Effect of Cholestyramine on an Oral Single-Dose Quinine Pharmacokinetics in Healthy Volunteers

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Master of Science Thesis in Pharmacology

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Thesis Title	Effect of Cholestyramine on an Oral Single-Dose Quinine Pharmacokinetics in Healthy Volunteers
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ชื่อวิทยานิพนธ์	ผลของยาโคเลสไทรามีนต่อเภสัชจลนศาสตร์ของยาควินินเมื่อให้โดยการรับประทานครั้งเดียวในอาสาสมัครสุขภาพปกติ
ผู้เขียน	นายอนันต์ กลีบแก้ว
สาขาวิชา	เภสัชวิทยา
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บทคัดย่อ

ควินินเป็นสารสกัดจากเปลือกต้นซิงโคนา (chinchona) ปัจจุบันองค์การอนามัยโลก(WHO)แนะนำให้ใช้เป็นยารักษาผู้ป่วยมาลาเรียที่ติดเชื้อ *Plasmodium falciparum* ที่ดื้อยาคลอโรควินและเป็นยาที่ได้รับการเลือกใช้ (drug of choice) ในการรักษาผู้ป่วยมาลาเรียที่มีภาวะแทรกซ้อน (complicated malaria) นอกจากนี้ยังนิยมใช้ในการรักษาผู้ป่วยที่เป็นตะคริวที่ขาตอนกลางคืน (nocturnal leg cramps) ส่วนยาโคเลสไทรามีนเป็นยาที่ใช้ในการรักษาผู้ป่วยที่มีไขมันในเลือดสูง (Type IIA และ IIB) มีรายงานการศึกษาพบว่า ยาโคเลสไทรามีนสามารถจับกับยาต่างๆได้หลายชนิดทั้งยาที่มีคุณสมบัติเป็นกรดและเบส มีผลทำให้การดูดซึมยาเหล่านั้นในระบบทางเดินอาหารลดลง ดังนั้นในกรณีที่มีการใช้ยาโคเลสไทรามีนและยาควินินร่วมกัน ยาโคเลสไทรามีนอาจมีผลเปลี่ยนแปลงเภสัชจลนศาสตร์ของยาควินิน ซึ่งอาจจะส่งผลถึงประสิทธิภาพในการรักษาของยาควินินได้ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาถึงผลของยาโคเลสไทรามีนต่อเภสัชจลนศาสตร์ของยาควินินในกรณีที่ให้ยาทั้งสองชนิดนี้ร่วมกันทันที ในกรณีที่ให้ยาควินินก่อนยาโคเลสไทรามีน 1 ชั่วโมงและในกรณีให้ยาควินิน

ภายหลังได้รับยาโคเลสไทรามีน 1 ชั่วโมง เปรียบเทียบกับการได้รับยาควินินชนิดเดียว ในอาสาสมัครชายไทยปกติจำนวน 8 คน จากผลการศึกษาและได้ทดสอบทางสถิติโดยใช้วิธีวิเคราะห์ความแปรปรวน พบว่าไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญของค่าเภสัชจลนศาสตร์ต่างๆของยาควินินในทุกแผนการทดลองเมื่อเปรียบเทียบกับการได้รับยาควินินเพียงชนิดเดียวที่ระดับความเชื่อมั่น 95 % ผลจากการศึกษาครั้งนี้แสดงให้เห็นว่าการให้ยาควินินร่วมกับยาโคเลสไทรามีนในขนาดที่ใช้ในการรักษาครั้งเดียวนั้นไม่น่าจะเกิดปฏิกิริยาระหว่างยาแต่อย่างใด

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ABSTRACT

Quinine is a cinchona alkaloid derived from the bark of the cinchona tree. It is currently recommended by the World Health Organization (WHO) for the treatment of chloroquine-resistant *Plasmodium falciparum* malaria and is the drug of choice for the treatment of complicated and/or cerebral malaria. It is also widely prescribed as the treatment of choice for nocturnal leg cramps. Cholestyramine is the drug of choice for the treatment of type IIA and IIB hyperlipoproteinemia. It has been reported to bind many drugs. This binding is a non-selective process and is observed with a variety of drugs possessing different chemical properties. Therefore, cholestyramine may interfere with the gastrointestinal absorption of quinine. So the alteration in the pharmacokinetic parameters of quinine may result in the efficacy of quinine. The objective of this study was to study the effect of cholestyramine on an oral single-dose quinine pharmacokietics in eight Thai healthy male volunteers receiving quinine and cholestyramine simultaneously, quinine 1 hour before or after cholestyramine compared to the administration of quinine alone. The pharmacokinetic parameters were determined from plasma quinine

concentration during 48 hour period using high performance liquid chromatography (HPLC). Statistical analysis using analysis of variance (ANOVA) indicated that there were no significant differences ($P<0.05$) in all trial-phases compared to a control phase. Thus, the present results could suggest that quinine and cholestyramine coadministration according to the study design is not likely to produce pharmacokinetic interactions.

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LIST OF ABBREVIATIONS

g	= gram
ml	= milliliter
µg	= microgram
l	= liter
kg	= kilogram
r	= correlation coefficient
hr	= hour
min	= minute
wk	= week
°C	= degree Celsius
%	= percent
®	= trade name
nm	= nanometer
cm	= centimeter
µm	= micrometer
µl	= microliter
C_{max}	= maximal plasma concentration
T_{max}	= time to maximal plasma concentration
Ke	= elimination rate constant
$t_{1/2}$	= elimination half-life
Ka	= absorption rate constant
$t_{1/2}$ (abs)	= absorption half-life
Cl/f	= apparent oral clearance

LIST OF ABBREVIATIONS (Continued)

AUC	= area under the concentration-time curve
Vd/f	= apparent volume of distribution
S.D.	= standard deviation
V/V	= volume by volume
yr	= year
P	= P value

CHAPTER 1

INTRODUCTION

Malarial infection is caused by unicellular animals, class *Sporozoa*, genus *Plasmodium*. More than 120 species have been found but only 4 species are the cause of malarial disease in humans i.e. *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae and Plasmodium ovale. Plasmodium falciparum* is the most dangerous form of human malaria. The most severe or uncomplicated malaria such as malaria with renal failure or cerebral malaria is caused by *Plasmodium falciparum* (Looareesuwan, 1990).

Malaria remains the world's most devastating human infection (Tracy and Webster, 1996), with 2,117 million persons (42 % of the world population) settle down at the outbreak of malarial infection (WHO, 1989). Approximately, 1.5 to 2.7 million people, mainly young children in Africa, were killed by *Plasmodium falciparum* each year (WHO, 1995).

Ten years ago, about 300,000 clinical cases of malarial infection were found in Thailand with the mortality rate of 2,000-3,000 cases per year. In 1994, clinical cases were approximately 100,000 and in 1995, the mortality rate decreased to 1,000 cases per year. However, the figure may be less than the actual one because some patients may treat the infection by themselves (Looareesuwan, 1995)

Chloroquine and primaquine are used to treat uncomplicated malarial infection in patients who were infected with *Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale,* whereas patients with uncomplicated malarial infection caused by *Plasmodium falciparum* are treated with chloroquine and sulfadoxine-pyrimethamine. In the resistant cases to these drugs, quinidine, mefloquine combined with sulfadoxine-pyrimethamine and artesunate can be used. However, quinine is the most widely used (Looareesuwan, 1990).

Quinine is a cinchona alkaloid derived from the bark of the cinchona tree which is indigenous to certain regions of South America. Justus Hasskar, Netherland's botanist, brought the seeds of cinchona from Bolivia and Peru to cultivate in Indonesia where most of the world's drugs supply comes from (Looareesuwan, 1990). The World Health Organization currently recommends quinine to be used for the treatment of chloroquine-resistant *Plasmodium falciparum* malaria and pernicious malaria (WHO, 1990). The number of chloroquine-resistant malaria treated in the United States is increasing not only more cases of malaria occurring, but also more proportion of insensitive cases to chloroquine which spreads through out the endemic areas of the world (Shann et al., 1985). Quinine is also widely prescribed as the treatment of choice for the nocturnal leg cramps (Mackie and Davidson, 1995) and it is the most effective pharmacological treatment available (McGee, 1990).

Cholestyramine is a strongly basic anion-exchange resin consisting of styrene-divinylbenzene copolymer with quaternary ammonium

functional groups in the chloride form (Swinyard, 1985). It is effective in the treatment of cholestasis-related pruritis (Datta and Sherlock, 1963), and has been used in the treatment of diarrhea following intestinal resection (Hofmann and Poley, 1969), hyperoxaluria (Smith, et al., 1972) and hyperthyroid Graves' disease (Mercado, et al., 1996). It is widely used for the treatment of hypercholesterolemia. This resin is water insoluble, inert to digestive enzymes in the intestinal tract and is not absorbed. The resin may interfere with the gastrointestinal absorption of several drugs such as some basic drugs such as propranolol (Hibbard et al., 1984) and acidic drugs such as warfarin (Hunninghake and Pollack, 1977).

A knowledge of the pharmacokinetics of quinine is essential for the rational design of regimens for the treatment of falciparum malaria. Recently, pharmacokinetic factors resulting in subtherapeutic quinine plasma concentrations (less than 10 mg/l) were attributed to the fatal outcome of a patient who was given full parenteral doses of quinine for the treatment of cerebral malaria (Looareesuwan, et al., 1990). Cholestyramine may interfere the gastrointestinal absorption of quinine when used in combination with quinine. The alteration of the pharmacokinetics of quinine may result in the treatment failure.

The purposes of this investigation were to study the effect of cholestyramine on an oral single-dose quinine pharmacokinetics in healthy volunteers. Due to the lack of interaction data between cholestyramine and quinine, the present study may be the guidance and

useful data for decision making in case of coadministration of quinine and cholestyramine.

CHAPTER 2

LITERATURE REVIEW

Quinine

Quinine, the chief alkaloid of cinchona, is one of the oldest drugs in the Pharmacopoeia and has been used in treatment of malaria since 1630 (Salako and Sowunmi, 1992). The term cinchona was given by Linnaeus in 1742. The bark was probably brought to Europe by Jesuits in 1631 or 1632 and again in 1645. In 1820, the structure of quinine was identified by Pelletier and Caventou and the complete synthesis of quinine was achieved in 1945 (Krishna and White, 1996). Quinine has been used as an antimalarial agent for at least 350 years and has a potent schizontocidal effect against all *Plasmodium* species (Tracy and Webster, 1996).

CHEMICAL AND PHYSICAL PROPERTIES

Synonyms : 6'- methoxycinchonan-9-ol

Molecular weight : 324.41

pKa : 8.4

Solubility ; 1 g dissolves in 1,900 ml H_2O, 0.8 ml alcohol

Quinine contains a quinoline group attached through a secondary alcohol linkage to a quinuclidine ring. A methoxy side chain is attached

to the quinoline ring and a vinyl to the quinuclidine (Tracy and Webster, 1996).

Karle, et al. (1992) and Oleksyn, 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 (9C)O-H bonds relative to each other, are crucial to antimalarial activity.

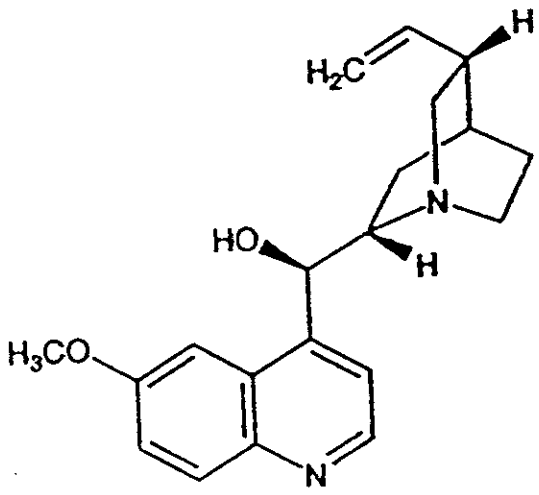

Figure 1 Molecular structure of quinine

PHARMACOKINETIC PROPERTIES

In Normal Volunteers

1. Absorption

Quinine is extremely bitter and many find it unpalatable, although, it is well absorbed by mouth in both healthy subjects and patients with uncomplicated malaria (Hall, et al., 1973). Taggart, et al. (1948) considered quinine to be almost completely absorbed, since less than 5 % of the dose was recovered in the feces. In the former case absorption occurs mainly from the upper small intestine (Tracy and Webster, 1996). Intramuscular injection of quinine is also well absorbed and causes little or no local discomfort or tissue necrosis (Chongsuphajaisiddhi, et al., 1983; Shann, et al., 1985). In adult healthy subjects, the time to peak plasma drug concentration (T_{max}) is 1-4 hr (Karbwang, et al., 1993a; Paintaud, et al., 1993; Wanwimolruk, et al., 1991a; Wanwimolruk, et al., 1986; Wanwimolruk and Chalcroft, 1991; Salako and Sowunmi, 1992; Wanwimolruk, et al., 1991b; Jamaludin, et al., 1988). The peak plasma concentration (C_{max}) for therapeutic dose of quinine sulfate, 600 mg administered by oral route, is 4.1-5.6 mg/l (Wanwimolruk, et al., 1991a; Wanwimolruk, et al., 1986; Wanwimolruk, et al., 1991b; Jamaludin, et al., 1988). The bioavailability is approximately 76-90 % (Paintaud, et al. , 1993; Hall, et al., 1973) and is similar for hydrochloride, sulfate and ethylcarbonate salts (Jamaludin, et al., 1988). There is no difference when oral drug is given as capsules or tablets (Hall, et al., 1973) and there is no

difference in the rate of absorption in young and elderly subjects (Wanwimolruk, et al., 1991b).

2. Distribution

The volume of distribution of quinine in normal subjects was 1.8 l/kg (White, 1985; Dyer, et al., 1994). Karbwang, et al. (1993a) reported that the pharmacokinetic properties of intravenous quinine are adequately described by a two compartment open model with a mean volume of the central compartment (V_C) was 0.3 (range from 0.2-0.9) l/kg and a total mean apparent volume of distribution was 3.1 (range from 1.8-4.3) l/kg, which is similar to the study of Davis, et al. (1988) and White, et al. (1983). However, oral and intramuscular quinine could be described by a one compartment open model (Waller, et al., 1990; Supanaranond, et al., 1991; Dyer, et al., 1994).

3. Plasma Protein Binding of Quinine

Plasma protein binding expressed as the percentage of bound quinine was 69-92 % (Wanwimolruk and Denton, 1992) and alpha 1- acid glycoprotein (AAG) is the most important binding protein, with a high-affinity, low-capacity binding profile (Hensbrork, et al., 1996).

4. Elimination

The clearance of quinine varied between 0.084 l/hr/kg in healthy individuals and was reduced to approximately 0.062 l/hr/kg in elderly

subjects (Wanwimolruk, et al., 1991b; Salako and Sowunmi, 1992). 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 hr (Wanwimolruk, et al., 1991b; Salako and Sowunmi, 1992; Karbwang, et al., 1993a; Wanwimolruk, et al., 1991a; Karbwang, et al., 1993b; Paintaud, et al., 1993).

In Malaria

Malarial infection alters quinine pharmacokinetics. The disease causes a reduction in its volume of distribution and systemic clearance. The consequent elevation of plasma drug concentration is proportional to the severity of disease (White, et al., 1982).

In Uncomplicated Malaria

1. Absorption

In uncomplicated falciparum malaria, plasma quinine concentrations following oral quinine sulfate (10 mg/kg) are significantly higher during the acute infection compared to convalescence, the mean plasma concentration in acute phase is 8.4 mg/l compared to 5.7 mg/l in convalescence (Supanaranond, et al., 1991). Estimated oral systemic

bioavailability exceeds 80 % in uncomplicated malaria (Sabcharoen, et al., 1982).

2. Distribution

The volume of distribution in 15 adult Thai patients with uncomplicated falciparum malaria was 0.78 l/kg in acute illness and increased to 1.57 l/kg in convalescence (in 10 of the same patients)(Supanaranond, et al., 1991). White, et al. (1982) reported that the volume of distribution in 13 patients with uncomplicated falciparum malaria was 1.62 l/kg in acute phase and increased to 2.74 l/kg in convalescence.

3. Elimination

White (1985) reported that the total systemic clearance of quinine is approximately 0.084 l/hr/kg in uncomplicated malaria and 0.054 l/hr/kg in cerebral malaria.

In Severe Malaria

Severe malaria results in a reduction of systemic clearance of the drug, presumably on the basis of reduced hepatic blood flow and a subsequent decrease in the metabolism of quinine (Tracy and Webster, 1996; White, et al., 1982).

