# Effect of Azole Antimycotics (Ketoconazole and Itraconazole) on the Pharmacokinetics and Pharmacodynamics of a Single Oral Dose of Quinine in Healthy Volunteers

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Master of Science Thesis in Pharmacology

Prince of Songkla University

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| ชื่อวิทยานิพนธ์ | ผลของยาต้านเชื้อรากลุ่มอะโซล (คีโตโคนาโซล และไอทราโคนาโซล) ต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาควินินขนาดรับประทานครั้งเดียวในอาสาสมัครสุขภาพปรกติ |
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## บทคัดย่อ

ควินินเป็นยาที่ใช้ในการรักษามาเลเรียที่มีอาการรุนแรงและมีภาวะแทรกซ้อน เช่น มาเลเรียขึ้นสมองรวมทั้งฟัลซิพารัมมาเลเรียที่ดื้อต่อยาคลอโรควิน ช่วงความเข้มข้นของยาในการรักษาแคบและมีความเป็นพิษที่รุนแรงโดยเฉพาะผลต่อหัวใจและหลอดเลือด ควินินถูกแปรรูปที่ตับเป็นส่วนใหญ่โดยเอนไซม์ CYP3A4 ส่วนคีโตโคนาโซลและไอทราโคนาโซลเป็นยาต้านเชื้อรากลุ่มอะโซลที่มีผลต่อเชื้อราหลายชนิดและมีฤทธิ์ยับยั้งเอนไซม์ CYP3A4 ซึ่งจะทำให้ยาที่ให้ร่วมกันถูกกำจัดออกจากร่างกายได้ช้าลงและอาจก่อให้เกิดอาการพิษได้ ดังนั้นในกรณีที่มีการใช้ยาต้านเชื้อรากลุ่มอะโซลร่วมกับควินินจึงอาจทำให้เกิดความเป็นพิษจากควินินได้ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของคีโตโคนาโซลและไอทราโคนาโซลต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของควินิน โดยทำการศึกษาในอาสาสมัครสุขภาพปรกติจำนวน 9 คน โดยให้รับประทานยาควินินขนาด 300 มิลลิกรัมใน 3 กรณี (ก) ควินินอย่างเดียว (ข) และ (ค) คีโตโคนาโซลขนาด 400 มิลลิกรัมหรือไอทราโคนาโซลขนาด 200 มิลลิกรัมวันละครั้งเป็นเวลา 4 วันก่อนรับประทานยาควินินในวันที่ 4 ทำการวัดระดับความเข้มข้นของยาควินินในพลาสมาภายใน 48 ชั่วโมงหลังจากได้รับยาควินินโดย HPLC เปรียบเทียบความแตกต่างของค่าเภสัชจลนศาสตร์และเภสัชพลศาสตร์โดยใช้ Student 's *t*-test พบว่าการให้คีโตโคนาโซลร่วมกับควินินทำให้ค่าอัตราการกำจัดยา (CL/f), ค่าคงที่ของการกำจัดยา (Ke) และค่าปริมาตรการกระจายตัวของยา (Vd/f) ลดลง 2.2 เท่า (0.17 0.08 vs

0.077 ± 0.032 l/hr/kg ; $P < 0.01$), 1.6 เท่า (0.08 ± 0.02 vs 0.05 ± 0.02 $hr^{-1}$ ; $P < 0.01$) และ 1.3 เท่า (1.97 ± 0.61 vs 1.53 ± 0.27 l/kg ; $P < 0.01$) ตามลำดับ ค่าครึ่งชีวิตของการกำจัดยา ($t_{1/2}$), ค่าพื้นที่ใต้กราฟ (AUC), ค่าความเข้มข้นสูงสุด และเวลาที่ความเข้มข้นของยาสูงสุด ($T_{max}$) เพิ่มขึ้น 1.7 เท่า (9 ± 2.13 vs 15.26 ± 4.9 hr ; $P < 0.01$), 2 เท่า (36.06 ± 14 vs 74.76 ± 26.9 mg/l.hr; $P < 0.01$), 1.3 เท่า (2.35 ± 0.52 vs 3.14 ± 0.54 ; $P < 0.01$) และ 1.6 เท่า (1.76 ± 0.59 vs 2.8 ± 0.83 hr ; $P < 0.01$) ตามลำดับเมื่อเปรียบเทียบกับควินินอย่างเดียว ส่วนการให้ไอทราโคนาโซลร่วมกับควินินทำให้ค่า AUC และ $t_{½}$ เพิ่มขึ้น 2 เท่า (36.06 ± 14 vs 70.8 ± 35.76 mg/l.hr ; $P < 0.01$) และ 1.7 เท่า (9 ± 2.13 vs 15.4 ± 7.2 hr ; $P < 0.01$) ตามลำดับ, ค่า CL/f และ Ke ลดลง1.8 เท่า (0.17 ± 0.08 vs 0.096 ± 0.067 l/hr/kg ; $P < 0.01$) และ 1.6 เท่า (0.08 ± 0.02 vs 0.051 ± 0.02 $hr^{-1}$; $P < 0.01$) ตามลำดับ ในการศึกษาครั้งนี้คีโตโคนาโซลและไอทราโคนาโซลอาจจะยับยั้งการทำงานของเอนไซม์ CYP3A4 ซึ่งเป็นเอนไซม์ที่สำคัญในการแปรรูปยาที่ตับ และนอกจากนี้ทั้งควินินและคีโตโคนาโซลต่างก็มีผลในการยับยั้งการทำงานของ P-glycoproteins ซึ่งอาจทำให้คีโตโคนาโซลแข่งขันกับควินินในการจับกับ P-glycoproteins ในขณะที่ควินินกำลังเกิดการแพร่กระจายตัวซ้ำมีผลให้ Vd/f ลดลงอย่างมีนัยสำคัญ สำหรับเภสัชพลศาสตร์ที่ทำการศึกษาในครั้งนี้พบว่าโดยส่วนใหญ่แล้วค่าความดันโลหิต, อัตราการเต้นชีพจรและค่า $QT_c$ ก่อนทำการทดลองและหลังรับประทานยาควินนินในทุกกรณีไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางคลินิก

อย่างไรก็ตามขนาดของควินินที่ใช้ในทางคลินิกมากกว่าขนาดที่ใช้ในการทดลองครั้งนี้ 2 เท่าดังนั้นจึงอาจเป็นไปได้ว่าการใช้ยาควินินร่วมกับคีโตโคนาโซลหรือไอทราโคนาโซลร่วมกันในขนาดรักษาปกติอาจทำให้เกิดการเปลี่ยนแปลงทางเภสัชพลศาสตร์ได้ จึงควรพิจารณาวัดระดับยาควินินเพื่อป้องกันอาการพิษที่อาจจะเกิดขึ้นจากยาควินิน

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## ABSTRACT

Quinine is recommended for treatment of chloroquine-resistant *P. falciparum* malaria. It is an important drug of choice for treatment of complicated/or cerebral malaria. Therapeutic window is narrow and its toxicity is serious, especially cardiotoxicity. The major pathway of metabolism is via CYP3A4 isozyme. Ketoconazole and itraconazole, the azole antifungal agents, have broad spectrum antifungal activity, and are potent inhibitors for CYP 3A4 isozyme. They reduced the clearance of many drugs in humans resulted in drug-drug interactions and toxicities. So, concomitant administration of azole antifungal agents and quinine may lead to quinine toxicity. The objective of this study is to examine the effect of ketoconazole or itraconazole on the pharmacokinetics and pharmacodynamics of quinine in 9 healthy volunteers after receiving quinine 300 mg as an oral single dose in 3 occasions: (a) quinine alone (b) and (c) after pretreatment

with ketoconazole 400 mg or itraconazole 200 mg, respectively given orally once daily for 4 days prior to quinine administration on day 4. The plasma quinine concentrations during 48 hours after dosing were measured using High Performance Liquid Chromatography (HPLC). Statistical analysis using Student's *t*-test indicated that when quinine and ketoconazole were coadministered, the apparent oral clearance (CL/f), the elimination rate constant (Ke) and the apparent volume of distribution (Vd/f) were reduced by 2.2-fold (0.17 ± 0.08 vs 0.077 ± 0.03 l/hr/kg ; $P < 0.01$), 1.6-fold (0.08 ± 0.02 vs 0.05 ± 0.02 $hr^{-1}$ ; $P < 0.01$) and 1.3-fold (1.97 ± 0.61 vs 1.53 ± 0.27 l/kg ; $P < 0.01$), respectively. The elimination half-life ($t_{1/2}$), the area under the concentration time curves (AUC), peak plasma concentration ($C_{max}$) and the time to peak ($T_{max}$) increased by 1.7-fold (9 ± 2.13 vs 15.26 ± 4.9 hr; $P < 0.01$) and 2-fold (36.06 ± 14 vs 74.76 ± 26.9 mg/l.hr; $P < 0.01$), 1.3-fold (2.35 ± 0.52 vs 3.14 ± 0.54 ; $P < 0.01$) and 1.6-fold (1.76 ± 0.59 vs 2.8 ± 0.83 hr; $P < 0.01$), respectively. After pretreatment with itraconazole resulted in an increase of AUC and t½ by 2-fold (36.06 ± 14 vs 70.8 ± 35.76 mg/l.hr; $P < 0.01$) and 1.7-fold (9 ± 2.13 vs 15.4 ± 7.2 hr; $P < 0.01$), respectively. The Ke and CL/f reduced by 1.6-fold (0.08 ± 0.02 vs 0.051 ± 0.02 $hr^{-1}$; $P < 0.01$) and 1.8-fold (0.17 ± 0.08 vs 0.096 ± 0.067 l/hr/kg; $P < 0.01$), respectively. According to the present study, the alteration of quinine pharmacokinetic parameters may result from the inhibition of CYP 3A4 mainly in the liver by ketoconazole and itraconazole. In addition, both ketoconazole and quinine were inhibitors of many P-glycoprotein substrates, therefore, ketoconazole may compete with quinine to bind with P-glycoproteins during the

redistribution phase leading to a significant reduction of Vd/f. For the pharmacodynamic study, most of all blood pressures, pulse rates and $QT_c$ intervals were not clinically significant altered when compared with before study, and with all quinine phases.

However, concomitant use of quinine with ketoconazole or itraconazole in clinical practice may produce serious drug interactions because the usual dose of quinine in malaria treatment is 2-fold higher than the quinine dose used in this study. Thus, monitoring of quinine concentration must be considered in some cases.

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Tippawan Suvarnaraksha

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## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ng | = | nanogram |
| μg | = | microgram |
| mg | = | milligram |
| g | = | gram |
| kg | = | kilogram |
| μl | = | microliter |
| ml | = | milliliter |
| l | = | liter |
| nM | = | nanomolar |
| μM | = | micromolar |
| mM | = | millimolar |
| M | = | molar |
| min | = | minute |
| hr | = | hour |
| $C_{max}$ | = | maximum plasma concentration |
| $T_{max}$ | = | time to reach the maximal plasma concentration |
| Ka | = | absorption rate constant |
| Ke | = | elimination rate constant |
| $t_{½}$ | = | elimination half-life |

## LIST OF ABBREVIATIONS (continued)

| | | |
|---|---|---|
| CL/f | = | apparent oral clearance |
| Vd/f | = | apparent volume of distribution |
| C.V. | = | coefficient of variation |
| S.D. | = | standard deviation |
| r | = | correlation coefficient |
| *P* | = | P value |
| % | = | percent |
| ® | = | trade name |
| eg. | = | exampli gratia |
| etc. | = | et cetera |
| i.e. | = | id est |
| M.W. | = | molecular weight |
| vol/vol/vol/vol | = | volume by volume by volume by volume |
| w/v | = | weight by volume |
| m V.F.S. | = | millivolt full scale |
| iv | = | intravenous |
| °C | = | degree Celcieus |
| vs | = | versus |

# CHAPTER 1

## INTRODUCTION

Malarial infection is caused by unicellular animals, class *Sporozoa* genus *Plasmodium.* More than 120 species of *Plasmodium* have been found but only 4 species are the cause of malarial disease in humans i.e. *Plasmodium falciparum* (*P. falciparum*), *P. vivax, P. ovale* and *P. malariae. P. falciparum* is the most dangerous form of human malaria results in severe or complicated malaria such as malaria with renal failure or cerebral malaria. (Looareesuwan and Chongsuphajaisiddhi, 1994)

Malaria is the important health problem in the world including Thailand. Half of the world populations settle down at the outbreak of malarial infection. The person at risk is estimated to be around 2.4 billion, and estimated death due to the disease is 1.1-2.7 million yearly; mostly (90%) in children under 5 years of age in Africa and South of the Sahara (WHO, 2000). Ten years ago in Thailand it was found that patients with malaria were more than 300,000 cases each year with the mortality rate of 2,000-3,000 cases per year. In 1994, it was found that patients with malaria were about 100,000 cases. However, the data may be less than the actual one because some malarial infections may treat the infection by themselves (Looareesuwan, 1995).

Chloroquine and primaquine are used to treat uncomplicated malarial infections in patients infected with *P. vivax, P. malariae* and *P. ovale*,

whereas patients infected with *P. falciparum* are treated with chloroquine and sulfadoxine-pyrimethamine. Quinidine, quinine, mefloquine and artesunate can be used when *P. falciparum* is resistant to chloroquine and sulfadoxine-pyrimethamine (White, 1988). Quinine is the cinchona alkaloid widely used for blood schizontocide in patients infected with *P. falciparum* and resistant to chloroquine both complicated and severe malaria. (Hall, 1976 ; Krishna and White, 1996). Severity of infection is the criterion for making decision of route of quinine administration. For the chloroquine resistant falciparum malaria, quinine is used in a dose of 10 mg/kg (600 mg) orally 3 times a day for 7 days in combination with Fansidar® (25 mg pyrimethamine and 500 mg sulfadoxine per tablet) or 250 mg tetracycline or doxycycline, whereas in severe cases 10 mg/kg quinine dihydrochloride (highest dose not more than 600 mg) mixed with 300 ml normal saline is intravenously infused in 1-2 hours as loading dose and followed by 10 mg/kg every 8 hours for 7 days, the drug should be changed to oral administration as soon as the patient can take medication orally. However, in areas of quinine resistant falciparum malaria, 20 mg/kg of quinine infusion in 2 hours as loading dose is required (Karbwang and Cross, 1997). Quinine is also prescribed as the treatment of choice for the nocturnal leg cramps (Mackie and Davidson, 1995).

In healthy volunteers after oral administration, quinine was rapidly and completely absorbed, and the time to peak plasma concentration was between 1-4 hours. Quinine was 69-92% bound in human plasma (Wanwimolruk and Denton, 1992), and $\alpha_1$-acid glycoprotein is the important binding protein (Van Henbroek *et al.,* 1996). Volume of distribution was

about 2 l/kg. Quinine was mainly metabolized in the liver by oxidative biotransformation catalyzed by CYP3A4 (Barrow *et al.,* 1980 ; White, 1985). The major metabolite is 3-hydroxyquinine, and the unchanged drug is excreted in urine by 20% of the dose (Krishna and White, 1996). Elimination half-life ($t_{1/2}$) of quinine and the effective concentration in plasma are 10-18 hours and 5-10 mg/l, respectively (Frake *et al.*, 1987). The pharmacokinetics of quinine is altered by many factors such as genetics, disease status and drug coadministration. Toxicity occurred when prolonged treatment and plasma concentrations are equal or more than 10 mg/l (Powell and McNamara, 1972). Side effect of quinine such as cinchonism was found in patients with plasma concentration higher than 5-12 mg/l (Painisko and Keystone, 1990). The symptoms of cinchonism include tinnitus, vertigo, transient. loss of hearing, nausea, vomiting, abdominal pain, dysphoria, headache and blurred vision (Painisko and Keystone, 1990). The QRS interval may be prolonged when total plasma quinine exceeds 10 mg/l (Dollery, 1999). The symptoms of severe toxicities are decrease in blood pressure and irregular heart rate (Boland *et al.,* 1985 ; Dyson *et al.*, 1985). However, electrocardiogram changes can be seen in healthy volunteers with quinine concentrations around 5 mg/l (Karbwang *et al.,* 1993b).

Azole antimycotics (ketoconazole and itraconazole) are broad spectrum of antifungal activity. The important drugs in this group are ketoconazole, itraconazole and fluconazole which have indication for the treatment of *Candida albicans, Candida tropicalis, Candida globata Cyptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum,*

*Coccidiodes immitis* and *dermatophytes* (Bennett, 1996). The side effects depend on dosage such as 400 mg/day result in nausea and vomiting, increase in triglyceride, reduce in potassium, rash and asymtomatic elevation of hepatic enzymes i.e. alanine aminotransferase, aspatate aminotransferase and gamma-glutamyltransferase (Janssen and Symoens, 1983). Azole antimycotics inhibit enzyme cytochrome P450s that metabolize many drugs in humans. Ketoconazole and itraconazole are potent inhibitors of isozyme CYP3A4 (Jalava *et al.*, 1997 ; Olkkola *et al.*, 1994 ; Varhe, Olkkola and Neuvonen., 1994). The results of CYP3A4 inhibition produce an increase in plasma concentration of drug coadministered such as triazolam, terfenadine and felodipine (Varhe *et al.*, 1994 ; Crane and Shih, 1993 ; Jalava *et al.*, 1997), which may be life threatening.

Itraconazole inhibited metabolism of quinidine *in vitro* by inhibition of CYP3A4 and 2C9 (Zhao *et al.*, 1997). Recently, Mirghani *et al.* (1999) showed that CYP3A4 is important for the 3-hydroxylation of quinine in humans. However, the effect of itraconazole on quinine pharmacokinetics in healthy volunteers has not been reported. Since quinine has a narrow therapeutic index, therefore coadministration of some azole antimycotics (ketoconazole or itraconazole) with quinine may cause a significant increase in plasma concentrations of quinine, and leads to side effects. Normally, azole antifungal agents require long term in the treatment of fungal infection. The more increase in number of patients with HIV infection, the more increase in using antifungal agents. Moreover, evidence of malaria is increased not only transmission by infective female mosquito bites but also by coinfection with

malaria and HIV injecting drug users (Bastos *et al.,* 1999). Therefore, quinine is possibly coadministered with ketoconazole or itraconazole in clinical practice. The purposes of this study were to observe and compare the effects of azole antimycotics (ketoconazole and itraconazole) on the pharmacokinetics and pharmacodynamics of a 300 mg single oral dose of quinine in healthy volunteers. The present study may be the guidance and useful data for making decision in case of coadministration of quinine with ketoconazole or itraconazole.

# CHAPTER 2

## LITERATURE REVIEW

### Quinine

Quinine, the chief alkaloid derived from the bark of cinchona tree, has been used in malaria suppression and treatment for more than 350 years. The principal areas producing cinchona are central Africa, India and Indonesia. Linneus gave the term cinchona in 1742. Jesuits probably brought the bark to Europe in 1631 or 1632 and again in 1645. In 1820, the structure of quinine was identified by Pelletier and Carventon (Krishna and White, 1996 ; Tracy and Webster, 1996) and complete synthesis of quinine was achieved in 1945 by Robert B. Woodward (Meesuk, 1978).

Quinine is recommended for the treatment of chloroquine-resistant *P. falciparum* malaria and is an important drug of choice for treatment of complicated and/or cerebral malaria. Besides treatment of malaria, quinine is also prescribed for nocturnal leg cramps in general practice (Tracy and Webster, 1996).

### Chemical and Physical Properties

Chemical structure : $C_{20}H_{24}N_2O_2$

Synonyms : 6′- methoxycinchonam-9-ol

Molecular weight : 324.4

pKa :

Quinuclidinyl group : 4.1

Quinolone group : 8.5

Solubility :

in ethanol : 1 in 1 (w/v)

in water : very low

(Dollery, 1999)

Quinine contains a quinoline group attached through a secondary alcohol linkage to a quinuclidine ring. A methoxy side chain is attacted to the quinoline ring and a vinyl to the quinuclidine (Figure 1) (Tracy and Webster, 1996).

Karle *et al.* (1992) described that the conformation around atoms C-8 and C-9 of the cinchona alkaloids, particularly the direction of the aliphatic N-H and (9) O-H bonds relative to each other, are crucial to antimalarial activity.

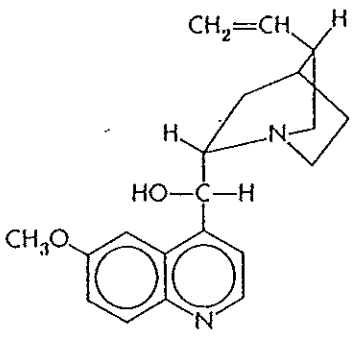


Figure 1 Chemical Structure of Quinine

## 1. Pharmacodynamic Properties

### 1.1 Mechanism of Action

The mode of action of quinine is still unclear. However, quinine is known to depress many enzyme systems. Quinine is highly concentrated and accumulates in the acid food vacuoles of malarial parasites. It inhibits the parasite enzyme hemepolymerase. This enzyme allows the incorporation of heme, which is toxic to the parasite, into insoluble (and apparently inert) crystalline material called hemozoin (Dollery 1999). Another possible of its mechanism is to form a hydrogen bond complex with double stranded DNA that inhibits strand separation, transcription and protein synthesis (Tracy and Webster, 1996 ; Goldsmith, 1996 ; Painisko *et al.,* 1990).

### 1.2 Antimalarial Action

Quinine is a rapidly acting and highly effective blood schizontocide against four malarial parasites. The drug is gametocidal for *P. vivax, P. ovale* and *P. malariae* but not effective against for *P. falciparum* gametocytes. Quinine has no effect on sporozoites or the liver stages of the parasite (Goldsmith, 1996).

### 1.3 Pharmacologic Effects

#### 1.3.1 Central Nevous System

Therapeutic doses of quinine have few effects on the central nervous system (CNS) other than to cause analgesia and antipyresis. The discovery that cinchona lowered the fever of malarial patients quickly led to its use in all forms of febrile illnesses. However, quinine is not a potent or particular effective antipyretic.

### 1.3.2 Cardiovascular System

The action of quinine on cardiovascular system includes $Na^+$ current blockade and multiple cardiac $K^+$ currents blockade. As a consequence of its $K^+$ channel blocking actions, quinine prolongs action potentials in most cardiac cells. This effect is most prominent at slow rates. Quinine also produces α-adrenergic receptor blockade and vagal inhibition. Quinine, given intravenously as a bolus dose, can sometimes cause alarming and even fatal hypotension. However, in therapeutic doses it has little effect on the normal heart or blood pressure in humans (Webster, 1991).

### 1.3.3 Skeletal Muscle

It increases the tension response to a single maximum stimulus delivered to the muscle directly or through the nerve, and increases the refractory period of muscle so that the response to tonic stimulation is diminished. The excitability of the motor endplate region decreases as a result in responses to repetitive nerve stimulation and to acetylcholine was reduced. Thus, quinine can antagonize the actions of physostigmine on skeletal muscle as effectively as does curare. Quinine may cause symptomatic relief of myotonia congenita. This disease is the pharmacological antithesis of myasthenia gravis. Thus, quinine may produce alarming respiratory distress and dysphagia in patients with myasthenia gravis.

Quinine has a slight oxytocic action on the gravis uterus, especially in the third trimester (Goldsmith, 1996 ; Looareesuwan, 1995).

