation

A large number of drugs, including methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines, undergo metabolic activation by P450 enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic haem of P450, called metabolic intermediate (MI) complex, so that the P450 is sequestered in a functionally inactive state. MI complexation can be reversed, and the catalytic function of ferric P450 can be restored by *in vitro* incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site. Other *in vitro* methods by which the ferrous complex can be disrupted include irradiation at 400 to 500 nm or oxidation to the ferric state by the addition of potassium ferricyanide. Dissociation or displacement of the MI complex

results in the reactivation of P450 functional activity. However, in *in vivo* situations, the MI complex is so stable that the P450 involved in the complex is unavailable for drug metabolism, and synthesis of new enzymes is the only means by which activity can be restored. The nature of the MI complexation is, therefore, considered to be quasi-irreversible (Lin and Lu, 1998).

3.3 Irreversible Inactivation of P450

Drugs containing certain functional groups can be oxidised by P450 to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site. Because metabolic activation is required for enzyme inactivation, these drugs are classified as mechanism-based inactivators or suicide substrates. The mechanism-based inactivation of P450 may result from irreversible alteration of haem or protein, or a combination of both. In general, modification of the haem group invariably inactivates the P450, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer and oxygen activation, are modified.

3.3.1 Haem Alkylation

Drugs containing terminal double-bond (olefins) or triple-bond (acetylenes) can be oxidised by P450 to radical intermediates that alkylate the prosthetic haem group and inactivate the enzyme. The evidence for haem alkylation includes the demonstration of equimolar loss of enzyme and haem, as well as the isolation and structural characterisation of the haem adducts. Heam alkylation is initiated by the addition of activated oxygen to the internal carbon of the double or triple bond and is terminated by binding to haem

pyrrole nitrogen. It is interesting to note that linear acetylenes react with the nitrogen of pyrrole ring A of CYP2B1 in liver microsomes of phenobarbital-induced rat, whereas linear olefins with the nitrogen of pyrrole ring D.

3.3.2 Covalent Binding to Apoprotein

The best known example of inactivation of P450 through protein modification by a suicide inactivator is that of chloramphenicol. The dichloroacetamido group is oxidised to an oxamyl moiety that acylates a lysine residue in the P450 active centre. This acylation event interferes with the transfer of electrons from P450 reductase to the haem group of the P450 and thereby prevents catalytic turnover of the enzyme. The inactivation by chloramphenicol is not uniform for all P450s. Studies with rat liver microsomes revealed that CYP2B1, CYP2C6 and CYP2C11 are susceptible to inactivation by chloramphenicol, whereas CYP1A1 and CYP1A2 are resistant (Halpert et al., 1985).

4. Mechanisms of Induction of P450

One of the intriguing aspects of the P450 is that some of these enzymes, but not all, are inducible. Human CYP1A1, CYP2C9, CYP2E1 and CYP3A4 are known to be inducible. Unlike CYP inhibition, which is an almost immediate response, P450 induction is a slow regulatory process that can reduce drug concentrations in plasma, and may compromise the efficacy of the drug in a time-dependent manner. Unless care is taken in study design, the pharmacokinetic and clinical consequences of P450 induction are often overlooked in clinical studies.

Although the phenomenon of P450 induction has been known for more than 4 decades, only in recent years we have begun to uncover the mechanisms involved in induction. From a biological point of view, induction is an adaptive response that protects the cells from toxic xenobiotics by increasing the detoxification activity. While in most cases P450 induction is the consequence of an increase in gene transcription, some nontranscriptional mechanisms also are known to be involved.

In drug therapy, there are 2 major concerns related to P450 induction. First, induction will result in a reduction of pharmacological effects caused by increased drug metabolism. Secondly, induction may create an undesirable imbalance between "toxification" and "detoxification". Like a double-edged sword, induction of drug metabolising enzymes may lead to a decrease in toxicity through acceleration of detoxification, or to an increase in toxicity caused by increased formation of reactive metabolites. Depending upon the delicate balance between detoxification and activation, induction can be a beneficial or harmful response (Lin and Lu, 1998).

5. Clinical Implications

5.1 Inhibition of P450

The clinical relevance of drug inhibition will depend on a number of considerations. One of the most important considerations is the therapeutic index of the drug. Patients receiving anticoagulants, antidepressants or cardiovascular drugs are at a much greater risk than patients receiving other kinds of drugs because of the narrow therapeutic index of these drugs.

Although most interactions that can occur with these agents are manageable, usually by appropriate dosage adjustment, a few are potentially life threatening.

For example, coadministration of terfenadine, an antihistamine agent, and ketoconazole led to fatal ventricular arrhythmias in some patients. Terfenadine is a widely used histamine H_1 receptor antagonist. It is metabolised extensively by CYP3A4 in humans to form 2 metabolites by *N*-dealkylation and hydroxylation. After oral administration of a 60 mg dose, terfenadine is usually undetectable in plasma because of extensive first pass metabolism. Concurrent administration of drugs that inhibit terfenadine metabolism can result in an excessive increase in plasma concentration of terfenadine (Honig et al., 1993 ; Yun et al., 1993 ; Woosley et al., 1993).

Clinical data showed that itraconazole and erythromycin also impair the metabolism of terfenadine. Because CYP3A4 represents a major P450 isoform in human liver, and because CYP3A4 has a broad spectrum of substrate specificity, it is likely that many other drugs are capable of inhibiting terfenadine metabolism. Because of its undesirable properties, terfenadine was recently withdrawn from sale or had its use restricted in several countries (Crane and Shih, 1993 ; Honig et al., 1992).

Inhibition can also reduce clinical efficacy, if the drug is a prodrug requiring metabolic activation to achieve its effects and activation is blocked.

5.2 Induction of P450

Usually, metabolites are less pharmacologically active than the parent drug and, therefore, enzyme induction results in a reduction in pharmacological effect because of increased drug metabolism. In some cases, the metabolites

formed during biotransformation may be chemically reactive, so that enzyme induction may result in increased toxicity caused by the increased production of the toxic metabolites.

Rifampicin is one of the most potent enzyme inducers known to humans. It induces several P450 isoforms, including CYP2C and CYP3A. Clinical studies in healthy volunteers demonstrated a reduction in the thrombin time and a corresponding decrease in the plasma half-life of warfarin following treatment with rifampicin (Lin and LU, 1998). Heimark et al. (1987) have shown that the reduction in hypoprothrombinaemic response of warfarin by rifampicin was caused by increased clearance of both warfarin enantiomers.

Another clinically important interaction with rifampicin involves the concomitant administration of oral contraceptives, which has been reported to result in menstrual disturbance and unplanned pregnancies. The increased metabolism of both estrogenic and progesterogenic components of oral contraceptives is believed to be the underlying mechanism (Back et al., 1979). A 4-fold increase in the rate of hydroxylation of estradiol and ethinylestradiol in patients treated with rifampicin was associated with an increase of P450 content in liver biopsies (Bolt et al., 1975).

Rifampicin also increases the metabolism of cyclosporin in patients, resulting in low blood concentrations of the immunosuppressive agent. The subtherapeutic blood concentration of cyclosporin caused by coadministration of rifampicin have frequently resulted in acute allograft rejection (Daniels et al., 1984 ; Campana et al., 1996).

Although enzyme induction generally reduces the pharmacological effect because of increased drug metabolism, sometimes the formed metabolites has the same pharmacological activity as the parent drug. Thus, the clinical consequences of enzyme induction will be determined by the relative reactivity of the parent drug and the formed metabolite.

Table 1 Major human liver cytochrome P450 (CYP) enzymes (Lin and Lu, 1998)

CYP	Drug substrate	Marker substrate/reaction	Inhibitor	Inducer
1A2	Paracetamol(acetaminophen), caffeine, ondansetron, phenacetin, tacrine, tamoxifen, theophylline	Phenacetin, O-de-ethylation	Furafylline	Smoking, charred food
2A6	Coumarin, nicotine	Coumarin, 7-hydroxylation	Dithiocarb sodium (diethyldithiocarbamate)	
2C9	Diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (S)-warfarin	Tolbutamide, methyl hydroxylation	Sulfaphenazole	Barbiturates, rifampicin
2C19	Diazepam, (S)-mephenytoin, omeprazole, pentamidine, propranolol, (R)-warfarin	(S)-mephenytoin, 4-hydroxylation		
2D6	Bufuralol, codeine, debrisoquine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	Bufuralol, 1-hydroxylation	Quinidine, ajmaline	
2E1	Paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	Chlorzoxazone,6-hydroxylation	Dithiocarb sodium	Alcohol,isoniazid
3A4	Benzphetamine, clarithromycin, codeine, cyclosporin, dapsone, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	Testosterone, 6β-hydroxylation	Gestodene, troleandomycin, L-754,394, ketoconazole, itraconazole	Barbiturates, rifampicin, dexamethasone, carbamazepine

CHAPTER 3

MATERIALS AND METHODS

Chemicals and Reagents

The standard mefloquine HCl (Ro 21-5998/001) and mefloquine metabolite (Ro 21-5104) were kindly donated from F-hoffmann-La Roche Ltd., Basel, Switzerland. Mefloquine (Larium®, 250 mg/tablet, Lot No.B230) and rifampicin (Rifagen®, 300 mg/capsule, Lot No. 98558) were purchased from F-hoffmann-La Roche Ltd., Bangkok, Thailand and General Drugs House Co. Ltd., Bangkok, Thailand, respectively. An HPLC grade of acetonitrile and methanol were bought from J.T. Baker Inc., Phillipsburg, USA. Anhydrous sodium sulfate (analytical grade), 85% phosphoric acid (analytical grade), and zinc sulphate (pro analytical grade) were purchased from Merck Darmstadt, Germany and Carlo Erba, Italy, respectively. Water was purified for HPLC by the Milli Q Water Purification System (Millipore, Milford, MA, USA.)

Instrumentation

The HPLC system consisted of a Jasco PU-980 pump, the Waters 717 plus Autosampler (Waters Associates, Milford, MA., USA.) and a Jasco UV 975 detector. Detection was done with the variable-wavelength UV detector set at 222 nm and peak area was measured by a Jasco 807-IT integrator (Tokyo, Japan). A Jasco recorder attenuation was set at 16 mV.F.S. and chart

speed was 2 mm/min. Separation was achieved on a reversed-phase Novapak C_{18} column (3.9 mm x 150 mm HPLC column, particle size 4 μm) (Waters Associates, Milford, MA., USA.). A guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

Methods

1. Subjects

Seven Thai male volunteers aged 24-35 yr, weighing 57-72 kg were enrolled in this study. None were smokers. The subjects were considered to be healthy based on medical history, physical examination, and essential laboratory tests (complete blood count, renal and liver function tests). All subjects were asked to avoid from taking any drugs which were not included in this study, smoking, alcoholic beverages and coffee at least 1 week prior to and during an entire course of the study. Each volunteer was given a detailed explanation of the purpose and protocol of this study, and each gave a written consent which was approved by the Ethics Committee, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand.