1. Absorption

In cerebral malaria, White, et al. (1983) reported that 13 of 15 patients who received a loading dose (20 mg/kg quinine dihydrochloride, infused over 4 hr) had plasma concentrations exceeding 10 mg/l.

2. Plasma Protein Binding

The mean percentage of unbound quinine was significantly lower in patients with malaria (11 %) than in healthy subjects (Wanwimolruk and Denton, 1992). The increasing of AAG during the malarial attack could prevent quinine toxicity in the presence of high quinine plasma level (Mansor, et al., 1990; Silamut, et al., 1985; Mansor, et al., 1991).

3. Distribution

The total apparent volume of distribution in 18 severe malarial patients was 1.18 l/kg and it was lowest when compared with normal volunteers (approximately 3.1 l/kg) and uncomplicated malaria (approximately 1.67 l/kg) (White, 1985).

4. Elimination

White (1985) found that the total systemic clearance of quinine is approximately 0.054 l/hr/kg in cerebral malaria. The mean terminal elimination half-life was 18 hr in cerebral malaria (White, 1987). The reduction of clearance of quinine in severe malaria is caused by the

decrease in hepatic blood flow (White, et al., 1982; Tracy and Webster, 1996). So the plasma concentrations of quinine in patients with malarial infection were higher than normal subjects and were proportional to the severity of disease (White, et al., 1982). At the same time, the increased AAG could prevent toxicity of the higher quinine concentration (Mansor, et al., 1990; Mansor, et al., 1991).

In Children With Malaria

1.Absorption

In young children with cerebral malaria, the peak plasma quinine concentration after rapid intravenous injection (4 mg/kg of the quinine dihydrochloride after dilution with saline, injected manually into the side arm of the drip over exactly 4.0 min) was 12.3 mg/l, 43 % higher than in adults given the same regimen; this was due to a small apparent volume of distribution (Winstanley, et al., 1993) and in severe falciparum malaria, the high dose regimen (20 mg/kg loading dose) infused over 2 hr produced peak plasma quinine concentration of 15.3 mg/l (Pasvol, et al., 1991).

2. Distribution

The volume of distribution in children after the end of the 4 hr intravenous infusion of quinine dihydrochloride was 1.51 l/kg and after intramuscular injection was 1.3 l/kg (Shann, et al., 1985). Henabroek, et

al. (1996) described that the volume of distribution in six children less than two years of age with severe malaria after received intravenous quinine dihydrochloride was 1.04 l/kg and in ten children less than two years of age with severe malaria after received intramuscular quinine dihydrochloride was 1.32 l/kg.

3. Elimination

The total clearance of quinine in young children with malaria was varied between 0.438 l/hr/kg to 0.08 l/hr/kg (Sabchareon, et al., 1982; Pasvol, et al., 1991; Winstanley, et al., 1994). The terminal elimination half-life in children with falciparum malaria by intravenous infusion at the dose of 10 mg/kg ranged from 9 to 11 hr whereas, the value in the controls (convalescent patients) was 3 to 7 hr and it neither depends on the route nor the duration of infusion of the drug (Sabchareon, et al., 1982). Pasvol, et al. (1991) studied the pharmacokinetics of quinine in African children with severe malaria and reported that patients who were received high dose (20 mg/kg loading, then 10 mg/kg every 12 hr by intravenous infusion over 2 hr) the elimination half-life was 12.5 hr and in low dose (10 mg/kg loading, then 5 mg/kg every 12 hr by intravenous infusion over 2 hr) was 8.9 hr and high dose (20 mg/kg loading, then 10 mg/kg every 12 hr by intramuscular) was 15.7 hr.

In Pregnancy

There are no pharmacokineic data from healthy pregnant women, but there are significant differences in the third trimester of pregnancy when compared with other adults in the acute phase of malaria (Looareesuwan, et al., 1987). The volume of distribution values were lower, the elimination half-life were shorter, and total clearance was reduced.

Biotransformation

The biotransformation of quinine occurred in liver by oxidative reaction (Barrow, et al., 1980; Wanwimolruk, et al., 1991a; White, 1985). To date, at least seven possible metabolites of quinine have been identified in human urine, and 3-hydroxy-quinine has been identified as the major metabolite of quinine (Liddle, et al., 1981; Wanwimolruk, et al., 1995b). Approximately 20 % of an oral dose being excreted unchanged in the urine and more than 50 % of the metabolic clearance of quinine was carried out through the hepatic microsomal enzymes : cytochrome P450 (CYP) 3A4 (Krishna and White, 1996). CYP 450 1A which is induced in smokers may also be involved in quinine metabolic clearance because smoking increases quinine clearance by 77 % when compared with non-smokers (Wanwimolruk, et al., 1993).

Tissue distribution

White, et al. (1982) described that the cerebrospinal fluid (CSF) quinine concentration in cerebral malaria was 7 % compared with quinine concentration in plasma and the mean CSF/plasma ratio was inversely correlated with the total plasma concentration and Silamut, et al. (1985) reported that the CSF/free quinine ratio in 18 patients with cerebral malaria was 0.55 ± 0.33 which suggests that quinine dose not freely cross the blood-brain barrier.

In Renal Failure

Though, urinary quinine clearance comprises only 20 % of total clearance in healthy subjects, but Donadio, et al. (1986) reported that the plasma concentration of 6 patients with falciparum malaria in acute phase 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.

Drug Interaction

Activated Charcoal

Lockey and Bateman (1989) reported that oral activated charcoal decreased quinine half-life from 8.23 hr to 4.55 hr and increased oral quinine clearance by 56 % in seven normal volunteers.

Cimetidine and Ranitidine

Cimetidine pretreatment significantly reduced the apparent oral clearance of quinine (0.182 l/hr/kg to 0.133 l/hr/kg) since cimetidine reduces hepatic blood flow and inhibits the biotransformation of the drugs eliminated by the hepatic monooxygenase enzyme system (White, et al., 1982). In addition, the mean elimination half-life was increased 49 % because quinine is considered a low clearance drug which is relatively insensitive to change in hepatic blood flow, so this interaction would be due to inhibition of the hepatic monooxygenase enzyme system by cimetidine (Wanwimolruk, et al., 1986) but had no significant effect on the clearance or half-life of quinine.

Oral Contraceptive

Wanwimonruk, et al. (1991a) reported that there were no significant differences in the maximum plasma concentration and the time to peak concentration between the subjects who used oral contraceptives (seven of the healthy Thai women had been taking an oral contraceptive steroids preparation for at least 6 month before the study and continued to take the contraceptive pill throughout the course of the study) and the control subjects. The mean elimination half-life of quinine in the oral contraceptive use group was similar to the control group.

Cytochrome P450 2D6

The cytochrome P450 2D6 is unlikely to be involved in the major metabolic pathway of quinine because the mean elimination half-life of quinine in the poor metabolizer was similar to the extensive metabolizer and the oral clearance was not significantly different between two groups (Wamwimolruk and Chalcroft, 1991).

Patients with Hepatitis

The pharmacokinetic parameters of quinine were not different between the acute phase of hepatic infection and recovery phase, in contrast, the terminal elimination half-life was prolonged in acute hepatitis (17 hr) and recovery (15 hr) compared to healthy subjects (10 hr) (Karbwang, et al., 1993).

Rifampicin

Wanwimolruk, et al. (1995a) demonstrated that oral pretreatment with rifampicin 600 mg daily for 2 weeks in nine healthy Thai male resulted in decreasing of C_{max} by 45 %, mean clearance (Cl/f) by 621 %, unbound clearance (Cl_u/f) by 687 % and increasing the elimination half-life by 49.5 % compared to quinine alone.

PHARMACODYNAMIC PROPERTIES

Mechanism of Action

The specific mechanism of antimalarial action of quinine is still unclear. However, quinine is known to depress many enzyme systems. It also forms a hydrogen bonded complex with double-stranded DNA that inhibits strand separation, transcription and protein synthesis (Goldsmith, 1992).

Antimalarial Action

Quinine is a rapidly acting, highly effective blood schizonticide against the four malarial parasites. The drug is gametocidal for *Plasmodium vivax* and *Plasmodium ovale* but not very effective against *Plasmodium falciparum* gametocytes. Quinine has no effect on sporozoites or the liver stages of any of the parasites (Goldsmith, 1992).

Clinical Uses

A. Parenteral Treatment of Severe Falciparum Malaria

In patients with previously untreated severe malaria an initial loading dose of quinine should be given (Krishna and White, 1996). An initial dose of 20 mg/kg loading dose given over four hours, following by 10 mg/kg every eight hours for a total of seven days was found to be safe and effective (White, et al., 1983). Parenteral quinine is available as a

dihydrochloride salt and should be given in a dose of 7 mg/kg infused at a constant rate over 30 min, followed by 10 mg/kg infused over 4 hour (Davis, et al., 1990). Wattanagoon, et al. (1986) suggested that when intravenous infusion is not possible an intramuscular quinine loading dose (quinine dihydrochloride 20 mg salt or 16.7 mg base/kg followed by three or four eight hourly maintenance dose of 10 mg salt or 8.3 mg base/kg) injected into the anterior thigh is an effective means of starting treatment in patients with moderately severe falciparum malaria who can not swallow tablets.

B. Oral Treatment of Falciparum Malaria Resistant to Chloroquine

Quinine is currently recommended for the treatment of chloroquine-resistant *Plasmodium falciparum* malaria and is the drug of choice for the treatment of complicated and/or cerebral malaria (WHO, 1990). Quinine sulfate 600 mg every 8 hours combined with tetracycline 250 mg every 6 hours for 7 days is one of the choice in adult with uncomplicated falciparum malaria (Looareesuwan, 1995).

C. Other Uses

Quinine is widely prescribed as the treatment of choice for nocturnal leg cramps (Mackie and Davidson, 1995) and McGee (1990) reported that quinine has been suggested as the most effective pharmacological treatment available. Doses of quinine ranging from a single 300 mg tablet of the bisulfate salt (equivalent to 178 mg quinine

base) to 600 mg (two tablets) of quinine sulfate (representing 496 mg base) are prescribed commonly for cramp (Dyer, et al., 1994).

Therapeutic Range

The blood concentrations of quinine required for optimum parasiticidal effect are not known with certainly, but most investigators have aimed to achieve a total plasma levels over 8-15 mg/l in severe drug resistant malaria (White, 1988). After standard therapeutic doses, peak plasma levels of quinine may reach 15 to 20 mg/l in severely ill Thai patients without causing major toxicity (Tracy and Webster, 1996). In addition, plasma quinine concentrations exceeding 10 mg/l as the minimum inhibitory concentration have been required for some strains of *Plasmodium falciparum* cultured from patients in Thailand (White, et al., 1983).

Adverse Effects

1. Gastrointestinal Effects

Quinine is an irritant to the gastric mucosa and often causes nausea, vomiting or epigastric pain (Goldsmith, 1992).

2. Cinchonism

Mild toxic effect known as cinchonism, may also occur at therapeutic doses. Quinine produces a characteristic symptom complex

when plasma concentrations exceed 5 mg/l (as they invariably do during the treatment of falciparum malaria) (Powell and McNamara, 1972). These symptoms are called cinchonism and consist of nausea, vomiting, dizziness, diarrhea and headache (White, 1988) and it usually develops when plasma levels of quinine exceed 7-10 mg/l; in some patients, however, symptoms may occur at lower plasma levels (Goldsmith, 1992).

3. Hematologic Effects

Hemolysis directly attributable to quinine occur in 0.05 % of people treated for acute malaria; it may occur in glucose-6-phosphate dehydrogenase-deficient persons. Leukopenia, agranulocytosis, thrombocytopenic purpura. Henoch-Schonlein purpura and hypoprothrombinemia are rare (Goldsmith, 1992). Quinine has a peripheral vasodilator action and has been associated with marked postural hypotention in patients being treated for acute falciparum malaria (Kofi-Ekue, et al., 1987; Supanaranond, et al., 1993). It has been postulated that this effect is mediated the inhibition of the action of aldosterone and angiotensin (Hadjokas and Goodfriend, 1991).

4. Hypoglycemia

Hypoglycemia is the most important adverse effect of quinine at therapeutic dose (White, et al., 1983; Okitolonda, et al., 1987). Quinine stimulates insulin release from pancreatic islet cells, both *in vitro* and in volunteers and patients (White, et al., 1983; Davis, et al., 1993) and may

cause recurrent hyperinsulinaemic hypoglycemia, in addition, quinine-induced hyperinsulinaemia is one of several factors contributing to the pathogenesis of hypoglycemia in severe falciparum malaria (White, 1988).

5. Severe Toxicity

Severe toxicity is rare but it can occur in the cases of over doses and it is closely related to plasma concentration. Bateman, et al. (1985) investigated 16 patients with quinine poisoning; four had no clinical evidence of quinine toxicity (admission plasma quinine concentration range from 4.7 to 9.3 mg/l), three had mild clinical toxicity as evidenced by symptoms of cinchonism with no objective defects in vision (admission plasma quinine concentration range from 9.1 to 9.7 mg/l) and the other nine had more severe quinine poisoning with ocular features including peripheral field constriction, dilated pupils, reduced acuity or blindness (admission plasma quinine concentration range from 12.4 to 23.5 mg/l). Three from nine patients had cardiac arrhythmia (admission plasma quinine concentration range from 14.6 to 23.5 mg/l and one from nine died (admission plasma quinine concentration was 20.4 mg/l, he was a two-years-old boy) and they concluded that plasma quinine concentrations above 10 mg/l are associated with an increasing risk of permanent ocular damage or death, and plasma concentrations above 16 mg/l were associated with cardiac arrhythmia. In the cases of chronic therapy, toxic effects of quinine generally occur with levels of more than

or equal to 10 mg/l (Powell and McNamara, 1972). Rheeder and Sieling (1991) reported permanent blindness in a young man given 5 doses of quinine 600 mg in less than 24 hr. After vomiting the first dose, administration was repeated, resulting in confusion, disorientation and drowsiness, with bipolar headache, malaise, tinitus and the perception of yellow spots in the visual field. Three further doses were given in the next 12 hr (total dose 3,000 mg). On waking, the patient was blind. Lethal doses may be around 8 g (Bolan et al., 1985). Quinine intoxication following doses of 4 to 12 g is characterized by seizures and coma. Early symptoms are mild visual and hearing complains. A principal sign is the sudden onset of bilateral pupil dilatation (Phillips-Howard and Kuile, 1995).

Cholestyramine

Cholestyramine is the chloride salt of a basic anion exchange resin. It is a high molecular weight (more than 1,000,000) copolymer of 98 % polystyrene and 2 % divinylbenzene containing approximately 4 meq of fixed quaternary ammonium groups per gram of dry resin. The resin is administered as the chloride salt, which exchanges for other anions in the tract with a greater affinity for the positively charged functional groups on the resin (Blanchard and Nairn, 1968). The compound is not absorbed in the tract and it is hydrophilic but insoluble in water (Goldstein and Brown, 1991).

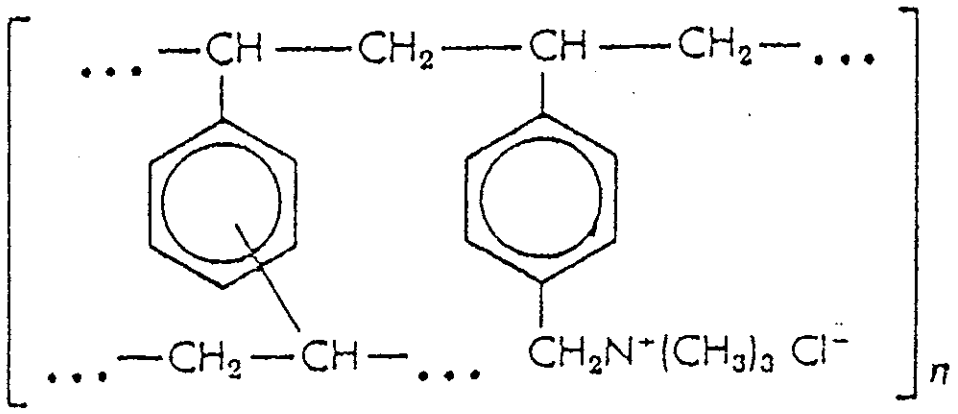

Figure 2 Molecular structure of cholestyramine

Indication

Cholestyramine was originally used to control pruritus in patients with elevated concentrations of plasma bile acid due to cholestasis. While this remains a valid use of the drug, greater interest now centers on the ability of this agent to lower concentrations of plasma LDL-cholesterol (Hashim and Van-Itallie, 1965) and it is the drug of choice for the treatment of patients with hypercholesterolemia caused by increased LDL-cholesterol levels without concurrent hypertriglyceridemia (type II A and type II B hyperlipoproteinemia) (Ast and Fishman, 1990). Shakir, et al. (1993) reported that cholestyramine could be useful in the treatment of hyperthyroidism in patients with exogenous thyrotoxicosis and from the study of Mercado, et al. (1996) they concluded that cholestyramine represents an effective and well-tolerated adjunctive therapy in patients with hyperthyroid Graves' disease, and it produces a more rapid and complete decline in thyroid hormone levels in these patients. Aside from these, it has been suggested that it may be useful in treatment of poisoning with digitoxin (Caldwell and Greenberger, 1971), acetaminophen (Dordoni, et al., 1973; Siegers and Moller-Hartmann, 1989), methotrexate (McAnena, et al., 1987), digoxin (Henderson and Solomon, 1988) and amiodarone (Goddard and Whorwell, 1989).