**2. Oral Treatment of Falciparum Malaria Resistant to Chloroquine**

Quinine is currently recommended for the treatment of chloroquine-resistant *P. falciparum* malaria and the drug of choice for the treatment of complicated and/or cerebral malaria (WHO, 1990). In adults with uncomplicated falciparum malaria are quinine sulphate 600 mg every 8 hours combined with tetracycline 250 mg every 6 hours for 7 days (Karbwang and Cross, 1997 ; Looareesuwan, 1995).

**3. Other Use**

Quinine is widely used as a treatment for nocturnal leg cramps. The dose of quinine is 200 to 300 mg before retiring (Mackie and Davidson, 1995; Tracy and Webster, 1996).

**4. Therapeutic Range**

After standard therapeutic dose, peak plasma levels of quinine may reach 15-20 mg/l in severely ill Thai patients without major toxicity (Tracy and Webster, 1996). The blood concentration of quinine required for optimum parasiticidal effect are not certainly known, but most investigators have aimed to achieve a total plasma level over 8-15 mg/l more than 7 days in severe drug-resistant malaria and should not result in toxicity in severe falciparum malaria (White, 1988).

## 5. Adverse Effects

### 5.1 Gastrointestinal Effect

Most quinine salts taken orally have an extremely bitter taste and irritant to the gastric mucosa and often cause nausea, vomiting or epigastric pain (Painsko and Keystone, 1990).

### 5.2 Cinchonism

Cinchonism is the common symptom, which frequently occurs when plasma quinine levels exceed 5 mg/l. These symptoms are tinnitus, vertigo, transient loss of hearing, nausea, vomiting, abdominal pain, dysphoria, headache and blurred vision (Painsko and Keystone, 1990) and it usually develops when plasma levels of quinine exceed 7-10 mg/l; in some patients, however, symptoms may occur at lower plasma level (Goldsmith, 1996).

### 5.3 Hematologic Effects

Quinine induced immune thrombocytopenic purpura followed by hemolytic uremic syndrome, which were directed against the platelet glycoprotien complexes GPI/IX and GPIIb/IIIa, endothelial cells and leukocytes (Glynne *et al.,* 1999). Another report of hemolysis directly attributable to quinine occurs in 0.05% of people treated for acute malaria; it may also occur in glucose-6-phosphate dehydrogenase-deficient patients. However, leukopenia, agranulocytosis, thrombocytopenic purpura, Henock-Schonlein purpura and hypoprothrombinemia are rare.

Quinine has a peripheral vasodilator action and has been associated with marked postural hypotension in patients being treated for acute falciparum malaria. This symptom occurs when infusions are rapid

(Supanaranond *et al.,* 1991 and Kofi- Ekue *et al.,* 1988). It was postulated that this effect is mediated by the inhibition of the action of aldosterone and angiotensin (Hadjokas and Goodfriend, 1991).

**5.4 Hypoglycemia**

Hypoglycemia occurs commonly during intravenous therapeutic doses of quinine (White *et al.,* 1983b). Quinine stimulates insulin release from pancreatic beta cell, both in healthy subjects and patients (White *et al.,* 1983b ; Dyer *et al.,* 1994) and may cause recurrent hyperinsulinaemic hypoglycemia. This important complication is marked with pregnancy and severe disease.

**6. Severe Toxicity**

In the recommended doses, serious adverse effects are infrequent. While in patients on chronic therapy, toxic effects of quinine generally occur with levels of more than 10 mg/l (Powell and McNamara, 1972). Boland *et al.* (1985) suggested that patients with ocular toxic effects are likely to have plasma quinine level 15 mg/l after ingestion and more than 10 mg/l after 10 hours. In cases study of Dyson *et al* (1985) since 1953-1983 of the 46 patients with toxicity was found that visual loss occurred in concentration above 10 mg/l, 8 patients developed visual symptom, blindness in six, appreciable constriction of the peripheral fields in one and abnormal color vision in one. Blindness developed 4.5-14 hours after ingestion. Of 71 further patients reported during 1963-1983, cardiovascular toxicity was unusually mild and was found in 24 patients. Two of the three deaths reported, however, were due to arrthymia and ventricular tachycardia occurred in one patient, who

has the highest plasma concentration (23.5 mg/l). The arrhythmia ended spontaneously eight hours after ingestion. Twenty-four hours after ingestion the patient developed gross pulmonary edema. Electrocardiograms from 17 other patients all showed some abnormality. Arrhythmia were associated with plasma concentration above 16 mg/l. Quinine intoxication following doses of 4 to 12 g is characterized by seizures and coma. Early symptoms are mild visual and hearing complaints. A principal sign is the sudden onset of bilateral pupil dilatation. Lethal doses may be around 8 g (Phillips-Howard and Knile, 1995).

## 7. Pharmacokinetics Properties

### 7.1 In Normal Volunteers

#### 7.1.1 Absorption

Quinine was well absorbed after oral administration in both healthy subjects and patients with uncomplicated malaria, the bioavailability is approximately 76-90 % (Hall *et al.,* 1973 ; White, 1985 ; Paintaud *et al.*, 1993). There is no difference when oral drug is given as capsules or tablets (Hall *et al.*, 1973). Taggast *et al.* (1988) considered the quinine tablet almost completely absorbed, since less than 5% of the drug was recovered in the faces. The absorption occurs mainly from the upper small intestine (Tracy and Webster, 1996). In adult healthy subjects, the time to peak plasma drug concentration ($T_{max}$) was 1-4 hours (Karbwang *et al.,* 1993b ; Paintaud *et al.*, 1993 ; Wanwimolruk *et al.*, 1991 ; Salako and Suwanmi, 1992). The peak plasma concentration ($C_{max}$) for the therapeutic dose of quinine

sulphate, 600 mg administered orally was 4.1-5.6 mg/l (Wanwimolruk *et al.*, 1991 ; Jamaludin *et al.*, 1988) and the rate of absorption in young and elderly subjects were similar (Wanwimolruk *et al.*, 1991).

### 7.1.2 Distribution

The volume of distribution of quinine in normal subjects was 1.8 l/kg (White *et al.*, 1983a ; Dyer *et al.*, 1994). Karbwang *et al.* (1993b) showed that pharmacokinetic properties of intravenous quinine were adequately described by a two-compartment open model with a mean volume of central compartment ($V_c$) 0.3 l/kg (range 0.2 - 0.9) and a total mean apparent volume of distribution was 3.2 l/kg (range 1.8 - 4.6), which is similar to the study of Davis *et al.* (1988). Whereas, oral and intramuscular quinine could be described by a one-compartment open model (Supanaranond *et al.*, 1991 ; Dyer *et al.*, 1994).

The ratio of cerebrospinal fluid to free plasma quinine was 0.55 $\pm$ 0.33, which suggests that quinine does not freely cross the blood brain barrier (Silamut *et al.*, 1985).

### 7.1.3 Plasma Protien Binding of Quinine

In healthy subjects, plasma protein binding expressed as the percentage quinine was 69-92% (Wanwimolruk and Denton, 1992). Quinine is mainly bound to $\alpha_1$-acid glycoprotien with a high affinity, low capacity binding profile in plasma but also to a less extend with albumin (Van Hensbroek *et al.*, 1996 ; Mihaly *et al.*, 1987).

### 7.1.4 Elimination

Quinine undergoes extensive hepatic biotransformation, less

than 20% of an administered dose were excreted unchanged (Tracy and Webster, 1996). Wanwimolruk *et al.* (1995) showed that the metabolites of quinine were at least seven possible metabolites detected in human urine. Three of these were identified as 2′-oxoquininone, quinine glucuronide and 3-hydroxyquinine. The major metabolic pathway of quinine has been shown to be 3-hydroxylation mediated mainly by human CYP3A4 (Mirghani *et al.*, 1999 ; Zhao and Ishizaki., 1997 ; Zhang *et al.*, 1997).

The clearance of quinine varies between 0.072 and 0.24 l/hr/kg in healthy individuals and was reduced to approximately 0.062 l/hr/kg in elderly subjects (Krishna and White, 1996). White (1985) reported that the total systemic clearance of quinine is approximately 0.15 l/hr/kg in adult subjects. The mean terminal elimination half-life in healthy subjects was 10-13 hours (Wanwimolruk *et al.,* 1991 ; Salako and Sowanmi, 1992 ; Karbwang *et al.*, 1993b ; Paintaud *et al.,* 1993).

## 7.2 In Uncomplicated Malaria

### 7.2.1. Absorption

The rate of quinine absorption in acute phase compared to convalescence phase was not significantly different. When given orally, it was well absorbed and oral bioavailability of quinine sulphate probably exceeds 80% (Supanaranond *et al.*, 1991; Sabchareon *et al.*, 1982). The concentration at steady state was achieved in day 2 after receiving standard dose of quinine.

### 7.2.2 Distribution

The volume of distribution in uncomplicated falciparum

malaria was smaller than convalescence (Sabchareon *et al.*, 1982). The contraction in volume of distribution may be related to several factors such as dehydration, obstruction of the capillary bed by parasited red cells and alterations in tissue binding (White *et al.*, 1982).

### 7.2.3 Elimination

White *et al.* (1982) studied in 13 uncomplicated falciparum malarial patients and found that the quinine CL is 1.35 ± 0.6 ml/min/kg, and White (1985), the total clearance of quinine is approximately 0.084 l/hr/kg (1.4 ml/min/kg) in uncomplicated malaria and 0.054 l/hr/kg (0.9 ml/min/kg) in cerebral malaria. Thus the quinine clearance reduces relative to severity of disease because the liver function in malarial infection is decrease.

## 7.3 In Severe Malaria

In severe malaria, the systemic clearance of quinine is reduced, presumably on the basis of reduced hepatic blood flow and a subsequent decrease in the metabolism of quinine (Tracy and Webster, 1996 ; Looareesuwan, 1995 ; White *et al.*, 1982).

### 7.3.1 Absorption

In cerebral malaria, patients who received a loading dose (20 mg/kg quinine hydrochloride, infused over 4 hours) has plasma concentrations exceeding 10 mg/l and time to reach a peak is 48 ± 22 hours after treatment began (White *et al.,* 1983a).

### 7.3.2 Plasma Protein Binding

The mean percentage of unbound quinine was significantly

lower in patients with cerebral malaria (7.2%) than uncomplicated malaria and healthy persons (Silamut *et al.,* 1985). Plasma $\alpha_1$-acid glycoprotein concentrations were consistently raised in acute malaria and cerebral malaria may be prevent quinine toxicity in the presence of high quinine plasma concentration (Wanwimolruk and Denton, 1992 ; Mansor *et al.*, 1991 ; Silamut *et al.*, 1991).

### 7.3.3 Distribution

Quinine mean total apparent volumes of distribution (Vd) in 25 cerebral malarial patients were 1.18 l/kg; this was significantly lower than uncomplicated malaria (1.67 l/kg) (White *et al.*, 1982).

### 7.3.4. Elimination

In cerebral malaria, a reduction in systemic clearance 0.054 l/kg and prolonged half-life (18 hr) resulted from impaired hepatic metabolism. Malarial infection reduced the concentration of cytochrome P450 in liver microsomes (Dollery, 1999 ; White *et al.*, 1982).

## 7.4 In Children with Malaria

There are significant pharmacokinetic differences between adults and children. In children, volume of distributions was smaller and elimination half-life was shorter than adult (Sabchareon *et al.*, 1982).

### 7.4.1 Absorption

Quinine was rapidly and completely absorbed either by intramuscular or nasogastric administration (Van Hensbroek *et al.,* 1996 ; Shan *et al.*, 1985). Sabchareon *et al.* (1982) found that the highest mean serum quinine

concentration (22.5 nM/ml) in acute malaria is higher than in convalescent children (10.17 nM/ml) after oral administration (10 mg/kg). The time to reach peak plasma concentration after oral administration is 2 to 4 hours, whereas after intravascular infusion there were no significant differences.

The peak plasma quinine concentration after rapid intravenous dosing (4.0 mg of the salt /kg body weight) in 4 min was 12.3 mg/l, which was 43% higher than in adults given the same regimen (Winstanley *et al.*, 1993).

### 7.4.2 Distribution

The mean volume of distribution in children with falciparum malaria after intravenous infusion, intramuscular and nasogastric administration of quinine dihydrochloride was 1.51, 1.29 and 1.33 l/kg, respectively. There was no significant difference in the volume of distribution of quinine among children with cerebral and uncomplicated malaria (Shann *et al.*, 1985). Van Henbroek *et al.* (1996) studied in children under 2 years and found that the mean volume of distribution after intravenous and intramuscular administration was 1.04 l/kg.

### 7.4.3 Elimination

The elimination half-life in falciparum malaria by intravenous infusion at the dose of 10 mg/kg range from 9 to 12 hours which were longer than in the convalescence (3.2 to 7.55 hours) and it neither depends on the route nor the duration of infusion of the drug although total clearance values are similar to adult (Sabchareon *et al.*, 1982).

## 7.5 In Pregnancy

Pregnant women with falciparum malaria in the third trimester

showed significant pharmacokinetic differences when compared with other adults in the acute phase of malarial infection. The values of volume of distribution were smaller (0.96 ± 0.27 l/kg), elimination half-lives were shorter (11.3 ± 4.3 hr) and total clearance was reduced (1.22 ± 0.77 ml/min/kg). 8 women delivered of live infant while taking quinine had placental cord plasma quinine concentrations from 1 to 4.6 mg/l, which correlated significantly with maternal plasma quinine concentrations. Heart blood from fetus aborted at term has a plasma quinine concentrations of 2.8 mg/l. Breast milk quinine concentrations and milk to plasma ratio were 0.5 to 3.6 mg/l and 0.11-0.53, respectively in 25 women who were breast-feeding and had taken oral quinine sulphate for 1-10 day (Phillips *et al.*, 1986).

### 7.6 In Renal Failure

The urinary quinine clearance comprises only 20% of total clearance in healthy subjects. Donadio *et al.* (1968) reported that the plasma concentration of 6 patients with falciparum malaria in acute phase of renal failure was higher and they concluded that the dose of quinine in renal failure should be reduced by one-half to two-thirds to avoid potential toxicity.

## 8. Drug Interaction

### 8.1 Antimalarial and Non-antimalarial Drugs (*in vitro*)

Of the twenty-three tested compounds incubated with liver microsomes and quinine, 13 exhibited an inhibitory effect on quinine 3-hydroxylation. All of these drugs inhibited the liver microsomal

metabolism of quinine in a concentration dependent manner, but the magnitude of the inhibition differed among them. The inhibitory rank order were as follows: ketoconazole > doxycycline > omeprazole > primaquine > tetracycline = troleandomycin > nifedipine > erythromycin > verapamil > cimetidine > diltiazem > oleandomycin > hydralazine. The antimalarial drugs, doxycycline, primaquine and tetracycline, inhibited quinine 3-hydroxylation with mean $IC_{50}$ values of 17, 20 and 29 μM, respectively. Non-antimalarial drugs, calcium-antagonists (verapamil, nifedipine and diltiazem), macrolide antibiotics (troleandomycin, erythromycin, and oleandomycin), ketoconazole, omeprazole and cimetidine are inhibitors/substrates of CYP3A4. Ketoconazole was a potent inhibitor of quinine metabolism with mean $IC_{50}$ values of 0.026 μM. A significant interaction may occur when these drugs are administered concomitantly with quinine *in vivo* (Zhao and Ishizaki, 1997).

**8.2 Etoposide (*in vitro*)**

Etoposide, an anticancer agent with a broad range of antitumor activity, is claimed to be metabolized largely *via* CYP3A4 by 3′-demethylation in human liver microsomes and its metabolite has the same antitumor activity. Quinine and etoposide were metabolized mainly by the same human CYP isoform. Etoposide showed the inhibitory of microsomal metabolism of quinine 3-hydroxylation in a concentration–dependent manner with a mean $IC_{50}$ values of 65 μM and the mean maximum inhibition produced by etoposide (100 μM) on the 3-hydroxylation of quinine was about 60% compared with the control. Etoposide 3′-demethylation was also inhibited by quinine in a concentration-related manner with a mean $IC_{50}$ values of

90 μM. The mean maximum inhibition produced by quinine (100 μM) on 3′-demethylation of etoposide was about 52% compared with control. An excellent correlation ($r = 0.947$, $p < 0.01$) between quinine 3-hydroxylase and etoposide 3'-demethylase activities in 6 different human liver microsome was observed. Two inhibitors of CYP3A4, ketoconazole (1 μM) and troleandomycin (100 μM), inhibited quinine 3-hydroxylation by about 90% and 80%, and etoposide 3'-demethylation by about 75% and 65%, respectively. Conclude that quinine and etoposide mutually inhibit the metabolism of each other (Zhao *et al.*, 1997)

**8.3 Cimetidine**

After cimetidine (1 g/day) pretreatment for 7 days there was a significant reduction in the apparent oral clearance of quinine from 0.182 ± 0.063 to 0.133 ± 0.055 l/hr/kg. This was reflected in a 49% (range 17 to 90%) increased in the mean elimination half-life from 7.6 ± 1.3 to 11.3 ± 3.7 hours. A reduction in clearance of quinine during the treatment of cimetidine found in this study would be due to inhibition of the hepatic mixed-function oxidase system by cimetidine (Wanwimolruk *et al.*, 1986).

**8.4 Cigarette Smoking**

The mean $AUC_{0-\infty}$ in smokers was significantly less than non-smokers. Cigarette smoking increased quinine clearance by 77%, The mean unbound clearance (CLu/f) of quinine were significantly greater, mean half-life was significantly shorter than non-smokers. The mean 48 hours recovery (% of dose) of unchanged quinine in the urine was lower in the smokers.

Quinine is extensively metabolized by hepatic oxidative biotransformation and is considered a low clearance drug with a narrow therapeutic window and it is well known that cigarette smoking predominately induced the P450 enzyme family. Cigarette smoking enhanced hepatic metabolism of quinine, total quinine clearance was increased but renal clearance not altered (Wanwimolruk *el al.*, 1993).

Table 1 has shown the pharmacokinetic parameters in some published studies in healthy volunteers after oral administration of quinine.

**Table 1** Some published pharmacokinetics data of quinine given orally in male healthy subjects

| No. of subject | Mean age ± S.D. | Dose (mg) | $C_{max}$ (μg/ml) | $T_{max}$ (hr) | $t_{1/2}$ (hr) | Vd (l/kg) | CL/f (l.hr/kg) | AUC (mg/l.hr) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 63.1 ± 6.5 | 600 | 3.7 ± 0.8 | - | 19.9 ± 6.3 | 1.7 ± 0.56 | 0.06 ± 0.02 | - | Dyer, 1994 |
| 9 | 30.3 ± 3.4 | 600 | 4.6 ± 1.0 | 2.5 | 11.1 ± 3 | - | 0.14 ± 0.05 | 66 ± 20 | Wanwimolruk, 1995 |
| 6 | 37-50 | 600 | 3.45 | 1.6 | 9.7 | 2.78 | 0.17 | - | Auprayoon, 1995 |
| 7 | 21-29 | 600 | 2.7 ± 0.5 | 2.8 ± 1.4 | 11.4 ± 2.7 | 2.5 ± 1.4 | 0.17 ± 0.02 | 30 ± 3.5 | Suwanmi and Salako, 1996 |
| 8 | 28.5 ± 3.1 | 600 | 5.68 ± 2.48 | 1.35 ± 0.53 | 10.07 ± 1.20 | 1.91 ± 0.6 | 0.13 ± 0.05 | 90.23 ± 43.39 | Ridtitid *et al.*, 1998 |
| 9 | 16-37 | 300 | 2.35 ± 0.52 | 1.76 ± 0.59 | 9 ± 2.13 | 1.97 ± 0.61 | 0.17 ± 0.09 | 36.06 ± 14 | Present Study |

## Azole Antimycotics

Azole antifungal agents are the synthetic compounds with one or more five-membered ring, where each ring contains either 2 (imidazoles) or 3 (triazoles) nitrogen atoms. The azoles antifugal are less toxic than amphotericin B and effective in many different fungi (Lyman and Walsh, 1992). Imidazoles and triazoles share the same antifungal spectrum and mechanism of action. The systemic triazoles are more slowly metabolized and have less effect on human sterol synthesis than do the imidazoles. Of the drugs now on the market in the United States, clotrimazole, miconazole, ketoconazole, econazole, butoconazole, oxiconazole, and sulconazole are imidazoles; terconazole, itraconazole, and fluconazole are triazoles (Bennett, 1996).

## Mechanism of Action

The azole antifungal agents are inhibitors of the biosynthesis of ergosterol, a major component of the cell membrane of yeast and fungal cells. It replaces the precursor lanosterol, which is a substrate of the fungal cytochrome P450 enzyme, lanosterol 14-α-demethylase, which catalyses the conversion of lanosterol to ergosterol. Imidazoles and triazoles thus impair the biosynthesis of ergosterol for cytoplasmic membrane and lead to the accumulation of 14-α-methylsterol. On the molecular level, one of the nitrogen atoms of the azole ring is thought to bind to the heam moiety of the fungal cytochrome P450 enzyme lanosterol 14-α-methylase, thereby interrupting the conversion of lanosterol to ergosterol. These methylsterols

may disrupt the close packing of acyl chains of phospholipids, altering the permeability of fungal cell walls, impairing the functions of certain membrane-bound enzyme systems and inhibiting growth (Lyman and Walsh, 1992 ; Bennett, 1996 ; Dollery, 1999).

**Ketoconazole**

Ketoconazole is a synthetic imidazole derivative (Figure 2), the first orally absorbable antifungal azole, was introduced in 1970 (Lyman and Walsh, 1992). It offered a number of significant advantages, including its broad spectrum of antifungal activity and wide tissue distribution. But strong inhibitory effect on cyclosporin oxidase and testosterone 6β-hydroxylase activity in human (Baldwin *et al.,* 1995).

**Chemical and Physical Properties**

Chemical structure : $C_{26}H_{28}Cl_2N_4O_4$ (Figure 2)

Synonyms : *cis*-l-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)
-2-(1*H*-imidazol-l-ylmethyl)-1,3-dioxolam-4-yl]
methoxy]phenyl]piperazine

Molecular weight : 531.4

pKa : 6.51, 2.94

Solubility :

in alcohol : 1 in 54 (w/v)

in water : almost insoluble

Octanol/water partition coefficient : 5400 (pH 11.8)

(Dollery, 1999)

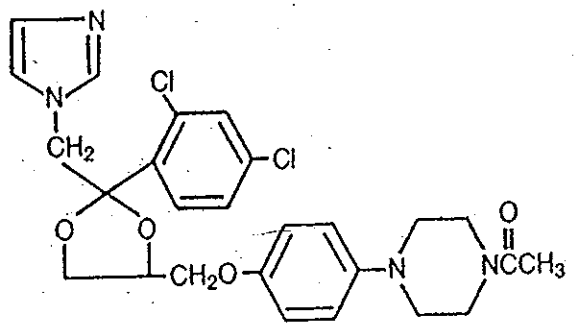

**Figure 2** Chemical Structure of Ketoconazole

## 1 Pharmacokinetics

### 1.1 Absorption

Ketoconazole is a lipophilic with poor water solubility except at low pH (pH < 3). Ketoconazole was well absorbed after oral administration although there is large inter-and intraindividual variation in peak serum concentration after the same dose and absorption increased in the presence of a meal. Antacid or $H_2$ antagonists reduced absorption of ketoconazole, thus should take these drug at least 2 hours before ketoconazole (Van Der Meer *et al.*, 1980).