2. Protocol

The study was an open-label, two-phase cross-over design with a 2-month washout period.

Phase 1

In the morning after an overnight fast, each subject received a single oral dose of 500 mg mefloquine (2 tablets of 250 mg mefloquine tablet). The

drug was administered with a glass of water (200 ml) under supervision. No food was taken at least 2 hours after ingestion of the drug.

A catheter was inserted into a forearm vein for the collection of blood samples, and was maintained patent using 1 ml of a dilute heparin solution (100 units/ml) after each sample. Venous blood samples (5 ml) were collected in heparinized tubes before drug administration and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hours, and 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49 and 56 days post-drug administration. Samples were centrifuged not later than 30 minutes after collection, and the plasma was separated and srored at -20°C until analysis.

Phase 2

After 2 months of being drug free, all subjects received rifampicin capsule at an oral dose of 600 mg (2 capsules of 300 mg rifampicin capsule) once daily before breakfast for 7 days prior to mefloquine administration. In the morning of day 1 (after rifampicin pretreatment for 7 days), after an overnight fast, all subjects ingested a 600 mg rifampicin 2 hours before an oral administration of 500 mg mefloquine. Venous bloods samples were collected at the time interval before and after mefloquine administration as previously done in phase 1. However, rifampicin was continued to orally administered 600 mg daily before breakfast from day 1 to day 7 and then 600 mg twice weekly from day 8 to day 56.

3. Sample Analysis

The plasma mefloquine and mefloquine metabolite concentrations were measured by a high performance liquid chromatographic (HPLC) method (Bergqvist, 1988) with some modification as follows.

3.1 Mobile Phase

The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) and adjusted to pH 3.07 with 85% phosphoric acid. The mobile phase was freshly prepared daily and was filtered through 0.45 micropore filtered paper (Nyron 66), then degases by sonification for 10 minutes before using. The flow rate was 1 ml/min. All analysis were performed at room temperature (about 25 ± ÎC)

3.2 Stock Standard Solution

A stock standard solution at a concentration of 400 µg/ml was prepared by dissolving 4 mg of standard mefloquine in 10 mmol/l hydrochloric acid and standard mefloquine metabolite dissolved in 10 mmol/l sodium hydroxide solution. All solutions were adjusted to 10 ml in a 10 ml volumetric flask. The stock solutions were stable for at least 6 months at 4°C (Edstein, et al., 1991). Working standard solutions used to prepare a calibration curve day by day were prepared by appropriate dilution of the stock standard solution with a blank plasma (Appendix-1).

3.3 Calibration Curve

Calibration curves were prepared by adding a standard mefloquine and mefloquine metabolite solution to blank human plasma so that the final concentrations in plasma were 62.5, 125, 250, 500, 1,000 and 2,000 ng/ml. The calibration curves for mefloquine and mefloquine metabolite were linear in the range of 62.5 to 2,000 ng/ml. The average coefficient of variations (CV) should be less than 10%. The lower detection limit for mefloquine and mefloquine metabolite was 50 ng/ml.

3.3.1 Recovery

Potential loss of mefloquine and metabolite during the precipitation by acetonitrile was determined by comparing the peak area of mefloquine and metabolite precipitated from plasma samples in the range of 62.5-2,000 ng/ml and the equal concentration of standard mefloquine prepared in mobile phase. The percent recovery was calculated as following

$$\frac{\text{Peak area of standard mefloquine or metabolite in plasma}}{\text{Peak area of standard mefloquine or metabolite in mobile phase}} \times 100$$

3.3.2 Precision and Variability

To determine intra-day precision and variability, the standard mefloquine was spiked in blank plasma at low (125 ng/ml), medium (500 ng/ml) and high (2,000 ng/ml) concentrations and 10 replications of each were carried out on one day. All should be of ±10% of spiked value (actual value) and the CV (CV = SD/Mean normalised peak area ratio x 100) of each concentration should be < 10%.

To determine inter-day precision and variability, the standard mefloquine was spiked in blank plasma at low (125 ng/ml), medium (500 ng/ml) and high (2,000 ng/ml) concentrations and each concentration was carried out on 10 different days. Accuracy should be of ±10% of spiked value (actual value) and the CV of each concentration should be <10%.

3.4 Sample preparation

A 200 µl of plasma sample or spiked standard plasma was used and 50 µl of 0.2 M zinc sulphate solution was added dropwise to polypropylene tubes containing sample plasma or spiked standard plasma and vortex mixing for 30

seconds. A 500 µl volume of acetonitrile was then added dropwise during vortex-mixing for 30 seconds. After 15 minutes the tubes were centrifuged at 10,000 g for 7 minutes. The supernatant (600 µl) was transfered into polypropylene tubes and evaporated to dryness at 55°C for 15-20 minutes under a stream of nitrogen. The residue was reconstituted in 300 µl of mobile phase via ultrasonication (3 minutes) and 100 µl of the solution was injected into the HPLC system (Appendix-2). The chromatographic conditions used in this study were good to separate mefloquine and mefloquine metabolite from other endogenous substances in plasma.

4. Data Analysis

4.1 Pharmacokinetic Calculations

The following parameters were calculated by using Winnonlin® software program, 1995.

The maximum plasma mefloquine concentration (C_{max}), the time to reach C_{max} (T_{max}), the absorption rate constant (Ka), the absorption half-life ($t_{1/2abs}$), the elimination rate constant (Ke), the elimination half-life ($t_{1/2}$) and the area under the concentration-time curve (AUC).

The apparent oral clearance (Cl/f) was calculated as dose/(AUC x body weights).

The apparent volume of distribution (Vd/f) was calculated as Cl/f divided by Ke.

4.2 Statistical Analysis

All results were expressed as means ± S.D. Differences in mefloquine and mefloquine metabolite pharmacokinetic parameters among control and

treatment groups were tested for statistical significance by Student's t-test with $P < 0.05$ taken as the minimum level of significant.

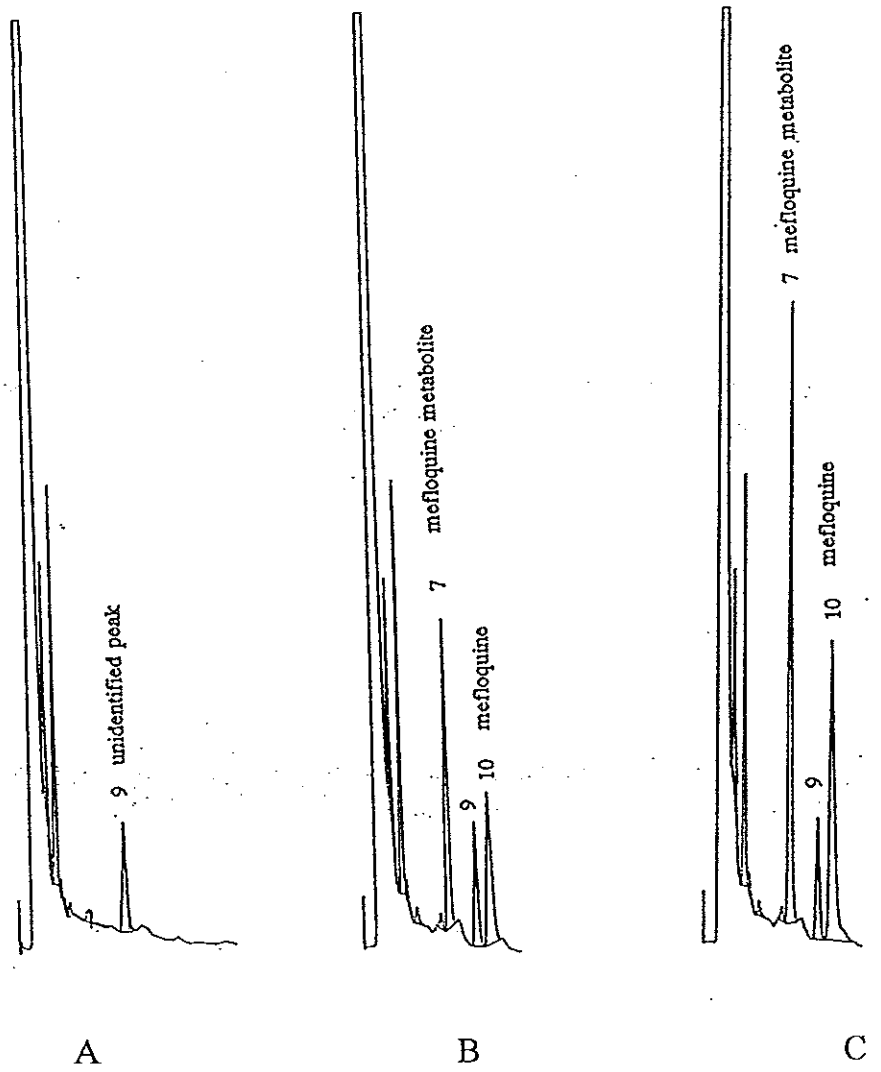

Figure 6 Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma ; (B) spiked with standard mefloquine and mefloquine metabolite, 500 ng/ml ; (C) spiked with standard mefloquine and mefloquine metabolite, 1,000 ng/ml. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S., respectively.

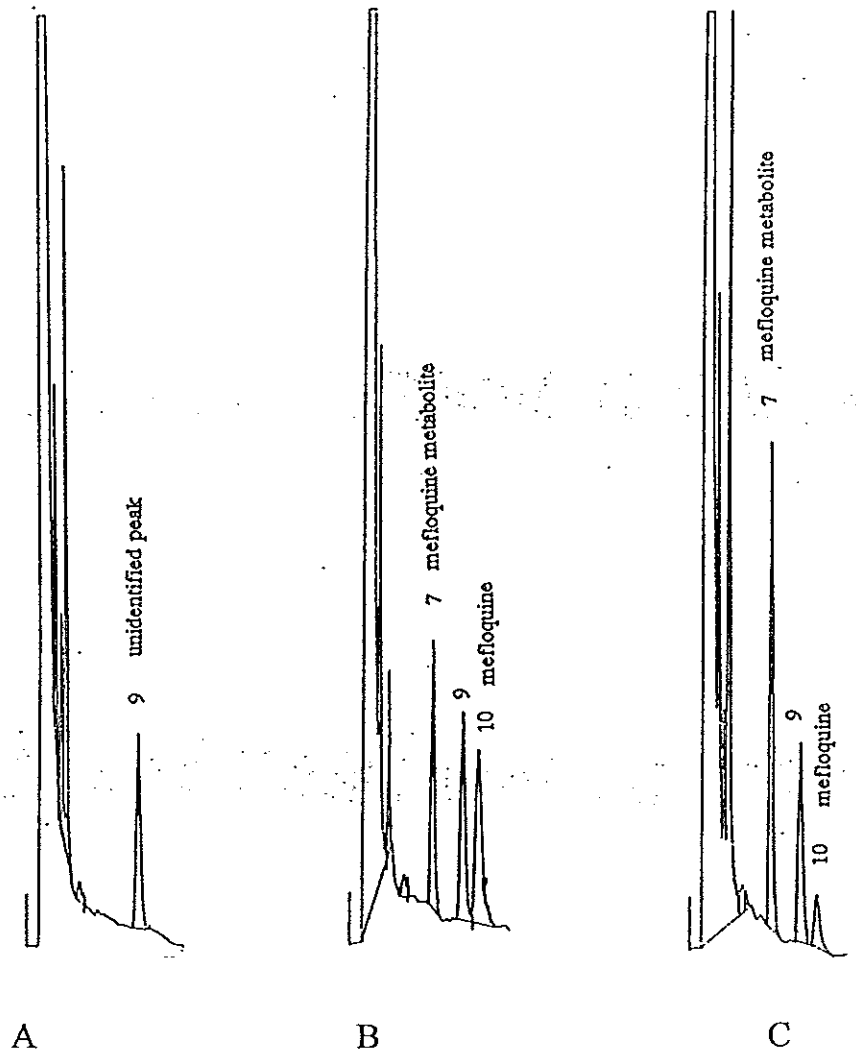

Figure 7 Representative chromatograms of 100 µl human plasma samples. Key : (A) blank human plasma ; (B) and (C) plasma obtained from a subject without rifampicin treatment, 48 and 672 hr, respectively after an oral administration of 500 mg mefloquine. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S.,respectively.