Dosage and Administration

Orally, the recommended initial adult dose is 1 packet or 1 spoonful (containing 4 gram of cholestyramine resin in 9 g of the drug) 3

times daily before meals. Dosage may then be adjusted as required to meet the patient's clinical situation; however, the total daily dosage should not exceed 24 gram. The usual maintenance dosage is 4 g 3 or 4 times daily before meals and at bedtime. The dosage for infants and children has not been established.

The powder should always be mixed with water or other fluids before ingestion.

Mechanism of Action

The highly charged quaternary ammonium salts of cholestyramine have a complex mechanism of action that includes non specific binding of the bile acid pool within the small intestine and resul in decrease of the absorption of bile acids into the systemic circulation.

Efficacy

Lipid Research Clinic Program (1984) reported that cholestyramine decreased in total cholesterol by 13.4 % and LDL- cholesterol 20.3 % in patients consuming 16 g of cholestyramine per day and Brensike, et al. (1984) showed that cholestyramine decreased in LDL-cholesterol by 26 % in patients receiving 6 g of cholestyramine four times daily. Sweeney. et al. (1991) demonstrated that cholestyramine powder and bar are equally effected in yielding significant reductions in total cholesterol (17 % and 16 % decrease, respectively). The reductions in LDL-cholesterol were also significant, with a 29 % reduction in the powder group and a 28 %

reduction in the bar group. HDL-cholesterol increased slightly in both groups, but this was not significant.

Adverse Effect

Since, cholestyramine is not absorbed from the gastrointestinal tract, it may be the safest drugs currently available for the treatment of hyperlipoproteinemia. The principal disadvantages of cholestyramine are the gastrointestinal tract side effects, the inconvenience of preparation and its organoleptic properties. All these factors contribute to poor patient acceptability and compliance and may have contributed to the delay in physician acceptance that elevated cholesterol levels are a definite risk factor to be actively treated (Stein, et al., 1990). It has been used for more than 20 years without serious side effects (Berkowitz, 1964). However, from the study of Brensike, et al. (1984) they reported that the adverse effects of cholestyramine in patients who received daily dosage of 24 g for five years were abdominal pain 3.4 %, belching/bloating 5.1 %, constipation 5.1 %, heartburn 5.1 %,drowsiness 8.5 %, itching 1.7 %, leg cramps 8.5 %, nervousness 8.5 %, rash 1.7 % and weakness 5.1 % where placebo were 0, 5.3, 3.5, 0, 1.8, 0, 1.8, 5.3, 0, and 0 %, respectively. Levy, et al. (1972) suggested that the usage of cholestyramine may result in the malabsorption of fat-soluble vitamins, especially at doses above 24 g/day but for vitamin D, the result of the study from Hoogwerf, et al. (1992) indicated that long-term cholestyramine administration (dosage 24 g/day) dose not have any apparent adverse effects on availability of vitamin D (a

clinical trial was carried out over a period of 7 to 10 years). Folic acid deficiency has been reported in children, sometimes requiring supplementation (West and Lloyd, 1975).

Drug Interaction

Because it is not absorbed from the gastrointestinal tract, it may interfere with the gastrointestinal absorption of several drugs. There are many pharmacokinetics interaction studies between cholestyramine and other drugs.

Paracetamol

Dordoni, et al. (1973) reported that the absorption of 2-g dose of paracetamol was markedly reduced to 62 % by the simultaneous oral administration of cholestyramine but was only slightly reduced (16 %) when cholestyramine was given 60 minutes after the paracetamol.

Chlordecone

Boylan, et al. (1978) reported that cholestyramine binds chlordecone (Kepone®) in the intestinal of male Sprague-Dawley rats, increases its excretion into the feces, and decreases its contents in the tissues.

Hydrocortisone

In 10 healthy subjects, cholestyramine reduced the plasma cortisol (35 %) concentration after oral hydrocortisone and it might delay as well as reduce the intestinal absorption of this drug (Johansson, et al., 1978).

Propranolol

Hibbard, et al. (1984) reported that the peak plasma concentration of propranolol and 4-hydroxypropranolol reduced from 102.6 ng/ml to 76.6 ng/ml and 11.2 ng/ml to 8.7 ng/ml, respectively when cholestyramine one dose (8 g) was given concomitantly with propranolol compared to propranolol alone and when the two doses regimen of cholestyramine (an additional dose of cholestyarmine was given 12 hr prior to the ingestion of propranolol) the peak plasma concentration of propranplol and 4-hydroxypropranolol reduced from 104.1 ng/ml to 46 ng/ml and 9.6 to 4.6 ng/ml, respectively.

Warfarin

Cholestyramine increased plasma warfarin clearance by 30 % and reduced elimination half-life by 38 % (Renowden, et al., 1985).

Hydrochlorothiazide

A single 8 g dose of cholestyramine 2 hr before or after hydrochlorothiazide significantly decreased the amount of hydrochlorothiazide excreted unchanged in the urine over 24 hr by 65 %

and 36 %, respectively and multiple dose of cholestyramine significantly altered hydrochlorothiazide kinetics, including reductions in AUC by 32 % and C_{max} by 31 % (Hunninghake, et al., 1985).

Methotrexate

McAnena, et al. (1978) reported that the addition of cholestyramine to an elemental liquid diet improves survival and reduces gastrointestinal toxicity following methotrexate administration, by binding methotrexate in bile and reducing the delay in systemic clearance of the drug and they suggested that cholestyramine may be of clinical benefit in patients receiving high dose methotrexate regimens as an adjunct to leucovorin rescue.

Digoxin

The serum digoxin concentration declined rapidly and the digoxin half-life decreased by 73.6 % while cholestyramine was administered, in patient with digoxin intoxication. All signs and symptoms of toxic reaction subsided during the period of cholestyramine therapy, which correlated with the decline in digoxin concentrations (Henderson and Solomon, 1988)

Non-Steroidal Anti-Inflammatory Drugs (NSAID)

Naproxen

Calvo and Dominguez-Gil (1984) reported that cholestyramine shows a marked affinity for naproxen and the intensity of this is governed by the pH values and it causes an important delay in the incorporation of naproxen into the systemic circulation, though no significant modifications are seen to take place in any other pharmacokinetic parameters of the drug.

Tenoxecam and Piroxicam

Guentert, et al. (1988) reported the influence of multiple doses of cholestyramine (10 days) on the single dose pharmacokinetics of tenoxecam and piroxicam in eight healthy young volunteers in which they found that the average values of half-life were reduced from 67.4 hr to 31.9 hr by tenoxecam and from 46.8 hr to 28.1 hr by piroxicam.

Sulindac

In the concurrent phase (4 g of cholestyramine twice daily for 2 days and on the second day, the subjects concomitantly took 400 mg of sulindac with the morning dose of cholestyramine), the AUC and peak plasma concentration for sulindac decreased by approximately 77 % and for its metabolite (sulindac sulfide), the AUC and peak plasma concentration for sulindac sulfide decreased by approximately 84 % and

73 %, respectively. The half-life was found to decreased by 67 % and AUC decreased by 56 % when the drugs are administered in the staggered fashion (sulindac 3 hours before cholestyramine) compared to sulindac alone and for sulindac sulfide the half-life decreased by 70 % and the AUC decreased by 55 % in the staggered fashion compared to sulindac sulfide alone (Malloy, et al., 1994).

Meloxicam

Busch, et al. (1995) studied the effect of cholestyramine on the pharmacokinetics of single IV doses of meloxecam in healthy male volunteers and found that the mean terminal phase elimination half-life was reduced from 19.5 hr to 12.7 hr and the clearance was approximately 50 % higher when multiple doses of cholestyramine followed the intravenous drug administration than when the drug was given alone.

Furosemide

Neuvonen, et al. (1988) found that the absorption of frusemide, an acidic drug with a pKa of 3.8 (40 mg) concomitant administration with cholestyramine (8 g) was diminished by 95 % compared with frusemide alone (the peak plasma concentration for frusemide was reduced from 1.2 mg/l to 0.05 mg/l).

Glipizide

The absorption of glipizide (a secondary-generation sulfphonylurea) was reduced by cholestyramine 29 % (measured as the AUC from 0 to 10 hr) and peak plasma concentration was lowered by 33 % in six male volunteers who were received 5 mg glipizide with 8 g cholestyramine compared to glipizide with water (Kivisto and Neuvonen, 1990).

Lorazepam and Lorazepam Glucuronide

Herman and Chaudhary (1991) showed that *in vitro* study, cholestyramine bound to lorazepam by 24 % and lorazepam glucuronide by 74 %, in addition, these values were independent of substrate concentration, and buffers of pH 5 and pH 6 had no significant effect.

Imipramine

In six depressed patients who received chronic treatment with imipramine and received a 5 days course of cholestyramine of a dosage of 4 g three times daily. Cholestyramine treatment was associated with an average of 23 % decrease in plasma imipramine levels (Spina, et al., 1994) although, *in vitro*, cholestyramine has been shown to bind extensively to imipramine, the degree of adsorption varied from a 79 % at pH 1 to 44 % at pH 4 and to 62 % at pH 6.5 (Bailey, 1992).

Anticonvulsant Drugs

Valproate

Malloy, et al. (1996) demonstrated that when valproic acid was given concurrently with cholestyramine, there was a significant decrease in peak plasma concentration of valproic acid by approximately 15 %, and the AUC exhibited a 21 % decrease, compared to valproic acid alone where as staggering the 2 drugs led to no significant decrease in AUC and peak plasma concentration of valproic acid compared to when valproic acid was given alone.

CHAPTER 3

MATERIALS AND METHODS

Chemicals and Reagents

Standard quinine sulfate and quinidine hydrochloride were purchased from Aldrich Chem. Co. and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. Quinine sulfate (300 mg tablet) was obtained from The Government Pharmaceutical Organization, Bangkok, Thailand. Cholestyramine (Questran® light Lot No. B602721) was obtained from Bristol-Myers Squib Pharmaceuticals Pty. Ltd. Victoria, Australia. Acetonitrile (HPLC-grade) and Triethylamine (analytical grade) were obtained from J.T. Baker Inc. Phillipsburg, U.S.A. and Fluka, Messerschmittstr, 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 a Jasco PU-980 pump, the automated injection system was Waters 717 plus Autosampler (Waters Associates, Milford, MA, U.S.A.). The detector was Jasco 821-FP intelligent Spectrofluorometer (Japan Spectroscopic Co, Ltd). The integrater was the Jasco model 807-IT (Tokyo, Japan). A μ-Bondapak C_{18} (30 cm x 3.9 mm I.D., particle size 10 μm, Waters Associates) was

used as the column. A guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

Methods

1. Subjects

Ethical approval for this study was obtained from the Human Ethical Committee, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. All subjects gave written informed consent. Eight healthy Thai men, aged 26-35 yr, weighing 55-64 kg, participated in the study. All subjects were non-smoker, non-alcoholic. Medication was stopped for at least 1 month before and during the entire period of study. Prior to the study, a medical history, a physical examination, and a full hematological and biochemical screening were performed in each subject.

2. Protocol

Four phases of studies were a 4 x 4 Latin square designed with 2 weeks wash-out period . In phase 1, each subject received a single 600 mg dose of quinine sulfate (479 mg quinine base) orally with approximately 200 ml of water followed by a serial blood draw. In phase 2, each subject received quinine sulfate 600 mg with 200 ml of water concomitantly with 8 g of cholestyramine (Questran®) mixed with 200 ml water and a serial blood draw was performed. In phase 3, each subject received 600 mg quinine sulfate with 200 ml of water 1 hr before taking 8 g of

cholestyramine, and a serial blood draw was performed. In phase 4, each subject was orally given 8 g of cholestyramine 1 hr before administration of quinine sulfate 600 mg.

Blood Sample Collection

Quinine sulfate was administered after an over night fast, an indwelling heparin-lock catheter was placed in a vein in the forearm of each subject. Serial blood samples (5ml) were drawn immediately before quinine sulfate administration and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 and 48 hr post quinine sulfate administration. Plasma was separated from blood samples by centrifugation at 1,000 g for 15 minutes and aliquots of the plasma were stored at -20°C until analysis.

3. Analytical Methods

Samples were analyzed by high performance liquid chromatography for quinine and quinidine by methods previously described by Lehmann, et al. (1986); Edstine, 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 of deionized water and the pH was adjusted to 2.5 with 85 % phosphoric acid, the mixture was added with deionized water to 930 ml and 70 ml of acetonitrile (1 : 91.4 : 7 : 0.6 %, v/v, triethylamine, deionized water, acetonitrile and 85 % phosphoric acid, respectively) was added. The mixture of mobile phase was filtered through 0.45 micropore filtered paper and degased before using.

The mobile phase was pumped at 1.5 ml/min. and the eluent was monitored with fluorescence detector at room temperature.

3.2 Stock Standard Solutions

The standard powders of quinine sulfate and quinidine hydrochloride (10 mg) were dissolved in 1 ml of methanol and were added with the mobile phase to a final volume of 10 ml and they were diluted to 100 μg/ml by the mobile phase for stock standard solutions and stored at -20° C.

3.3 Calibration Curves

The calibration curves were prepared by adding a stock standard solutions (100 μg/ml of quinine and quinidine in mobile phase) to blank human plasma and the final concentrations of both drugs were 1, 5, 10 and 15 μg/ml. The calibration curves for both drugs by using peak area was linear in the range 1 to 15 μg/ml. The average coefficient of variation

(CV) less than 6 %. The lower detection limit for quinine sulfate was 20 ng/ml.

3.3.1 Recovery

Potential loss of quinine during the precipitation by acetonitrile was determined by comparing the peak area of quinine precipitated from plasma sample in the range of 1-15 μg/ml and the equal concentration of standard quinine prepared in mobile phase. The potential loss of the internal standard determined by the same method. The percent recovery was calculated as following

$$\frac{\text{peak area of standard quinine or quinidine in plasma x 2}}{\text{peak area of standard quinine or quinidine in mobile phase}} \times 100$$

3.3.2 Precision and Variability

To determine intra-day precision and variability, the standard quinine was spiked in blank plasma at concentration 1, 5, 10, and 15 μg/ml and 10 replications were carried out on one day. All should be of ± 10 % of spiked value and the CV of each concentration should be less than 10 %.

To determine inter-day precision and variability, the standard quinine was spiked in blank plasma at concentration 1, 5, 10, and 15 μg/ml and each was carried out on 10 different days. Accuracy should be

of ± 10 % of spiked value and the CV of each concentration should be less than 10 %.

3.3.3 Sample Preparation

A 100 µl of internal standard (5 µg/ml of quinidine hydrochloride in mobile phase) was added to 400 µl of plasma. The mixture was precipitated with acetonitrile 500 µl. After mixing for 30 second and centrifuging at 14,000 rpm for 15 min, the 20 µl supernatant was injected by an automated injection.

4. Data Analysis

4.1 Pharmacokinetic Calculations

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

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

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

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

4.2 Statistical Analysis

All results are expressed as means ± S.D. Differences in quinine pharnacokinetic parameters among control and three treatments were tested for statistical significance by analysis of variance (ANOVA) with P value less than 0.05 taken as the minimum levels of significant. Duncan's multiple range test was used to test for significant differences between means.