Peak serum concentrations of ketoconazole occur within 3 hours of administration and are proportional to dose (Daneshmend *et al.*, 1981).

### 1.2 Distribution

The drug is rapidly and widely distributed throughout the body in animal and human. However, the volume of distribution was only 0.36 l/kg (Van Tyle, 1984). Ketoconazole is extensively bound in human whole blood (99%), with 84% to plasma proteins, largely albumin and 15% to erythrocytes (Heel *et al.*, 1982). Penetration into saliva is high and detectable; penetration into CSF occurs only in the presence of inflamed meninges (Brass *et al.*, 1982). And concentrations attainable are inadequate for treatment of fungal meningitis (Graybill *et al.*, 1980). In dogs, the drug has been detected in breast milk at 22% of peak plasma values and it is therefore contraindicated in nursing mothers.

### 1.3 Elimination

Ketoconazole is extensively metabolized in the liver, the major route of elimination being as metabolites in bile. In three human volunteers given $^{3}$H-ketoconazole 2.5 mg/kg about 70% of the administered dose was excreted within 4 days (57% in faeces and 13% in urine). Of the faecal radioactivity 20-65% was due to unchanged drug and 2-4% of urinary radioactivity (Gascoigne *et al.*, 1981). There may be enterohepatic circulation because the double peaks plasma concentrations, seen at higher doses of ketoconazole (Brass *et al.*, 1982). It has been suggested that the elimination of ketoconazole was impaired in liver disease.

The major metabolic reactions in humans are oxidation of the imidazole ring followed by degradation of the oxidized imidazole, oxidative

degradation of piperazine ring and aromatic hydroxylation (Danesmend and Warnock, 1988). Ketoconazole itself appears to be oxidized by CYP3A.

The elimination half-life appeared to be dose dependent, increasing with increasing dose and after repeated dosing (Daneshmend *et al.*, 1983). With an oral dose of 200 mg the range of mean ketoconazole half-life 1.51 to 4 hours. At higher dose (400 and 800 mg) the mean half-life were 3.7 hours (range from 1.3 to 11.6 hours) (Maksymink *et al.*, 1982).

**2 Therapeutic use**

2.1 Systemic mycoses: paracoccidioidomycosis, coccidioidomycosis, candidiosis and histoplasmosis.

2.2 Severe chronic mucocutanous candidosis

2.3 Disabling candidal chronic paronychia

2.4 Severe mycoses of the gastrointestinal tract not responsive or resistant to other therapy

2.5 Chronic vaginal candidosis not responsive to other therapy

2.6 Prophylaxis in immunosupressed patients

2.7 Culturally determined dermatophyte infections unresponsive to other therapy

2.8 Treatment of dermatophyte (ringworm) infections, cutaneous candidosis, pityriasis versicolor, seborrheric dermatitis and pityriasis capitis (dandruff) caused by *Pityrosporum spp.*

**Mode of Use**

The treatment of systemic mycosis and dermatophyte infections is normally 200 mg ketoconazole daily for 14 days, or longer if clinical response is poor. The dose may be increased to 400 mg daily if response to 200 mg daily is poor. However, it may be wise to measure plasma levels before increasing the dose (maximum 8 mg/kg daily) (Dollery, 1999).

**3 Adverse Effect**

The most frequent dose-limiting side effects of ketoconazole therapy are nausea and vomiting (Dismukes *et al.*, 1983). They occur in approximately 10% of patients receiving 400 mg/day but increase to more than 50% in pateints receiving more than 800 mg/day (Sugar *et al.*, 1987). Pont *et al.* (1984) found that when given ketoconazole in conventional dose (400 mg/day), transiently blocks of testosterone synthesis and adrenal response to corticotrophin were noted. Higher therapeutic doses (800 to 1200 mg/day), even once daily, caused more prolonged blockade. Oligospermia and azospermia occurred after prolonged therapy. Impotence and decreased libido were found. Gynecomastia appeared in high dose more commonly than with lower dose. Depressed response to corticotrophin was pronounced. Urine cortisol excretion was depressed. The blockade appear related to the serum ketoconazole concentration. Trachtenberg (1984) observed in 13 patients with symptomatic stage $D_2$ prostatic cancer administered 400 mg ketoconazole orally every 8 hours and found that by 24 hours of treatment serum testosterone decreased to the castrate level and the adrenal androgens,

androstenedione and dehydroepiandrosterone, also decreased significantly. After one week of treatment clinical response was evident in all patients. Pain was improved and serum prostatic acid phosphatase reached the normal range. The patients have been followed for 3 to 10 months without relapse. Approximately 10% of females report menstrual irregularities (Bennet, 1996) and 1 in 10,000 patients receiving ketoconazole associated with hepatotoxicity and 2 to 8% had some abnormal elevation of serum transminase and usually reversible if treatment is stopped. The risk of hepatotoxicity increases with longer duration of treatment; courses of greater than 14 days should only be given after consideration of risks (Lewis *et al.*, 1984).

## 4. Drugs Interaction

### 4.1 Oral Anticoagulants

A patient had been treated with warfarin for three years for a pulmonary embolism, and later received ketoconazole 200 mg twice daily for chronic vaginal thrush infection. After three weeks of treatment with ketoconazole she complained of subcutaneous bruising and reported to the clinic, whereas platelet count and liver function tests gave normal results. Treatment of ketoconazole was stopped, warfarin dosage reduced. Over the next three weeks her wafarin control was restabilised at previous level (Smith, 1984).

### 4.2 Chlordiazepoxide (Benzodiazepine)

Chlordiazepoxide is extensively oxidized in the liver with little urinary excretion of the parent drug. Ketoconazole impaired chlordiazepoxide

clearance from plasma. After a single dose of ketoconazole, there was a 20% decrease in clearance and 26% decrease in volume of distribution without evidence of inhibition of drug metabolism. These changes apparently were not related to ketoconazole dose. After repetitive dosing with ketoconazole, chlordiazepoxide clearance decreased by 38% and was associated with reduced concentrations of its first oxidative metabolite, N-desmethylchlordiazepoxide. It was concluded that ketoconazole inhibits at least one subset of the hepatic mixed-function oxidase system, but not generally (Brown *et al.*, 1985).

**4.3 Tirilazad**

Tirilazad mesylate is a membrane lipid peroxidation inhibitor that shows efficacy in reducing the damaging effects of lipid peroxidation on the cell membrane triggered by brief periods of ischemia. Tirilazad is highly metabolized after intravenous administration in healthy volunteers. It was postulated that the limited bioavailability was due to extensive first-pass metabolism in the liver. The major pathways of tirilazad metabolism in man are mediated by the CYP3A. Pretreatment with ketoconazole for 7 days results in increased mean tirilazad mesylate AUC by 67% and 309% for intravenous and oral administration, respectively. Mean AUC for active reduced metabolite of tirilazad (U-89678) were increased 472% and 720% by ketoconazole administration with iv and oral tirilazad, respectively, whereas increases of more than 10-fold in mean U-87999 (another active metabolites) AUC. Ketoconazole increased the bioavailability 20.9% by decreasing the first-pass liver and gut wall metabolism of tirilazad mesylate in similar

degrees. These results indicate that ketoconazole inhibits the metabolism of three compounds (tirilazad, U-89678 and U-87999), which suggests that all of the compounds are substrates for CYP3A (Fleishaker *et al.*, 1996).

### 4.4 Nisoldipine

Nisoldipine is a calcium antagonist of the 1,4-dihydropyridine class. It reduces vascular resistance and blood pressure by inhibiting calcium uptake of myocardial and smooth muscle cells. Nisoldipine is extensively metabolized by the cytochrome P450 system, with isoenzyme CYP3A4 catalyzing the dehydrogenation of the dihydropyridine ring. Pretreatment with and concomitant administration of ketoconazole resulted in a 24-fold and 11-fold increase in mean AUC and $C_{max}$ of nisoldipine, respectively compare with nisoldipine alone. The interaction is likely to be caused by inhibition of first-pass metabolism, although an effect on systemic clearance cannot be roule out, because the terminal elimination phase was not assessable after treatment with nisoldipine alone. The parallel increases in plasma concentration of the metabolite 2-hydroxyisobutyl (M9) and parent drug indicate that side-chain hydroxylation of nisoldipine in contrast to the oxidation of the dihydropyridine ring is not mediated by CYP3A4 (Heinig *et al.*, 1999).

### 4.5 Triazolam

Triazolam is a short-acting hypnotic having an average $t_{1/2}$ of 2 to 4 hours. After oral administration, triazolam is metabolized during its absorption (first-pass) and elimination phase by CYP3A4. Triazolam commonly causes amnesia. Nine healthy volunteers received 400 mg ketoconazole, 200 mg itraconazole, or matched placebo orally once a day for

4 days. On day 4, each ingested a single 0.25 mg dose of triazolam. Ketoconazole and itraconazole increased AUC of triazolam by 22-fold and 27 fold, $C_{max}$ by 3-fold, and $t_{1/2}$ by 6-fold and 7-fold, respectively. All pharmacodynamic effects revealed a significant difference between the antimycotics and placebo phases. Ketoconazole and itraconazole seriously affects the pharmacokinetics of triazolam and increase the intensity and duration of its effects by inhibition of CYP3A4 during the absorption and elimination phases of triazolam (Varhe *et al.*, 1994).

**4.6 Midazolam**

Midazolam is a benzodiazepine that is used clinically for conscious sedation. It is specifically metabolized by CYP3A to one predominant metabolite (1′-hydroxymidazolam); it has a short half-life. After ketconazole therapy, AUC of midazolam increased 5-fold after intravenous midazolam administration and 16-fold after oral midazolam administration. Intrinsic clearance decreased by 84%. Total bioavailability increased from 25% to 80%. The intestinal component of midazolam bioavailability increased to a greater extent than hepatic component. In the control phase, female subjects had greater midazolam clearance values than the male subjects. Ketoconazole caused marked inhibition of CYP3A activity that was greater in the intestine than liver for midazolam biotransformation (Tsunoda *et al.*, 1999). Olkkola *et al.* (1994) indicated that ketoconazole and itraconazole also increased the AUC by 10 to 15-fold and mean peak concentration 3- to 4-fold compared with placebo phase. In psychomotor tests, the interaction was statistically significant until at least 6 hours after drug administration. Inhibition of the

CYP3A by ketoconazole and itraconazole may explain the observed pharmacokinetic interaction (Olkkola, Backman and Neuvonen, 1994).

### 4.7 Quinine

Mirghani *et al.* (1999) showed the effect of ketoconazole on quinine pharmacokinetics, it (which inhibits CYP3A4) significantly decreased the mean apparent oral clearance of quinine by 31%, whereas coadministration with fluvoxamine (which inhibits CYP1A2 and to some extent CYP2C19) had no significant effect on the mean apparent oral clearance of quinine. Coadministration of ketoconazole also decreased the mean AUC of 3-hydroxyquinine, whereas coadministration with fluvoxamine increased 3-hydroxyquinine AUC significantly.

CYP3A4 is important for the 3-hydroxylation of quinine *in vivo*. On the other hand, CYP1A2 had no significant effect on this metabolic pathway.

### 4.8 Reboxetine

Reboxetine is a specific norepinephrine reuptake inhibitor that is licensed in several European contries for treatment of depression. It is metabolized by CYP3A4. Eleven healthy volunteers received 4 mg reboxetine orally on the 2$^{nd}$day of a 5 days regimen of 200 mg ketoconazole once daily in a crossover design. Ketoconazole increased R, R(-)-reboxetine and S, S (+)-reboxetine (more active reboxetine enantiomers) mean AUC by 58% and 43%, respectively ($P < 0.02$). Oral clearance of both enantiomers were consequently decreased 34% and 24%, respectively by ketoconazole ($P < 0.05$). Mean terminal half-life after administration of ketoconazole (21.5 and 18.9 hours) were significantly longer than after reboxetine alone (14.8 and

14.4 hours; $P < 0.005$). The AUC ratio for R, R (-)-reboxetine to S, S (+)-reboxetine was reduced by ketoconazole administration (12.76 after ketoconazole versus 2.39; $P < 0.003$).

Ketoconazole decreased clearance of both reboxetine enantiomers. Although the adverse effect profile for reboxetine was not altered by ketoconazole, the results of this study suggest that caution should be taken and that a reduction in reboxetine dose should be considered when the two drugs are coadministered (Herman *et al.*, 1999).

**4.9 Amprenavir**

Amprenavir is a new human immunodeficiency virus (HIV)-1 protease inhibitor. In human microsomes, CYP3A4 is primarily responsible for amprinavir metabolism. Subjects received amprenavir 1200 mg, ketoconazole 400 mg and amprenavir 1200 mg plus ketoconazole 400 mg. Each treatment was separated by 14 days result in increased amprenavir $AUC_{0-\infty}$ by 31% and reduced its $C_{max}$ by 16%. Peak serum concentrations of amprenavir are decreased when given with ketoconazole, and the absorption profile is shifted to the right. Whether this reflects as combined effect on gastrointestinal CYP3A4 and P-glycoprotein inhibition of one excretory rout in preference to the other remains to be determined. Amprenavir increased the $AUC_{0-\infty}$ of ketoconazole by 44% and increased $t_{1/2}$ and $C_{max}$ by 23% and 19%, respectively. Both agents resulted in substantial inhibition of erythromycin breath test (ERMBT), a specific maker for hepatic CYP3A activity. However the ERMBT did not correlate with clearance of amprenavir, has did the reduction in ERMBT by ketoconazole predict the magnitude of its effect on

amprenavir. Suggest that gastrointestinal P-glycoprotein and CYP3A4 contribute substantially to overall clearance of amprenavir. Coadministration of ketoconazole and amprenavir results in a significant increase in AUC for both agents, but the changes are not likely to be clinically important (Polk *et al.*, 1999).

**Itraconazole**

Itraconazole was first synthesized in 1980. It is a water insoluble, lipophilic triazole analogue. When given orally, it was 5 to 100 times more active than ketoconazole (Heeres, Backx and Van Cutsem, 1984). In addition, unlike ketoconazole, it was active in aspergillosis, meningeal cryptococcosis and sporotrichosis and more desirable pharmacokinetic profile and less toxicity (Warnock, 1989).

**1. Chemical and Physical Properties**

Chemical structure : $C_{35}H_{38}Cl_2N_8O_4$ (Figure 3)

Synonyms : 4-[4-[4-[4-[[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4 -triazol-l-ylmethyl)-1,3-dioxolam-4-yl]methoxy] phenyl]- 1- piperazinyl] phenyl]-2,4- dihydro-2- (1-methylpropyl)-3*H*-1,2,4 triazol-3-one

Molecular weight : 705.6

pKa : 3-4

Solubility : in dimethyl sulfoxide: >1 in 100 (w/v)
in alcohol: 1 in 1,000 (w/v)
in water: <1 in 1,000,000 (w/v)

Octanol/water partition coefficient : 46,000 (pH8.1)

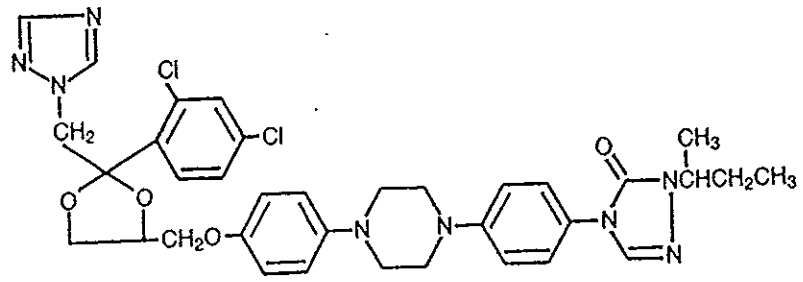

**Figure 3** Chemical Structure of Itraconazole

## 2. Pharmacokinetics Properties

The pharmacokinetic of itraconazole in humans are characterized by a good oral absorption, extensive tissue distribution, with tissue concentrations many times higher than those in plasma, an elimination half-life of about 1 day, and transformation in to a number of metabolites

### 2.1 Absorption

Following oral administration, itraconazole is rapidly and extensively absorbed with absolute systemic bioavialability of 55%. Itraconazole is only ionized at low pH, such as in the gastric milieu. The plasma concentration time curves of itraconazole in healthy volunteers were a wide interindividual and steady-state pharmacokinetics of 200 mg itraconazole once daily and twice daily in 6 healthy volunteers. The $T_{max}$ and AUC observed for the once daily dosage was 1.1 mg/l and 15.4 mg/l.hr, respectively and for the twice a day dosage 2.0 mg/l and 39.3 mg/l.hr. Thus, dosage increase between 100,

200 and 400 mg daily produced non-linear increases in the AUC, suggesting that the possibility of saturable metabolic processes (Hardin *et al.*, 1988). Mean peak concentrations of 0.02 mg/l are attained when a 100 mg dose is administered during fasting, while peak concentrations of 0.18 mg/l are attained when the drug is administered after feeding (Wishart, 1987).

**2.2 Distribution**

As with ketoconazole, itraconazole is highly protein bound (> 99%), with only 0.2% available as free drug (Heykants *et al.*, 1989). Tissue concentrations are 2 to 5 times higher than those in plasma. Itraconazole penetrates into the cerebrospinal fluid, urine and peritoneum at low concentration, probably reflecting the low concentrations found free in plasma. Penetration into skin and appendages occur at a low rate but accumulates in skin blister fluid and the horny layer for more than 10 days and the distal nail plates for 7 or more months, being delivered via both the nail bed and the matrix. The volume of distribution (10.7 l/kg) of itraconazole is large (Schafer-Korting, 1993).

**2.3 Elimination**

The drug has a prolonged clearance time, with a $t_{1/2}$ of 15 to 20 hours following a single dose, and 30 to 35 hours following multiple dosing (Hardin *et al.*, 1988).

The main site of metabolism is the liver and involves oxidative degradation of dioxoland, piperazine and triazole ring and oxidative or N-dealkylation of the 1-methylpropyl substituent. More than 30 metabolites were formed. One important metabolite, hydroxyitraconazole, has similar

antifungal activity *in vitro* to the parent drug (Mikami *et al.*, 1994). It has a half-life of about 12 hours and the peak plasma concentration and area under concentration time curve for this metabolite are approximately double to those of the parent drug. It is likely that hydroxyitraconazole is formed largely by presystemic metabolism during absorption (Heykants *et al.*, 1989).

The drug is extensively metabolized in the liver and excreted in the urine accounted for 35% of the dose given to tree volunteers and fecal excretion accounted for further 54%. Less than 1% of the active metabolites are excreted in the urine. Metabolism of the drug is not altered by renal dysfunction, hemodialysis or continuous peritoneal dialysis (Boelaert *et al.*, 1988).

Itraconazole appears to be a good substrate of CYP3A in humans and inducers of this isoenzyme such as phenobarbital and rifampin increased the drug metabolism.

**3. Mode of Use**

The recommended itraconazole dosage for superficial fungal infections is 100 mg once daily at mealtime for: 15 days in patients with tinea corporis/ crusris; 30 days, tinea pedis/manuum; 4-8 weeks, tinea capitis and a minimum of 3 to 6 months, onychomycoses. In pityriasis versicolor, vaginal candidiasis and fungal keratitis the recommended dosage is 200 mg once daily for 5 days, 3 days and 3 weeks, respectively. The initial dose in systemic mycoses is 200 mg daily increased to 400 mg daily in 1 or 2 divided doses when oral absorption is questionable and/or response is inadequate. In children the

recommended dose is 3 to 5 mg/kg/day. Itraconazole is contraindicated in pregnancy (Grant and Clissold, 1989).

**4. Adverse Effect**

Itraconazole is well tolerated at 200 mg daily. Most of the adverse reaction reported are transient, and include gastrointestinal disturbances, dizziness, headache, depressed libido (with normal testosterone levels and leukopenia (Graybill *et al.*, 1990). The drug has a low incidence of hepatotoxicity, with less than 3% of patients experiencing transient elevations in liver function tests (Cauwenbergh *et al.*, 1987). It has no effect on testicular or adrenal steroidogenesis (Van Cauteren *et al.*, 1987). In a series of 189 patients receiving 50 to 400 mg/day, nausea and vomiting were recorded in 10%, hypertriglyceridemia in 9%, hypokalemia in 6%, increased serum aminotransferase in 5%, rash in 2%, and at least one side effects in 39% (Tucker *et al.*, 1990). Profound hypokalemia has been seen in patients receiving 600 mg or more daily (Sharkey *et al.*, 1991). Doses of 300 mg twice daily have led to other side effects, including adrenal insufficiency, lower limb edema, hypertension and in one patient rhabdomyosis (Sharkey *et al.*, 1991). The evidence of side effects increase with duration of treatment.

**4. Drug Interaction**

**4.1 Lovastatin**

Lovastatin is an inactive lactone pro-drug converted *in vivo* to the corresponding open hydroxy acid, lovastatin acid, which is a competitive inhibitor of HMG-CoA reductase, the rate-limiting step in cholesterol

synthesis. Skeletal muscle toxicity is a rare side effect of HMG-CoA reductase inhibitor. Lovastatin is metabolized in the liver by CYP 3A4. Coadministration of lovastatin with itraconazole, the $C_{max}$ and the AUC of lovastatin were increased more than 20-fold ($P < 0.001$). The mean $C_{max}$ of the active metabolite, lovastatin acid, was increased 13-fold (range, 10 to 23-fold) and the $AUC_{(0-24)}$ 20-fold. Itraconazole greatly increase plasma concentrations of lovastatin and lovastatin acid by inhibiting CYP3A4 mediated metabolism probably explains the increased toxicity of lovastatin (Neuvonen and Jalava, 1996).

**4.2 Buspirone**

Buspirone is a non-benzodiazepine anxiolytic agent that acts as a partial agonist at serotonin receptor of $5HT_{1A}$ type. The oral bioavailability of buspirone is very low as a result of first-pass metabolism. Pretreatment of itraconazole 200 mg/day for 4 days increased the mean area under the plasma concentration-time curve from time zero to infinity ($AUC_{(0-\infty)}$) of buspirone about 19-fold ($P < 0.05$) compared with placebo. The mean $C_{max}$ of buspirone was increased about 13-fold ($P < 0.01$) by itraconazole. These interactions were evident in each subject, although a striking interindividual variability in the extent of the elimination half-life of buspirone was not prolonged by itraconazole. The greatly elevated plasma buspirone concentrations resulted in increased ($P < 0.05$) pharmacodynamic effects and in side effects of buspirone. The interaction caused by inhibiting its CYP3A4 mediated first-pass metabolism (Kivisto *et al.*, 1997).

### 4.3 Felodipine

Felodipine, a dihydropyridine calcium antagonist, is extensively metabolism by CYP3A4. Nine healthy volunteers received 200 mg itraconazole orally once a day for 4 days. On day 4, each ingested a single 5 mg oral dose of felodipine. On average, itraconazole increased the $C_{max}$ of felodipine nearly 8-fold ($P < 0.001$), the $AUC_{(0-\infty)}$ about 6-fold ($P < 0.001$) and the elimination half-life 2-fold ($P < 0.05$). The decrease in blood pressure and the increase in heart rate were significantly greater during the itraconazole phase. Itraconazole greatly increases plasma concentrations and effects of felodipine by inhibition of CYP3A4 during the first-pass and elimination phase of felodipine (Jalava *et al.*, 1997).