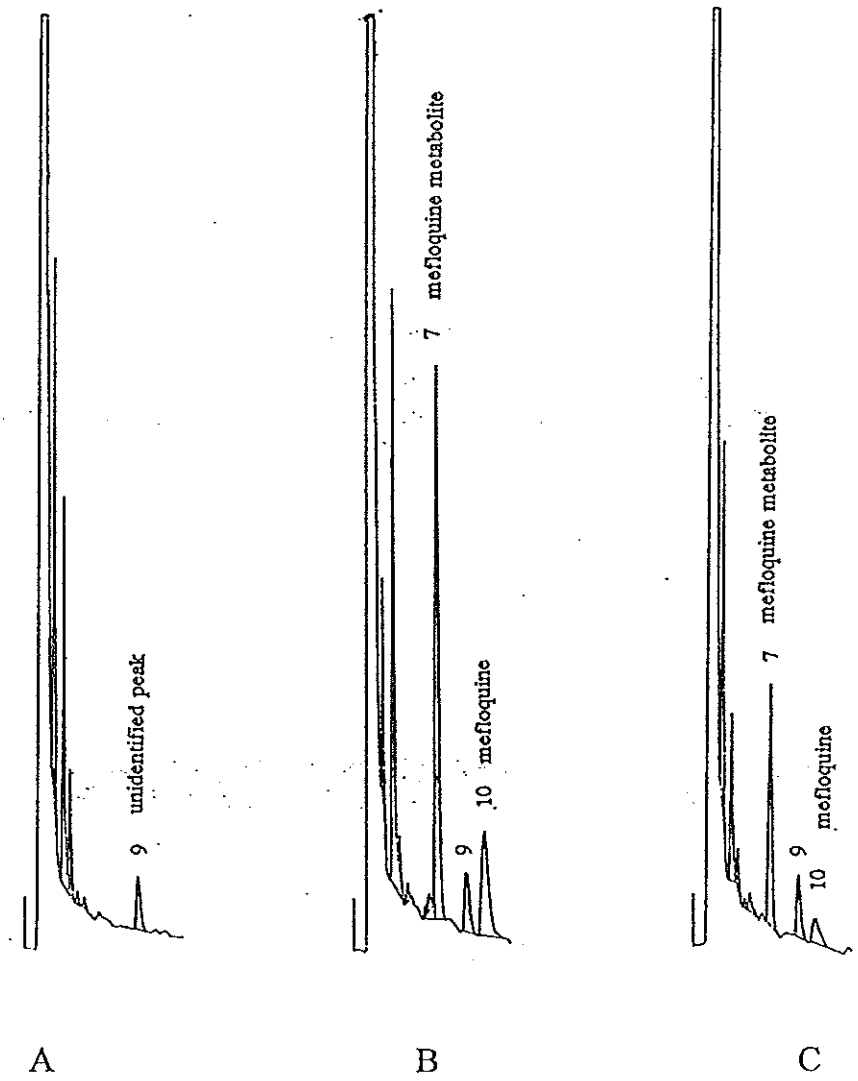

Figure 8 Representative chromatograms of 100 μl human plasma samples. Key : (A) blank human plasma ; (B) and (C) plasma obtained from a subject treatment with rifampicin, 48 and 672 hr, respectively after an oral administration of 500 mg mefloquine. The mobile phase consisted of 50 mmol/L sodium sulfate-methanol-acetonitrile (50 : 34 : 16 vol/vol/vol) pH 3.07 at a flow rate of 1.0 ml/min. Chart speed and attenuation were 2 mm/min and 32 mV F.S., respectively.

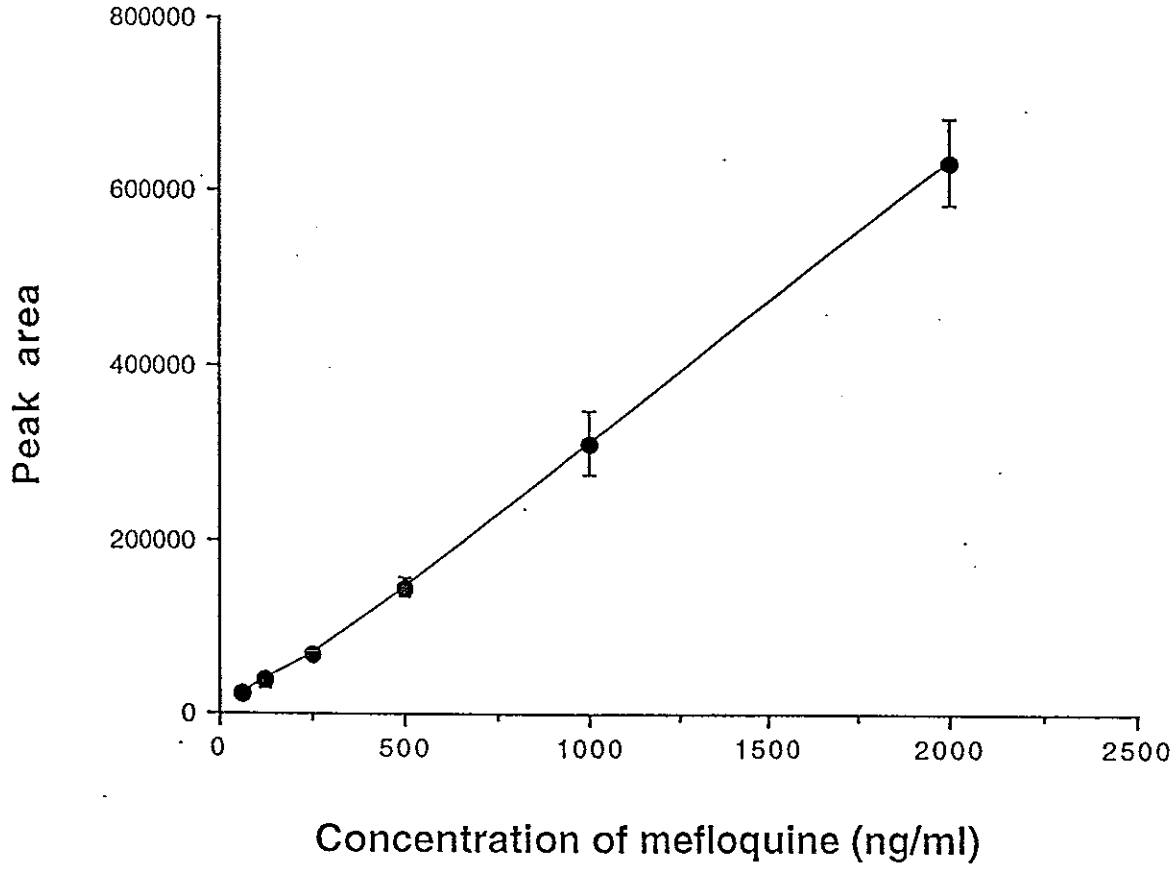

Figure 9 Mean calibration curve of standard mefloquine in plasma, correlation coefficient (r) = 0.999

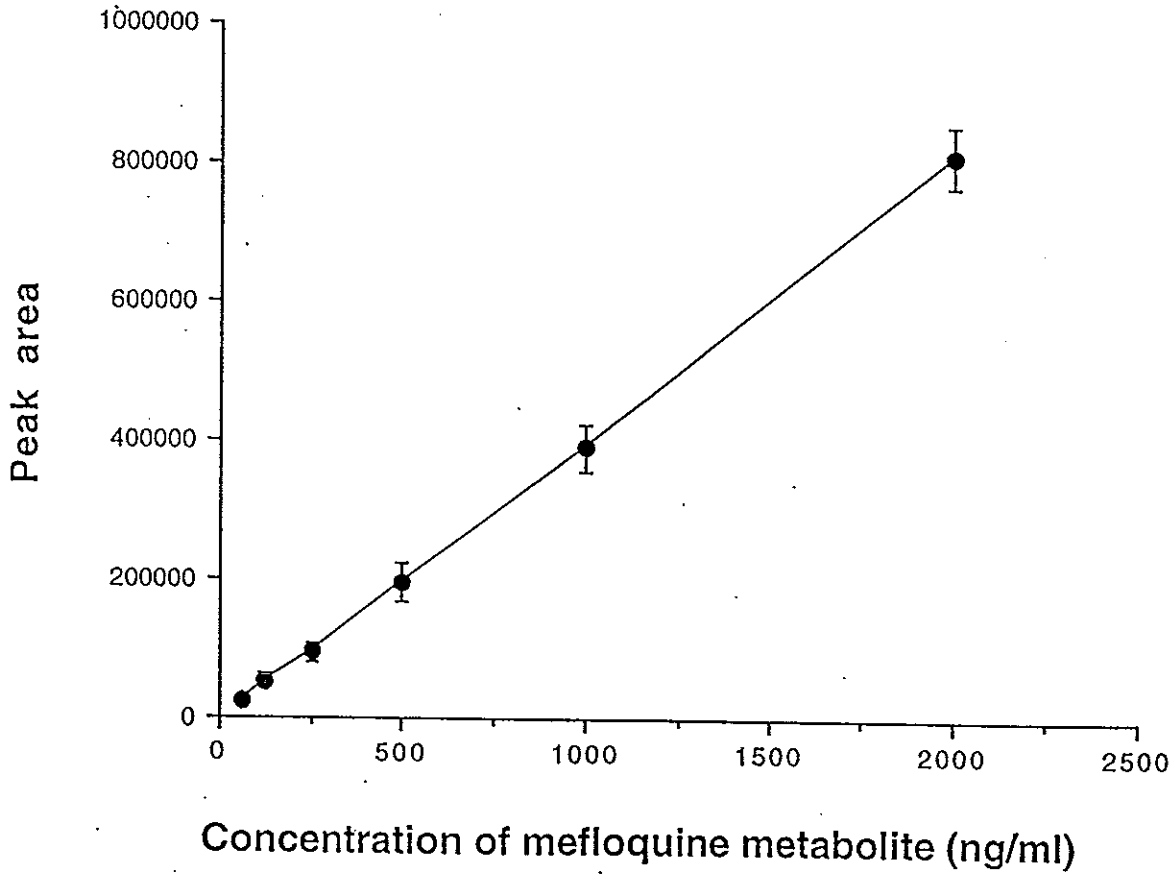

Figure 10 Mean calibration curve of standard mefloquine metabolite in plasma, correlation coefficient (r) = 0.999