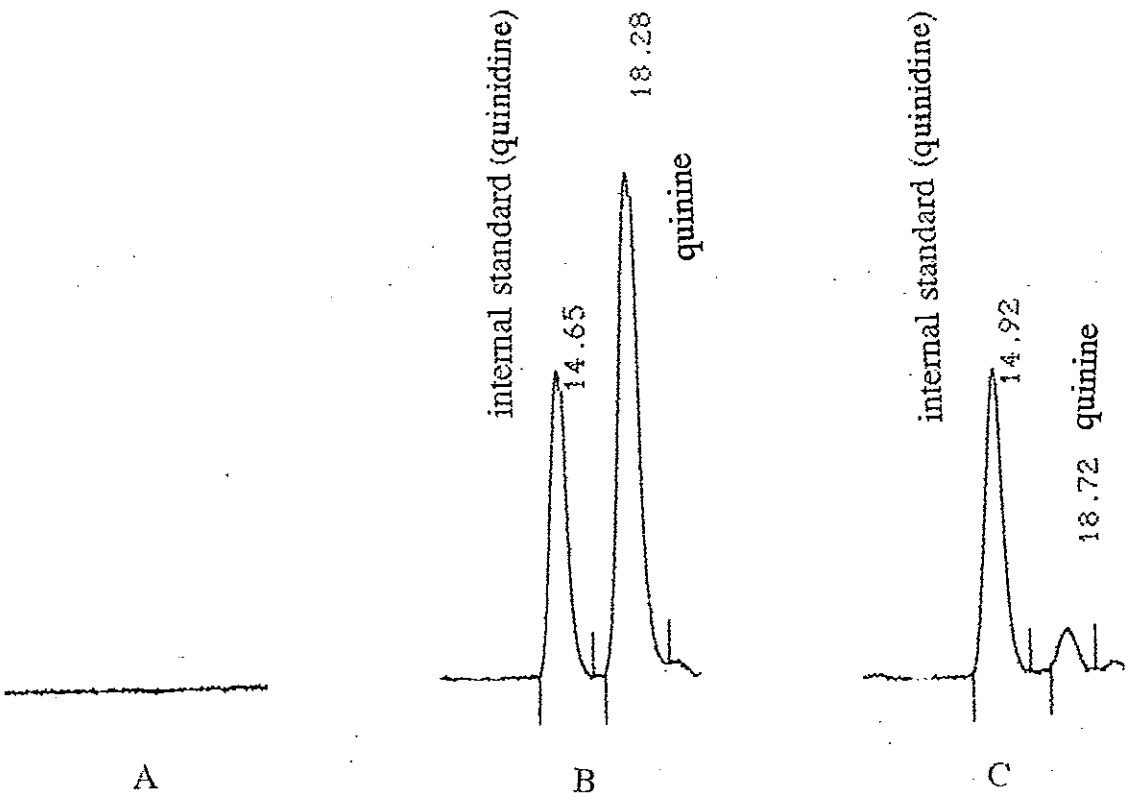

Figure 3 Chromatograms of a standard quinine and quinidine (internal standard) in a 20 µl human plasma sample. (A) blank; (B) spiked with a standard quinine 4 µg/ml and an internal standard (quinidine 5 µg/ml); (C) spiked with a standard quinine 0.5 µg/ml and an internal standard. Flow rate was 1.5 ml/min. Chart speed was 2 mm/min. Attenuation was 8 mV F.S.

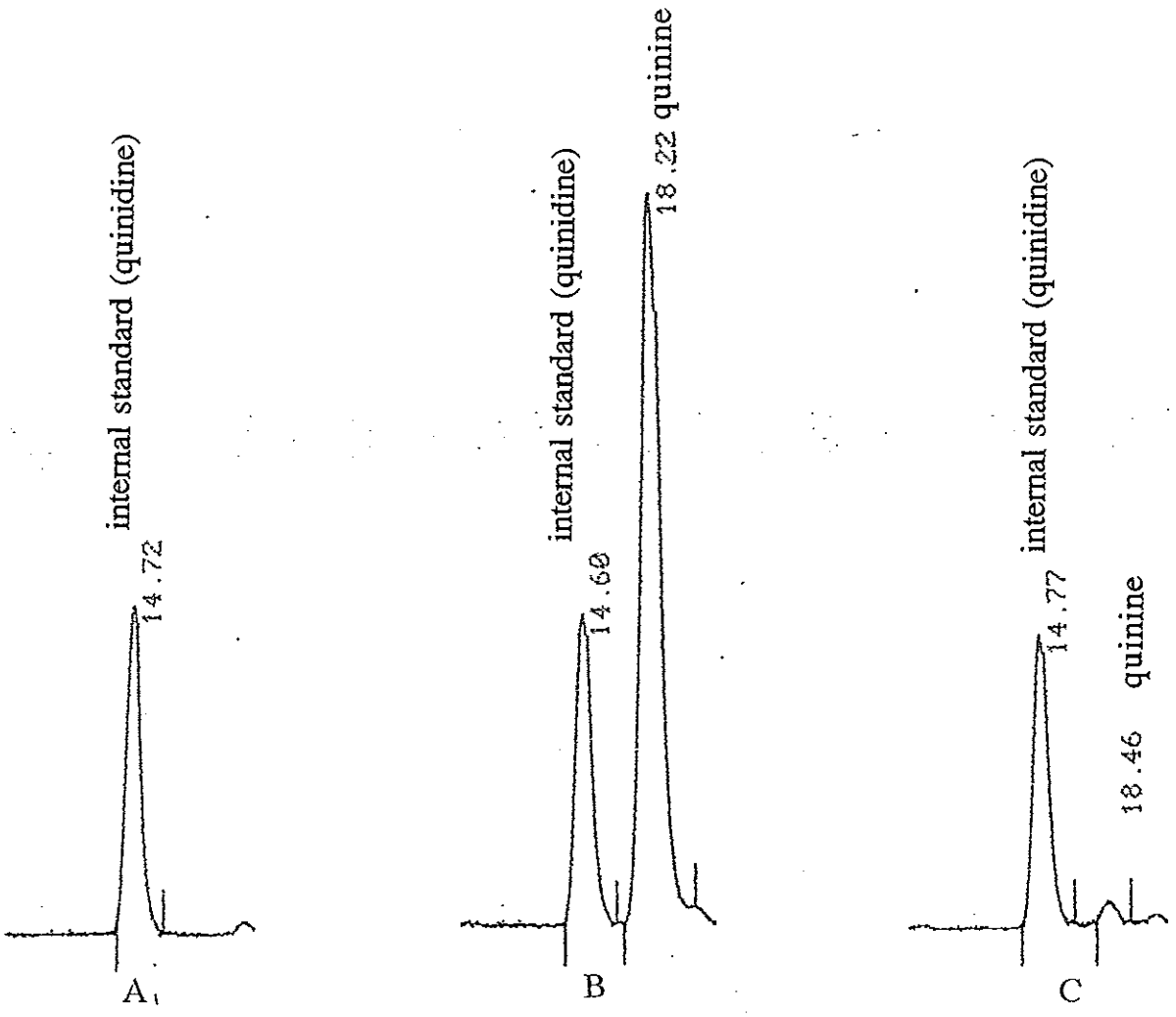

Figure 4 Chromatograms of quinine in a 20 µl human plasma sample of healthy subject after orally given 600 mg quinine sulfate alone. (A) before ingestion of quinine; (B) 1 hr after receiving quinine; (C) 48 hr after receiving quinine. Flow rate was 1.5 ml/min. Chart speed was 2 mm/min. Attenuation was 8 mV F.S.

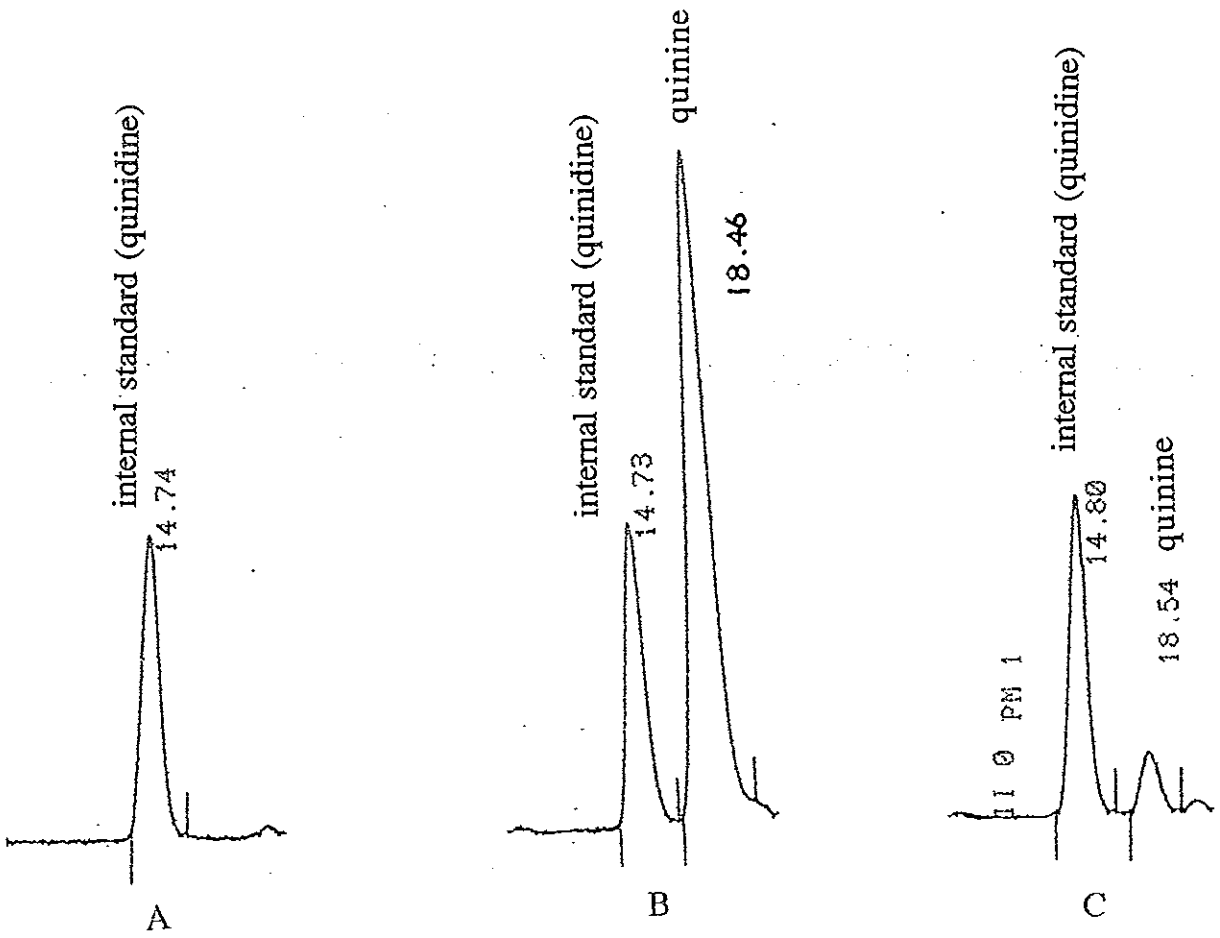

Figure 5 Chromatograms of quinine in a 20 μl human plasma sample of healthy subject receiving 600 mg quinine sulfate concomitant with 8 g of cholestyramine. (A) before treatment; (B) 1 hr after treatment; (C) 48 hr after treatment. Flow rate was 1.5 ml/min. Chart speed was 2 mm/min. Attenuation was 8 mV F.S.

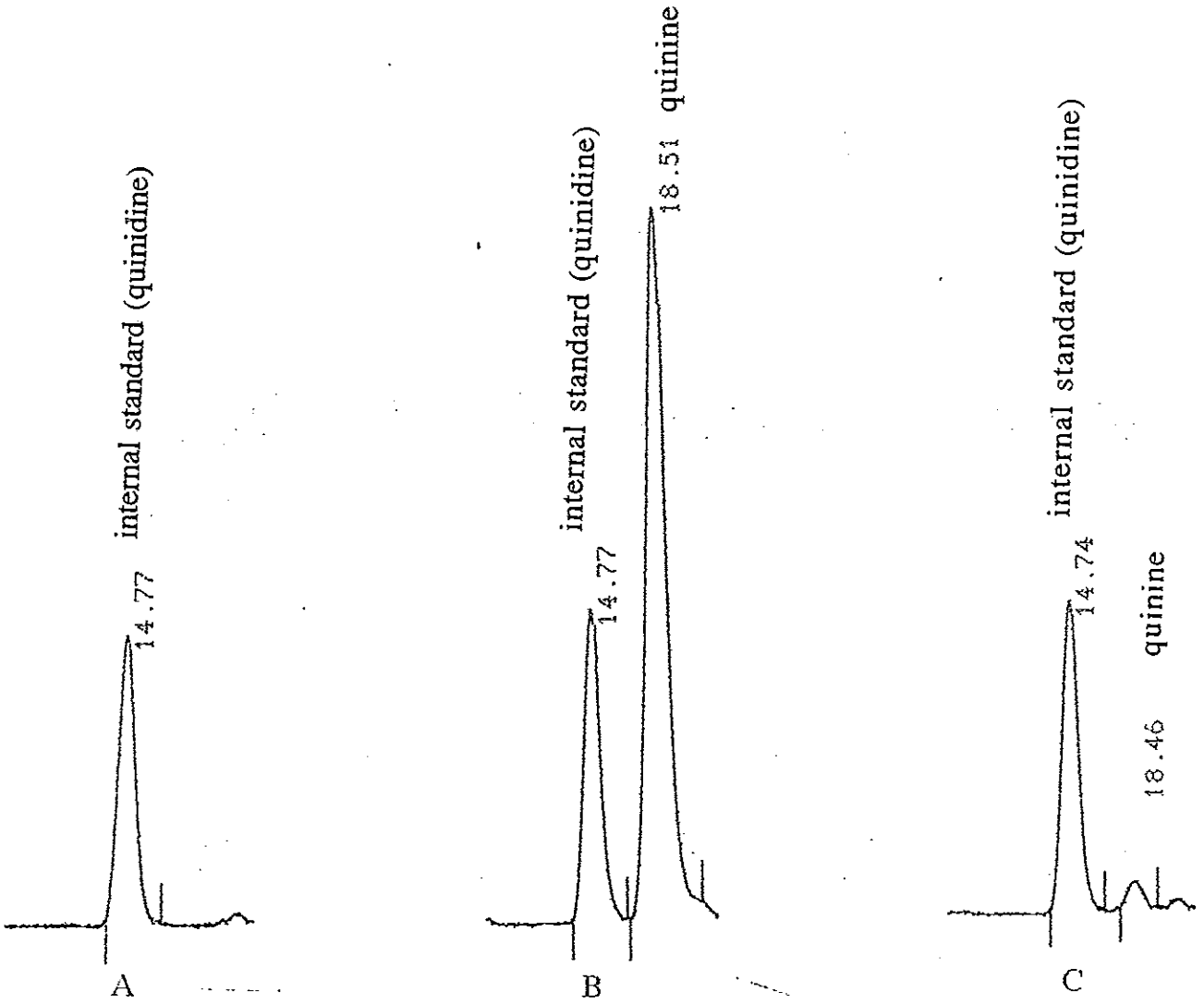

Figure 6 Chromatograms of quinine in a 20 μl human plasma sample of healthy subject receiving 600 mg quinine sulfate 1 hr before cholestyramine. (A) before treatment; (B) 1 hr after treatment; (C) 48 hr after treatment. Flow rate was 1.5 ml/min. Chart speed was 2 mm/min. Attenuation was 8 mV F.S.