### 4.4 Quinidine

The oxidation of quinidine to 3-hydroxyquinidine is a specific marker reaction for CYP3A4 activity *in vitro*. Concomitant administration of diclofenac (a CYP2C9 substrate) reduced the partial clearance of quinidine by N-oxidation by 27%, while no effect was found for other pharmacokinetic parameters of quinidine. Concomitant administration of disulfiram (an inhibitor of CYP2E1) did not alter any of the pharmacokinetic parameters of quinidine. Concomitant administration of itraconazole reduced quinidine total clearance, partial clearance by 3-hydroxylation and partial clearance by N-oxidation by 61, 84 and 73%, respectively. The renal clearance was reduced by 60% and termination half-life increased by 35%. Concomitant administration of grapefruit juice reduced the total clearance of quinidine and its partial clearance by 3-hydroxylation and N-oxidation by 15, 19 and 27%,

respectively. The elimination half-life of quinidine was increased by 19%. Concomitant administration of erythromycin reduced the total clearance of quinidine and its partial clearance by 3-hydroxylation and N-oxidation by 34, 50 and 33%, respectively. $C_{max}$ was increased by 39%. The cafeine metabolic index was reduced by 25% (Damkier, Hansen and Brosen, 1999).

**4.5 Quinidine**

Quinidine is eliminated mainly by CYP3A4-mediated metabolism. Itraconazole 200 mg was ingested once a day for 4 days. A single 100 mg oral dose of quinidine sulphate was ingested on day 4. On the average, the peak plasma concentration of quinidine increased to 2.6-fold and the area under the concentration-time curve of quinidine increased to 2.4-fold by itraconazole. The elimination half-life of quinidine was prolonged 1.6-fold and the area under the 3-hydroxyquinidine/quinidine ratio-time curve decreased to one-fifth ($P < 0.001$) by itraconazole. The renal clearance of quinidine decreased 50% by itraconazole, whereas the creatinine clearance was uneffected. The $QT_c$ interval correlated with the concentrations of quinidine during both itraconazole and placebo phases ($r^2 = 0.71$ and $r^2 = 0.79$, respectively).

Itraconzole increases plasma concentrations of oral quinidine, probably by inhibiting the CYP3A4 during the first-pass and elimination phase of quinidine. The decreased renal clearance of quinidine might be the result of the inhibition of P-glycoprotein-mediated tubular secretion of quinidine by itraconazole (Kaukonen *et al.*, 1997).

### 4.6 Terfenadine

Terfenadine is a widely used histamine $H_1$ receptor antagonist. It is metabolized extensively by CYP3A4 in humans to form 2 metabolites by N-dealkylation and hydroxylation. The case reported after concomitant 60 mg terfenadine orally twice a day with 200 mg itraconazole orally twice a day result in prolonged cardiac QT interval, syncope, and ventricular fibrillation. Serum concentrations of terfenadine were 96 ng/ml on the $2^{nd}$ day of hospital day and more than 10 ng/ml on day $15^{th}$, which normal subjects taking normal doses (60 mg twice a day) of terfenadine, levels of unchanged terfenadine in plasma are usually very low or undetectable (Crane and Shih, 1993).

### 4.7 Clarithromycin

Clarithromycin is the new macrolide antibiotic, it is appears to be a key drug for prophylaxis and treatment of *Mycobacterium avium complex* (MAC) infection. Three patients negative for human immunodeficiency virus infection were admitted for pulmonary MAC and aspergillosis infections. They were treated with different drug combinations, but all regimens included clarithromycin for MAC and itraconazole for aspergillosis. All patients experienced an increase in clarithromycin concentrations and clarithromycin: 14-OH-clarithromycin ratio compared with expected range values. They had no clinical side effects. The time course suggested a possible interaction between clarithromycin and itraconazole, presumably through itraconazole's effects on CYP3A4 activity. A bidirectional interaction can not be ruled out. The data suggest that when necessary, these two drugs can be administered

together safely. Further investigation is necessary to determine the extent and clinical consequences of coadministration in humans (Auchair *et al.*, 1999).

### 4.8 Bupivacaine

Bupivacaine is an amide-type local anaesthetic administered as a racemic mixture of two optically active enantiomers, R-bupivacaine and S-bupivacaine. R-bupivacaine is mainly responsible for the cardiotoxicity of bupivacaine, but it has also greater clearance compared with S-bupivacaine. Pretreatment of 200 mg orally itraconazole, once daily for 4 days, on day 4 racemic bupivacaine 0.3 mg/kg was given intravenous over 60 minutes. 21% and 25% reduces plasma concentrations of R- and S-bupivacaine, respectively, while it had no significant effect on other Pharmacokinetics variables of the enantiomers. Reduction of bupivacaine clearance by itraconazole probably increases the steady-state concentration of bupivacaine enantiomers by 20-25%. This should be taken into account in the concomitant use of bupivacaine and itraconazole, although the interaction seems to be of limited clinical significant (Palkama *et al.*, 1999).

## Cytochromes P-450

### 1. Introduction

Enzymes in the liver and other tissues catalyze xenobiotic biotransformation. Most of the enzymes have been classified as belonging to phase I or phase II pathway of metabolism. Phase I reaction involve hydrolysis, reduction, and oxidation. These reactions expose or introduce a functional group (-OH, $-NH_2$, -SH or –COOH). 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. Phase II biotransformation reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis) and conjugation with amino acids (such as glycine, taurine and glutamic acid).

In phase I pathway, cytochromes P-450 (P450s) are the most active among drug-metabolizing enzymes. These enzymes are also principally responsible for activation of procarcinogens and promutagens. Most clinically used drugs are metabolized to some degrees by P450s.

P450s have been classified based on principally amino acid sequence identity. Families are designated by an Arabic number, with all members of a particular family having more than 40% identity in amino acid sequence. A subfamily consists of enzymes in which the amino acid sequence is more than 55% identical. These are designated by a capital letter. An Arabic numeral is used to represent the individual enzyme. P450s are named CYP followed family, subfamily and individual enzymes, respectively.

The cytochromes P450 are a superfamily of hemoprotiens that are the terminal oxidized of the mixed function oxidase system. These found in animals, plants, yeast and bacteria. These enzymes are embedded in the lipid bilayer of smooth endoplasmic reticulum (microsomes).

The membrane localization is ideally suited for the function of P450s in metabolizing hydrophobic chemicals. These P450s have been referred to as mixed function monooxygenase because they add an atom of oxygen to numerous structurally-diverse substrates. A simplified scheme of oxidative cycle is presented in Figure 4. In the P450s catalytic cycle, the enzyme binds to its substrate (Step 1) and the heme ion is reduced from a valency of +3 to +2 by an electron transferred from NADPH via another flavoprotein called NADPH-P450 oxidoreductase 9 (Step 2). Then $O_2$ binds to the heme and is reduced by another electron (Step 3). A series of reaction occurs that result in splitting of $O_2$, production of $H_2O$ and oxidation of the substrate (Step 4). (Correia, 1998).

**2. Human Hepatic Cytochrome P450s (CYPs)**

Presently, the P450 subfamily consists of 17 CYP gene families in humans (de Wildt *et al.*, 1999). Three main P450 gene family, CYP1, CYP2 and CYP3 are responsible for the vast majority of drugs metabolism and account for at least 70% of the total P450 content in humans liver sample. Although the CYP1 and CYP3 gene families are relatively simple (i.e. CYP1A and CYP3A), the CYP2 gene family is comprised of many subfamilies (e.g., CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, etc).

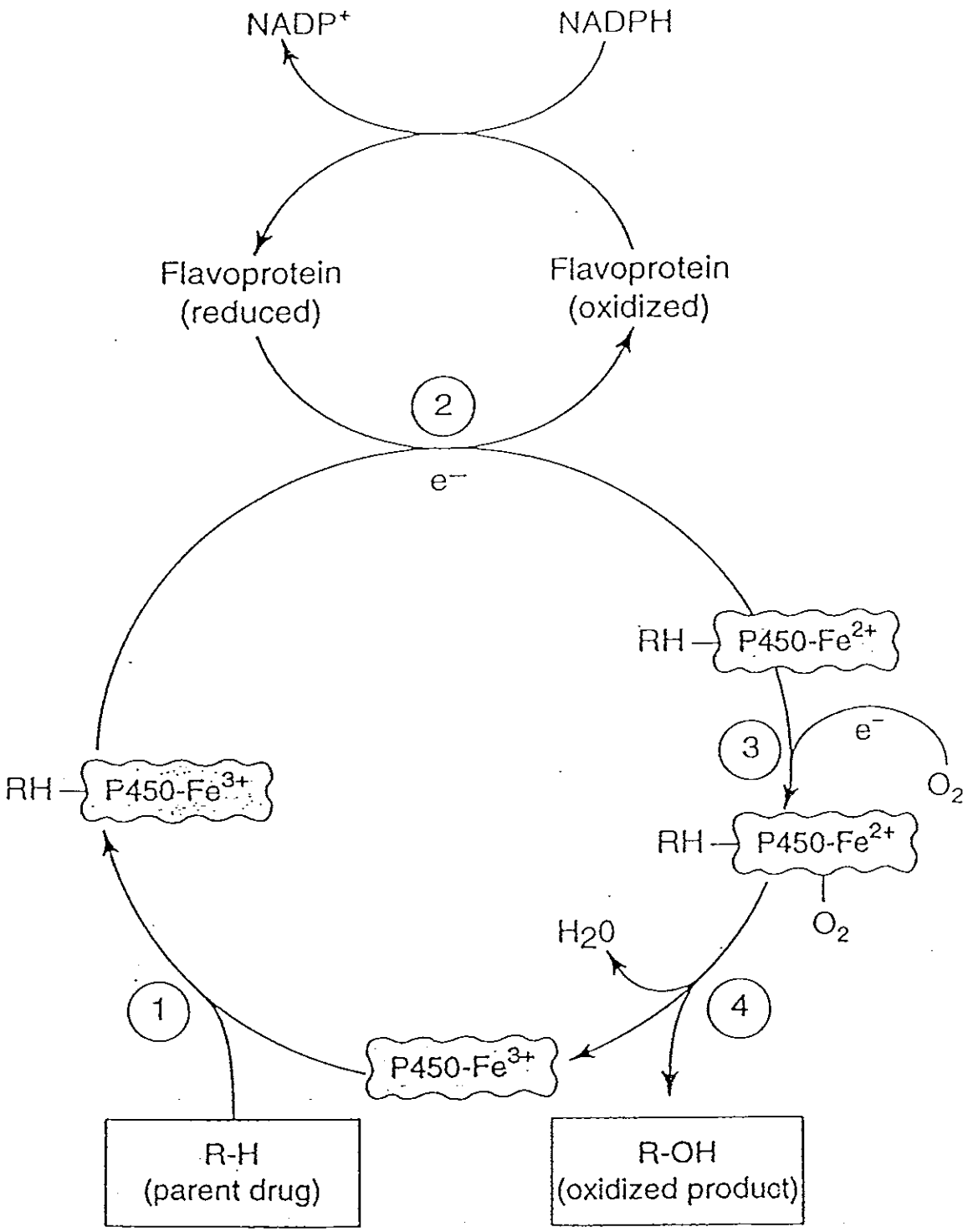


**Figure 4** Cytochromes P-450 cycle in drug oxidations (Correia, 1998 : 52)

These isoforms have the same oxidizing center (the heme iron), but different by their protien structures (Lin and Lu, 1998).

For different P450, the entry of the substrate into the active site and the direct interaction of amino acids in the active site with the substrate govern specificity control.

### 3. Mechanisms of Induction of Cytochrome P450s

Inducers of cytochrome P450s increase the rate of xenobiotic biotransformation (Batt *et al.*, 1994). Enzyme induction occurs when a drug stimulates the synthesis of more enzyme protien, enhancing the enzyme's metabolizing capacity. It is somewhat difficult to predict the time course of enzyme induction because several factor, including drug half-lives and enzyme turnover, determine the time course of induction (Cupp and Tracy, 1998 ; Tanaka, 1998a). One of the intriguing aspects of the CYP 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 immediately response, CYP 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 CYP induction are often overlooked in clinical studies.

Although the phenomenon of CYP induction has been known for more than 4 decades, only in recent years that have begun to uncover the mechanisms involved in induction. From 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 CYP 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 CYP 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 metabolizing 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).

**4. Mechanisms of Inhibition of Cytochrome P450s**

Enzyme inhibition usually involves competition with another drug for the enzyme binding site. This process usually begins with the first dose of the inhibitor, and onset and offset of inhibition correlate with the half-lives of the drugs involved (Cupp and Tracy, 1998).

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 heme 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 CYP enzyme activity, step (i), (iii) and (vi) are particularly vulnerable to inhibition.

The mechanism of CYP inhibition can be divided grossly into three categories: reversible inhibitions, quasi-irreversible inhibitions and irreversible inhibitions. Among these, reversible inhibition is probably the most common mechanism responsible for the documented drug interactions (Lin and Lu, 1998; Halpert, 1995).

**4.1 Reversible Inhibition**

Many of the potent reversible CYP inhibitors are nitrogen-containing drugs, including imidazole, pyridines and quinolines. These compounds can not only bind to the prosthetic heme iron, but also to the lipophilic region of the protein. Inhibitor that simultanously 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 the lone electron pair on the nitrogen and the prostatic heme iron. For example, both ketoconazole and cimetidine are imidazole-containing compounds that interact with ferric CYP at its sixth axial ligand position. The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the

hexacoordinated heme by a strong ligand. However, cimetidine is a relatively weak reversible inhibitor of CYP, an apparent result of an intrinsic low binding affinity to microsomal CYP. This latter property is most probably because of the low lipophilicity of cimetidine. On the other hand, ketoconazole, a potent CYP inhibitor, has a high lipophilicity. Similarly, fluconazole contains a triazole that binds to the prosthetic heme iron but is a weak reversible CYP inhibitor, again due mainly to its low lipophilicity.

The quinoline is another class of nitrogen heterocycles that exhibit potent CYP inhibition. Quinidine and its diastereoisomer quinine, both, which are potent reversible inhibitors of debrisoquine 4-hydroxylation, involve a reaction catalyzed by the CYP2D subfamily.

### 4.2 Quasi-Irreversible Inhibition via Metabolic Intermediate Complexation

A large number of drugs, including methylene dioxybenzenes, alkylamines, macrolide antibiotics and hydrazines, undergo metabolic activation by CYP enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic heme of CYP called metabolic intermediate (MI) complex, so that CYP is sequestered in a functionally inactive state. MI complexation can be reversed, and the catalytic function of ferric CYP can be restored by the highly lipophilic compounds that displace the metabolic intermediate from the active site. Other 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 reactivation of

CYP functional activity. However, *in vivo* situations, the MI complex is so stable that the CYP 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.

### 4.3 Irreversible Inactivation of CYP

Drugs containing functional groups can be oxidized by CYP 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 CYP may result from irreversible alteration of heme or protein, or a combination of both. In general, modification of the heme group invariably inactivates the CYP, 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.

#### 4.3.1 Heme Alkylation

Drugs containing terminal double-bound (olefins) or triple-bond (acetylenes) can be oxidized by CYP to radical intermediates that alkylate the prosthetic heme group and inactivate the enzyme. The evidence for heme alkylation includes the demonstration of equimolar loss of enzyme and heme, as well as the isolation and structural characterization of the heme adducts. Heme 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 heme

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 react with the nitrogen of pyrrole ring D.

### 4.3.2 Covalent Binding to Apoprotein

The best known example of inactivation of CYP through protein modification by a suicide inactivator is that of chloramphenicol. The dichloroacetamido group is an oxidized to an oxamyl moiety that acylates a lysine residue in the CYP active center. This acylation event interferes with the transfer of electrons from CYP reductase to the heme group of the CYP and thereby prevent catalytic turnover of the enzyme. The inactivation by chloramphenicol is not uniform for all CYPs. Studies with rat liver microsomes revealed that CYP2B1, CYP2C6 and CYP2C11 are susceptible to inactivation by chloramphenicol, whereas CYP1A1 and CYP1A2 are resistant.

Although terminal acetylenes have been known to alkylate the prosthetic heme group, some terminal acetylene compounds, such as 2-ethylnylnaphthalene, inactivate CYP by binding covalently to the protein with little loss of the heme group. 2-Ethylnylnapthalene is converted by CYP2B1 to a ketene, which modifies an active site peptide that includes Thr-302, a highly conserved residue known to play a role in oxygen activation.

Oxidation of sulphur groups in drug molecules can result in the modification of the CYP protein. A variety of sulphur compounds inactivate CYP by binding covalently to protein after the enzyme oxidatively activates them. CYP inactivation by sulphur compounds is believed to be involved with

sulphur oxidation that generates reactive sulphur metabolites. Tienilic acid, a substituted thiophene, is oxidized by yeast-expressed human CYP2C9 to a reactive metabolite, presumably a thiophene sulphoxide that binds covalently to the CYP apoprotein.

The protein modification is caused by formation of a sulphur reactive metabolite, rather than formation of hydrodisulphides (RSSH). Although covalent binding of the protein can be partially prevented by glutathione, the activity of the enzyme inactivated by tienilic acid cannot be restored by glutathione. In addition, diallyl sulphide, a flavour component of garlic, is known to be a potent suicide inhibitor of CYP2E1. The mechanism by which diallyl sulphide inhibits CYP2E1 involves initial oxidation at sulphur to give diallyl sulphone, which then undergoes metabolic activation on 1 or other terminal olefin groups to produce the ultimate reactive species (Lin and Lu, 1998 ; Halpert, 1995).

## 5. Clinical Implications

### 5.1 Induction of Cytochrome P450s

Usually, metabolites are less pharmacologically active than 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. The short half-life of rifampicin results in enzyme induction (CYP3A4, CYP2C), apparent within 24 hours,

whereas phenobarbital, which has a half-life 3-5 days, requires about 1 week for induction (CYP3A4, CYP1A2, CYP2C) to become apparent. These enzyme induction reactions also occur with smoking and long-term alcohol or drugs consumption and can reduce the duration of action of a drug by increasing its metabolic elimination. All these drugs, the clinically most problematic drugs involves the rifampicin series (rifampin, rifapentine and rifabutine) and includes antiepileptic drugs such as phenobarbital, carbamazepine and phenytoin and antituberculous drugs. The CYP1A2 enzyme can be induced by exposure to polycyclic aromatic hydrocarbons, such as are found in char-grilled foods and cigarette smoke. Most human CYP2C and CYP3A subfamily proteins are induced by barbiturates, while human CYP2E1 is inducible by ethanol and isoniazid, although the mechanism involved is complex (Tanaka, 1998a).

### 5.2 Inhibition of Cytochrome P450s

The clinical incident of drug inhibition will depend on a number of considerations. One of the most important considerations is the therapeutic index of the drug. Patients received 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.

For example, ketoconazole and itraconazole are known a potent inhibitor of CYP3A4. In clinical study, ketoconazole and itraconazole increased plasma concentration and effect of midazolam, but ketoconazole is more potent than itraconazole (Olkkola *et al.*, 1994). Coadministration of terfinadine, an antihistamine agent, and ketoconazole led to fatal ventricular

arrhythmias in some patients. Terfinadine is widely used histamine $H_1$ receptor antagonist. It is metabolized extensively by CYP3A4 in humans to form 2 metabolites by N-dealkylation and hydroxylation. After oral administration of 60 mg dose, terfinadine is usually undetectable in plasma because of extensive first-pass metabolism. Concurrent administration of drugs that inhibit terfinadine metabolism can result in an excessive increase in plasma concentration of terfinadine (Honig *et al.*, 1993; Yun *et al.*, 1993; Woosley *et al.*, 1993).

**6. CYP3A Subfamily**

Enzymes of the CYP3A subfamily appear to be responsible for the metabolism of the widest range of drugs and endogenous compound in human. CYP3A is the most abundant of the human hepatic cytochromes, accounting for nearly 30% in adult liver and small intestine (Watkins *et al.*, 1987). Activity of CYP3A is variable among individuals, but these are no evidence of genetic polymorphism. Significant amounts of CYP3A are present in the gastrointestinal tract (Von Moltke *et al.*, 1995). CYP3A subfamily consists of at least 3 isoforms: CYP3A4, CYP3A5 and CYP3A7 (de Wildt *et al.*, 1999).

CYP3A4 is the most abundantly expressed CYP and accounts for approximately 25% of the total CYP content in human adult liver and small intestine (Dresser, David Spence and Bailey, 2000 ; Lin and Lu, 1998) . CYP3A5 is 85% homologous to CYP3A4. It is expressed at a much lower level than CYP3A4 in the liver, but is predominant isoform in the lung and

stomach and is present in the small bowel and renal tissue (Dresser, David Spence and Bailey, 2000). CYP3A7 is the major CYP isoform detected in human embryonic fetal and newborn liver and does not appear to be present in adult, (Dresser, David Spence and Bailey, 2000 ; de Wildt *et al.*, 1999).

In human liver, CYP3A4 immunoreactivity was detected in midzonal and centrilobular regions (Ratanasavanh *et al.*, 1991), whereas intestinal CYP3A occurs in the enterocytes lining the lumen of the small intestine (Watkins *et al.*, 1987). Inoue *et al.* (1992) assigned the CYP3A4 gene to chromosome 7 at band q22.1. The gene is divided in to 13 exons and 12 introns with a length of approximately 27kb (Hashimoto *et al.*, 1993). The therapeutic important drugs of CYP3A4 substrates are such as erythromycin, midazolam, triazolam, cyclosporin, lidocaine and nifedipine (Leeder and Kearns, 1997). The endogenous compounds metabolized by human 3A subfamily including the 6β-hydroxylation of testosterone, cortisol, progesterone and androstanediol; the 2- and 4-hydroxylations of estradiol; and the 16α-hydroxylation of dehydroepiandrosterone 3-sulfate (DHEA-s) (Wrighton and Stevens, 1992). CYP3A4 also metabolizes procarcinogens such as sterigmatocystin and aflatoxin B1 (Shimada and Guengerich, 1989).

Table 2 is a list of representative substrates, inhibitors and inducers of CYP3A4.