Table 2. The intra-assay variance of three different mefloquine concentrations in plasma[a]

Concentration (ng/ml)	Mean peak area ±S.D. (n=10)	CV (%)[b]
125	38921.30 ± 1965.68	5.05
500	168109 ± 10238.19	6.09
2,000	499670.50 ± 26723.31	5.35

[a]Various concentrations of standard mefloquine were added to drug- free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

Table 3. The intra-assay variance of three different mefloquine metabolite concentrations in plasma[a]

Concentration (ng/ml)	Mean peak area ±S.D. (n=10)	CV (%)[b]
125	48458.70 ± 2916.45	6.02
500	201760.70 ± 8832.44	4.38
2,000	761501.50 ± 28131.88	3.69

[a]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

Table 4. The inter-assay variance of three different mefloquine concentrations in plasma[a]

Concentration (ng/ml)	Mean peak area ±S.D. (n=10)	CV (%)[b]
125	43194.00 ± 2997.319	6.94
500	158723.40 ± 12795.86	8.06
2,000	693740.80 ± 41991.50	6.05

[a]Various concentrations of standard mefloquine were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

Table 5. The inter-assay variance of three different mefloquine metabolite concentrations in plasma[a]

Concentration (ng/ml)	Mean peak area ±S.D. (n=10)	CV (%)[b]
125	51517 ± 3494.20	6.78
500	196899.30 ± 13552.92	6.88
2,000	859808 ± 60323.96	7.02

[a]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation as described in the text

[b]Standard deviation divided by mean, expressed in percent

Table 6. Relative percent recovery of standard mefloquine in human plasma

concentration (ng/ml)	peak area in mobile phase[a] (mean ± SD) (n=5)	peak area in plasma[b] (mean ± SD) (n=5)	% Recovery[c]
125	53996.2 ±2648.62	53606.0 ± 2651.27	99.05
500	171408.4 ± 26825.18	183195.86 ± 39554.86	105.99
2,000	667511.0 ± 55517.27	736840.11 ± 66430.56	110.50

[a]Various concentrations of standard mefloquine in mobile phase were directly injected.

[b]Various concentrations of standard mefloquine were added to drug-free human plasma samples prior to precipitation

[c]Mean peak area in plasma divided by mean peak area in mobile phase, expressed in percent

Table 7. Relative percent recovery of standard mefloquine metabolite in human plasma

concentration (ng/ml)	peak area in mobile phase[a] (mean ± SD) (n=5)	peak area in plasma[b] (mean ± SD) (n=5)	% Recovery[c]
125	69871.80 ± 8069.35	65171.12 ± 5687.77	94.63
500	284487.20 ±34753.64	284061.34 ± 18930.93	100.48
2,000	1137291.60±49516.12	1194742.56 ±98092.11	105.16

[a]Various concentrations of standard mefloquine metabolite in mobile phase were directly injected

[b]Various concentrations of standard mefloquine metabolite were added to drug-free human plasma samples prior to precipitation

[c]Mean peak area in plasma divided by mean peak area in mobile phase, expressed in percent

CHAPTER 4

RESULTS

Assay Validation

The assay validation of our experimental method showed that the standard curve was linear in the mefloquine and metabolite concentration range of 62.5-2,000 ng/ml (Figure 11). The correlation coefficient (r) and the coefficient of variation (CV) were 0.999 (Figures 9-10) and 3.69-8.06% (Tables 2-5), respectively. The recovery of mefloquine and metabolite in plasma was 99.05-110.50 (Table 6) and 94.63-105.16% (Table 7), respectively. The chromatograms showed that a peak of mefloquine and its metabolite were well separated from the other peaks in plasma (Figures 6-8). There was no interference from the peak of rifampicin in this analytical method.

The mean plasma concentrations of mefloquine and its metabolite after receiving mefloquine alone declined monoexponentially and were fitted to a one compartment open model (Figure 12). The summary of pharmacokinetic data of mefloquine in the present study were compared to other published data in human volunteers (Table 14). The pharmacokinetic parameters (C_{max}, T_{max}, Vd/f, Cl/f and AUC) of mefloquine in subjects receiving mefloquine alone were similar to the previous reports.

Adverse Effects

Seven adult male healthy Thai volunteers were enrolled and completed in this study. No serious side effects were observed after taking 500 mg of mefloquine. One subject reported gastrointestinal discomfort and one subject reported headache during rifampicin co-administration. The symptoms occurred for a few days, and were not required a specific treatment. However, all subjects were well tolerated to all drugs throughout the study. No marked laboratory abnormalities occurred in any subjects, and physical examinations revealed no abnormal findings at the end of the study.

Pharmacokinetics

The mean plasma concentration-time profile of mefloquine and its metabolite were shown in Figure 11. The pharmacokinetic parameters (mean ± S.D.), estimated from the plasma concentration-time data of mefloquine were presented in Table 8. The results showed that AUC, Ke, $t_{1/2}$, C_{max} and Cl/f were significantly different between parameters of mefloquine alone and mefloquine after rifampicin treatment while there were no significant difference in Ka, $t_{1/2}$(abs), T_{max} and Vd/f. The values (mean ± S.D.) of C_{max}, T_{max}, $t_{1/2}$, Vd/f, Cl/f and AUC in subjects receiving mefloquine alone were 855.63 ± 168 ng/ml, 8.15 ± 2.91 hr, 305.31 ± 47.15 hr, 9.44 ± 1.87 l/kg, 0.0214 ± 0.0038 l/hr/kg and 373.73 ± 57.47 mg/l.hr, respectively ; in rifampicin treated subjects they were 695.67 ± 56.63 ng/ml, 8.67 ± 3.92 hr, 113.43 ± 49.71 hr, 10.89 ± 1.36 l/kg, 0.08 ± 0.03 l/hr/kg and 119.77 ± 54.94 mg/l.hr, respectively.

The pharmacokinetic data of mefloquine metabolite were summarized in Table 9, the C_{max}, T_{max} and $t_{1/2}$ were significantly different between those groups receiving mefloquine alone and mefloquine after rifampicin treatment but the Vd/f, Cl/f and AUC were not significantly different. The values (mean ± S.D.) of C_{max}, T_{max}, $t_{1/2}$, Vd/f, Cl/f and AUC of mefloquine metabolite in subjects receiving mefloquine alone were 813.16 ± 297.96 ng/ml, 220.62 ± 69.75 hr, 506.66 ± 127.64 hr, 8.12 ± 3.45 l/kg, 0.0116 ± 0.0051 l/hr/kg and 786.42 ± 285.40 mg/l.hr, respectively ; in rifampicin treated subjects they were 1,194.45 ± 249.10 ng/ml, 52.48 ± 28.81 hr, 307.45 ± 56.90 hr, 6.28 ± 1.87 l/kg, 0.01486 ± 0.00607 l/hr/kg and 549.88 ± 170.32 mg/l.hr, respectively. In addition, a large inter-individual variation in C_{max}, T_{max}, $t_{1/2}$ and AUC were also observed.

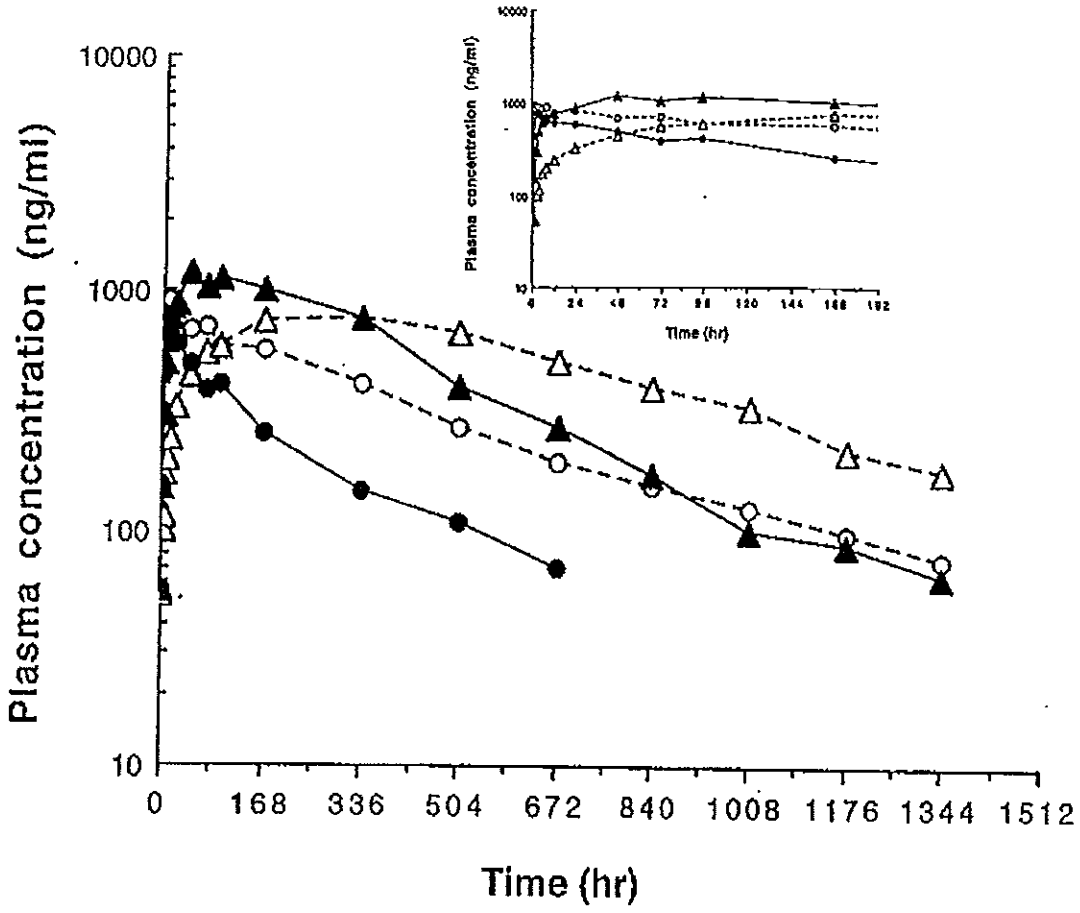

Figure 11 Semi-logarithmic mean plasma mefloquine and mefloquine metabolite concentrations after a single oral dose of 500 mg mefloquine administration alone (O---O : Mefloquine; △---△ : Mefloquine metabolite) and after rifampicin coadministration (●—● : Mefloquine + Rifampicin; ▲—▲ : Mefloquine metabolite + Rifampicin) in 7 normal volunteers.