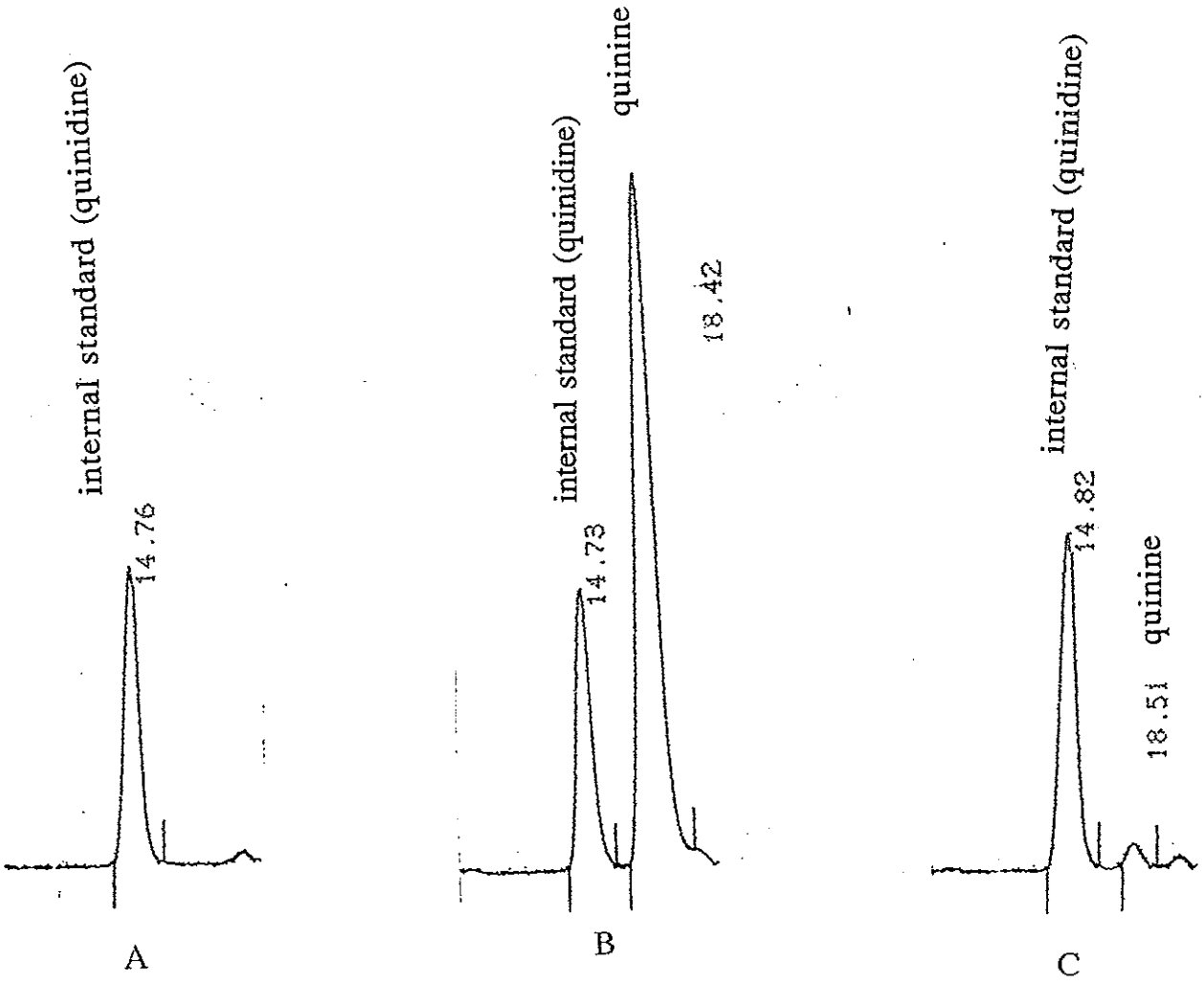

Figure 7 Chromatograms of quinine in a 20 μl human plasma sample of healthy subject receiving 600 mg quinine sulfate 1 hr after 8 g of cholestyramine. (A) before treatment; (B) 1 hr after treatment; (C) 48 hr after treatment. Flow rate was 1.5 ml/min. Chart speed was 2 mm/min. Attenuation was 8 mV F.S.

Table 1 **The intra-assay variance of four different quinine concentrations in mobile phase**

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	238745.55 ± 1498.36	0.63
5	1162332.70 ± 24442.57	2.10
10	2401726.33 ± 30529.25	1.27
15	3631772.33 ± 54997.51	1.50

Table 2 **The inter-assay variance of four different quinine concentrations in mobile phase**

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	239185.55 ± 3647.94	1.52
5	1170190.00 ± 40510.27	3.46
10	2386257.00 ± 30784.15	1.29
15	3558103.00 ± 61535.41	1.73

[a]Various concentrations of standard quinine were directly injected into HPLC system

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

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

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	126638 ± 476.59	0.38
5	605608.33 ± 5846.76	0.96
10	1246261.33 ± 2777.96	0.22
15	1927033.5 ± 11457.25	0.59

Table 4 The inter-assay variance of four different quinine concentrations in plasma

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	121993.25 ± 2665.63	2.78
5	596455.36 ± 8087.51	2.56
10	1180296.8 ± 30225.2	1.56
15	1775392.6 ± 49302.91	2.18

[a]Various concentrations of standard quinine were added to drug-free plasma sample prior to precipitation as ratio 1:1

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

Table 5 Relative recovery of standard quinine in plasma

Concentration[a] (μg/ml)	Peak area in mobile phase[b] (Mean ± S.D.) (n=10)	Peak area in plasma[b] (Mean ± S.D.) (n=10)	% Recovery
1	239185.55 ± 3647.94	243986.50 ± 5331.26	102.01
5	1170190 ± 40510.27	1192910.72 ± 16175.02	101.94
10	2386257 ± 30.784.15	2360593.60 ± 60450.40	98.92
15	3558103 ± 61535.41	3550785.2 ± 98605.82	99.79

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

[b] Various concentrations of standard quinine were added to drug-free plasma sample prior to precipitation and the area values from the integrater were multiple by 2 (because the standard samples were diluted 2 times)

Table 6 **The intra-assay variance of four different quinidine concentrations in mobile phase**

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	231619.58 ± 7737.87	3.34
5	1142661.56 ± 10304.67	0.90
10	2262406.52 ± 60666.23	2.68
15	3391689.00 ± 14197.29	0.42

Table 7 **The inter-assay variance of four different quinidine concentrations in mobile phase**

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	238558.25 ± 7679.82	3.22
5	1139099.90 ± 21494.42	1.89
10	2275757.80 ± 38848.52	1.71
15	3418946.30 ± 78591.33	2.30

[a]Various concentrations of standard quinine were added to drug-free plasma sample prior to precipitation as ratio 1:1

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

Table 8 The intra-assay variance of four different quinidine concentrations in plasma

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	111488.59 ± 907.15	0.81
5	581648.00 ± 2917.52	0.50
10	1167555.00 ± 2472.04	0.21
15	1744093.50 ± 10148.10	0.59

Table 9 The inter-assay variance of four different quinidine concentrations in plasma

Concentration[a] (μg/ml)	Mean peak area ± S.D. (n=10)	CV[b](%)
1	113813.70 ± 3541.63	3.11
5	587226.25 ± 16675.21	2.84
10	1162004.20 ± 35948.87	3.09
15	1751602.50 ± 79300.00	4.53

[a]Various concentrations of standard quinine were directly injected into HPLC system

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

Table 10 Relative recovery of standard quinidine in plasma

Concentration[a] (μg/ml)	Peak area in mobile phase[b] (Mean± S.D.) (n=10)	Peak area in plasma[b] (Mean± S.D.) (n=10)	% Recovery
1	238558.25 ± 7679.82	227627.4 ± 7083.26	95.42
5	1139099.9 ± 21494.42	1174452.5 ± 33350.42	103.10
10	2275757.8 ± 38848.52	2324008.4 ± 71897.75	102.12
15	3418946 ± 78591.33	3503205.00 ± 158599.98	102.46

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

[b] Various concentrations of standard quinine were added to drug-free plasma sample prior to precipitation and the area values from the integrater were multipled by 2 (because the standard samples were diluted 2 times).

CHAPTER 4

RESULTS

Adverse Effects

All subjects of four trial-phases were well tolerated either following a single oral dose of 600 mg quinine sulfate or given concurrently with 8 g of cholestyramine. Therefore, eight healthy volunteers participated and completed in this study. However, mild and self-limiting adverse effects were noted in one subject of quinine alone phase and concomitant phase during the fourth hour after drug ingestion. One subject reported palpitation for approximately 4 hr after the ingestion of 600 mg quinine sulfate and another subject reported abdominal discomfort after a single oral dose of 600 mg quinine sulfate given immediately with 8 g of cholestyramine. However, these side effects were disappeared within 4 hr and not required specific treatment.

Pharmacokinetics

The mean plasma quinine concentrations of the four trial-phases are shown in Figure 9. The pharmacokinetic parameters of quinine for each treatment are illustrated in Table 12-15, and all mean pharmacokinetic parameters of four treatments are summarized in Table 16. There were no significant differences in all pharmacokinetic parameters among the four groups. Values of $AUC_{0-\alpha}$ were slightly

decreased from 90.23 ± 43.39 μg/ml.hr in subjects receiving quinine alone to 80.25 ± 32.13, 77.36 ± 35.71, and 77.86 ± 28.32 μg/ml.hr in subjects receiving quinine and cholestyramine simultaneously, quinine 1 hr before cholestyramine, and in quinine 1 hr after cholestyramine, respectively.

The other pharmacokinetic parameters such as the elimination rate constant (Ke) in subjects receiving quinine alone, quinine and cholestyramine simultaneously, quinine 1 hr before cholestyramine, and quinine 1 hr after cholestyramine were 0.07 ± 0.01, 0.07 ± 0.01, 0.08 ± 0.01, and 0.07 ± 0.01 hr^{-1} (mean ± S.D.), respectively. The elimination half-lives ($t_{1/2}$) were 10.07 ± 0.53, 9.49 ± 1.69, 8.83 ± 1.61, and 9.61 ± 1.00 hr; the maximum quinine concentrations (C_{max}) were 5.68 ± 2.48, 5.64 ± 2.46, 5.51 ± 2.10, and 5.25 ± 1.84 μg/ml; the time to maximum concentrations (T_{max}) were 1.35 ± 0.53, 1.02 ± 0.32, 1.23 ± 0.88, and 1.11 ± 0.23 hr; the apparent oral clearances (Cl/f) were 0.13 ± 0.05, 0.15 ± 0.05, 0.16 ± 0.07, and 0.14 ± 0.04 l/hr/kg; the apparent volume of distributions (Vd/f) were 1.91 ± 0.60, 1.94 ± 0.53, 1.96 ± 0.71, and 1.97 ± 0.41 l//kg; the lag times were 0.27 ± 0.10, 0.31 ± 0.13, 0.21 ± 0.08, and 0.24 ± 0.13 hr; the absorption rate constants (Ka) were 7.64 ± 10.23, 10.24 ± 10.68, 9.78 ± 6.26, and 5.47 ± 2.40 hr^{-1}; and the half-life of absorptions ($t_{1/2}$ abs) were 0.19 ± 0.11, 0.11 ± 0.06, 0.20 ± 0.20, and 0.14 ± 0.05 hr, in subjects receiving quinine alone, quinine and cholestyramine simultaneously, quinine 1 hr before cholestyramine, and quinine 1 hr after cholestyramine, respectively.

The assay validation of the experimental method demonstrated that the coefficient of variation for intra- and inter-assay variance of four different quinine concentrations in mobile phase was ranged 0.63-2.10 % and 1.29-3.46 %, respectively (Table 1-2). The coefficient of variation for intra- and inter-assay variance of four different quinine concentrations in plasma was ranged 0.22-0.96 % and 1.56-2.78 %, respectively (Table 3-4). The coefficient of variation for intra- and inter-assay variance of four different internal standard concentrations (quinidine) in mobile phase was ranged 0.42-3.34 % and 1.71-3.22 %, respectively (Table 6-7). The coefficient of variation for intra- and inter-assay variance of four different internal standard concentrations (quinidine) in plasma was ranged 0.21-0.81 % and 2.84-4.53 %, respectively (Table 8-9). The linearity of the standard range of 0.5-16 μg/ml was used as the standard curve for each day. It composed of 400 μl of standard quinine in plasma, 100 μl of internal standard in mobile phase and 500 μl of acetonitrile, and it was linear with the correlation coefficient (r) of 0.9998 (Figure 8) and the coefficient of variation (CV) was 1.88-8.49 % (averaged 3.27) (Table 11). The recovery of standard quinine and quinidine in plasma were ranged 98.92-102.01 % and 95.42-103.10 %, respectively (Table 5 and Table 10).

The semi-logarithmic mean plasma quinine concentration - time profile (Figure 10) and quinine plasma concentration - time profile from one subject (Figure 11) receiving quinine alone that the plasma

concentration declined monoexponentially and were fitted to a one compartment open model.

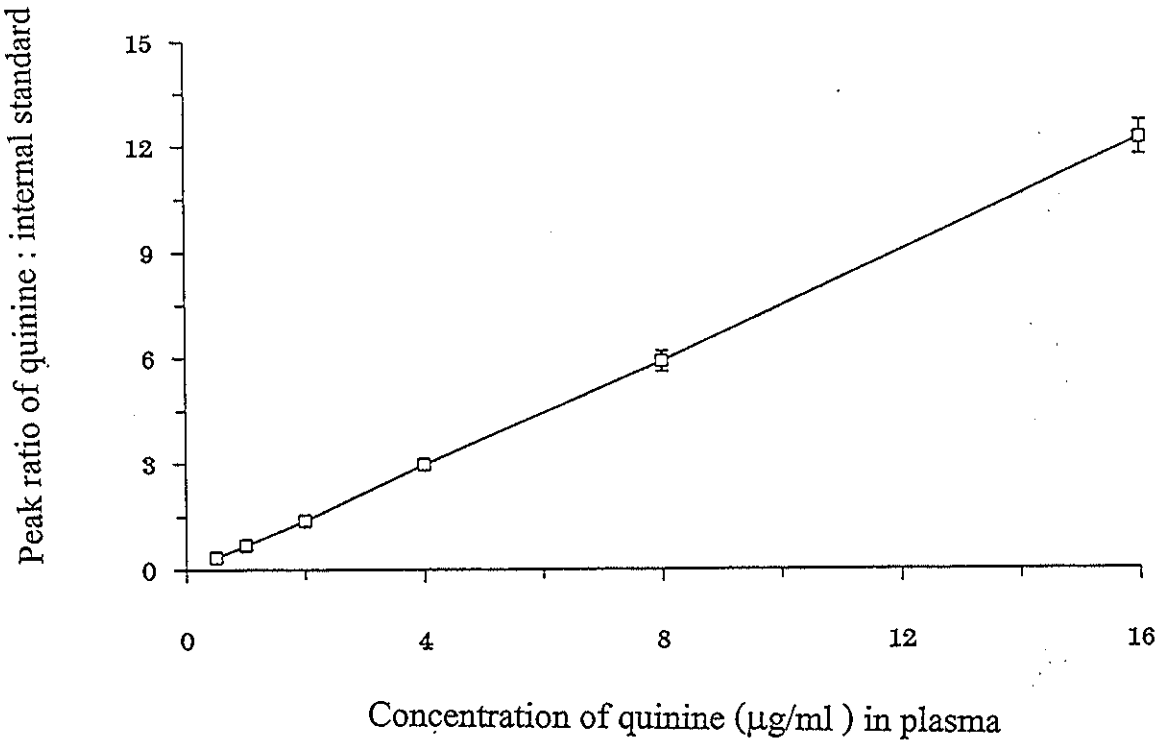

Figure 8 Correlation between peak ratio and concentration of quinine in plasma, correlation coefficient (r) = 0.9998

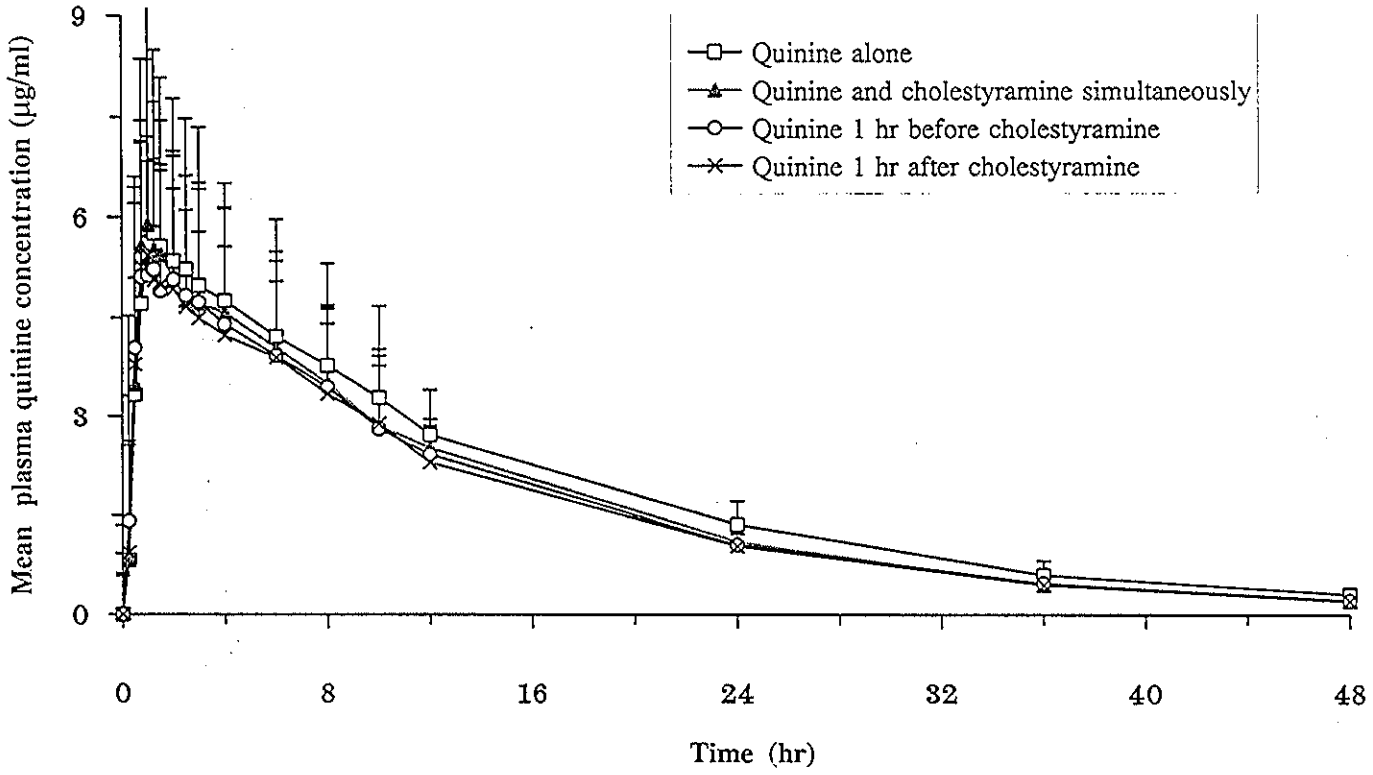

Figure 9 **Mean plasma quinine concentrations in 8 healthy volunteers following an oral administration of 600 mg qinine sulfate alone (□); or with 8 g of cholestyramine (▲); or given 1 hr before (O); or after (X) 8 g of cholestyramine. Each point represents mean ± S.D.**