**Table 2** List of representative substrates, inhibitors and inducers of CYP3A4 (Venkatakrishnan *et al.*, 2000 ; Von Moltke *et al.*, 1995)

| Substrate | Inhibitor | Inducer |
|---|---|---|
| **Immunosuppressants** | **Grapefruit juice** | Rifampicin |
| cyclosporin | bergamottin | Phenobarbital |
| tacrolimus | dihydroxyber-gamottin | Carbamazepine |
| **Hypnosedatives** | **Diethylstilbestrol** | Phenytoin |
| midazolam | gestodene | Primidone |
| triazolam | **$H_2$ antihistamines** | Ritonavir |
| alprazolam | cimetidine | Nevirapine |
| brotizolam | **Tricyclic antidepressants** | Dexamethasone |
| **Calcium antagonists** | fluoxetine | |
| nifedipine | **Antiarrhythmic agents** | |
| felodipine | amiodarone | |
| diltiazem | **Calcium antagonists** | |
| **Antiarrhythmic agents** | diltiazem | |
| amiodarone | | |
| quinidine | | |
| lidocaine | | |

**Table 2** (continued)

| Substrate | Inhibitor | Inducer |
|---|---|---|
| **Anti-infectives** | **Anti-infectives** | |
| erythromycin | troleandomycin | |
| quinine | erythromycin | |
| ritonavir | clarithromycin | |
| saquinavir | clotrimazole | |
| amprenavir | ketoconazole | |
| **Antineoplastic agents** | itraconazole | |
| etoposide | fluconazole | |
| infosfamide | indinavir | |
| vinblastine | amprenavir | |
| **Synthetic opioids** | nelfinavir | |
| fentanyl | ritonavir | |
| alfentanil | **Proton pump inhibitor** | |
| sufentanil | omeprazole | |
| **The nonsedating** | | |
| **antihistamines** | | |
| terfenadine | | |
| loratadine | | |
| astemizole | | |

# CHAPTER 3

# MATERIALS AND METHODS

## Chemicals and Reagents

Standard quinine and quinidine hydrochloride were purchased from SIGMA® Chemical Co. (St Louis, MO, U.S.A.). Quinine sulphate (300 mg tablet Lot No. 98098) was obtained from General drug House Co., Ltd., Bangkok Thailand. Ketoconazole (Nizoral®, Lot No. B181297) and itraconazole (Sporal®, Lot No. 384037) were obtained from OLIC (Thailand) Limited, under license of Janssen Pharmaceutica Ltd. Acetonitrile (HPLC-grade) and Triethylamine (analytical grade) were obtained from J.T. Baker Inc. Phillipsburg, U.S.A. and Fluka, Messerchmittstr, Switzerland, respectively. Water was purified for HPLC by the Milli Q Water Purification System (Millipore, Milford, MA, U.S.A).

## Equipments

The HPLC system composed of Waters 510 pump and the automated injection system, Waters 717 plus Autosampler (Waters Associates, Milford, MA, U.S.A.). The detector was Jasco 821-FP intelligent Spectrofluorometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan). The integrator was the Jasco model 807-IT (Japan Spectroscopic Co., Ltd., Tokyo, Japan). A μ-Bondapak $C_{18}$ (30 cm x 3.9 mm I.D., particle size 10 μm, Waters Associates, Milford,

MA, U.S.A) was used as the column. A Guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

## Methods

### 1. Subjects

The Human Ethics Committee Faculty of Science, Prince of Songkla University, Hat-Yai, Thailand, approved the study. All subjects gave written informed consent before study. Nine Thai healthy male volunteers, aged 16-37 years old, weighing 47-70 kilograms (mean weight 61.3 $\pm$ 7.52 kgs) participated in the study. Prior to the study, a medical history, physical examination, standard biochemical and hematological screening were performed in each subject. Neither any drugs were taken in the month preceding nor during the study. All of them are non-smokers. Drinking of alcoholic beverages, coffee and tea are not allowed at least 2 weeks prior to and during the entire period of study.

### 2. Protocol

Three phases of the studies were randomized crossover study designed with two weeks wash out period. In each phase, three subjects received a single 300 mg dose of quinine sulphate orally with 150 ml of water. The rest of two groups (3 subjects of each group) received 400 mg ketoconazole (Nizoral®) or 200 mg itraconazole (Sporal®) orally with breakfast and 150 ml of water for 4 days and followed by a single 300 mg dose of quinine

sulphate orally in the study day 4. A serial blood draw was done in the study day 4 before and after a single oral dose of 300 mg quinine sulphate ingestion. All volunteers were overnight fast before the study day 4.

**2.1. Blood Sample Collection**

The heparin-lock catheter was placed in a forearm vein of each subject. In the study day 4 after administration of 300 mg quinine sulphate, serial blood samples (5 ml) were drawn immediately before and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3, 4, 6, 8, 24 and 48 hours after quinine sulphate administration. Plasma was separated from blood sample by centrifugation at 1,000 g for 15 minutes and aliquots of the plasma were stored at –70 °C until analysis.

**2.2 EKG, Pulse Rate and Blood Pressure Monitoring**

The EKG, heart rate and blood pressure were monitored immediately before and at 0.5, 1.0, 1.5, 2.5, 4, 8 and 24 hours after 300 mg quinine sulphate administration.

**3. Analytical Methods**

Plasma samples were analyzed by high performance liquid chromatography for quinine and quinidine (internal standard) by methods previously described by Lehmann *et al.* (1986); Edsteine *et al.* (1990); and Supanaranond *et al.* (1991) with a slight modification using reverse-phase column (μ-Bondapak $C_{18}$) and a fluorescence detector (an excitation and emission wavelength were set at 340 and 425 nm, respectively).

### 3.1 Mobile Phase

A 10 ml of triethylamine was added to 900 ml deionized water and the pH was adjusted to 2.5 with 85% phosphoric acid. The mixture was added with deionized water to a final volume of 930 ml, and 70 ml of acetonitrile was added in the last step. The final component of mobile phase consisted of 91.4% of deionized water : 1% of triethylamine : 0.6% of 85% phosphoric acid : 7% of acetonitrile (vol/vol/vol/vol). The mobile phase was filtered through 0.45 micropore filtered paper (Nyron 66), then degassed by ultrasonicator for 9 minutes before using. The mobile phase was freshy prepared daily. The flow rate was 1.5 ml/min. All analysis were performed at room temperature (about 24 $\pm$ 2 °C).

### 3.2 Stock Standard Solution

Stock standard solution of quinine and quinidine hydrochloride at a concentration of 1000 μg/ml were prepared by dissolving 10 mg of standard quinine in 1 ml methanol and added with the mobile phase to a final volume of 10 ml in volumetric flask and covered it with foil to protect from light. Then, they were diluted to 100 μg/ml with the mobile phase for stock standard solutions and store at –20 °C.

Working standard solutions used to prepare a calibration curve were freshly prepared by appropriate dilution of the stock standard solution with blank plasma (Appendix-1).

### 3.3 Calibration Curve

Calibration curve was prepared by adding a standard quinine and internal standard quinidine solution to blank human plasma so that final

concentrations in plasma was 0.5, 1, 2.5, 5 and 10 μg/ml. The calibration curve plotted using peak height ratio of quinine to quinidine and concentration was linear in the concentration range of 0.5 to 10 μg/ml (Figure 9). The lower detection limit for quinine was 0.2 μg/ml.

### 3.3.1 Precision and Variability

To determine intra-day precision and variability, the standard quinine was spiked in blank plasma at the concentration of 1, 2.5, 5 and 10 μg/ml, and internal standard quinidine at concentration of 25 μg/ml was spiked in each concentration of quinine in plasma and eight replicates of each were carried out on one day.

Inter-day precision and variability was done as intra-day but carried out on different ten days. Accuracy should be of ± 10% of spiked value and the CV% of each concentration should be less than 10%.

### 3.3.2 Recovery

Potential loss of quinine during the precipitation by acetonitrile was determined by comparing the peak height of quinine from plasma sample in the concentration range of 1 to 10 μg/ml and the equal concentration of standard quinine prepared in the mobile phase. The potential loss of internal standard was also determined by the same method. The percent recovery was calculated as the following formula.

$$\% \text{ Recovery} = \frac{\text{peak height of quinine in plasma x1.5}}{\text{peak height of quinine in mobile phase}} \times 100$$

### 3.4 Sample preparation

Adding a 400 µl of quinine standard or sample and 100 µl internal standard (25 µg/ml quinidine hydrochloride in mobile phase) into a propylene tube, then vortex mixing for 30 seconds. Add acetonitrile 250 µl to the mixture and vortex mixing for 30 seconds. After 10 minutes the tube was centrifuged for 15 minutes. The supernatant (40 µl) were injected into the HPLC system by an automated injection.

## 4. Data Analysis

### 4.1 Pharmacokinetic Calculations

The following parameters were calculated by using Winnonlin® solftware program, 1995; the maximum plasma quinine concentration ($C_{max}$), the time to reach $C_{max}$ ($t_{max}$), the absorption rate constant (Ka), the elimination rate constant (Ke), the elimination half-life ($t_{1/2}$), the area under the concentration time curve (AUC) and lag time.

The apparent oral clearance (CL/f) was calculated as dose/(AUC x body weight). The apparent volume of distribution (Vd/f) was calculated as CL/f divided by Ke.

### 4.2 Pharmacodynamic study

Automatic Digital Blood Pressure Monitor, Model HEM-703C (Omron Corporation, Tokyo, Japan), was used to measure the systolic and diastolic blood pressure and pulse rate. The EKG was measured by polygraph and $QT_c$ was calculated by measurement QT interval divided by square root of R-R interval.

### 4.3 Statistical Analysis

All results were expressed as means ± S.D. Differences in quinine pharmacokinetic and phamacodynamic parameters among control and treatment groups were tested by analysis of variance (ANOVA) *P* value less than 0.05 taken as the minimum levels of significant. Student's pair *t*-test was used to test for significant differences between means.

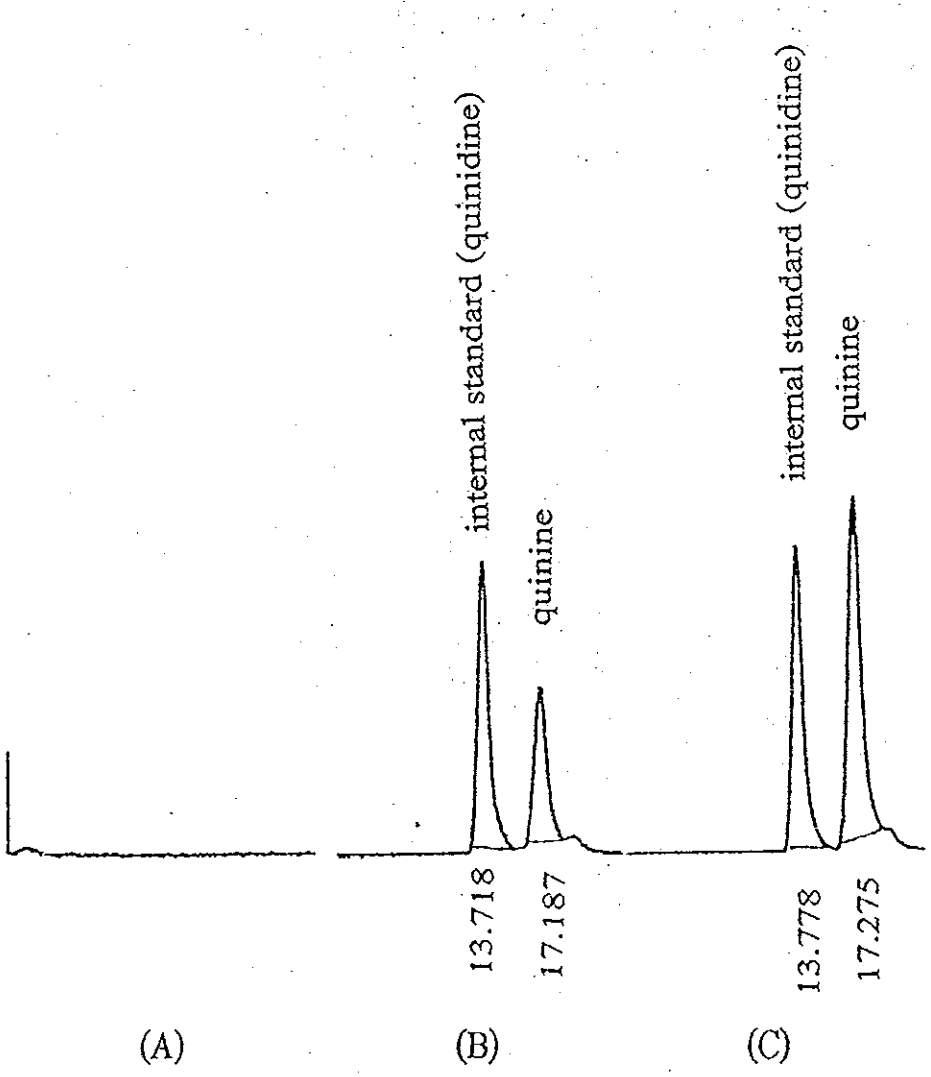


**Figure 5** Representative chromatograms of 40 µl human plasma samples. Key: (A) blank human plasma; (B) and (C) spiked with quinine 5 and 10 µg/ml, respectively. The mobile phase consisted of deionized water-triethylamine-85%phosphoric acid-acetonitrile (91.4: 1: 0.6: 7 vol/vol/vol/vol) pH 2.5 at a flow rate of 1.5 ml/min. Chart speed and attenuation were 1 mm/min and 128 mVF.S, respectively.

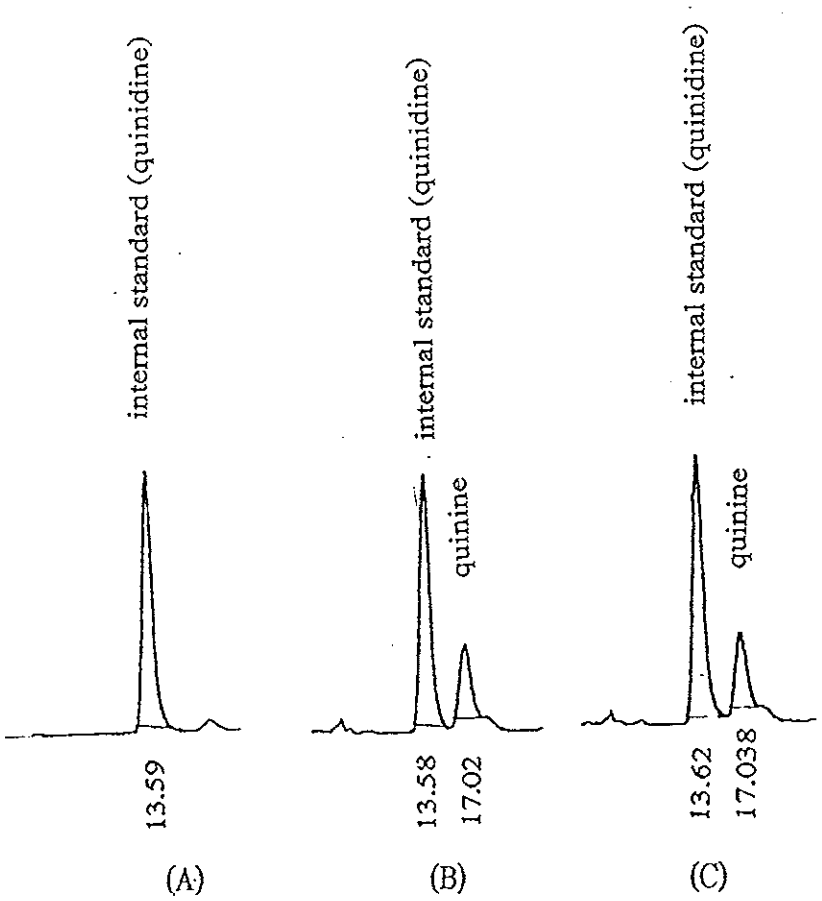


**Figure 6** Representative chromatograms of 40 µl human plasma samples. Key: (A) spiked with internal standard 25 µg/ml quinidine hydrochloride; (B) and (C) plasma obtained from a subject receiving 300 mg quinine alone at 0.75 and 2.5 hours, respectively. The mobile phase consisted of deionized water - triethylamine - 85%phosphoric acid - acetonitrile (91.4: 1: 0.6: 7 vol/vol/vol/vol) pH 2.5 at a flow rate of 1.5 ml/min. Chart speed and attenuation were 1 mm/min and 128 mV F.S., respectively.

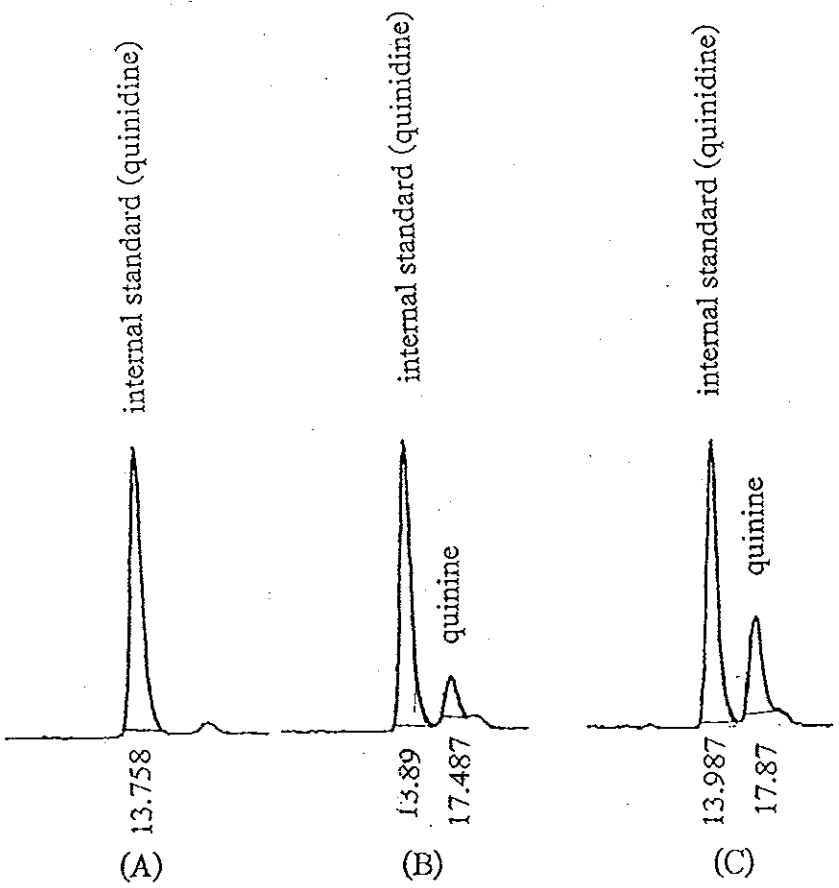


**Figure 7** Representative chromatograms of 40 µl human plasma samples. Key: (A) spiked with internal standard 25 µg/ml quinidine hydrochloride; (B) and (C) plasma obtained from subjects after pretreatment with 400 mg ketoconazole for 4 days at 0.75 and 2.5 hours, respectively after an oral administration of 300 mg quinine sulphate. The mobile phase consisted of deionized water-triethylamine-85% phosphoric acid-acetonitrile (91.4: 1: 0.6: 7 vol/vol/vol/vol) pH 2.5 at a flow rate of 1.5 ml/min. Chart speed and attenuation was 1 mm/min and 128 mVF.S., respectively.

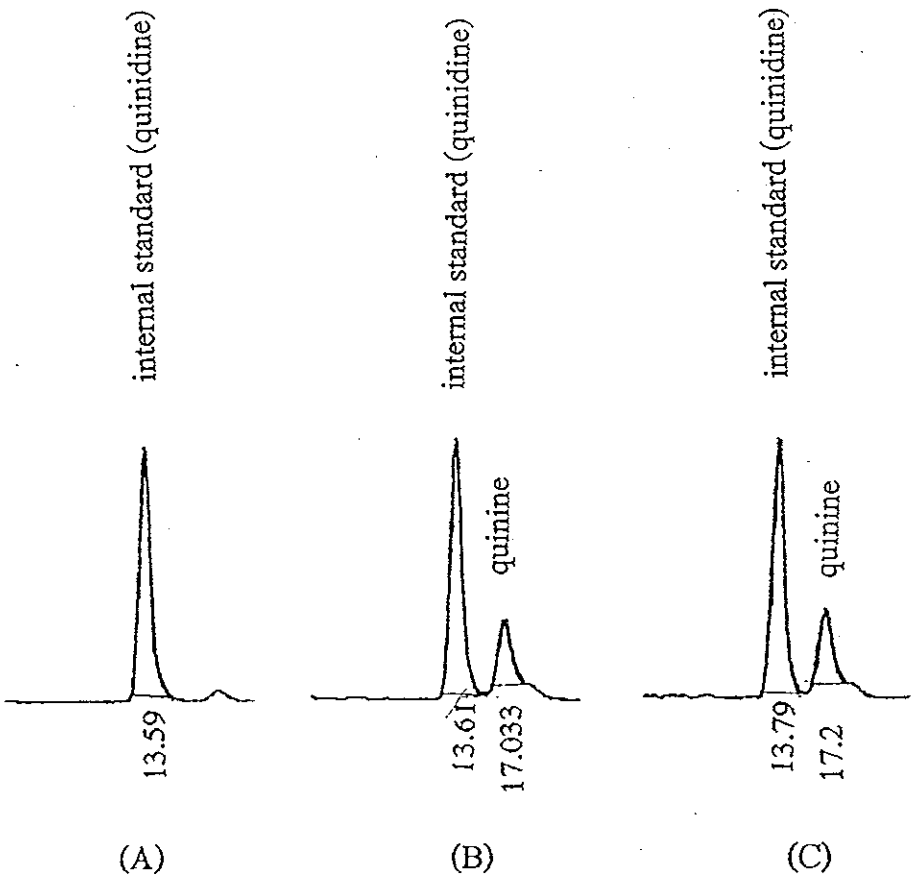


**Figure 8** Representative chromatograms of 40 μl human plasma samples. Key: (A) spiked with internal standard 25 μg/ml quinidine hydrochloride; (B) and (C) plasma obtained from a subject after pretreatment with 200 mg itraconazole for 4 days at 0.75 and 2.5 hours, respectively after an oral administration of 300 mg quinine sulphate. The mobile phase consisted of deionized water-triethylamine-85% phosphoric acid-acetonitrile (91.4: 1: 0.6: 7 vol/vol/vol/vol) pH 2.5 at a flow rate of 1.5 ml/min. Chart speed and attenuation was 1 mm/min and 128 mVF.S, respectively.

**Table 3** The intra-assay variance of four different quinine concentrations in human plasma

| Concentration[a] (μg/ml) | Mean peak height ratio of quinine to quinidine ± S.D. (n = 8) | CV(%)[b] |
|---|---|---|
| 1 | 0.13 ± 0.002 | 1.59 |
| 2.5 | 0.32 ± 0.011 | 3.48 |
| 5 | 0.66 ± 0.020 | 3.03 |
| 10 | 1.34 ± 0.014 | 1.05 |

[a]Various concentrations of standard quinine and internal standard quinidine 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** Inter-assay variance of four different quinine concentrations in human plasma

| Concentration[a] (μg/ml) | Mean peak height ratio of quinine to quinidine ± S.D. (n = 10) | CV(%)[b] |
|---|---|---|
| 1 | 0.15 ± 0.098 | 6.67 |
| 2.5 | 0.34 ± 0.018 | 5.31 |
| 5 | 0.66 ± 0.031 | 4.69 |
| 10 | 1.30 ± 0.051 | 3.92 |

[a]Various concentrations of standard quinine and internal standard quinidine 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** Relative percent recovery of standard quinine in human plasma

| Concentration (μg/ml) | Mean peak height in mobile phase[a] ± S.D. (n = 5) | Mean peak height in human plasma[b] ± S.D. (n = 5) | % Recovery[c] |
|---|---|---|---|
| 1 | 5.8 ± 0.11 | 3.48 ± 0.109 | 89.38 |
| 2.5 | 13.4 ± 0.42 | 8.40 ± 0.96 | 94.03 |
| 5 | 26.1 ± 1.08 | 16.00 ± 0.35 | 91.95 |
| 10 | 51.8 ± 2.25 | 31.64 ± 1.01 | 91.62 |

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

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

[c]Mean peak height of quinine in plasma multiplied by 1.5 and divided by mean peak height of quinine in mobile phase, expressed in percent.