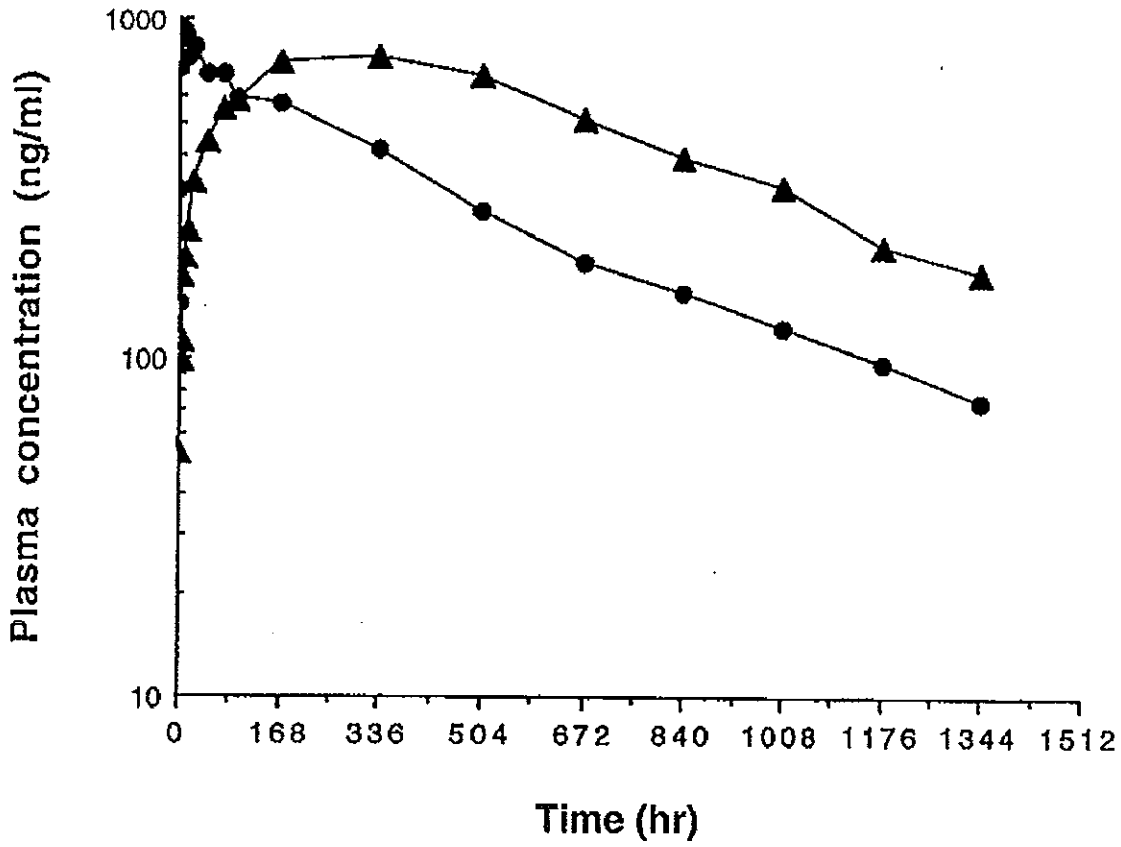

Figure 12 The representative semi-logarithmic mean plasma mefloquine (●—●) and mefloquine metabolite (▲—▲) concentration-time profile after a single oral administration of 500 mg mefloquine.

Table 8 Pharmacokinetic parameters (mean ±S.D.) of mefloquine in seven subjects receiving a single oral dose of 500 mg mefloquine alone and during rifampicin coadministration.

Parameter	Mefloquine alone	Mefloquine + Rifampicin	Paired student's t-test
Age (yr)	32 ± 3.96	32 ± 3.96	-
Weight (kg)	63 ± 5.13	63 ± 5.13	-
AUC (mg/l.hr)	373.73 ± 57.47	119.77 ± 54.94	$p < 0.01$
Ka (hr^{-1})	0.79 ± 0.26	0.62 ± 0.30	NS
Ke (hr^{-1})	0.00231 ± 0.00034	0.00693 ± 0.00239	$p < 0.01$
$t_{1/2}$ (abs)(hr)	1.00 ± 0.43	1.41 ± 0.80	NS
$t_{1/2}$ (hr)	305.31 ± 47.15	113.43 ± 49.71	$p < 0.01$
T_{max} (hr)	8.15 ± 2.91	8.67 ± 3.92	NS
C_{max} (ng/ml)	855.63 ± 168.00	695.67 ± 56.63	$p < 0.05$
Vd/f (l/kg)	9.44 ± 1.87	10.89 ± 1.36	NS
Cl/f (l/hr/kg)	0.0214 ± 0.0038	0.08 ± 0.03	$p < 0.01$

NS ; no significant differences from control

Table 9 Pharmacokinetic parameters (mean ± S.D.) of mefloquine metabolite in seven subjects receiving a single oral dose of 500 mg mefloquine alone and during rifampicin coadministration.

Parameter	Mefloquine alone	Mefloquine + Rifampicin	Paired student's t-test
Age (yr)	32 ± 3.96	32 ± 3.96	-
Weight (kg)	63 ± 5.13	63 ± 5.13	-
AUC (mg/l.hr)	786.42 ± 285.40	549.88 ± 170.32	NS
Ka (hr^{-1})	0.012 ± 0.006	0.09 ± 0.05	$p < 0.01$
Ke (hr^{-1})	0.00143 ± 0.00029	0.00232 ± 0.00044	$p < 0.01$
$t_{1/2}$ (abs)(hr)	72.96 ± 39.47	10.89 ± 7.80	$p < 0.01$
$t_{1/2}$ (hr)	506.66 ± 127.64	307.45 ± 56.90	$p < 0.01$
T_{max} (hr)	220.62 ± 69.75	52.48 ± 28.81	$p < 0.01$
C_{max} (ng/ml)	813.16 ± 297.96	1194.45 ± 249.10	$p < 0.05$
Vd/f (l/kg)	8.12 ±3.45	6.28 ± 1.87	NS
Cl/f (l/hr/kg)	0.0116 ± 0.0051	0.01486 ± 0.00607	NS

NS ; no significant differences from control

Table 10 Pharmacokinetic parameters of mefloquine in subjects receiving a single oral dose of 500 mg mefloquine alone

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)
1	26	58	401.95	0.946	0.0018	0.73	385.00	6.58	747.98	11.38	0.02
2	34	70	342.85	0.392	0.0020	1.77	346.50	13.50	674.87	10.3	0.02
3	35	61	434.48	0.811	0.0024	0.85	288.75	7.19	1028.24	7.84	0.02
4	27	57	365.67	0.796	0.0028	0.87	247.50	7.13	995.95	8.64	0.02
5	32	69	384.92	0.982	0.0022	0.71	315.00	6.24	822.41	8.69	0.02
6	36	61	264.67	1.092	0.0025	0.63	277.20	5.56	667.03	12.12	0.03
7	34	65	421.60	0.484	0.0025	1.43	277.20	10.87	1052.89	7.11	0.02
x ± S.D.	32 ±3.96	63 ± 5.13	373.73 ± 57.47	0.79 ± 0.26	0.0023 ± 0.0003	1.00 ± 0.43	305.31 ± 47.15	8.15 ± 2.91	855.63 ± 168.00	9.44 ± 1.87	0.0214 ± 0.0038

Table 11 Pharmacokinetic parameters of mefloquine metabolite in subjects receiving a single oral dose of 500 mg mefloquine alone

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)
1	26	58	703.99	0.006	0.0016	105.00	433.13	285.38	709.93	7.72	0.012
2	34	70	549.95	0.019	0.0009	36.47	770.00	161.81	465.12	13.08	0.013
3	35	61	796.05	0.021	0.0015	33.00	462.00	135.93	983.94	6.78	0.010
4	27	57	1011.93	0.017	0.0013	41.01	533.08	163.07	1079.35	6.55	0.009
5	32	69	839.30	0.009	0.0013	79.65	533.08	253.47	799.93	6.45	0.009
6	36	61	370.70	0.009	0.0017	77.00	407.65	224.29	439.41	12.57	0.022
7	34	65	1232.99	0.005	0.0017	138.60	407.65	320.42	1214.42	3.68	0.006
x ± S.D.	32 ±3.96	63 ± 5.13	786.42 ± 285.40	0.012 ± 0.006	0.0014 ± 0.0003	72.96 ± 39.47	506.66 ± 127.64	220.62 ± 69.75	813.16 ±297.96	8.12 ± 3.45	0.012 ± 0.005

Table 12 Pharmacokinetic parameters of mefloquine in subjects receiving a single oral dose of 500 mg mefloquine during rifampicin coadministration.

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)
1	26	58	67.80	0.457	0.011	1.52	63.00	8.40	663.52	11.88	0.13
2	34	70	127.59	0.229	0.005	3.03	130.75	16.75	628.60	10.38	0.056
3	35	61	106.90	1.150	0.007	0.60	100.43	4.47	724.55	10.97	0.077
4	27	57	91.52	0.603	0.007	1.15	97.61	7.44	620.75	13.4	0.096
5	32	69	237.35	0.782	0.003	0.89	216.56	7.06	742.01	9.55	0.031
6	36	61	108.02	0.42	0.007	1.65	99.00	9.87	734.69	10.38	0.076
7	34	65	99.20	0.666	0.008	1.04	86.63	6.71	755.59	9.65	0.078
x ± S.D.	32 ±3.96	63 ±5.13	119.77 ±54.94	0.62 ±0.30	0.00693 ±0.00239	1.41 ±0.80	113.43 ±49.71	8.67 ±3.92	695.67 ±56.63	10.89 ±1.36	0.08 ±0.03

Table 13 Pharmacokinetic parameters of mefloquine metabolite in subjects receiving a single oral dose of 500 mg mefloquine during rifampicin coadministration.