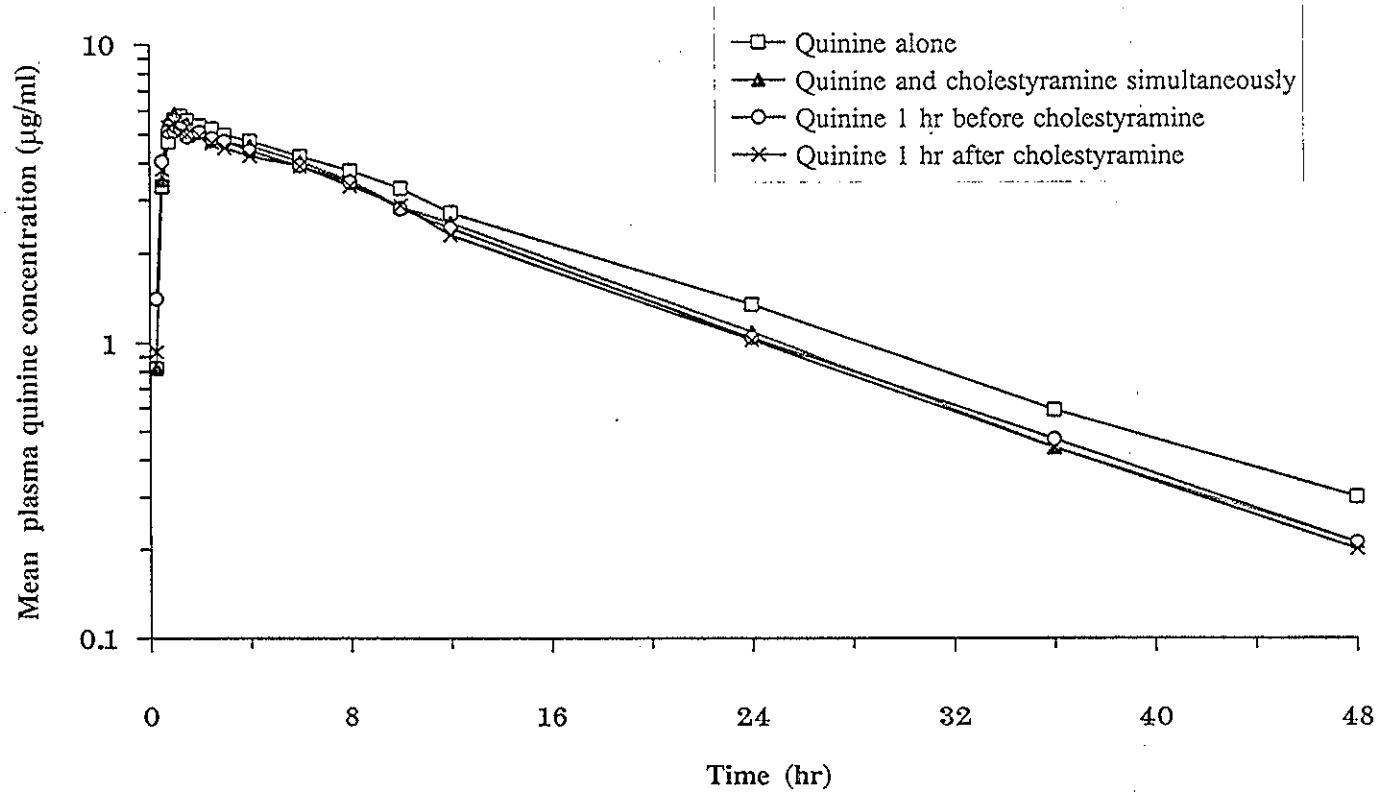

Figure 10 Semi-logarithmic mean plasma quinine concentrations in 8 healthy volunteers following an oral administration of 600 mg quinine sulfate alone (□); or with 8 g of cholestyramine(▲); or given 1 hr before (O); or after (x) 8 g of cholestyramine.

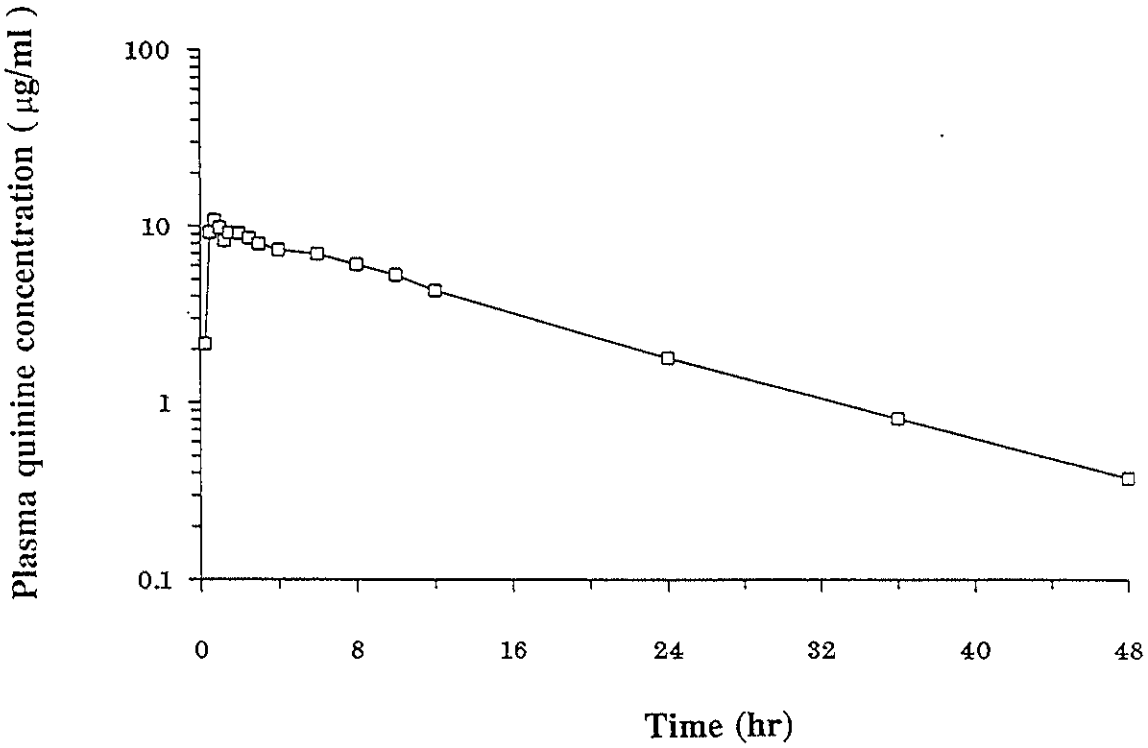

Figure 11 Semi-logarithmic plasma quinine concentration-time profile following an oral administration of a single 600 mg oral dose of quinine sulfate alone (subject number 3)

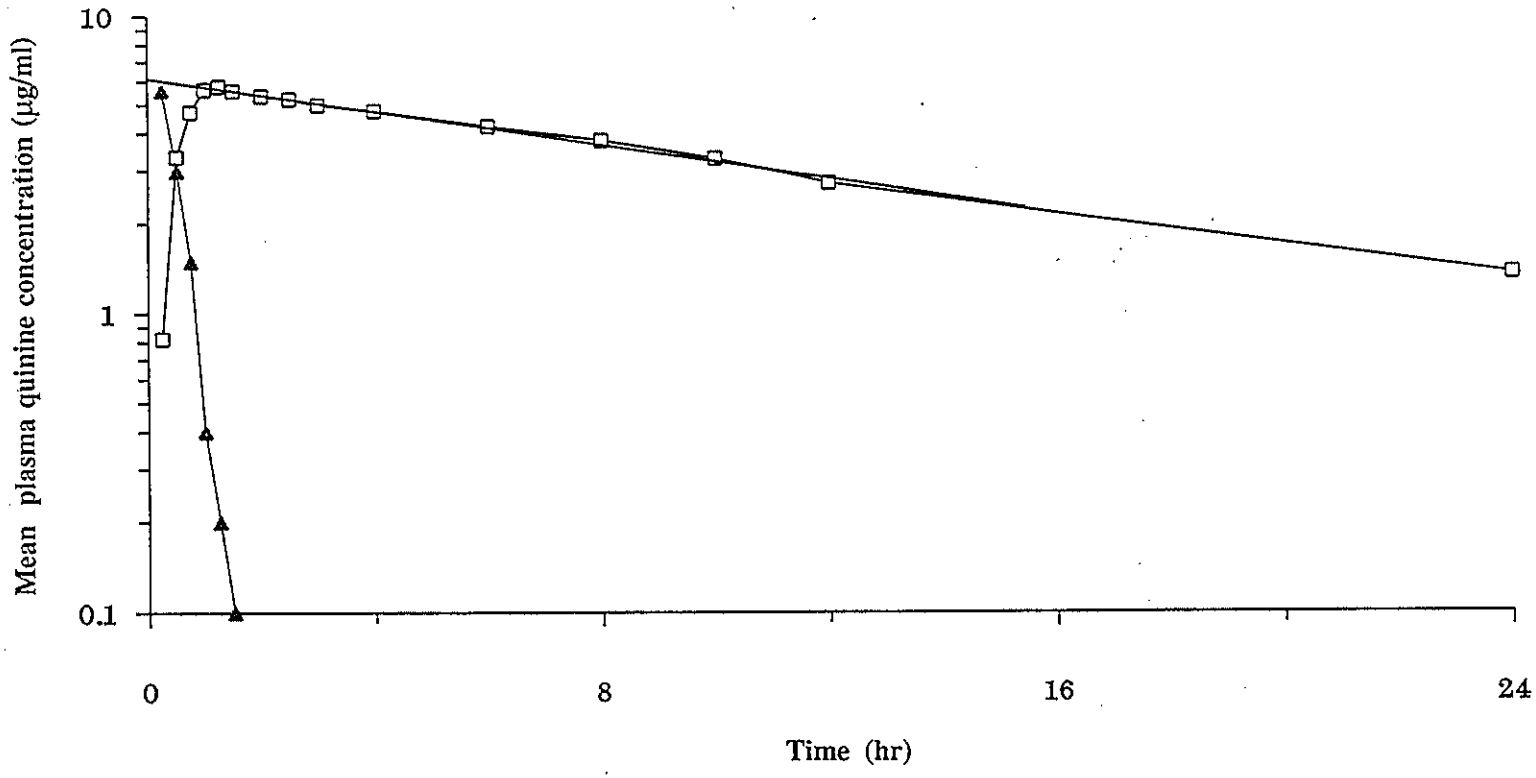

Figure 12 Semi-logarithmic mean plasma quinine concentrations-time profile in 8 healthy volunteers receiving a single 600 mg oral dose of quinine sulfate alone showing a lag-time

Table 11 **The relationship between the standard quinine concentration and peak ratio of quinine and internal standard (quinidine) in plasma of eight healthy volunteers.**

Concentration[a] (μg/ml)	Peak ratio of quinine / internal standard (quinidine)									
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Mean	S.D.
16	12.08	12.91	12.82	12.56	11.71	11.67	12.22	11.89	12.23	0.48
8	6.10	5.85	6.11	6.35	5.71	5.54	6.02	5.54	5.09	0.29
4	2.93	2.92	2.96	3.05	3.08	3.08	3.09	2.67	2.97	0.14
2	1.33	1.46	1.44	1.53	1.37	1.15	1.46	1.31	1.38	0.12
1	0.69	0.66	0.69	0.73	0.70	0.71	0.72	0.67	0.69	0.02
0.5	0.32	0.29	0.27	0.35	0.34	0.34	0.36	0.33	0.32	0.03

[a]Standard quinine 400 μl in each concentration + internal standard 100 μl + acetonitrile 500 μl

Table 12 **Pharmacokinetic parameters of quinine in subjects receiving a single oral dose of 600 mg quinine sulfate alone**

Subject No.	Age (yr)	Weight (kg)	$AUC_{0-\alpha}$ (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ug/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)	Lag time (hr)
1	29	56	54.35	5.74	0.074	0.12	9.38	1.2	3.8	2.66	0.197	0.43
2	28	58	61.51	2.27	0.069	0.3	10.07	1.8	3.8	2.44	0.168	0.21
3	25	55	177.74	2.19	0.07	0.32	9.91	1.81	11.09	0.87	0.061	0.19
4	27	54	64.32	32.3	0.076	0.02	9.14	0.43	4.81	2.27	0.172	0.24
5	28	60	73.44	2.27	0.072	0.3	9.56	1.97	4.75	1.89	0.136	0.41
6	35	61	116.91	3.94	0.054	0.17	12.87	1.33	5.93	1.56	0.084	0.23
7	30	56	116.1	3.19	0.066	0.22	10.27	1.44	7.21	1.4	0.092	0.2
8	26	64	57.47	7.79	0.074	0.09	9.34	0.82	4.08	2.2	0.163	0.22
Mean	28.50	58	90.23	7.46	0.07	0.19	10.07	1.35	5.68	1.91	0.13	0.27
S.D.	3.07	3.42	43.39	10.23	0.01	0.11	1.20	0.53	2.48	0.60	0.05	0.10

Table 13 **Pharmacokinetic parameters of quinine in subjects receiving a single oral dose of 600 mg quinine sulfate and 8 g of cholestyramine simultaneously**

Subject No.	Age (yr)	Weight (kg)	$AUC_{0-\alpha}$ (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ug/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)	Lag time (hr)
1	29	56	48.22	4.19	0.089	0.16	7.73	1.4	3.98	2.5	0.222	0.46
2	28	58	49.46	4.53	0.084	0.15	8.26	1.34	3.84	2.49	0.209	0.44
3	25	55	146.43	17.85	0.078	0.04	8.88	0.77	11.16	0.95	0.074	0.47
4	27	54	82.87	6.05	0.089	0.11	7.77	0.94	6.94	1.51	0.134	0.24
5	28	60	70.98	3.89	0.074	0.18	9.39	1.24	4.85	1.9	0.141	0.21
6	35	61	103.17	5.95	0.055	0.12	12.49	0.99	5.48	1.73	0.095	0.2
7	30	56	75.97	34.13	0.066	0.02	10.43	0.42	4.98	2.14	0.141	0.24
8	26	64	64.86	5.29	0.063	0.13	10.99	1.03	3.88	2.29	0.144	0.18
Mean	28.5	58	80.25	10.24	0.07	0.11	9.49	1.02	5.64	1.94	0.15	0.31
S.D.	3.07	3.42	32.13	10.68	0.01	0.06	1.69	0.32	2.46	0.53	0.05	0.13

Table 14 **Pharmacokinetic parameters of quinine in subjects receiving a single oral dose of 600 mg quinine sulfate 1 hr before administration of 8 g of cholestyramine**

Subject No.	Age (yr)	Weight (kg)	$AUC_{0-\alpha}$ (mg/l/hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ug/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)	Lag time (hr)
1	29	56	84.1	2.47	0.064	0.28	10.77	1.69	4.91	1.99	0.127	0.17
2	28	58	52.32	7.81	0.081	0.09	8.55	0.64	4.04	2.44	0.198	0.05
3	25	55	145.45	24.78	0.069	0.03	10.07	0.47	9.84	1.09	0.075	0.23
4	27	54	60.58	1.58	0.095	0.44	7.31	2.12	4.8	1.93	0.183	0.22
5	28	60	79.66	5.01	0.089	0.14	7.77	1.03	6.61	1.41	0.125	0.22
6	35	61	106.78	1.29	0.062	0.53	11.17	2.8	5.69	1.48	0.092	0.33
7	30	56	58.52	21.68	0.096	0.03	7.17	0.49	5.52	1.91	0.183	0.24
8	26	64	31.44	13.61	0.088	0.05	7.83	0.57	2.69	3.39	0.298	0.2
Mean	28.5	58	77.36	9.78	0.08	0.20	8.83	1.23	5.51	1.96	0.16	0.21
S.D.	3.07	3.42	35.71	9.26	0.01	0.20	1.61	0.88	2.10	0.71	0.07	0.08

Table 15 **Pharmacokinetic parameters of quinine in subjects receiving a single oral dose of 600 mg quinine sulfate1 hr after administration of 8 g of cholestyramine**

Subject No.	Age (yr)	Weight (kg)	$AUC_{0-\alpha}$ (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$(abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ug/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)	Lag time (hr)
1	29	56	56.75	4.65	0.078	0.15	8.89	1.3	4.13	2.42	0.189	0.41
2	28	58	58.76	4.24	0.077	0.16	9.01	1.19	4.19	2.29	0.176	0.23
3	25	55	141.51	10.79	0.071	0.06	9.8	0.69	9.68	1.08	0.077	0.22
4	27	54	67.88	6.85	0.076	0.1	9.06	0.89	4.93	2.15	0.164	0.23
5	28	60	74.18	3.93	0.072	0.18	9.64	1.43	4.95	1.87	0.135	0.4
6	35	61	90.47	5.24	0.061	0.13	11.37	1.07	5.23	1.78	0.109	0.21
7	30	56	77.33	4.81	0.065	0.14	10.71	1.14	4.72	2.13	0.138	0.23
8	26	64	55.96	3.22	0.083	0.22	8.38	1.15	4.2	2.02	0.167	0
Mean	28.5	58	77.86	5.47	0.07	0.14	9.61	1.11	5.25	1.97	0.14	0.24
S.D.	3.07	3.42	28.32	2.40	0.01	0.05	1.00	0.23	1.84	0.41	0.04	0.13

Table 16 Mean pharmacokinetic parameters of quinine in the present study (Mean ± S.D.)