# CHAPTER 4

# RESULTS

## Assay Validation

The assay validation of this experimental method showed that the calibration curve was linear in the quinine concentration range of 0.5 to 10 μg/ml (Figure 9) with the correlation coefficient (r) of 0.999. The coefficient of variations (CV) for intra-assay and inter-assay were 1.05 to 3.48% and 3.57 to 6.67%, respectively (Table 3-4). The recovery of standard quinine in blank plasma was ranged from 89.38 to 94.03% (Table 5). The chromatograms illustrated that the peak of quinine was well seperated from the peak of quinidine, used as internal standard, and the other peaks in plasma (Figure 5-8). There was no interference from the peak of itraconazole and ketoconazole in the chromatograms of this method. The mean plasma concentration-time profiles of quinine after receiving quinine alone and pretreatment with ketoconazole or itraconazole were fitted to one compartment open model (Figure 10).

## Pharmacokinetics

The pharmacokinetic parameters showed wide interindividual variations. The mean plasma concentration-time profiles of quinine after pretreatment with ketoconazole or itraconazole were shown in Figure 10. All

The results indicated that AUC, $t_{1/2}$, Ke, , $C_{max}$, $T_{max}$, Vd/f and CL/f of quinine after pretreatment with 400 mg ketoconazole for 4 days were significantly different when compared with the administration of quinine alone, whereas only the parameters AUC, Ke, $t_{1/2}$, and CL/f of quinine after pretreament with 200 mg itraconazole for 4 days were significantly different when compared with the administration of quinine alone. The values (mean ± S.D.) of AUC, Ka, Ke, $t_{1/2}$, $T_{max}$, $C_{max}$, Vd/f and CL/f after administration of quinine alone were 36.06 ± 14 mg/l.hr, 2.63 ± 1.7 $hr^{-1}$, 0.08 ± 0.02 $hr^{-1}$, 9 ± 2.13 hr, 1.76 ± 0.59 hr, 2.35 ± 0.52 mg/l, 1.97 ± 0.61 l/kg and 0.17 ± 0.09 l/hr/kg, respectively, whereas those after pretreatment with 400 mg ketoconazole and 200 mg itraconazole for 4 days were 74.76 ± 26.9 mg/l.hr, 1.85 ± 0.72 $hr^{-1}$, 0.05 ± 0.02 $hr^{-1}$, 15.26 ± 4.9 hr, 2.8 ± 0.83 hr, 3.14 ± 0.54 mg/l, 1.53 ± 0.27 l/kg and 0.077 ± 0.032 l/hr/kg, respectively; and 70.8 ± 35.76 mg/l.hr, 2.09 ± 1.41 $hr^{-1}$, 0.051 ± 0.02 $hr^{-1}$, 15.4 ± 7.2 hr, 2.23 ± 0.87 hr, 2.84 ± 0.76 mg /l, 1.75 ± 0.62 l/kg and 0.096 ± 0.067 l/hr/kg, respectively. All pharmacokinetic parameters of quinine after pretreatment with ketoconazole were not significantly different from parameters of quinine after pretreatment with itraconazole.

**Pharmacodynamics** (Blood pressure, pulse rate and $QT_c$)

Blood pressure, pulse rate and EKG were also monitored in this study. In the phase of quinine alone, all pharmacodynamic parameters were not significantly different from control, except the decreased in pulse rates at 0.5 and 1.5 hours (73 vs 68, $P < 0.05$)

After pretreatment with ketoconazole or itraconazole, the blood pressure at time measured in all treatments were not significantly different from control, except the systolic blood pressure at 0.5 hour (116 vs 109, $P < 0.05$) after pretreatment with itraconazole and the diastolic blood pressure at 2.5 hour (74 vs 66, $P < 0.05$) after pretreatment with ketoconazole were significantly different from control (Figure 11; Table 7). Pulse rates were significantly different between group of quinine alone and pretreatment with ketoconazole at 0.5, 1, 2.5, 4 and 24 hours (68 vs 62, 71 vs 63, 79 vs 72, 78 vs 71 and 72 vs 64, respectively $P < 0.05$) (Figure 12; Table 8). The $QT_c$ in all phases were not significantly different, (Figure 13; Table 9). However, none of the subjects complained of heart irregularity and syncope after quinine administration in any phases.

**Adverse effect**

Three of nine subjects complained of nausea after the administration of ketoconazole, but they did not require any specific treatment. However, all subjects were well tolerated and completed the study. Other adverse effects were not reported in this study.

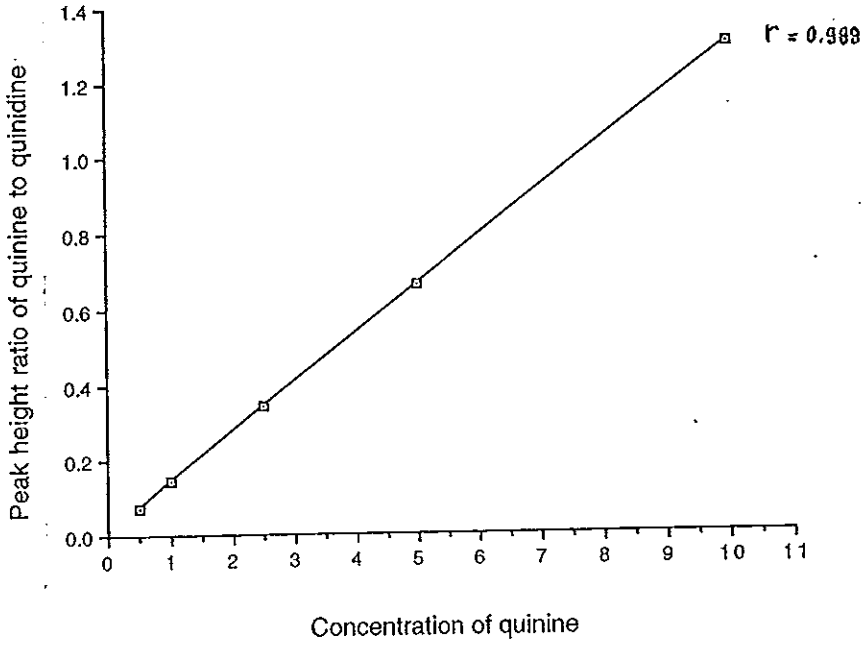


**Figure 9** Mean calibration curve of standard quinine in plasma.

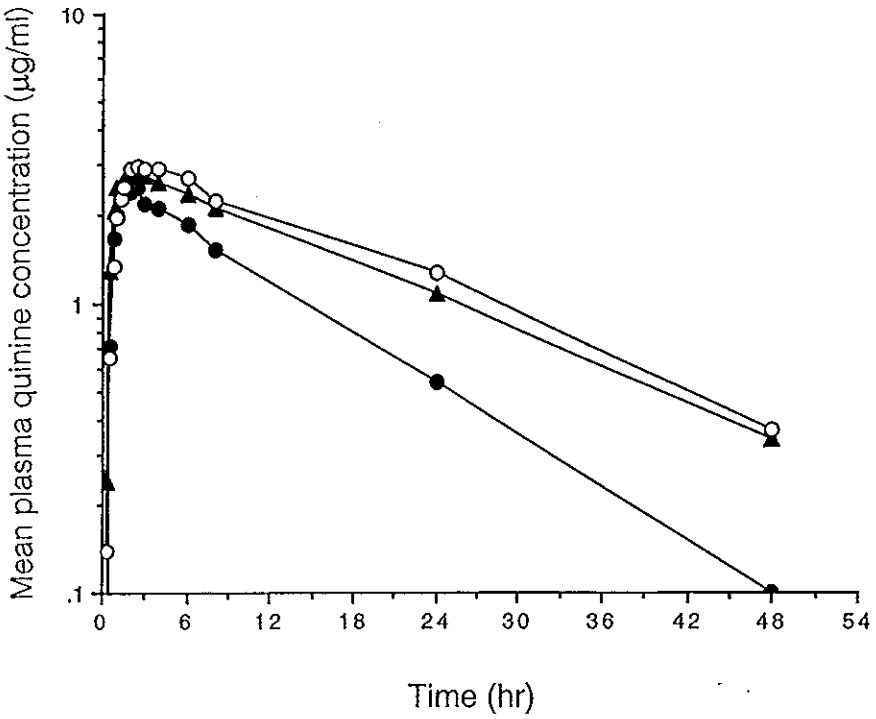


**Figure 10** Semi-logarithmic mean phasma quinine concentration at 0-48 hours after a single oral dose of 300 mg quinine alone ( ○ ) and pretreatment with 400 mg ketoconazole ( ● ) or 200 mg itraconazole (▲) orally for 4 days.

**Table 6** Pharmacokinetic parameters (mean ± S.D.) of quinine in nine subjects recieving a single oral dose of 300 mg quinine alone and after pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

| Parameter | Quinine alone | Quinine + Ketoconazole [a] | Quinine+ Itraconazole [a] |
|---|---|---|---|
| AUC (mg/l.hr) | 36.06 ± 14 | 74.76 ± 26.9** | 70.80 ± 35.76** |
| Ka ($hr^{-1}$) | 2.63 ± 1.7 | 1.85 ± 0.72 | 2.09 ± 1.41 |
| Ke($hr^{-1}$) | 0.08 ± 0.02 | 0.05 ± 0.02** | 0.051 ± 0.02* |
| $t_{1/2}$(hr) | 9.00 ± 2.13 | 15.26 ± 4.9** | 15.40 ± 7.2** |
| $T_{max}$ (hr) | 1.76 ± 0.59 | 2.80 ± 0.83** | 2.23 ± 0.87 |
| $C_{max}$ (mg/l) | 2.35 ± 0.52 | 3.14 ± 0.54** | 2.84 ± 0.76 |
| Vd/f (l/kg) | 1.97 ± 0.61 | 1.53 ± 0.27** | 1.75 ± 0.62 |
| CL/f (l/hr/kg) | 0.17 ± 0.09 | 0.077 ± 0.03** | 0.096 ± 0.067** |

*P < 0.05, **P < 0.01, significantly different compared with the administration of quinine alone.

[a] There were no significant differences in all pharmacokinetic parameters in quinine - ketoconazole phase compared to those of quinine - itraconazole phase.

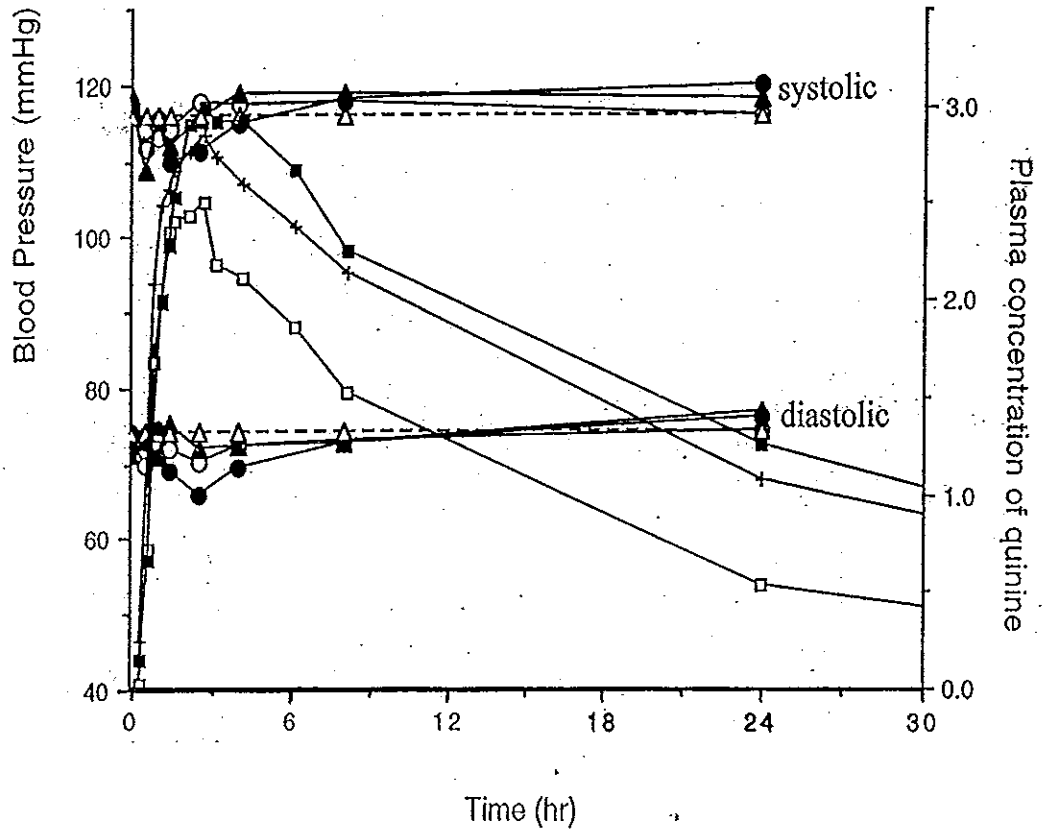


**Figure 11** The mean systolic and diastolic blood pressure before (control, Δ) and after receiving a single oral dose of quinine alone (○), and pretreatment with 400 mg ketoconazole (●) or 200 mg itraconazole (▲) orally for 4 days. Compared with plasma concentrarion in various times after receiving a single oral dose of quinine alone (□) and pretreatment with 400 mg ketoconazole (■) or 200 mg itraconazole (×) orally for 4 days.

**Table 7** The systolic and diastolic blood pressure (mmHg, Mean ± S.D.) before (control) and after receiving a single oral dose of 300 mg quinine alone, and pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

| Time (hr) | Systolic / Diastolic blood pressure (mmHg) | | | |
|---|---|---|---|---|
| | Control | Quinine alone | Quinine + Ketoconazole[a] | Quinine + itraconazole[a] |
| 0 | 116 ± 8 / 74 ± 7 | 116 ± 8 / 74 ± 7 | 117 ± 12 / 72 ± 12 | 118 ± 9 / 71 ± 7 |
| 0.5 | - | 112 ± 8 / 70 ± 7 | 117 ± 12 / 74 ± 12 | 109 ± 10* / 73 ± 7 |
| 1 | - | 113 ± 8 / 72 ± 9 | 116 ± 13 / 75 ± 13 | 115 ± 9 / 71 ± 8 |
| 1.5 | - | 114 ± 7 / 72 ± 6 | 110 ± 11 / 69 ± 11 | 112 ± 5 / 75 ± 8 |
| 2.5 | - | 117 ± 8 / 70 ± 8 | 111 ± 9 / 66 ± 8* | 116 ± 7 / 72 ± 9 |
| 4 | - | 116 ± 6 / 72 ± 9 | 115 ± 10 / 70 ± 9 | 119 ± 5 / 72 ± 8 |
| 8 | - | 118 ± 10 / 73 ± 9 | 118 ± 10 / 73 ± 11 | 119 ± 8 / 73 ± 8 |
| 24 | - | 117 ± 7 / 74 ± 7 | 120 ± 7 / 76 ± 10 | 118 ± 8 / 77 ± 7 |

*$P < 0.05$, significantly different compared with control

[a]There were no significant differences in all blood pressure in quinine - ketoconazole or itraconazole phase at the time measured compared with quinine alone.

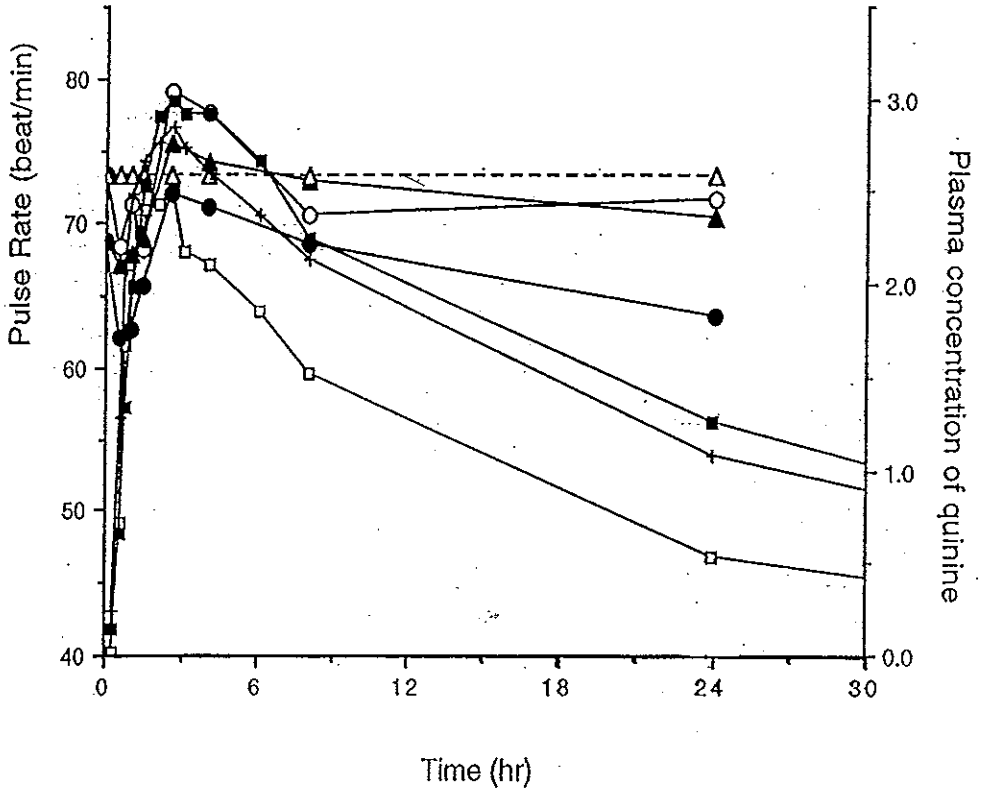


**Figure 12** The pulse rate (beat/min, mean ± S.D.) before (control, Δ ), after receiving 300 mg a single oral dose of quinine alone ( ○ ), pretreatment with 400 mg ketoconazole ( ● ) or 200 mg itraconazole ( ▲ ) orally for 4 days.Compared with plasma concentrarion in various times after receiving a single oral dose of quinine alone ( □ ) and pretreatment with 400 mg ketoconazole ( ■ ) or 200 mg itraconazole ( × ) orally for 4 days.

**Table 8** The pulse rate (beat/min, mean ± S.D.) before (control) and after receiving 300 mg a single oral dose of quinine alone and after pretreatment with 400 mg ketoconazole or 200 mg itraconazole orally for 4 days.

| Time (hr) | Pulse rate (beat/min) | | | |
|---|---|---|---|---|
| | Control | Quinine alone | Quinine+ Ketoconazole[a] | Quinine + Itraconazole[a] |
| 0 | 73 ± 10 | 73 ± 10 | 69 ± 9 | 69 ± 9 |
| 0.5 | - | 68 ± 6* | 62 ± 5*† | 67 ± 6 |
| 1 | - | 71.25 ± 9.9 | 63 ± 6**† | 68 ± 6 |
| 1.5 | - | 68.22 ± 3.38* | 66 ± 5* | 69 ± 7 |
| 2.5 | - | 79 ± 7.62 | 72 ± 7†† | 75 ± 6 |
| 4 | - | 77.67 ± 5.87 | 71 ± 4†† | 74 ± 5 |
| 8 | - | 70.56 ± 6.69 | 69 ± 6 | 74 ± 9 |
| 24 | - | 71.67 ± 7.19 | 64 ± 7**† | 70 ± 7 |

*$P < 0.05$, **$P < 0.01$, significantly different compared with control.

†$P < 0.05$, ††$P < 0.01$, significant difference compared with quinine alone at the time measured.

[a]There were no significant differences in pulse rates between quinine-ketoconazole and quinine-itraconazole phase at the time measured.

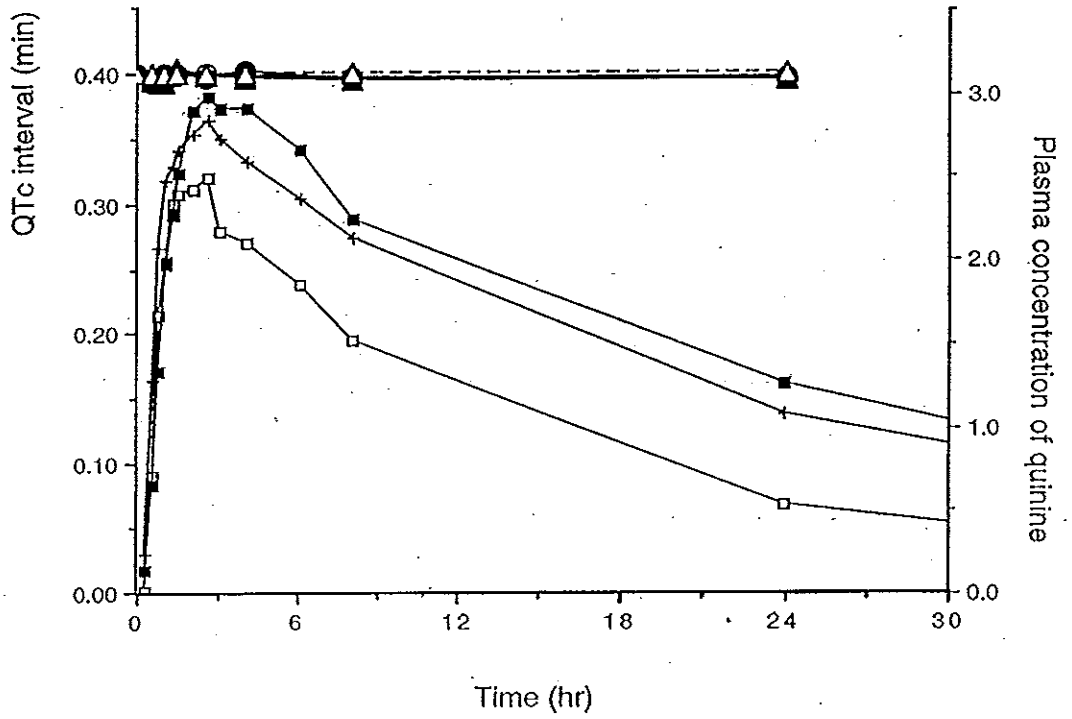


**Figure 13** The $QT_c$ (mean ± S.D.) interval before (control, Δ ) and after receiving a single oral dose of 300 mg quinine alone ( ○ ) and pretreatment with 400 mg ketoconazole ( ● ) or 200 mg itraconazole ( ▲ ) orally for 4 days. Compared with plasma concentrarion in various times after receiving a single oral dose of quinine alone ( □ ) and pretreatment with 400 mg ketoconazole ( ■ ) or 200 mg itraconazole ( × ) orally for 4 days.

**Table 9** The $QT_c$ (mean ± S.D.) interval before (control) and after receiving a single oral dose of 300 mg quinine alone, pretreatment with 400 mg ketoconazole and 200 mg itraconazole orally for 4 days.

| Time (hr) | $QT_c$ interval (min) | | | |
|---|---|---|---|---|
| | Control | Quinine alone | Quinine + Ketoconazole[a] | Quinine + Itraconazole[a] |
| 0 | 0.40 ± 0.01 | 0.40 ± 0.01 | 0.40 ± 0.01 | 0.40 ± 0.01 |
| 0.5 | - | 0.4 0 ± 0.01 | 0.40 ± 0.08 | 0.40 ± 0.02 |
| 1 | - | 0.4 0 ± 0.01 | 0.40 ± 0.01 | 0.39 ± 0.02 |
| 1.5 | - | 0.4 0 ± 0.01 | 0.40 ± 0.01 | 0.40 ± 0.01 |
| 2.5 | - | 0.40 ± 0.11 | 0.40 ± 0.01 | 0.40 ± 0.01 |
| 4 | - | 0.40 ± 0.01 | 0.40 ± 0.01 | 0.40 ± 0.01 |
| 8 | - | 0.40 ± 0.01 | 0.39 ± 0.01 | 0.40 ± 0.01 |
| 24 | - | 0.40 ± 0.01 | 0.39 ± 0.01 | 0.40 ± 0.01 |

[a]There were no significant differences in $QT_c$ in quinine - ketoconazole or quinine - itraconazole phase at the time measured compared with quinine alone.