Subject No.	Age (yr)	Weight (kg)	AUC (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)
1	26	58	553.94	0.055	0.0019	12.72	364.74	63.03	970.35	7.84	0.016
2	34	70	581.98	0.119	0.0023	5.82	301.30	33.76	1229.81	5.38	0.012
3	35	61	572.67	0.165	0.0028	4.21	251.09	25.23	1474.33	5.19	0.014
4	27	57	433.44	0.072	0.0029	9.63	238.97	46.03	1124.34	6.8	0.020
5	32	69	886.05	0.026	0.0019	27.07	364.74	109.66	1366.51	4.31	0.008
6	36	61	325.39	0.124	0.0026	5.57	266.54	31.64	790.23	9.54	0.025
7	34	65	831.87	0.062	0.0019	11.18	364.74	58.01	1405.61	4.91	0.009
x ± S.D.	32 ±3.96	63 ±5.13	549.88 ±170.32	0.09 ±0.05	0.0023 ±0.0004	10.89 ±7.80	307.45 ±56.90	52.48 ±28.81	1194.45 ±249.10	6.28 ±1.87	0.015 ±0.006

Table 14 Mefloquine pharmacokinetic data were compared to other published data

Data	Sources					
	Karbwang 1991	Karbwang 1992	Crevoisier 1997	Mansor 1989	Sunbhanich 1997	Present study[a]
Subjects	8 men	8 men	20 men	10 men	10 men	7 men
Age (yr)	24-51	25-52	-	-	20-40	24-35
Dose (mg)	750	750	750	500	500	500
Route	oral	oral	oral	oral	oral	oral
C_{max} (ng/ml)	1228 ± 223	1161 ± 120	868 ± 204	1010	555.46 ± 117.41	855.63 ± 168
T_{max} (hr)	6 ± 3	5.6 ± 2.8	36 ± 30	5.7	12.54 ± 7.35	8.15 ± 2.91
$t_{1/2}$ (days)	17.7 ± 2.51	19.7 ± 3.2	17.17 ± 4.58	16.1	9.6 ± 1.45	12.72 ± 1.96
Vd/f (l/kg)	19.4 ± 3.03	19.6 ±4.0	-	17.6	15.58 ± 3.56	9.44 ± 1.87
Cl/f (l/hr/kg)	0.032 ± 0.005	0.029 ± 0.004	-	0.032	0.051 ± 0.026	0.0214 ± 0.0038
AUC (mg/l.hr)	446.4 ±51.36	480 ± 91.2	461 ± 123	-	225.18 ± 109.76	373.73 ± 57.47

[a]Data obtained from subjects receiving mefloquine alone

CHAPTER 5

DISCUSSION AND CONCLUSION

Mefloquine, a quinoline methanol derivative of quinine, is an antimalarial drug which is effective for all species of malarial parasites infecting humans, including multidrug-resistant *Plasmodium falciparum,* and was first introduced for clinical use in Thailand in late 1984 (Nosten, et al., 1991). It is given both as a prophylactic and as a therapeutic agent (Crevoisier, et al., 1997). Mefloquine is relatively well tolerated and has the advantage of a single day regimen. It has ideal properties for prophylactic use (Nosten and Price, 1995). During the past years, there have been several failures of mefloquine treatment, it is not clear whether this represents genuine resistance (Karbwang, et al., 1991). Rifampicin is a potent enzyme inducer of hepatic drug metabolism in humans by CYP 2C8-10 and CYP 3A, mainly CYP 3A4 (Venkatesan, 1992) and is known to interact with a number of drugs such as warfarin, digitoxin, dapsone, chlorpropamide, metoprolol, theophylline, antipyrine, hexobarbitone and cyclosporine (Venkatesan, 1992). It has been shown that rifampicin decreases the elimination half-life of quinine and quinidine, a diastereoisomer of quinine (Wanwimolruk, et al., 1995a). Mefloquine is structurally related to quinine (Nosten and Price, 1995) and cytochrome P450 3A4 is important for the 3-hydroxylation of quinine in humans *in vivo* (Mirghani, et al., 1999). In addition, CYP3A4 is the principle

isozyme induced by rifampicin (Venkatesan, 1992). In the pharmacokinetic point of view, there is the possibility of a pharmacokinetic interaction between mefloquine and rifampicin in humans *in vivo*. Therefore, we investigated the effect of rifampicin on the pharmacokinetics of mefloquine in healthy Thai male volunteers.

In the present study, the profile of plasma mefloquine concentrations and the derived pharmacokinetic parameters in healthy subjects receiving a single oral dose of 500 mg mefloquine were similar to those previously reported. (Table 14) (Karbwang, et al., 1991 ; Karbwang, et al., 1992 ; Crevoisier, et al., 1997 ; Mansor, et al., 1989 and Sunbhanich, et al., 1997). The mean peak plasma mefloquine concentrations in each study were depended on the oral doses of mefloquine. Nevertheless, the variations of these pharmacokinetic parameters may be influenced by inter-individual variation and environmental factors (e.g., sex, races, diet, smoking, coffee and alcoholic drinking). In this study, there were 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 Karbwang et al. (1988a).

The semi-logarithmic plots of the plasma mefloquine concentration-time curve (Figure 12) shows 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 concentration-time curve

(AUC), the elimination rate constant (Ke), the elimination half-life ($t_{1/2}$), the maximum plasma concentration (C_{max}) and the apparent oral clearance (Cl/f) of the subjects receiving rifampicin treatment were significantly different from those receiving mefloquine alone. These findings suggest that rifampicin alters the pharmacokinetics of mefloquine if the two drugs are concomitantly administered.

In a long term rifampicin coadministration (~56 days), oral clearance (Clo) of mefloquine increased by about 4-fold (273.83%), the C_{max} decreased from 855.63 ng/ml to 695.67 ng/ml (18.46%), the $t_{1/2}$ was shorter about 2.5-fold (62.85%) and the AUC decreased about 3-fold (67.95%) from control. In addition, the $t_{1/2}$ of mefloquine metabolite decreased about 1.5-fold (39.31%) and T_{max} decreased about 4-fold (76.21%) when rifampicin and mefloquine were coadministered. The efffect of rifampicin on the pharmacokinetics of mefloquine may be mediated by induction of the hepatic mixed function oxidase system by rifampicin which were similar to those occuring with quinine (Wanwimolruk, et al., 1995b) since mefloquine has a chemical structure related to quinine and quinidine. Significant quantities of CYP 3A4 are found in small bowel enterocytes and liver (Villikka, et al., 1997). CYP 3A4 is the most abundantly expressed CYP and accounts for approximately 30 to 40% of the total CYP content in human adult liver and small intestine (Wildt, et al., 1999). Since rifampicin is a potent inducer of CYP 3A4 in both liver and small bowel enterocytes, the obvious explanation of the strong significant interaction observed between rifampicin and mefloquine in this study was based on CYP 3A4 activity

induced by rifampicin. The decrease in mefloquine concentrations after a rifampicin coadministration was due to induction of metabolic transformation of mefloquine in the liver rather than in the gut wall, as T_{max} of mefloquine was not significantly affected by rifampicin coadministration. Moreover, $t_{1/2}$ of mefloquine was significantly decreased by rifampicin coadministration. Accordingly, the decrease in bioavailability of mefloquine after rifampicin coadministration is the result of an increase in metabolism in the liver rather than in the small bowel enterocytes.

Rifampicin is a potent inducer of hepatic drug metabolism, as evidenced by a proliferation of smooth endoplasmic reticulum and an increase in the cytochrome P450 content in the liver. The induction is a highly selective process and not every drug metabolised via oxidation is affected (Venkatesan, 1992).

The hepatic microsomal enzyme cytochrome P450 3A4 is responsible for metabolizing both quinine and quinidine (Krishna and White, 1996). Quinidine (dextrorotatory stereoisomer of quinine) elimination is enhanced by phenobarbitone, phenytoin and rifampicin and reduced by the hepatic CYP inhibitors including cimetidine, amiodarone, verapamil and possibly erythromycin (Gonzalez and Idle, 1994 and Spinler, et al., 1995). Cimetidine reduces the clearance and prolongs the elimination $t_{1/2}$ of mefloquine in a manner similar to quinine (Sunbhanich, et al., 1997).

Many clinical studies and case reports have shown that rifampicin can enhance metabolism of several drugs, and most of these interactions are of clinical

importance. The pharmacokinetics of warfarin, quinidine, propranolol, verapamil, norethindrone, ethinylestradiol, prednisolone, tolbutamide, cyclosporin, and ketoconazole were all significantly altered by rifampicin, thus dosages of these drugs will be increased or use of alternative compounds to maintain adequate clinical responses when these medications are coadministered with rifampicin (Venkatesan, 1992).

In vitro minimum inhibitory concentrations of mefloquine have been reported as 10^{-7} M (41.48 ng/ml) to $1.7x10^{-7}$ M (70.51 ng/ml) (Suebsang, et al., 1986) and plasma levels 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 coadministration with rifampicin were 855.63 ± 168 ng/ml and 695.67 ± 56.63 ng/ml, respectively which are adequate for a schizontocidal activity for *Plasmodium falciparum* malaria.

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 *P.falciparum* parasitaemia (Crevoisier, et al., 1997). Recently, it has been estimated that 99% and 95% prophylactic efficacy can be achieved at mefloquine blood concentrations of 915 ng/ml and 620 ng/ml, respectively (Lobel, et al., 1993). In the present results, plasma concentrations of mefloquine in subjects administered mefloquine alone and rifampicin treatment were 855.63 ± 168 ng/ml

and 695.67 ± 56.63 ng/ml, respectively which is higher than the 95% prophylactic efficacy, but there was an increase in clearance of mefloquine from 0.0214 ± 0.0038 l/hr/kg to 0.08 ± 0.03 l/hr/kg and a decrease in $t_{1/2}$ from 305.31 ± 47.15 hr to 113.43 ± 49.71 hr when coadministration with rifampicin. These results may lead to a prophylaxis failure, increase risk of treatment failure and spread of drug resistance because mefloquine is given as a single dose regimen. However, concentrations in plasma associated with successful response and treatment failure must vary considerably depending on the intrinsic susceptibility of the parasites (Karbwang and White, 1990).

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. Differences in absorption may contribute an explanation for some of the failures which can occasionally occur during prophylaxis, although failures are due not only to inadequate drug blood concentration (variation in kinetics, non compliance) but also to drug resistance of the parasites (Crevoisier, et al., 1997). The pharmacokinetic parameters of mefloquine after administration of a generic tablet of mefloquine and the reference tablet showed significant differences ($P < 0.05$). The C_{max} and AUC values of the test formulation were significantly lower and the T_{max} value was considerably longer than the respective values of the reference formulation (Weidekamm, et al., 1998). Therefore, if mefloquine and

rifampicin were coadministered, mefloquine should be administered with high fat diet and qualified drug should be used to maximise therapeutic outcome.

In conclusion, in a long term rifampicin treatment it has shown that rifampicin markedly decreased the plasma concentration of mefloquine and significantly increased the clearance and decreased the elimination half-life of mefloquine. These effects may be mainly due to the induction of CYP3A4 isozyme by rifampicin. Therefore, mefloquine and rifampicin should not be coadministered in order to maximise therapeutic efficacy and prevent a risk of resistant of *P. falciparum.* Moreover, the present results also indicate that CYP 3A4 is important for the metabolism of mefloquine in humans *in vivo*.