Study design	Pharmacokinetic parameters											
	Age (yr)	Weight (kg)	$AUC_{0-\alpha}$ (mg/l.hr)	Ka (hr^{-1})	Ke (hr^{-1})	$t_{1/2}$ (abs) (hr)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (μg/ml)	Vd/f (l/kg)	Cl/f (l/hr/kg)	Lag time (hr)
1. quinine alone	28.5 ± 3.07	58 ± 3.42	90.23 ± 43.39	7.46 ± 10.23	0.07 ± 0.01	0.19 ± 0.11	10.07 ± 1.20	1.35 ± 0.53	5.68 ± 2.48	1.91 ± 0.60	0.13 ± 0.05	0.27 ± 0.10
2. quinine and cholestyramine simultaneously	28.5 ± 3.07	58 ± 3.42	80.25 ± 32.13	10.24 ± 10.68	0.07 ± 0.01	0.11 ± 0.06	9.49 ± 1.69	1.02 ± 0.32	5.64 ± 2.46	1.94 ± 0.53	0.15 ± 0.05	0.31 ± 0.13
3. quinine 1 hr before cholestyramine	28.5 ± 3.07	58 ± 3.42	77.36 ± 35.71	9.78 ± 9.26	0.08 ± 0.01	0.20 ± 0.20	8.83 ± 1.61	1.23 ± 0.88	5.51 ± 2.10	1.96 ± 0.71	0.16 ± 0.07	0.21 ± 0.08
4. quinine 1 hr after cholestyramine	28.5 ± 3.07	58 ± 3.42	77.86 ± 28.32	5.47 ± 2.40	0.07 ± 0.01	0.14 ± 0.05	9.61 ± 1.00	1.11 ± 0.23	5.25 ± 1.84	1.97 ± 0.41	0.14 ± 0.04	0.24 ± 0.13

Table 17 Quinine pharmacokinetics data are compared to other published data

Data	Sources					
	Dyer 1994	Wanwimolruk 1995a	Auprayoon 1995	Sowunmi and Salako 1996	Babalola 1996	Present study[a]
Subjects	10 men	9 men	6 men	7 men	4 men	8 men
Age (yr)	63.1 ± 6.5	30.3 ± 3.4	37-50	21-29	22-44	28.5 ± 3.7
Dose (mg)	600	600	600	600	600	600
Route	oral	oral	oral	oral	oral	oral
C_{max} (µg/ml)	3.7 ± 0.8	4.6 ± 1.0	3.45	2.7 ± 0.5	2.88 ± 0.16	5.68 ± 2.48
	-	(2.9 - 6.4)	(2.25-3.91)	-	(2.57 - 3.16)	(3.8 - 11.09)
T_{max} (hr)	-	2.5	1.6	2.8 ± 1.4	2.75 ± 0.2	1.35 ± 0.53
	-	(1.5 - 10.0)	(0.8-2)	-	(2 - 3)	(0.43 - 1.97)
$t_{1/2}$ (hr)	19.9 ± 6.3	11.1 ± 3	9.7	11.4 ± 2.7	12.8 ± 2.29	10.07 ± 1.20
	-	(6.6 - 16.3)	(7.8-17.2)	-	(6.75 - 17.32)	(9.14 - 12.87)
Vd/f (l/kg)	1.7 ± 0.56	-	2.78	2.5 ± 1.4	.	1.91 ± 0.60
	-	-	(1.49-3.38)	-	.	(0.87 - 2.66)
Cl/f (l/hr/kg)	0.06 ± 0.02	0.14 ± 0.05	0.17	0.17 ± 0.02	.	0.13 ± 0.05
	.	(0.08 - 0.15)	(0.1-0.24)	-	.	(0.058 - 0.193)
AUC 0-α (l/hr/kg)	.	66 ± 20	-	30.2 ± 3.5	59 ± 13.33	90.23 ± 43.39
	.	(32 - 105)	-	-	(28.92 - 93.64)	(54.35 - 177.74)

Data obtained from subjects receiving quinine alone

CHAPTER 5

DISCUSSION

Quinine is one of the oldest drug in pharmacopoeia, which has been used in the treatment of malaria since 1963. It was recommended by the World Health Organization (WHO) for the treatment of chloroquine-resistant *Plasmodium falciparum*. It is also widely prescribed as the treatment of choice for nocturnal leg cramps. Quinine has a narrow therapeutic index and its toxicity is serious, especially cardiotoxicity. Cholestyramine is the drug of choice for hypercholesterolemia (type ΠA and ΠB). Previous studies reported that it interfered drug absorption, both acid and basic drugs, in the gastrointestinal tract. The binding of drugs by cholestyramine has resulted in some clinically important drug interaction. A variety of drugs which gastrointestinal absorption may be reduced by cholestyramine included thyroxine (Northcutt, et al., 1969), digitoxin (Caldwell and Greenberger, 1971), acetaminophen (Dordoni, et al., 1973), phenprocoumon (Meinertz, et al., 1977), chlordecone (Boylan, et al., 1978), warfarin (Jahnchen, et al., 1978), hydrochlorothiazide (Hinninghake and Hibbard, 1986), hydrocortisone (Johansson, et al., 1978), loperamide (Ti, et al., 1978), vancomycin (King and Barriere, 1981), methotrexate (McAnena, et al., 1987), tenoxicam and piroxicam, (Guentert, et al., 1988) digoxin, (Henderson and Solomon, 1988), furosemide (Neuvonen, et al., 1988), amiodarone (Goddard and

Whorwell, 1989), glipizide (Kivisto and Neuvonen, 1990) and imipramine (Spina, et al., 1994). Coadministration of quinine and cholestyramine may cause drug interaction. Therefore, we investigated the effect of cholestyramine on the pharmacokinetics of quinine when given concomitantly, given 1 hr before quinine or 1 hr after quinine compared to oral administration of quinine alone in eight healthy volunteers.

The profile of plasma quinine concentration and the derived pharmacokinetic parameters in this study were similar to those previously reported following oral quinine sulfate (Table 17) (Dyer, et al., 1994; Wanwimolruk, et al., 1995a; Auprayoon, et al., 1995; Balabola, et al., 1996; Sowunmi and Salako, 1996). In this present study there were considerable interindividual variability in plasma concentration profiles, and as a consequence there were large variations in the derived pharmacokinetic parameters as previous reports such as Jamaludin, et al. (1988) and Sowunmi and Salako (1996).

The semi-logarithmic plots of plasma quinine concentration-time curves (Figure 10 and Figure 11) showed that the data were well described by a one compartmental open model with first-order kinetics for both absorption and elimination which was similar to the study of Alvan, et al. (1991); Dyer, et al. (1994) and Supanaranond, et al. (1991).

The $AUC_{0-\alpha}$ value was slightly decreased from 90.23 $\pm$ 43.39 μg/ml.hr in subjects receiving quinine alone to 80.25 $\pm$ 32.13, 77.36 $\pm$ 35.71, and 77.86 $\pm$ 28.32 μg/ml.hr in subjects receiving quinine and cholestyramine simultaneously, in subjects receiving quinine 1 hr before

cholestyramine, and in subjects receiving quinine 1 hr after cholestyramine, respectively, and the other pharmacokinetic parameters were also shown no significant differences among 4 trial-phases.

Cholestyramine is a highly charged quaternary ammonium resin and is administered as the chloride salt. It may alter both the rate and total absorption of a variety of drugs. The binding of cholestyramine to other drug is not a selective process and is observed with a variety of drugs possessing different chemical properties (Malloy, et al., 1996). The interference with the gastrointestinal absorption of most of certain drugs, usually acids in nature, by cholestyramine seems to be serious. Some acidic drugs show a decrease in absorption. Robinson, et al. (1971) reported that a separation of the time of dosing of cholestyramine and warfarin by 3 hrs were associated with a significant decrease in plasma warfarin levels, but a single dose of cholestyramine given 6 hrs after warfarin did not influence the absorption of warfarin (Kuentzel and Brunk, 1970). The absorption of acetaminophen was markedly reduced by 62 % (range 30-98%) over 120 min by the simultaneous oral administration of cholestyramine but was only slightly reduced (16 %, range 0-51%) when cholestyramine was given 60 min after acetaminophen (Dordoni, et al., 1973). Neuvonen, et al. (1988) reported that the bioavailability of furosemide was reduced by cholestyramine 95 %. Kivisto and Neuvonen (1990) found that the absorption of glipizide (measured as the AUC from 0-10 hr) was reduced by cholestyramine 29 % and peak plasma glipizide concentration was lowered by 33 %.

Renouden, et al. (1995) found that cholestyramine increased plasma warfarin clearance by 30% and reduced elimination half-life by a similar amount in five healthy male volunteers given a single intravenous dose of warfarin followed by cholestyramine (4 g three times a day). Malloy, et al. (1996) demonstrated that sulindac, a non-steroidal anti-inflammatory drug (NSAID) concurrently coadministered with 4 g of cholestyramine resulted in a decrease in AUC compared to sulindac alone (7.11 $\pm$ 3.25 vs 31.65 $\pm$ 7.94 μg/ml/hr, respectively). In some acidic drugs, cholestyramine altered only the delay of absorption such as in the study of Hunninghake and Pollack (1977) who reported that the absorption of aspirin and tolbutamide in 12 male volunteers who received cholestyramine 8 g 2 min prior to, and 6 and 12 hrs after administration of either single dose of aspirin and tolbutamide was not statistically different, these drugs were only delayed in absorption by cholestyramine. In contrast, the extent of absorption of some acidic drugs is unaffected by cholestyramine such as phenytoin (Callaghon, et al., 1983). Ion-exchange mechanism is the mechanism that take place between some acidic drugs. Calvo and Dominguez-Gil (1984) proposed that the cholestyramine-naproxen interaction is electrostatic in nature, the chloride ions of the resin being exchanged for the anions of the drug in solution. The interaction takes place between the carboxyl group of the ionized drug and the quaternary ammonium group of the positively charged resin. However, the extent and stability of the complex formed between the resin and anionic drugs is not related to acidity of the drug. Aspirin (pKa

3.5) is a stronger acid than cholate (pKa 6.4), but was not bound as tightly by the resin (Gallo, et al., 1965).

Neutral or non-ionic compounds should not interact with cholestyramine by ion-exchange mechanism, although they may be adsorbed by cholestyramine (Gallo, et al., 1965) such as digoxin which Neuvonen, et al. (1988) reported that cholestyramine reduced the absorption of digoxin by 30-40 % in six healthy volunteers who received 0.25 mg digoxin concomitantly with 8 g of cholestyramine.

Quinine is a basic drug with a pKa value for the charged nitrogen atoms in the quinuclidine ring of 4.0 and 8.6 (Silamut, et al., 1991). For basic drugs, there were a few reports shown that cholestyramine interfered the absorption, and Gallo, et al. (1965) suggested that cationic drugs could be adsorbed by cholestyramine, but the specific reaction would not be expected between cholestyramine and cationic drugs. Hibbard, et al. (1984) reported that one and two dosage regimen of cholestyramine administration was associated with a 43 % and 12 % respectively, reduction in propranolol AUC following cholestyramine. They suggested that the hydrophobic bonding was the major mechanism in complex formation. The charged of the monovalent cation propranolol, which possesses a high degree of lipophilicity was of minor importance. Bailey, et al. (1992) demonstrated that the adsorption *in vitro* study of the tricyclic antidepressants (amitriptyline, desipramine, doxepine, imipramine, and nortriptyline) onto cholestyramine was approximately 80 % for each of the tricyclic antidepressants but Spina, et al. (1994) reported

that cholestyramine (4 g three time a day for 5 days) in depressed patients was associated with an average 23 % decrease in plasma imipramine levels, whereas desipramine levels decreased only marginally. Neuvonen, et al. (1988) found that the absorption of carbamazepine (a substituted amide) was not decreased by cholestyramine and they mentioned that the interference with gastrointestinal absorption of the basic drug by cholestyramine seems to be minimal. In the present study cholestyramine was not likely to produce drug interaction with quinine in all of three study designs. However, the AUC and C_{max} seemed to be slightly decreased but not statistically different. It may be possible that cholestyramine primarily bound anionic drugs and there were either limited or reversible binding with neutral and basic drugs. Some clinical studied, however, indicated that the absorption of nonionic drugs can also be altered by cholestyramine (Hunninghake, et al., 1985). Further studies will need to be performed to study the effect of multiple doses of cholestyramine on the pharmacokinetics of quinine since the patients who were treated with cholestyramine take a long course in order to lower plasma cholesterol levels so the interaction may occur. In the present study, palpitation and abdominal discomfort were occurred in some subjects. However, these side effects were disappeared within 4 hr and not required specific treatment.

Conclusion, in the present study, there were no statistically significant differences in all trial-phases compared to a control phase.

Thus the single dose combination use of quinine and cholestyramine at therapeutic dose is not likely to produce pharmacokinetic interactions.

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APPENDIX

APPENDIX - 1

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

Stock A = Quinine 32 μg/ml in blank plasma

16 μg/ml = blank plasma 400 μl+400 μl of 32 μg/ml

8 μg/ml = blank plasma 400 μl+400 μl of 16 μg/ml

4 μg/ml = blank plasma 400 μl+400 μl of 8 μg/ml

2 μg/ml = blank plasma 400 μl+400 μl of 4 μg/ml

1 μg/ml = blank plasma 400 μl+400 μl of 2 μg/ml

0.5 μg/ml = blank plasma 400 μl+400 μl of 1 μg/ml

APPENDIX - 2

Protein precipitation

1. Take 400 µl of sample or standard solution
2. Add 100 µl of quinidine (internal standard, 5 µg/ml in mobile phase)
3. Add acetonitrile 500 µl
4. Shake
5. Centrifuge at 14,000 rpm for 15 min

APPENDIX - 3

Plasma concentrations of quinine (μg/ml) at 0-48 hr in subjects receiving a single oral dose of 600 mg quinine sulfate alone.