**Table 10** Pharmacokinetic parameters of a single oral dose of 300 mg quinine alone in nine healthy volunteers at time interval of 0-48 hours.

| Parameter | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| AUC (mg/l.hr) | 51.7 | 26.81 | 31.00 | 24.50 | 32.64 | 16.49 | 33 | 51.37 | 56.88 | 36.06 ± 14 |
| Ka ($hr^{-1}$) | 3.65 | 2.90 | 1.0 | 1.79 | 0.52 | 1.81 | 3.82 | 2.10 | 6.07 | 2.63 ± 1.7 |
| Ke($hr^{-1}$) | 0.057 | 0.11 | 0.09 | 0.09 | 0.075 | 0.105 | 0.075 | 0.064 | 0.06 | 0.08 ± 0.02 |
| $t_{1/2}$(hr) | 12.13 | 6.28 | 7.36 | 7.74 | 9.27 | 6.60 | 9.24 | 10.91 | 11.36 | 9.00 ± 2.13 |
| $T_{max}$ (hr) | 1.50 | 1.50 | 0.84 | 2.40 | 2.71 | 2.08 | 1.42 | 2.12 | 1.23 | 1.76 ± 0.59 |
| $C_{max}$ (mg/l) | 2.77 | 2.60 | 2.79 | 1.87 | 2.06 | 1.46 | 2.28 | 2.93 | 3.31 | 2.35 ± 0.52 |
| Vd/f (l/kg) | 1.67 | 1.69 | 2.29 | 2.18 | 1.79 | 3.33 | 2.07 | 1.51 | 1.24 | 1.97 ± 0.61 |
| CL/f (l/hr/kg) | 0.10 | 0.19 | 0.21 | 0.194 | 0.134 | 0.35 | 0.152 | 0.096 | 0.075 | 0.17 ± 0.08 |
| Lag time(hr) | 0.43 | 0.33 | 0.37 | 0.65 | 0.10 | 0.43 | 0.371 | 0.403 | 0.462 | 0.39 ± 0.14 |

**Table 11** Pharmacokinetic parameters of a single oral dose of 300 mg quinine after pretreatment with 400 mg ketoconazole orally for 4 days in nine healthy volunteers at time interval of 0-48 hours.

| Parameter | Subjects No. | | | | | | | | | Mean±S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| AUC (mg/l.hr) | 105.35 | 58.00 | 87.00 | 37.26 | 59.43 | 45.62 | 70.70 | 112.34 | 97.06 | 74.76 ± 26.9 |
| Ka ($hr^{-1}$) | 2.83 | 1.85 | 0.87 | 0.95 | 1.15 | 1.81 | 2.27 | 2.45 | 2.48 | 1.85 ± 0.72 |
| Ke($hr^{-1}$) | 0.034 | 0.05 | 0.043 | 0.073 | 0.05 | 0.066 | 0.068 | 0.031 | 0.034 | 0.05 ± 0.02 |
| $t_{1/2}$(hr) | 20.27 | 13.50 | 15.98 | 9.49 | 14.51 | 10.50 | 10.19 | 22.36 | 20.564 | 15.26 ± 4.9 |
| $T_{max}$ (hr) | 2.26 | 2.56 | 4.00 | 4.07 | 3.31 | 2.33 | 1.72 | 2.14 | 2.85 | 2.80 ± 0.83 |
| $C_{max}$ (mg/l) | 3.41 | 2.69 | 3.23 | 2.21 | 2.47 | 2.67 | 4.3 | 3.31 | 2.99 | 3.14 ± 0.54 |
| Vd/f (l/kg) | 1.38 | 1.72 | 1.70 | 1.78 | 1.52 | 1.91 | 1.04 | 1.42 | 1.31 | 1.53 ± 0.27 |
| CL/f (l/hr/kg) | 0.047 | 0.086 | 0.073 | 0.128 | 0.073 | 0.13 | 0.071 | 0.044 | 0.044 | 0.077 ± 0.032 |
| Lag time (hr) | 0.69 | 0.57 | 0.38 | 1.15 | 0.429 | 0.432 | 0.125 | 0.34 | 0.235 | 0.48 ± 0.30 |

**Table 12** Pharmacokinetic parameters of a single oral dose of 300 mg quinine and after pretreatment with 200 mg itraconazole orally for 4 days in nine healthy volunteers at time interval of 0-48 hours.

| Parameter | Subject No. | | | | | | | | | Mean±S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| AUC (mg/l.hr) | 82.37 | 44.08 | 68.98 | 34.00 | 61.96 | 23 | 77.09 | 129.86 | 116.78 | 70.80 ± 35.76 |
| Ka ($hr^{-1}$) | 4.39 | 4.13 | 1.69 | 0.68 | 3.39 | 1.73 | 2.82 | 1.47 | 1.51 | 2.09 ± 1.41 |
| Ke($hr^{-1}$) | 0.043 | 0.06 | 0.052 | 0.065 | 0.056 | 0.08 | 0.051 | 0.022 | 0.033 | 0.051 ± 0.02 |
| $t_{1/2}$(hr) | 16.29 | 11.67 | 13.47 | 10.66 | 12.29 | 8.66 | 13.59 | 31.50 | 20.81 | 15.40 ± 7.2 |
| $T_{max}$ (hr) | 1.23 | 1.46 | 2.38 | 3.84 | 1.53 | 1.99 | 1.75 | 3.15 | 2.74 | 2.23 ± 0.87 |
| $C_{max}$ (mg/l) | 3.34 | 2.57 | 3.19 | 1.73 | 3.22 | 1.59 | 3.65 | 2.70 | 3.56 | 2.84 ± 0.76 |
| Vd/f (l/kg) | 1.40 | 1.90 | 1.79 | 2.15 | 1.27 | 3.13 | 1.28 | 1.73 | 1.12 | 1.75 ± 0.62 |
| CL/f (l/hr/kg) | 0.06 | 0.114 | 0.093 | 0.14 | 0.071 | 0.25 | 0.065 | 0.038 | 0.037 | 0.096 ± 0.067 |
| Lag time (hr) | 0.166 | 0.43 | 0.243 | 0.015 | 0.31 | 0.127 | 0.3 | 0.261 | 0.155 | 0.22 ± 0.12 |

# CHAPTER 5

# DISCUSSION AND CONCLUSION

Quinine, the cinchona alkaloid, has been used for malarial treatment more than 350 years. It is recommended for treatment of chloroquine-resistant *P. falciparum* malaria, an important drug of choice for treatment of complicated and/or cerebral malaria (Tracy and Webster, 1996; WHO, 1990), and is also used for relief nocturnal leg cramps (Mackie and Davidson, 1995). Quinine has a narrow therapeutic window and its toxicity is serious, especially cardiotoxicity. It is mainly metabolized to 3-hydroxyquinine by CYP3A4 and its metabolism is inhibited by many drugs such as ketoconazole both *in vitro* (Zhao and Ishizaki, 1997) and *in vivo* (Mirghani *et al.*, 1999), etoposide (Zhao *et al.*, 1997), and cimetidine (Wanwimolruk *et al.*, 1986).

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

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

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

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

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

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

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

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

The present results revealed that after pretreatment with 400 mg ketoconazole for 4 days, the mean AUC increased by 107% (2-fold), the mean $T_{max}$ increased by 59% (1.6-fold), the mean $C_{max}$ increased by 34% (1.3-fold), the mean Ke reduced by 38% (1.6-fold), the mean $t_{1/2}$ increased by 70% (1.7-fold), the mean Vd/f increased by 22% (1.3-fold) and the mean CL/f reduced

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

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

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

There was significantly decreased in Vd/f in ketoconazole pretreated group. The alteration in Vd/f of quinine may caused by (a) increase plasma protein binding or decrease quinine tissue binding, (b) circulatory changes, (c) liver blood flow and (d) competitive bound with P-glycoprotein, which is

function as an efflux pump of some substrate of P-glycoprotein such as saquinavir, itraconazole, rhodamine and doxorubicin (Eagling *et al.*, 1999 ; Miyama *et al.*, 1998 ; Yamamoto *et al.*, 1999 ; Smit *et al.*, 1998)

(a) Ketoconazole increased quinine plasma protein binding or decrease tissue binding. Quinine and ketoconazole are extensively bound to plasma protein (69-92% and more than 99%, respectively), the major protein was $\alpha_1$-acid glycoprotein for quinine and albumin for ketoconazole, respectively. Less is known about quinine tissue binding or how ketoconazole affects it but Brown *et al.* (1985) showed ketoconazole had no effect on chlordiazepoxide (the major binding of albumin) binding to plasma protein.

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

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

(d) Competitive by bound with P-glycoprotein resulted that ketoconazole might limit accession of quinine to certain body compartments i.e. CSF, liver. Coadministration of ketoconazole with some substrates of P-glycoprotein demonstrates that ketoconazole inhibit P-glycoprotein leading to the accumulation of those P-glycoprotein substrates in

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

For the itraconazole-quinine interaction, the mean AUC of quinine after pretreatment with itraconazole was increased by 96% (2-fold), Ke decreased by 36% (1.6-fold), $t_{1/2}$ increased by 71% (1.7-fold) and CL/f decreased by 44% (1.8-fold). The plasma concentration time-curve of itraconazole in healthy volunteers was wide inter-individual variation (Hardin *et al.*, 1988). The mean AUC, Ke, $t_{1/2}$ and CL/f of quinine after pretreatment with itraconazole were significantly different from quinine alone, but there were no significant differences from those pretreated with ketoconazole. The mean AUC and $C_{max}$ after pretreatment with itraconazole were slightly less than pretreatment with ketoconazole, which is similar to the study of Olkkola, *et al.* (1994). Itraconazole also increased $t_{1/2}$ and decreased quinine clearance which was similar to the results previously reviewed, and Vd/f of quinine was slightly decreased. So, it indicated that itraconazole may competitive with quinine to bind with P-glycoprotein in similar manner of ketoconazole.

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

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

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

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

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

Our present results found that mean peak plasma concentration of quinine in subjects administered a single oral dose of 300 mg quinine alone was 2.35 μg/ml, and after pretreatment with ketoconazole and itraconazole

were 3.14 and 2.84 μg/ml, respectively. The intention of this study is to observe only quinine pharmacokinetics without producing any side effects. For these reasons, we used a 300 mg quinine dose instead of 600 mg dose and expected that after pretreatment with ketoconazole or itraconazole the peak plasma concentration of quinine would not be high enough to produce toxicities, especially cardiotoxicity.

Generally, the effective plasma quinine concentration was 8-15 μg/ml and after 600 mg oral dose of quinine sulphate the plasma concentration may reach 15-20 μg/ml. Mild toxicity usually occurred at plasma quinine concentration above 10 μg/ml (Powell and McNamara, 1972) and cardiovascular toxicity was observed when the plasma concentrations was above 16 μg/ml. Karbwang *et al.* (1993b) reported that the electrocardiogram may change in healthy volunteers with quinine concentrations around 5 μg/ml after 10 mg/kg intravenously infused. However, if we double the dose of quinine to 600 mg dose, the peak plasma quinine concentration after pretreatment with ketoconazole and itraconazole would be 6.28 and 5.7 μg/ml, respectively, which seem likely to produce electrocardiogram changes.

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

coadministration of quinine and azole antifungal agents (ketoconazole or itraconazole) in clinical practice.

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

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# APPENDIX

## APPENDIX-1

Preparation of standard quinine and internal standard quinidine in blank plasma for standard curve in each day.

Stock A = Quinine 1000 µg/ml

Stock B = Quinine 100 µg/ml

Stock C = Quinidine 1000 µg/ml

Stock D = Quinidine 100 µg/ml

Quinine

10 µg/ml = blank plasma 750 µl + 750 µl of 20 µg/ml

5 µg/ml = blank plasma 750 µl + 750 µl of 10 µg/ml

2.5 µg/ml = blank plasma 750 µl + 750 µl of 5 µg/ml

1 µg/ml = blank plasma 900 µl + 600 µl of 2.5 µg/ml

0.5 µg/ml = blank plasma 750 µl + 750 µl of 1 µg/ml

Quinidine

25 µg/ml = Mobile phase 1125 µl + 375 µl of 100 µg/ml quinidine

# APPENDIX-2

Protein precipitation method

500 µl plasma or spiked standard quinine (400 µl) and internal standard quinidine (100 µl)

vortex 30 seconds

↓

added dropwise 250 µl acetonitrile

↓

vortex 30 seconds

↓

kept for 10 minutes

↓

cetrifuged at 10,000 g for 15 minutes

↓

suppernatant was injected

# APPENDIX-3

Plasma concentrations of quinine (μg/ml) at time interval 0 - 48 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate alone.

| Time | Plasma concentration of quinine in subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.65 | 1.15 | 2.10 | 0 | 0.25 | 0 | 0.95 | 0.65 | 0.70 | 0.72 ± 0.65 |
| 0.75 | 1.75 | 1.90 | 2.75 | 0.50 | 0.65 | 1.00 | 1.90 | 1.60 | 3.00 | 1.68 ± 0.87 |
| 1 | 2.60 | 2.50 | 2.60 | 0.70 | 1.20 | 1.10 | 2.05 | 2.25 | 3.00 | 2.00 ± 0.80 |
| 1.25 | 2.95 | 2.60 | 3.40 | 1.50 | 1.65 | 1.15 | 2.40 | 2.50 | 3.00 | 2.35 ± 0.76 |
| 1.5 | 3.00 | 2.60 | 2.40 | 1.70 | 1.65 | 1.30 | 2.75 | 2.90 | 3.40 | 2.41 ± 0.71 |
| 2 | 3.00 | 2.55 | 2.40 | 2.00 | 1.75 | 1.40 | 2.20 | 3.10 | 3.45 | 2.43 ± 0.67 |
| 2.5 | 2.70 | 2.55 | 2.40 | 1.90 | 2.95 | 1.55 | 2.20 | 3.20 | 3.05 | 2.50 ± 0.54 |
| 3 | 2.65 | 2.15 | 2.10 | 1.70 | 1.75 | 1.35 | 2.05 | 2.95 | 3.05 | 2.19 ± 0.58 |
| 4 | 2.45 | 1.90 | 2.00 | 1.60 | 1.75 | 1.35 | 1.80 | 3.35 | 2.75 | 2.11 ± 0.63 |
| 6 | 2.45 | 1.80 | 1.80 | 1.50 | 1.65 | 1.05 | 1.70 | 2.05 | 2.70 | 1.86 ± 0.49 |
| 8 | 1.95 | 1.25 | 1.50 | 1.20 | 1.40 | 0.80 | 1.50 | 2.00 | 2.20 | 1.53 ± 0.45 |
| 24 | 0.90 | 0.30 | 0.40 | 0.30 | 0.60 | 0 | 0.40 | 1.10 | 0.80 | 0.53 ± 0.35 |
| 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# APPENDIX-4

Plasma concentrations of quinine (μg/ml) at time interval 0 - 48 hours in nine subjects after pretreatment with 400 mg ketoconazole (Nizoral®) for 4 days and receiving a single oral dose of 300 mg quinine sulphate on the study day 4.

| Time (hr) | Plasma concentration of quinine in subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0 | 0.14 ± 0.42 |
| 0.5 | 0.40 | 0 | 0 | 0 | 0.40 | 0.45 | 2.45 | 1.15 | 1.05 | 0.66 ± 0.79 |
| 0.75 | 0.60 | 0.95 | 1.20 | 0 | 0.55 | 0.95 | 3.80 | 2.15 | 1.9 | 1.34 ± 1.14 |
| 1 | 2.00 | 1.40 | 1.80 | 0 | 1.40 | 2.35 | 3.90 | 2.75 | 2.35 | 1.99 ± 1.07 |
| 1.25 | 3.00 | 2.10 | 1.90 | 0.30 | 1.70 | 1.95 | 4.00 | 3.20 | 2.4 | 2.28 ± 1.05 |
| 1.5 | 3.00 | 2.50 | 2.00 | 0.70 | 1.95 | 2.65 | 4.35 | 3.20 | 2.45 | 2.53 ± 1.00 |
| 2 | 3.60 | 2.6 | 2.80 | 1.40 | 2.30 | 2.75 | 4.85 | 3.20 | 2.65 | 2.91 ± 0.95 |
| 2.5 | 3.50 | 2.90 | 3.00 | 1.95 | 2.50 | 2.50 | 4.30 | 3.25 | 3.05 | 2.99 ± 0.67 |
| 3 | 3.30 | 2.70 | 3.10 | 2.10 | 2.60 | 2.50 | 3.80 | 3.35 | 3.05 | 2.93 ± 0.55 |
| 4 | 3.20 | 2.50 | 3.30 | 2.20 | 2.40 | 2.45 | 3.70 | 3.30 | 3.2 | 2.92 ± 0.53 |
| 6 | 3.00 | 2.30 | 3.00 | 2.00 | 2.20 | 2.20 | 3.60 | 2.95 | 2.85 | 2.68 ± 0.53 |
| 8 | 2.70 | 1.90 | 2.90 | 1.80 | 1.90 | 2.15 | 2.00 | 2.65 | 2.35 | 2.26 ± 0.41 |
| 24 | 1.80 | 0.90 | 1.50 | 0.60 | 1.10 | 0.50 | 1.65 | 1.70 | 1.6 | 1.26 ± 0.49 |
| 48 | 0.65 | 0.50 | 0.40 | 0 | 0.25 | 0 | 0 | 0.85 | 0.6 | 0.36 ± 0.32 |

# APPENDIX-5

Plasma concentrations of quinine (μg/ml) at time interval 0 - 48 hours in nine subjects after pretreatment with 200 mg itraconazole (Sporal®) for 4 days and receiving a single oral dose of 300 mg quinine sulphate on the study day 4.

| Time (hr) | Plasma concentration of quinine in subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 1.00 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0 | 0.65 | 0.24 ± 0.36 |
| 0.5 | 2.6 | 0.70 | 1.25 | 0.55 | 1.65 | 0.95 | 1.6 | 0.9 | 1.40 | 1.29 ± 0.62 |
| 0.75 | 3.20 | 2.00 | 1.95 | 0.95 | 2.65 | 1.25 | 3.05 | 1.30 | 2.40 | 2.08 ± 0.81 |
| 1 | 3.20 | 2.50 | 2.90 | 1.10 | 3.00 | 1.30 | 3.25 | 2.10 | 2.65 | 2.49 ± 0.81 |
| 1.25 | 3.90 | 2.40 | 2.50 | 1.30 | 3.20 | 1.45 | 3.25 | 2.15 | 3.05 | 2.58 ± 0.86 |
| 1.5 | 3.50 | 2.50 | 3.00 | 1.30 | 3.25 | 1.50 | 3.5 | 2.20 | 3.30 | 2.67 ± 0.86 |
| 2 | 3.20 | 2.50 | 3.00 | 1.40 | 3.20 | 1.55 | 3.9 | 2.65 | 3.50 | 2.77 ± 0.84 |
| 2.5 | 3.20 | 2.50 | 3.25 | 1.55 | 3.20 | 1.66 | 3.6 | 2.80 | 3.90 | 2.85 ± 0.81 |
| 3 | 2.90 | 2.60 | 2.95 | 1.65 | 2.95 | 1.70 | 3.55 | 2.70 | 3.40 | 2.74 ± 0.75 |
| 4 | 2.90 | 2.30 | 2.95 | 1.70 | 2.95 | 1.30 | 3.35 | 2.65 | 3.40 | 2.61 ± 0.71 |
| 6 | 2.60 | 1.90 | 2.90 | 1.60 | 2.45 | 1.25 | 2.9 | 2.50 | 3.35 | 2.38 ± 0.68 |
| 8 | 2.40 | 1.5 | 2.85 | 1.60 | 2.10 | 1.00 | 2.6 | 2.45 | 2.80 | 2.14 ± 0.64 |
| 24 | 1.40 | 0.70 | 1.00 | 0.50 | 1.00 | 0.30 | 1.25 | 1.70 | 1.90 | 1.08 ± 0.53 |
| 48 | 0.60 | 0.30 | 0 | 0 | 0.35 | 0 | 0 | 1.05 | 0.80 | 0.34 ± 0.39 |

# APPENDIX-6

Systolic and diastolic blood pressure at time interval 0 - 24 hours in nine subjects after receiving a single oral dose of 300 mg quinine sulphate alone.

| Time (hr) | Subject No. | | | | | | | | | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| 0 | 122 / 80 | 116 / 74 | 122 / 64 | 114 / 70 | 108 / 71 | 109 / 75 | 123 / 80 | 104 / 68 | 126 / 86 | 116 / 74 | 8 / 7 |
| 0.5 | 115 / 75 | 108 / 68 | 106 / 57 | 111 / 72 | 115 / 68 | 104 / 66 | 117 / 79 | 101 / 65 | 129 / 80 | 112 / 70 | 8 / 7 |
| 1 | 116 / 75 | 120 / 68 | 115 / 59 | 105 / 73 | 115 / 70 | 107 / 69 | 116 / 84 | 102 / 66 | 127 / 85 | 113 / 72 | 8 / 9 |
| 1.5 | 114 / 73 | 124 / 79 | 113 / 62 | 110 / 74 | 125 / 78 | 108 / 65 | 116 / 79 | 101 / 69 | 115 / 69 | 114 / 72 | 7 / 6 |
| 2.5 | 110 / 70 | 120 / 61 | 118 / 69 | 112 / 63 | 133 / 79 | 112 / 62 | 123 / 77 | 107 / 70 | 124 / 83 | 118 / 70 | 8 / 8 |
| 4 | 117 / 79 | 114 / 63 | 118 / 68 | 116 / 68 | 130 / 78 | 114 / 61 | 117 / 74 | 106 / 70 | 125 / 89 | 117 / 72 | 7 / 9 |
| 8 | 114 / 69 | 111 / 71 | 110 / 57 | 115 / 74 | 140 / 90 | 114 / 69 | 125 / 78 | 110 / 69 | 122 / 82 | 118 / 73 | 10 / 9 |
| 24 | 107 / 80 | 112 / 71 | 123 / 64 | 114 / 71 | 124 / 80 | 109 / 66 | 121 / 81 | 113 / 71 | 121 / 83 | 116 / 74 | 6 / 7 |

# APPENDIX-7

Systolic and diastolic blood pressure at time interval 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 400 mg ketoconazole (Nizoral®) for 4 days.