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APPENDIX

APPENDIX-1

Preparation of standard mefloquine in plasma blank of standard curve for each day

Stock A = mefloquine 400 μg/ml

Stock B = mefloquine 40 μg/ml

Stock C = mefloquine 4 μg/ml (4,000 ng/ml)

2000 ng/ml = blank plasma 200 μl + 200 μl of 4000 ng/ml

1000 ng/ml = blank plasma 200 μl + 200 μl of 2000 ng/ml

500 ng/ml = blank plasma 200 μl + 200 μl of 1000 ng/ml

250 ng/ml = blank plasma 200 μl + 200 μl of 500 ng/ml

125 ng/ml = blank plasma 200 μl + 200 μl of 250 ng/ml

62.5 ng/ml = blank plasma 200 μl + 200 μl of 125 ng/ml

APPENDIX-2

Protein precipitation method

200 µl plasma or spiked standard

↓

vortex 30 sec {added dropwise 50 µl Zinc sulphate (0.2M)}

↓

vortex 30 sec {added dropwise 500 µl acetonitrile}

↓

kept for 15 min

↓

centrifuged at 10,000 g for 7 min

↓

supernatant was transfered to another tube (600 µl)

↓

evaporated at 55-60°C for 15-20 min under a stream of nitrogen

↓

residue + 200 µl of MP via ultrasonication (3 min)

↓

100 µl injected

APPENDIX-3

Plasma concentrations of mefloquine (ng/ml) at 0-1344 hr in subjects receiving a single oral dose of 500 mg mefloquine alone.

Time	Subject No.								
(hr)	1	2	3	4	5	6	7	Mean	S.D.
0	0	0	0	0	0	0	0	0	0
0.5	146.97	0	64.04	138.94	298.45	340.85	0	141.32	131.06
1	397.98	133.24	188.19	452.26	570.21	329.68	107.72	311.33	174.67
2	696.12	934.22	591.03	854.4	680.35	987	289.06	719.31	238.24
3	842.19	949.44	1027.38	1000.74	979.97	1114.51	532.2	920.92	190.08
4	902.42	711.14	1038.56	912.71	1179.61	1018.13	603	909.37	197.81
6	617.61	748.95	1129.19	804.02	985.33	1198.62	674.08	879.69	226.90
8	589.06	679.48	1154.11	730.36	1188.71	1137.83	767.93	892.50	256.78
12	654.33	683.11	940.07	701.03	984.58	872.31	599.83	776.47	152.64
24	530.21	743.57	1074.73	838.6	896.18	1060.28	629.31	824.70	206.07
48	480.07	614.18	967.24	731.88	661.95	727.05	658.96	691.62	148.07
72	498.25	591.97	815.83	761.09	643.37	884.5	690.43	697.92	133.44
96	513.91	573.92	661.34	544.61	746.69	663.78	410.41	587.81	112.11
168	382.26	582.39	601.39	512.23	752.11	662.15	480.33	567.55	122.05
336	338.85	365.61	541.52	386.71	523.81	383.23	327.26	409.57	86.97
504	156	421.1	308.18	214.71	187.39	326.03	269.51	268.99	91.56
672	124.89	287.44	135.76	239.48	162.4	185.17	198.19	190.48	57.70
840	144.25	81.22	208.16	197.91	110.18	230.11	118.63	155.78	56.56
1008	121.16	71.52	119.17	148.63	0	240.42	108.24	115.59	61.79
1176	52.77	52.72	141.75	103.41	0	201.75	102.8	93.60	66.20
1344	52.79	0	166.72	105.41	0	116.12	61.11	71.74	61.77

APPENDIX-4

Plasma concentrations of mefloquine (ng/ml) at 0-1344 hr in subjects receiving a single oral dose of 500 mg mefloquine and 600 mg rifampicin.

Time	Subject No.								
(hr)	1	2	3	4	5	6	7	Mean	S.D.
0	0	0	0	0	0	0	0	0	0
0.5	0	0	73.43	0	0	216.59	0	55.71	75.40
1	181.43	0	210.72	167.91	120.61	365.65	0	154.61	120.92
2	323.89	174.06	565.61	601.39	363.52	842.12	173.41	434.86	246.46
3	630.2	750.67	871.87	957.33	676.31	1142.57	244.17	753.30	285.05
4	754.67	785.03	970.95	904.67	823.11	752.31	478.81	781.36	155.99
6	720.22	795.53	903.21	718.46	638.29	584.01	538.25	699.71	125.56
8	730.28	690.56	536.63	682.15	587.99	604.41	624.86	636.70	67.45
12	672.59	565.99	640.87	782.9	494.68	530.67	587.9	610.80	97.28
24	648.34	405.63	612.21	666.2	510.29	611.7	759.73	602.01	114.25
48	583.95	350.65	511.46	625.4	484.98	453.22	419.34	489.86	94.35
72	452.48	315.95	319.08	420.31	327.04	401.52	440.21	382.37	59.92
96	430.55	336.75	409.93	550.25	320.34	448.14	353.19	407.02	79.70
168	178.86	221.37	292.01	319.13	121.85	248.3	285.97	251.21	50.15
336	129.14	81.12	151.97	232.74	116.82	116.16	182.15	144.30	50.14
504	0	74.88	72.25	220.94	83.64	133.4	143.36	104.07	69.73
672	0	0	0	213.16	79.51	0	0	69.02	66.48
840	0	0	0	132.64	0	0	0	18.95	50.13
1008	0	0	0	76.56	0	0	0	10.94	28.94
1176	0	0	0	127.69	0	0	0	18.24	48.26
1344	0	0	0	52.06	0	0	0	7.44	19.68

APPENDIX-5

Plasma concentrations of mefloquine metabolite (ng/ml) at 0-1344 hr in subjects receiving a single oral dose of 500 mg mefloquine alone.

Time	Subject No.								
(hr)	1	2	3	4	5	6	7	Mean	S.D.
0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
2	0	170.27	0	0	0	0	0	24.32	64.36
3	68.13	266.75	88.19	101.61	85.01	52.77	0	94.64	82.88
4	98.61	195.06	169.37	93.06	114.41	65.12	53.34	112.71	52.23
6	111.34	343.95	281.43	141.26	164.18	110.44	64.6	173.89	101.26
8	73.68	134.9	168.47	187.13	305.03	379.58	143.25	198.86	106.34
12	176.66	305.87	409.23	272.35	216.03	175.99	112.48	238.37	99.12
24	181.35	502.72	521.83	412.64	276.48	253.99	152.54	328.79	150.38
48	214.23	334	525.9	392.37	667.82	612.47	304.41	435.89	169.20
72	350.96	853.49	821.59	501.53	677.1	346.99	294.64	549.47	234.67
96	348.21	397.05	724.84	522.87	885.78	720.58	412.19	573.07	205.15
168	480.33	1178.92	1022.88	688.42	823.06	623.52	385.08	743.17	285.52
336	401.48	770.37	1124.43	753.34	1212.38	745.86	383.03	770.13	318.26
504	369.49	653.95	702.87	814.09	1184.79	618.74	375.47	674.20	278.89
672	314.06	499.63	582.92	517.98	915.61	427.6	293.39	507.31	208.99
840	326.83	292.54	540	335.77	663.83	423.62	149.28	390.27	169.80
1008	190.08	298.86	377.58	343.84	604.18	310.4	126.49	321.63	152.47
1176	118.98	204.56	259.28	244.11	372.01	216.32	84.07	214.19	94.81
1344	133.3	135.38	184.36	272.73	288.91	164.69	68.1	178.21	78.94

APPENDIX-6

Plasma concentrations of mefloquine metabolite (ng/ml) at 0-1344 hr in subjects receiving a single oral dose of 500 mg mefloquine and 600 mg rifampicin.

Time	Subject No.								
(hr)	1	2	3	4	5	6	7	Mean	S.D.
0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0
1	0	89.2	0	92.26	68.53	0	0	35.71	45.16
2	167.14	317.72	103.64	103.98	232.9	52.91	80.85	151.31	94.66
3	340.26	607.15	272.58	259.63	313.26	121.17	178.15	298.89	155.64
4	533.76	910.73	355.28	352.15	532.39	444.36	319.25	492.56	203.74
6	630.87	1067.13	657.43	514.67	590.76	323.71	532.54	616.73	226.85
8	930.71	1181.97	596.71	554.49	620.84	439.53	586.84	701.58	259.57
12	1021.96	1255.78	603.54	456.03	771.29	495.82	706.85	758.75	289.84
24	1130.45	1174.71	871.71	560.38	1006.7	777.29	653.04	882.04	234.48
48	1371.21	1446.92	1265.88	1129.16	1461.99	816.11	799.05	1184.33	281.27
72	1017.55	1408.06	1133.42	1002.09	1164.96	812.37	711.19	1035.66	231.50
96	916.32	1389.17	989.91	1548.95	1328.42	963.17	737.47	1124.77	296.95
168	1086.56	1091.66	686.33	1294.29	1356.15	1023.98	578.53	1016.79	289.82
336	723.44	643.52	592.75	1170.84	1110.5	755.69	354.18	764.42	288.41
504	426.34	318.54	214.57	525.23	699.56	394.06	190.51	395.54	178.38
672	235.62	203.89	206.49	483.64	350.48	241.65	170.17	270.28	109.90
840	167.05	79.22	108.46	393.24	237.73	140.76	94.45	174.42	110.22
1008	94.91	0	74.44	220.02	127.89	84.02	57.64	94.13	67.91
1176	67.74	0	129.33	179.44	117.18	66.43	0	80.02	66.88
1344	0	0	53.34	219.76	88.47	0	0	51.65	81.95

APPENDIX-7

ที่ ทม 1209/ 698

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90110

หนังสือรับรองการศึกษาวิจัย

การศึกษาวิจัยเรื่อง : "การศึกษาปฏิกิริยาทางเภสัชจลนศาสตร์ระหว่างยาไรแฟมปิซินและยาเมโฟลควินในคน

ผู้วิจัย : นายแพทย์วิบูลย์ ฤทธิทิศ
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณา และเห็นชอบจากคณะกรรมการจริยธรรม ซึ่งเป็นคณะกรรมการพิจารณาโครงการวิจัย ตลอดจนติดตามผลในส่วนของการทดลองที่กระทำต่ออาสาสมัคร ของคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ ๒๐ พฤศจิกายน 2540

..... ประธานคณะกรรมการ
(รศ.เพริศพิชญ์ คณาธารณา)
รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา

..... กรรมการ
(นายแพทย์สมหมาย ปล่องสมบูรณ์)
ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์

.....กรรมการ
(รศ.ถาวร เกียรติทับทิว)
ภาควิชารัฐประศาสนศาสตร์ คณะวิทยาการจัดการ

.....กรรมการ
(นายแพทย์วิบูลย์ ฤทธิทิศ)
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