| Time | Subject No. | | | | | | | | | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| 0 | 122 / 76 | 112 / 74 | 110 / 59 | 115 / 71 | 131 / 89 | 101 / 55 | 129 / 85 | 101 / 64 | 129 / 76 | 117 / 72 | 12 / 11 |
| 0.5 | 115 / 76 | 126 / 86 | 101 / 57 | 117 / 77 | 133 / 86 | 102 / 59 | 122 / 89 | 92 / 58 | 120 / 78 | 114 / 74 | 13 / 13 |
| 1 | 114 / 76 | 122 / 86 | 101 / 58 | 116 / 69 | 128 / 83 | 100 / 57 | 129 / 85 | 94 / 55 | 121 / 86 | 116 / 75 | 13 /13 |
| 1.5 | 120 / 69 | 115 / 77 | 99 / 56 | 110 / 68 | 126 / 83 | 99 / 63 | 116 / 76 | 91 / 51 | 113 / 80 | 110 / 69 | 11 / 11 |
| 2.5 | 113 / 70 | 117 / 76 | 113 / 55 | 120 / 63 | 110 / 70 | 101 / 55 | 126 / 72 | 98 / 60 | 123 / 71 | 113 / 66 | 9 / 8 |
| 4 | 113 / 70 | 125 / 80 | 115 / 60 | 118 / 69 | 124 / 81 | 105 / 62 | 120 / 74 | 93 / 54 | 121 / 76 | 115 / 70 | 10 / 9 |
| 8 | 113 / 83 | 121 / 76 | 107 / 54 | 118 / 72 | 137 / 86 | 106 / 58 | 118 / 71 | 117 / 76 | 126 / 80 | 118 / 73 | 10 / 11 |
| 24 | 123 / 89 | 119 / 73 | 109 / 66 | 128 / 72 | 126 / 87 | 109 / 60 | 123 / 74 | 116 / 75 | 125 / 87 | 120 / 76 | 7 / 10 |

# APPENDIX-8

Systolic and diastolic blood pressure at time interval 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 200 mg itraconazole (Sporal®) for 4 days.

| Time | Subject No. | | | | | | | | | Mean | S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | | |
| 0 | 115 / 70 | 121 / 61 | 114 / 64 | 113 / 71 | 108 / 71 | 111 / 70 | 133 / 76 | 106 / 69 | 121 / 78 | 118 / 71 | 9 / 7 |
| 0.5 | 101 / 71 | 114 / 75 | 104 / 70 | 108 / 75 | 115 / 68 | 93 / 62 | 120 / 83 | 105 / 66 | 111 / 72 | 109 / 73 | 10 / 7 |
| 1 | 116 / 74 | 127 / 65 | 100 / 57 | 110 / 66 | 114 / 70 | 109 / 70 | 116 / 81 | 105 / 67 | 124 / 74 | 115 / 71 | 9 / 8 |
| 1.5 | 112 / 70 | 118 / 79 | 105 / 59 | 112 / 70 | 125 / 78 | 110 / 76 | 118 / 80 | 110 / 75 | 105 / 87 | 112 / 75 | 5 / 8 |
| 2.5 | 120 / 73 | 118 / 70 | 115 / 59 | 117 / 71 | 133 / 79 | 117 / 73 | 123 / 78 | 100 / 60 | 111 / 85 | 116 / 72 | 7 / 9 |
| 4 | 122 / 74 | 117 / 72 | 114 / 62 | 120 / 65 | 130 / 78 | 125 / 74 | 118 / 72 | 110 / 67 | 121 / 88 | 119 / 72 | 5 / 8 |
| 8 | 120 / 70 | 124 / 79 | 112 / 58 | 121 / 78 | 140 / 90 | 113 / 68 | 124 / 77 | 104 / 67 | 125 / 75 | 119 / 73 | 8 / 8 |
| 24 | 104 / 78 | 122 / 82 | 114 / 64 | 110 / 71 | 124 / 80 | 120 / 71 | 129 / 79 | 113 / 75 | 127 / 84 | 118 / 77 | 8 / 7 |

# APPENDIX-9

Pulse rate at time interval 0 - 24 hours in nine subjects after receiving a single oral dose of 300 mg quinine sulphate alone.

| Time | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 66 | 64 | 81 | 71 | 92 | 66 | 71 | 65 | 83 | 73 ± 10 |
| 0.5 | 63 | 65 | 72 | 62 | 80 | 64 | 69 | 65 | 76 | 68 ± 6 |
| 1 | 65 | 62 | 84 | 63 | 72 | 66 | 74 | 62 | 87 | 71 ± 10 |
| 1.5 | 67 | 66 | 75 | 65 | 71 | 67 | 71 | 76 | 65 | 69 ± 4 |
| 2.5 | 76 | 69 | 81 | 74 | 79 | 87 | 78 | 73 | 94 | 79 ± 8 |
| 4 | 72 | 71 | 85 | 70 | 79 | 81 | 79 | 76 | 86 | 78 ± 6 |
| 8 | 71 | 59 | 69 | 71 | 67 | 66 | 78 | 82 | 72 | 71 ± 7 |
| 24 | 70 | 59 | 74 | 70 | 75 | 66 | 69 | 78 | 84 | 72 ± 7 |

# APPENDIX-10

Pulse rate at time interval 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 400 mg ketoconazole (Nizoral®) for 4 days.

| Time | Subject no. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 73 | 59 | 69 | 68 | 81 | 67 | 70 | 63 | 70 | 69 ± 6 |
| 0.5 | 60 | 61 | 58 | 64 | 70 | 64 | 70 | 59 | 54 | 62 ± 5 |
| 1 | 64 | 65 | 60 | 59 | 74 | 61 | 65 | 57 | 56 | 63 ± 6 |
| 1.5 | 70 | 64 | 64 | 57 | 70 | 65 | 74 | 67 | 60 | 66 ± 5 |
| 2.5 | 68 | 70 | 76 | 70 | 84 | 62 | 77 | 67 | 75 | 72 ± 7 |
| 4 | 63 | 72 | 71 | 74 | 72 | 69 | 76 | 68 | 76 | 71 ± 4 |
| 8 | 62 | 65 | 69 | 66 | 78 | 70 | 76 | 71 | 61 | 69 ± 4 |
| 24 | 61 | 61 | 60 | 72 | 69 | 57 | 74 | 62 | 56 | 64 ± 7 |

# APPENDIX-11

Pulse rate at time interval 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 200 mg itraconazole (Sporal®) for 4 days.

| Time | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 67 | 68 | 67 | 61 | 92 | 70 | 86 | 67 | 65 | 69 ± 9 |
| 0.5 | 64 | 60 | 67 | 65 | 80 | 62 | 80 | 61 | 69 | 67 ± 6 |
| 1 | 69 | 64 | 66 | 58 | 72 | 70 | 74 | 62 | 71 | 68 ± 6 |
| 1.5 | 72 | 64 | 68 | 72 | 71 | 76 | 82 | 62 | 62 | 69 ± 7 |
| 2.5 | 67 | 75 | 70 | 70 | 79 | 73 | 85 | 76 | 75 | 75 ± 6 |
| 4 | 68 | 77 | 69 | 70 | 79 | 74 | 79 | 67 | 77 | 74 ± 6 |
| 8 | 75 | 87 | 72 | 69 | 67 | 68 | 88 | 64 | 69 | 74 ± 9 |
| 24 | 63 | 65 | 77 | 64 | 75 | 71 | 80 | 74 | 69 | 70 ± 7 |

# APPENDIX-12

Corected Q-T interval at time interval 0 - 24 hours in nine subjects after receiving a single oral dose of 300 mg quinine sulphate alone.

| Time (hr) | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0.41 | 0.40 | 0.40 | 0.40 | 0.41 | 0.39 | 0.40 | 0.39 | 0.41 | 0.40 ± 0.01 |
| 0.5 | 0.42 | 0.40 | 0.40 | 0.39 | 0.39 | 0.39 | 0.38 | 0.39 | 0.40 | 0.40 ± 0.01 |
| 1 | 0.40 | 0.40 | 0.41 | 0.39 | 0.39 | 0.39 | 0.38 | 0.39 | 0.40 | 0.40 ± 0.01 |
| 1.5 | 0.41 | 0.40 | 0.41 | 0.39 | 0.41 | 0.38 | 0.39 | 0.40 | 0.39 | 0.40 ± 0.01 |
| 2.5 | 0.41 | 0.40 | 0.40 | 0.39 | 0.40 | 0.38 | 0.38 | 0.40 | 0.41 | 0.40 ± 0.01 |
| 4 | 0.41 | 0.39 | 0.40 | 0.39 | 0.40 | 0.38 | 0.40 | 0.39 | 0.40 | 0.40 ± 0.01 |
| 8 | 0.41 | 0.39 | 0.40 | 0.38 | 0.40 | 0.39 | 0.39 | 0.40 | 0.40 | 0.40 ± 0.01 |
| 24 | 0.41 | 0.39 | 0.40 | 0.38 | 0.40 | 0.38 | 0.40 | 0.40 | 0.40 | 0.40 ± 0.01 |

# APPENDIX-13

Corrected Q-T interval at time interval 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 400 mg ketoconazole (Nizoral®) for 4 days.

| Time (hr) | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0.41 | 0.40 | 0.41 | 0.39 | 0.40 | 0.39 | 0.39 | 0.4 | 0.39 | 0.40 ± 0.01 |
| 0.5 | 0.38 | 0.38 | 0.39 | 0.40 | 0.40 | 0.40 | 0.40 | 0.4 | 0.39 | 0.39 ± 0.01 |
| 1 | 0.41 | 0.40 | 0.40 | 0.40 | 0.40 | 0.38 | 0.40 | 0.40 | 0.38 | 0.40 ± 0.01 |
| 1.5 | 0.40 | 0.40 | 0.39 | 0.41 | 0.41 | 0.40 | 0.40 | 0.40 | 0.38 | 0.40 ± 0.01 |
| 2.5 | 0.40 | 0.41 | 0.40 | 0.41 | 0.40 | 0.37 | 0.39 | 0.38 | 0.38 | 0.40 ± 0.01 |
| 4 | 0.41 | 0.40 | 0.38 | 0.41 | 0.41 | 0.41 | 0.39 | 0.39 | 0.38 | 0.40 ± 0.01 |
| 8 | 0.40 | 0.39 | 0.38 | 0.39 | 0.41 | 0.40 | 0.39 | 0.40 | 0.38 | 0.39 ± 0.01 |
| 24 | 0.41 | 0.39 | 0.38 | 0.39 | 0.40 | 0.40 | 0.39 | 0.40 | 0.38 | 0.39 ± 0.01 |

# APPENDIX-14

Corrected Q-T interval at 0 - 24 hours in nine subjects receiving a single oral dose of 300 mg quinine sulphate on the study day 4 after pretreatment with 200 mg itraconazole for 4 days.

| Time (hr) | Subject No. | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| 0 | 0.40 | 0.41 | 0.39 | 0.40 | 0.40 | 0.41 | 0.40 | 0.37 | 0.40 | 0.40 ± 0.01 |
| 0.5 | 0.42 | 0.39 | 0.38 | 0.38 | 0.40 | 0.38 | 0.41 | 0.40 | 0.40 | 0.40 ± 0.02 |
| 1 | 0.42 | 0.38 | 0.38 | 0.4 | 0.39 | 0.39 | 0.40 | 0.37 | 0.41 | 0.39 ± 0.02 |
| 1.5 | 0.42 | 0.39 | 0.39 | 0.41 | 0.41 | 0.39 | 0.40 | 0.39 | 0.41 | 0.40 ± 0.01 |
| 2.5 | 0.41 | 0.39 | 0.38 | 0.41 | 0.40 | 0.4 | 0.40 | 0.39 | 0.40 | 0.40 ± 0.01 |
| 4 | 0.42 | 0.39 | 0.38 | 0.40 | 0.40 | 0.39 | 0.40 | 0.40 | 0.40 | 0.40 ± 0.01 |
| 8 | 0.42 | 0.39 | 0.38 | 0.40 | 0.40 | 0.39 | 0.39 | 0.40 | 0.40 | 0.40 ± 0.01 |
| 24 | 0.42 | 0.4 | 0.38 | 0.40 | 0.40 | 0.39 | 0.39 | 0.39 | 0.40 | 0.40 ± 0.01 |

# APPENDIX-15

ที่ ทม 1210/461

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90110

**หนังสือรับรองการศึกษาวิจัย**

การศึกษาวิจัยที่ทำการทดลองในมนุษย์เรื่อง : ผลของยาต้านเชื้อรากลุ่มอะโซล(คีโตโคนาโซลและไอทราโคนาโซล) ต่อเภสัชจลนศาสตร์ และเภสัชพลศาสตร์ ของยาควินินขนาดรับประทานครั้งเดียวในอาสาสมัครสุขภาพปกติ

นักศึกษาปริญญาโทผู้ทำการวิจัย : น.ส.ทิพวัลย์ สุวรรณรักษ์
อาจารย์ที่ปรึกษาวิทยานิพนธ์ : รศ.นายแพทย์วิบูลย์ ฤทธิทิศ
ผศ.มาลินี วงค์นาวา
ผศ.นายแพทย์วีรวัฒน์ มหัทธนตระกูล

ได้ผ่านการพิจารณา และเห็นชอบจากคณะกรรมการจริยธรรม ซึ่งเป็นคณะกรรมการพิจารณาโครงการวิจัย ตลอดจนติดตามผลในส่วนของการทดลองที่กระทำต่ออาสาสมัคร ของคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ 31 สิงหาคม 2542

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

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

.....กรรมการ
(นายแพทย์สมหมาย ปลอดสมบูรณ์)
ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์

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

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

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

# ใบยินยอม

**1. ชื่อโครงการ :** ผลของยาต้านเชื้อรากลุ่มอะโซล(คีโตโคนาโซลและไอทราโคนาโซล)ต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาควินินขนาดรับประทานครั้งเดียวในอาสาสมัครสุขภาพปกติ

**2.** ข้าพเจ้า นาย.....นามสกุล.....อายุ.....ปี ยินยอมเป็นอาสาสมัครในโครงการศึกษาเรื่อง "ผลของยาต้านเชื้ออากลุ่มอะโซล (คีโตโคนาโซลและไอทราโคนาโซล) ต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาควินินขนาดรับประทานครั้งเดียวในอาสาสมัครสุขภาพปกติ"

**3. วัตถุประสงค์ของการศึกษา**

1) ศึกษาผลกระทบของการรับประทานยาต้านเชื้อรากลุ่มอะโซล (คีโตโคนาโซลและไอทราโคนาโซล)ต่อเภสัชจลนศาสตรและเภสัชพลศาสตร์ของยาควินิน ในอาสาสมัครสุขภาพปกติ

2) เป็นข้อมูลให้แพทย์ผู้สั่งใช้ยาตระหนักถึงปฏิกิริยาระหว่างยาต้านเชื้อรากลุ่มอะโซลกับยาควินินในกรณีที่ใช้ยาร่วมกันซึ่งอาจจำเป็นต้องเลี่ยงการใช้ร่วมกันหรือลดขนาดของยาลงเพื่อป้องกันอันตรายที่จะเกิดต่อร่างกาย

**4. วิธีการศึกษา**

4.1 อาสาสมัครที่เข้าร่วมโครงการต้องเป็นผู้ที่มีสุขภาพสมบูรณ์และแข็งแรง

4.2 เป็นอาสาสมัครเพศชาย อายุระหว่าง 20-45 ปี

4.3อาสาสมัครทุกคนต้องไม่ได้รับยาชนิดอื่นๆมาก่อนที่จะเริ่มทำการทดลองเป็นเวลาอย่างน้อย 1 เดือน

4.4 ก่อนเริ่มทำการทดลอง อาสาสมัครทุกคนจะได้รับการเจาะเลือดเพื่อตรวจความปกติ/ผิดปกติของเม็ดเลือดและค่าชีวเคมีของเลือดที่โรงพยาบาลสงขลานครินทร์หรือคลินิกเอกชน

4.5 ก่อนเริ่มทำการทดลอง ให้อาสาสมัครงดน้ำและอาหารมาอย่างน้อย 8 ชั่วโมง ในวันเริ่มทำการทดลองอาสาสมัครทุกคนจะได้รับประทานยาควินิน

ขนาด 600 มิลลิกรัม (ควินินซัลเฟต ผลิตโดยองค์การเภสัชกรรมขนาด 300 มิลลิกรัม 2 เม็ด) หลังรับประทานยาแล้วประมาณ 4 ชั่วโมงจึงจะยินยอมให้อาสาสมัครรับประทานอาหารได้ ส่วนการทดลองที่ 2 และ 3 อาสาสมัครจะได้รับยาคีโตโคนาโซลขนาด 400 มิลลิกรัม และไอทราโคนาโซลขนาด 200 มิลลิกรัมวันละ 1 ครั้งหลังรับประทานอาหารเช้าทันทีเป็นเวลา 4วันติดต่อกัน และในวันที่ 4 รับประทานยาทั้ง 2 ชนิดนี้ก่อนรับประทานยาควินินขนาด 600 มิลลิกรัมอย่างน้อย 1 ชั่วโมงตามลำดับ

4.6 เจาะเก็บเลือดที่บริเวณหลอดเลือดดำข้อพับด้านในของแขนครั้งละ 5 มิลลิลิตรในช่วงเวลา 48 ชั่วโมง โดยเจาะเก็บเลือดทันทีก่อนรับประทานยา และที่เวลา 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 24, 36 และ 48 ชั่วโมงหลังได้รับยาควินิน การเก็บเลือดนั้นจะทำการเจาะหลอดเลือดเพียงครั้งเดียวแล้วคาสาย catheter ไว้เพื่อเก็บตัวอย่างเลือดที่เวลาต่างๆ นำเอาเลือดที่ได้ไปปั่นเก็บพลาสมาทันทีและเก็บไว้ที่อุณหภูมิ -70 องศาเซลเซียสเพื่อทำการวิเคราะห์ต่อไป

**5. ผลข้างเคียงของการใช้ยาควินิน**

ส่วนใหญ่เกิดกับผู้ที่มีการตอบสนองไวเกินต่อยา (hypersensitivity) หรือได้รับยาขนาดสูงเกินไป อาการเป็นพิษที่พบได้แก่

1. ซิงโคนิซึม (cinchonism) เป็นกลุ่มอาการพิษที่ประกอบด้วย อาการปวดศรีษะ หูอื้อ ตาพร่า คลื่นไส้ อาเจียน ท้องเดิน อาการที่เกิดขึ้นมักจะพบในผู้ที่มีระดับยาในเลือดสูงกว่า 10-12 ไมโครกรัมต่อมิลลิลิตร

2. อาการระคายเคืองทางเดินอาหาร เช่น อาจทำให้เกิดการระคายเคืองของเยื่อบุทางเดินอาหาร มีอาการคลื่นไส้และปวดท้อง

3. ในคนที่ได้รับยาเกินขนาด เช่น ได้รับยาสูงกว่า 8 กรัมขึ้นไป อาจทำให้เสียชีวิตได้ เนื่องจากควินินเป็นยาที่มีอันตรายต่อระบบประสาท โดยเฉพาะกับระบบประสาทอัตโนมัติ

**6. ผลข้างเคียงของการใช้ยาคีโตโคนาโซล**

อาการข้างเคียงที่พบส่วนใหญ่ขึ้นอยู่กับขนาดยาที่ได้รับ ซึ่งจะมีอาการคลื่นไส้ เบื่ออาหาร และอาเจียน อาจมีจำนวนเสปิร์มและความรู้สึกทางเพศลดลงในบางราย เมื่อหยุดใช้ยาก็จะกลับเป็นปกติได้ อาการตับอักเสบเล็กน้อยแต่พบได้ไม่บ่อยและมักเกิดกับรายที่ใช้ยาเป็นระยะเวลานานๆ

**7. ผลข้างเคียงของไอทราโคนาโซล**

อาการข้างเคียงที่สำคัญได้แก่ อาการคลื่นไส้ เบื่ออาหารและอาเจียน ส่วนอาการข้างเคียงอื่นที่อาจพบได้เมื่อได้รับยาในขนาดสูงกว่า 400 มก. เป็นเวลานาน ได้แก่ ไตรกลีเซอร์ไรด์ในเลือดสูง โปแทสเซียมในเลือดต่ำ และเอนไซม์อะมิโนทรานสเฟอเรสในซีรั่มเพิ่มขึ้น ประสิทธิภาพการทำงานของไตลดลง บวมบริเวณช่วงล่างของลำตัว ความดันโลหิตสูง อาจพบอาการผื่นแพ้ความเป็นพิษต่อตับและมีการสลายของกล้ามเนื้อลาย

**8. ความรับผิดชอบต่ออาสาสมัครที่เข้าร่วมโครงการ**

หากอาสาสมัครที่เข้าร่วมโครงการทดลอง เกิดความผิดปกติทางด้านร่างกายอันเป็นผลสืบเนื่องมาจากการดลองนี้ ไม่ว่าสาเหตุใดก็ตามผู้ทำการทดลองต้องรับผิดชอบในการรักษาพยาบาลอาสาสมัครจนกว่ายาจะหมดฤทธิ์หรือไม่เกิน 3 เดือนหลังการทดลอง

**9. โอกาสในการซักถามและยกเลิกการเป็นอาสาสมัคร** : หากอาสาสมัครมีข้อสงสัยเกี่ยวกับการศึกษาครั้งนี้ อาสาสมัครมีสิทธิในการซักถามได้ทุกขั้นตอนและมีสิทธิยกเลิกการเป็นอาสาสมัครที่เข้าร่วมโครงการวิจัยได้เมื่อมีเหตุผลอันสมควร

**10.หากอาสาสมัครไม่ปฏิบัติตามเงื่อนไข** คณะผู้วิจัยมีสิทธิถอดถอนอาสาสมัครออกจากการร่วมโครงการ

**11. คำยินยอมเข้าร่วมโครงาการ** : ข้าพเจ้าได้อ่านและเข้าใจถึงวัตถุประสงค์ในการศึกษาครั้งนี้ และยินดีให้ความร่วมมืออย่างดีที่สุด

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

Subject name :.....

Subject Code :.....

## Azole antimycotics - Quinine interaction

(----------)

Date :.....

### I. Drug Information

Dose of .....:..... Route: ( )oral ( ) IM.

Drug producer/distributer.....

Manufacturing date :.....Lot No. :.....

### II. Subject information

Name :.....Code No. :.....

Address:.....

.....

Age :..... yrs Sex : ( ) male ( ) female

Body weight :.....Kgs Height :.....cms.

Smoking : ( )No ( )Yes :No. of cigarette/day.....

Alcohol consuming:( )No ( )Yes:No.of alcoholic drink(glass/day) .....

Contraceptive pill : ( )No ( )Yes

Drug : .....

Other information :.....

Subject name :.....

Subject code :.....

## III. Data

| No. | Sample code | Preferred time | Actual time | Time after dose (hr) | Blood pressure (mmHg) | | EKG | Quinine plasma conc. (μg/ml) |
|---|---|---|---|---|---|---|---|---|
| | | | | | SBP | DBP | | |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
| 12 | | | | | | | | |
| 13 | | | | | | | | |
| 14 | | | | | | | | |
| 15 | | | | | | | | |
| 16 | | | | | | | | |
| 17 | | | | | | | | |

## IV. Pharmacokinetic parameters

1. $K_{el}$ =.....$hr^{-1}$

2. $t_{1/2}$ =.....hr

3. AUC =.....mg.hr./L

4. Apparent CL =.....L/hr.

5. Apparent $V_d$ =.....L/kg

# VITAE

Name Miss Tippawan Suvarnaraksha

Birth Date April 6, 1975

Educational Attainment

| Degree | Name of Institution | Year of Graduation |
|---|---|---|
| Bachelor of Nursing | Prince of Songkla University | 1998 |