.....กรรมการ
(แพทย์หญิงสุวิณา รัตนชัยวงศ์)
ภาควิชาชีวเวชศาสตร์ คณะแพทยศาสตร์

.....กรรมการ
(ผศ.วุฒิพร พรหมชุนทอง)
ภาควิชาวาริชศาสตร์ คณะทรัพยากรธรรมชาติ

ใบยินยอม

1.ชื่อโครงการ : ผลของยาไรแฟมปีซินต่อเภสัชจลนศาสตร์ของยาเมโฟลควิน ในอาสาสมัครสุขภาพดีเมื่อให้โดยการรับประทานครั้งเดียว

2.ข้าพเจ้า นาย ..... นามสกุล ..... อายุ ..... ปี ยินยอมเป็นอาสาสมัครในโครงการศึกษาเรื่อง"ผลของยาไรแฟมปีซินต่อเภสัชจลนศาสตร์ของยาเมโฟลควินในอาสาสมัครสุขภาพดีเมื่อให้โดยการรับประทานครั้งเดียว"

3.วัตถุประสงค์ของการศึกษา

1.เพื่อศึกษาถึงการเปลี่ยนแปลงค่าทางด้านเภสัชจลนศาสตร์ของยาเมโฟลควินเมื่อให้ร่วมกับไรแฟมปีซิน

2.เพื่อเป็นข้อมูลและแนวทางในการรักษาผู้ป่วยในกรณีที่ได้รับยาทั้งสองชนิดนี้ร่วมกัน

4.วิธีการศึกษา

4.1 อาสาสมัครที่เข้าร่วมโครงการต้องเป็นผู้ที่มีสุขภาพสมบูรณ์และแข็งแรง

4.2 ใช้อาสาสมัครเพศชายอายุระหว่าง 20-35 ปี

4.3 อาสาสมัครทุกคนต้องไม่ได้รับยาชนิดอื่นๆมาก่อนที่จะเริ่มทำการทดลองเป็นเวลาอย่างน้อย 1 เดือน

4.4 ก่อนเริ่มทำการทดลอง อาสาสมัครทุกคนจะได้รับการเจาะเลือดตรวจความปกติหรือผิดปกติของเม็ดเลือดและค่าชีวเคมีของเลือดที่เซ็นทรัลแลบ

4.5 ให้อาสาสมัครงดอาหารมาก่อนอย่างน้อย 8 ชั่วโมงก่อนเริ่มทำการทดลอง ในการทดลองจะแบ่งออกเป็น 2 ตอนคือ

ตอนที่ 1 อาสาสมัครทุกคนจะต้องรับประทานยาเมโฟลควินขนาด 500 มิลลิกรัมครั้งเดียวในวันเริ่มทำการทดลอง

ตอนที่ 2 อาสาสมัครทุกคนจะได้รับยาไรแฟมปีซินขนาด 600 มิลลิกรัมต่อวันมาก่อนเป็นเวลา 7 วันและได้รับต่อไปตามแผนการทดลอง (protocol) จนครบ 56 วัน ส่วนยาเมโฟลควินจะให้อาสาสมัครรับประทานขนาด 500 มิลลิกรัมในวันที่ 7 (หลังรับประทานยาไรแฟมปีซินมาก่อน 7 วัน)

4.6 ในทั้ง 2 ตอนที่ทำการทดลอง จะทำการเจาะเลือด 5 มิลลิลิตรในช่วงเวลา 56 วันโดยเก็บเลือดที่เวลา 0 (ก่อนรับประทานยาเมโฟลควิน) และที่เวลา 0.5, 1, 2, 3, 4, 6, 8, 12, 24 ชั่วโมงและวันที่ 2, 3, 4, 7, 14, 21, 28, 35, 42, 49 และ 56 หลังรับประทานยาเมโฟลควิน การเก็บเลือดนั้นจะทำการเจาะเลือดเพียงครั้งเดียวแล้วคาสายสวน (catheter)ไว้เพื่อเก็บตัวอย่างเลือดใน 24 ชั่วโมงแรก ส่วนการเก็บเลือดในช่วงระยะหลังจะทำการเจาะเลือดที่บริเวณข้อพับของแขนโดยตรง นำเอาเลือดที่ได้ไปทำการปั่นแยกพลาสมาทันทีและเก็บไว้ที่อุณหภูมิ -20 องศาเซลเซียส เพื่อนำไปวิเคราะห์หาปริมาณยาเมโฟลควินต่อไป

5.ผลข้างเคียงจากการใช้ยาเมโฟลควิน

ส่วนใหญ่เกิดกับผู้ที่มีการตอบสนองต่อยาไวเกินหรือได้รับยาขนาดสูงเกินไปโดยเฉพาะอย่างยิ่งได้รับยามากกว่า 1,000 มิลลิกรัมขึ้นไป อาจทำให้เกิดอาการวิงเวียน ปวดศีรษะ การมองเห็นถูกรบกวน อาการบ้านหมุน หูอื้อ นอนไม่หลับ กระสับกระส่าย วิตกกังวล ซึมเศร้า และความคิดสับสน เป็นต้น

6. ผลข้างเคียงจากการใช้ยาไรแฟมปีซิน

อาการข้างเคียงที่พบได้แต่ไม่บ่อยนักได้แก่ มีผื่นขึ้น เกร็ดเลือดต่ำ และการทำงานของตับบกพร่อง เป็นต้น

7.ความรับผิดชอบต่ออาสาสมัครที่เข้าร่วมโครงการ

หากอาสาสมัครที่เข้าร่วมโครงการทดลอง เกิดอาการผิดปกติทั้งทางด้านร่างกายและจิตใจอันเป็นผลสืบเนื่องมาจากการทดลองนี้ ไม่ว่าจากสาเหตุใดก็

ตามผู้ทำการทดลองต้องรับผิดชอบในการรักษาพยาบาลอาสาสมัครจนกว่ายาจะหมดฤทธิ์หรือไม่เกิน 3 เดือนหลังการทดลอง

8.โอกาสในการซักถามและยกเลิกเป็นอาสาสมัครที่เข้าร่วมโครงการ

หากข้าพเจ้ามีข้อสงสัยเกียวกับการศึกษาครั้งนี้ ข้าพเจ้ามีสิทธิในการซักถามได้ทุกขั้นตอนและสามารถยกเลิกการเป็นอาสาสมัครที่เข้าร่วมโครงการได้เมื่อมีเหตุผลอันสมควร

9. หากอาสาสมัครไม่ปฏิบัติตามเงื่อนไข

คณะผู้วิจัยมีสิทธิถอดถอนอาสาสมัครออกจากการร่วมโครงการ

10. คำยินยอมเข้าร่วมโครงการ

ข้าพเจ้าได้อ่านและเข้าใจถึงวัตถุประสงค์ในการศึกษาครั้งนี้เป็นอย่างดีและยินดีให้ความร่วมมืออย่างดีที่สุด

.....	.....
(ลายเซ็นอาสาสมัคร)	วัน เดือน ปี
.....	.....
(ลายเซ็นพยาน)	วัน เดือน ปี
.....	.....
(ลายเซ็นแพทย์)	วัน เดือน ปี
.....	.....
(ลายเซ็นผู้วิจัย)	วัน เดือน ปี

แบบบันทึกประวัติและการตรวจร่างกาย
ของอาสาสมัครไทย

เลขที่.....

วันที่.....

1. ประวัติส่วนตัว

ชื่อ (นาย,นางสาว,นาง).....

อายุ.....ปี.....เดือน, เพศ : ชาย, หญิง

อาชีพ.....ที่อยู่.....

ชื่อผู้บันทึก.....วันที่.....

แพทย์ผู้ตรวจร่างกาย.....

2. ประวัติการเจ็บป่วย

2.1 <u>ประวัติการเจ็บป่วยในปัจจุบัน</u>

(1).....

(2).....

(3).....

2.2 <u>ประวัติการเจ็บป่วยในอดีต</u>

(1) เคยเจ็บป่วยเป็นอะไรมาก่อน? เมื่อไหร่? รักษาที่ไหน? หมอบอกว่าเป็นโรคอะไร?

.....

.....

(2) ประวัติการผ่าตัด

.....

(3) เคยเป็นโรคภูมิแพ้ (เช่น หืด, หวัดแพ้อากาศ, ลมพิษ, ผื่นคัน).....

.....

(4) เคยแพ้ยาบ้างไหม? ระบุชื่อยาและอาการของการแพ้ยาเป็นอย่างไร บ้าง.....

(5) เคยมีอาการตัวเหลืองตาเหลือง .....

.....

3. ประวัติการเจ็บป่วยในครอบครัว

3.1 <u>ประวัติโรคกรรมพันธุ์</u>

( ) (1) โรคภูมิแพ้ (หืด,ลมพิษ, หวัดเรื้อรัง, ไซนัสอักเสบ)

( ) (2) โรคเบาหวาน

( ) (3) โรคลมบ้าหมู

( ) (4) โรคเลือด (ธาลัสซีเมีย, ฮีโมฟีเลีย, ขาดเอนไซม์ G-6-P-D)

3.2 <u>โรคติดเชื้อ</u> เช่น วัณโรค, หัด, ไข้เลือดออก

4. ประวัติและอุปนิสัยส่วนตัว

( ) (1) ดื่มเหล้า วันละ.....เป็ก, แบน, ขวด/วัน

( ) (2) บุหรี่ วันละ.....มวน, ซอง/วัน

( ) (3) อาหารดิบ ระบุถ้ามี .....

( ) (4) ยา ระบุชื่อยาถ้ามี.....

5. การตรวจร่างกาย

Age.....(yr) Sex.....

Height.....(cm) Body weight.....kgs.

GA : .....

Vital sign : BT..... C, ...PR...../min

RR...../min,.....BP.....mm.Hg.

Skin : .....

Heart : .....

.....

Lung : .....

.....

Abdomen : .....

.....

.....

.....

Extremities : .....

.....

Neuroexamination :

Conciousness : ( ) poor

( ) fair

( ) good

Eyes : pupil diameter.....

Movement.....

RTL.....

Other.....

Reflexes : .....

.....

Muscle Power : .....

สรุปการตรวจร่างกาย

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ

6. การตรวจทางห้องปฏิบัติการ

6.1 CBC ผล.....

6.2 FBS ผล.....

6.3 Renal function test (BUN, Creatinine) ผล .....

6.4 Liver function test (SGOT, SGPT, ALP, Direct/indirect bilirubin, Albumin /globulin) ผล .....

7. สรุปผลของการตรวจร่างกายและทางห้องปฏิบัติการ

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ ระบุ (1).....

(2).....

(3).....

(4).....

VITAE

Name Miss Pinyada Chaipol

Birth Date August 24, 1973

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Nursing	Prince of Songkla University	1996