	Subject No.									
Time(hr)	1	2	3	4	5	6	7	8	Mean	S.D.
0	0	0	0	0	0	0	0	0	0	0
0.25	0.11	0.37	1.6	1.39	0.23	0.55	1.39	0.9	0.82	0.58
0.5	1.23	1.9	6.31	4.93	1.18	3.71	3.66	3.69	3.33	1.81
0.75	3.43	2.8	7.27	5.61	2.36	5.63	6.37	4.21	4.71	1.77
1	3.43	4.05	10.19	4.78	3.98	6.17	8.28	4.01	5.61	2.44
1.25	4	3.44	11.58	4.75	4.39	6.09	7.85	4.1	5.76	2.75
1.5	3.68	3.46	11.67	4.35	5.02	6	7.18	3.91	5.66	2.74
2	3.47	3.52	10.85	3.85	4.78	5.78	6.78	3.69	5.34	2.53
2.5	3.52	3.73	10.67	3.63	4.73	5.45	6.42	3.58	5.22	2.44
3	3.46	3.65	10	3.44	4.4	5.39	6.07	3.41	4.98	2.26
4	3.19	3.41	10.08	3.2	3.99	4.9	5.99	3.24	4.75	2.37
6	3.01	3.21	7.99	3.13	3.54	4.7	5.3	2.84	4.22	1.76
8	2.26	2.69	7.55	2.9	3.22	4.23	4.75	2.54	3.77	1.75
10	1.97	2.16	6.52	2.74	2.88	3.76	4.24	2.01	3.28	1.54
12	1.45	1.89	5.63	1.92	2.32	3.23	3.57	1.74	2.72	1.39
24	0.81	0.74	2.48	1.04	1.06	2.06	1.85	0.78	1.35	0.68
36	0.27	0.28	1.26	0.4	0.44	0.91	0.84	0.34	0.59	0.36
48	0.16	0.08	0.67	0.13	0.2	0.52	0.46	0.14	0.3	0.22

APPENDIX - 4

Plasma concentrations of quinine (μg/ml) at 0-48 hr in subjects receiving a single oral dose of 600 mg quinine sulfate and 8 g of cholestyramine simultaneously.

	Subject No.									
Time(hr)	1	2	3	4	5	6	7	8	Mean	S.D.
0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	0.75	0.69	0.73	1.37	1.71	1.28	0.82	0.62
0.5	0.71	1.01	4.69	5.01	3.68	4.58	5.08	3.18	3.49	1.75
0.75	2.56	2.96	11.42	8.36	4.67	5.65	4.91	4.05	5.57	2.96
1	4.23	3.81	12.02	7.87	4.58	5.55	5.18	3.86	5.89	2.8
1.25	4.17	4.03	10.96	6.89	4.63	5.17	4.64	3.66	5.52	2.41
1.5	4.06	3.82	10.37	6.72	4.64	5.24	4.87	3.81	5.44	2.21
2	3.75	3.73	9.67	5.89	4.76	5.17	4.31	3.52	5.1	2.01
2.5	3.31	3.06	8.93	5.4	4.64	5.1	4.1	3.49	4.75	1.89
3	3.47	3.45	9.02	5.3	4.34	4.81	3.8	3.47	4.71	1.87
4	3.16	3.26	8.73	4.88	4.31	4.88	3.88	3.43	4.57	1.81
6	2.83	2.8	7.65	4.41	3.6	4.27	3.68	3.12	4.04	1.58
8	2.32	2.3	6.67	3.81	3.19	4.07	3.06	2.57	3.5	1.44
10	1.84	1.92	5.38	3.06	2.61	3.21	2.61	2.19	2.85	1.13
12	1.39	1.5	5.01	2.91	2.03	3.1	2.3	1.91	2.52	1.17
24	0.74	0.57	1.62	1.65	0.75	1.48	1.09	0.81	1.09	0.43
36	0.29	0.16	0.74	0.51	0.31	0.61	0.51	0.39	0.44	0.19
48	0.15	0	0.35	0.28	0.12	0.32	0.24	0.22	0.21	0.11

APPENDIX - 5

Plasma concentrations of quinine (μg/ml) at 0-48 hr in subjects receiving a single oral dose of 600 mg quinine sulfate 1hr before administration of 8 g of cholestyramine.

	Subject No.									
Time(hr)	1	2	3	4	5	6	7	8	Mean	S.D.
0	0	0	0	0	0	0	0	0	0	0
0.25	0.1	3.31	3.67	0.69	1.13	0.37	0.62	1.37	1.41	1.35
0.5	1.45	3.81	10.36	1.37	4.91	1.46	6.28	2.69	4.04	3.12
0.75	5.5	4.74	9.91	2.5	6.75	2.66	6.18	2.61	5.11	2.57
1	5.27	3.93	9.5	3.75	7.29	3.13	5.67	2.49	5.13	2.34
1.25	4.99	3.58	9.47	5.34	6.71	4.87	4.75	2.58	5.28	2.08
1.5	4.61	3.68	8	4.94	6.27	4.64	4.64	2.41	4.9	1.66
2	4.54	3.49	8.67	5.11	5.98	5.95	4.36	2.44	5.07	1.89
2.5	4.56	3.27	8.34	4.48	5.65	6	4.06	2.29	4.83	1.85
3	4.38	3.55	8.41	4.45	5.24	5.58	3.98	2.26	4.73	1.8
4	4.2	3.2	7.8	4.05	5.17	5.07	3.54	2.17	4.4	1.69
6	3.75	2.79	7.55	3.33	4.08	4.91	3.07	1.86	3.92	1.72
8	4.02	2.41	5.99	2.68	3.69	4.43	2.97	1.37	3.44	1.42
10	2.8	1.88	5.07	2.32	3.21	3.81	2.37	1.04	2.81	1.24
12	2.28	1.44	4.54	2.18	2.66	3.2	2.19	0.87	2.42	1.11
24	1.34	0.65	1.51	1.09	0.93	1.63	0.89	0.32	1.04	0.44
36	0.6	0.24	0.8	0.33	0.37	0.88	0.38	0.15	0.47	0.26
48	0.29	0.06	0.32	0.16	0.16	0.47	0.12	0.08	0.21	0.14

APPENDIX - 6

Plasma concentrations of quinine (μg/ml) at 0-48 hr in subjects receiving a single oral dose of 600 mg quinine sulfate 1hr after administration of 8 g of cholestyramine

	Subject No.									
Time(hr)	1	2	3	4	5	6	7	8	Mean	S.D.
0	0	0	0	0	0	0	0	0	0	0
0.25	0.11	0.4	2.14	0.69	0.06	1.1	0.46	2.49	0.93	0.92
0.5	1.49	2.65	9.2	3.99	1.92	4	3.13	3.97	3.79	2.39
0.75	3.44	4.36	10.79	5.71	3.08	5.57	5.04	4.11	5.26	2.42
1	4.15	4.52	9.8	5.18	5.52	5.37	5.02	3.88	5.43	1.86
1.25	4	4.04	8.25	4.94	5.41	4.92	5.04	3.91	5.06	1.41
1.5	3.85	3.61	9.15	4.75	5.17	5.02	4.5	3.91	4.99	1.77
2	4.07	4.22	9.09	4.48	4.54	4.86	4.24	3.94	4.93	1.71
2.5	3.97	3.68	8.53	4	4.42	4.84	4.2	3.81	4.66	1.5
3	4	3.57	7.91	3.41	4.26	4.77	3.99	4.03	4.49	1.44
4	3.18	3.73	7.31	3.57	3.94	4.41	3.77	3.91	4.23	1.29
6	2.93	2.94	6.93	4.23	3.45	4.16	3.35	3.12	3.89	1.33
8	2.69	2.51	6.04	2.98	3.16	3.52	3.35	2.48	3.34	1.15
10	1.96	2.25	5.27	2.49	3.16	3.03	2.88	1.98	2.88	1.07
12	1.56	1.91	4.3	1.95	2.3	2.59	2.31	1.46	2.3	0.89
24	0.85	0.6	1.77	0.94	1.06	1.32	1.12	0.53	1.02	0.4
36	0.43	0.27	0.8	0.37	0.38	0.6	0.47	0.22	0.44	0.19
48	0.22	0.08	0.37	0.19	0.15	0.33	0.22	0.07	0.2	0.11

APPENDIX-7

ที่ ทม 1209/ 197

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90110

หนังสือรับรองการศึกษาวิจัย

การศึกษาวิจัยเรื่อง : "ผลของยาโคเลสไทรามีนต่อเภสัชจลนศาสตร์ของยาควินิน เมื่อให้โดยการรับประทานครั้งเดียว ในอาสาสมัครสุขภาพปกติ"

ผู้วิจัย : นายอนันต์ กลีบแก้ว
นักศึกษาปริญญาโท สาขาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณา และเห็นชอบจากคณะกรรมการจริยธรรม ซึ่งเป็นคณะกรรมการพิจารณาโครงการวิจัย ตลอดจนติดตามผลในส่วนของการทดลองที่กระทำต่ออาสาสมัคร ของคณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ แล้ว

ให้ไว้ ณ วันที่ ๒๐ พฤศจิกายน 2540

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

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

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

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

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

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

ใบยินยอม

1. ชื่อโครงการ : ผลของยาโคเลสไทรามีนต่อเภสัชจลนศาสตร์ของยาควินินเมื่อให้โดยการรับประทานครั้งเดียวในอาสาสมัครสุขภาพปกติ

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

3. วัตถุประสงค์ของการศึกษา

1) เพื่อให้ทราบถึงการเปลี่ยนแปลงค่าทางด้านเภสัชจลนศาสตร์ของยาควินินเมื่อให้ร่วมกับยาโคเลสไทรามีน

2) เพื่อเป็นข้อมูลและแนวทางในการรักษาผู้ป่วยในกรณีที่ได้รับยาทั้งสองชนิดนี้ร่วมกัน

4. วิธีการศึกษา

4.1 อาสาสมัครที่เข้าร่วมโครงการต้องเป็นผู้ที่มีสุขภาพสมบูรณ์และแข็งแรง

4.2 ใช้อาสาสมัครเพศชาย อายุระหว่าง 20-35 ปี

4.3 อาสาสมัครทุกคนต้องไม่ได้รับยาชนิดอื่นๆมาก่อนที่จะเริ่มทำการทดลองเป็นเวลาอย่างน้อย 1 เดือน

4.4 ก่อนเริ่มทำการทดลอง อาสาสมัครทุกคนจะได้รับการเจาะเลือดเพื่อตรวจความปกติ/ผิดปกติของเม็ดเลือดและค่าชีวเคมีของเลือดที่โรงพยาบาลสงขลานครินทร์

4.5 ก่อนเริ่มทำการทดลอง ให้อาสาสมัครงดอาหารมาก่อนอย่างน้อย 8 ชั่วโมง ในวันเริ่มทำการทดลอง อาสาสมัครทุกคนจะได้รับประทานยาควินินขนาด 600 มิลลิกรัม (ควินินซัลเฟต ผลิตโดยองค์การเภสัชกรรมขนาด 300 มิลลิกรัม 2 เม็ด) หลังจากได้รับยาแล้วประมาณ 4 ชั่วโมงจึงจะยินยอมให้อาสาสมัครรับประทานอาหารได้ ส่วนในการทดลองที่ 2, 3 และครั้งที่ 4 อาสาสมัครจะได้รับยาควินินร่วมกับยาโคเลสไทรามีนขนาด 8 กรัมผสมกับน้ำ 200 มิลลิลิตรพร้อมกัน

ก่อนรับประทานยาควินิน 1 ชั่วโมงและหลังรับประทานยาควินิน 1 ชั่วโมงตามลำดับ

4.6 เก็บเลือดครั้งละ 5 มิลลิลิตรในช่วงเวลา 48 ชั่วโมง โดยเก็บเลือดทันทีก่อนรับประทานยาและที่เวลา 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 และ 48 ชั่วโมง หลังได้รับยาควินิน การเก็บเลือดนั้นจะทำการเจาะหลอดเลือดเพียงครั้งเดียวแล้วคาสาย catheter ไว้เพื่อเก็บเอาตัวอย่างเลือดที่เวลาต่างๆกัน นำเอาเลือดที่ได้ไปปั่นเก็บพลาสมาทันทีและเก็บไว้ที่อุณหภูมิ - 20 องศาเซลเซียสเพื่อทำการวิเคราะห์ต่อไป

5. ผลข้างเคียงของการใช้ยาควินิน

ส่วนใหญ่เกิดกับผู้ที่มีการตอบสนองไวเกินต่อยา (hypersensitivity) หรือได้รับยาขนาดสูงเกินไป อาการเป็นพิษที่พบได้แก่

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

2. อาการระคายเคืองทางเดินอาหาร เช่น อาจทำให้เกิดการระคายเคืองของเยื่อบุทางเดินอาหาร มีอาการคลื่นไส้และปวดท้อง

3. ในคนที่ได้รับยาเกินขนาด เช่น ได้รับสูงกว่า 8 กรัมขึ้นไป อาจทำให้เสียชีวิตได้ เนื่องจากควินินเป็นยาที่อันตรายต่อระบบประสาท โดยเฉพาะกับระบบประสาทอัตโนมัติ

6. ผลข้างเคียงของการใช้ยาโคเลสไทรามีน

ผลข้างเคียงของการใช้ยาโคเลสไทรามีนได้แก่ อาการท้องอืด ท้องผูก ลำไส้อุดตันและถ้าได้รับยานี้เป็นเวลานานๆอาจเกิดภาวะขาดวิตามินเอ วิตามินดีและกรดโฟลิก (folic acid)

7. ความรับผิดชอบต่ออาสาสมัครที่เข้าร่วมโครงการ

หากอาสาสมัครที่เข้าร่วมโครงการทดลอง เกิดอาการผิดปกติทั้งทางด้านร่างกายและจิตใจอันเป็นผลสืบเนื่องมาจากการทดลองนี้ ไม่ว่าจากสาเหตุใดก็ตาม ผู้

ทำการทดลองต้องรับผิดชอบในการรักษาพยาบาลอาสาสมัครจนกว่ายาจะหมดฤทธิ์ หรือไม่เกิน 3 เดือนหลังการทดลอง

8. โอกาสในการซักถามและการยกเลิกการเป็นอาสาสมัคร : หากอาสาสมัครมีข้อสงสัยเกี่ยวกับการศึกษาครั้งนี้ อาสาสมัครมีสิทธิในการซักถามได้ทุกขั้นตอนและมีสิทธิยกเลิกการเป็นอาสาสมัครที่เข้าร่วมโครงการวิจัยได้เมื่อมีเหตุผลอันสมควร

9. หากอาสาสมัครไม่ปฏิบัติตามเงื่อนไข คณะผู้วิจัยมีสิทธิถอดถอนอาสาสมัครออกจากการร่วมโครงการ

10. คำยินยอมเข้าร่วมโครงการ : ข้าพเจ้าได้อ่านและเข้าใจถึงวัตถุประสงค์ในการศึกษาครั้งนี้เป็นอย่างดี และยินดีให้ความร่วมมืออย่างดีที่สุด

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

แบบบันทึกประวัติและการตรวจร่างกาย
ของอาสาสมัครไทย

เลขที่.....

วันที่.....

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1. ประวัติส่วนตัว

ชื่อ.....นามสกุล.....

อายุ.....ปี เพศ ( ) ชาย ( ) หญิง

น้ำหนัก.....กก. ส่วนสูง.....ซม.

อาชีพ.....ที่อยู่.....

2. ประวัติการเจ็บป่วย

2.1 <u>ประวัติการเจ็บป่วยในปัจจุบัน</u>

(1).....

(2).....

(3).....

2.2 <u>ประวัติการเจ็บป่วยในอดีต</u>

( ) เคยนอนพักรักษาตัวในโรงพยาบาล ระบุชื่อโรค.....

( ) เคยได้รับการผ่าตัด ระบุชื่อโรค.....

( ) เคยเป็นโรคภูมิแพ้

( ) เคยแพ้ยา ระบุชื่อยาและอาการ.....

( ) เคยมีอาการตัวเหลือง ตาเหลือง เมื่อปี.....

3. ประวัติการเจ็บป่วยในครอบครัว

3.1 <u>ประวัติโรคกรรมพันธุ์</u> มีญาติป่วยเป็นโรค

( ) โรคภูมิแพ้

( ) โรคเบาหวาน

( ) โรคลมบ้าหมู

( ) โรคเลือด.....

3.2 <u>โรคติดเชื้อ</u>

( ) วัณโรค

( ) ตับอักเสบ

( ) อื่นๆ

4. ประวัติและอุปนิสัยส่วนตัว

บุหรี่ ( ) ไม่สูบ ( ) สูบ : จำนวน.....มวน/วัน

สุรา ( ) ไม่ดื่ม ( ) ดื่ม : จำนวน.....แก้ว/วัน

ยาที่ใช้/รับประทานเป็นประจำ ระบุชื่อยา.....

5. การตรวจร่างกาย

GA : .....

Vital Sign : BT..... °C PR...../min

RR...../min BP.....mmHg

Skin : .....

Heart : .....

Lung : .....

Abdomen : .....

Extremities : .....

Neuroexamination :

Conciousness : ( ) poor ( ) fair ( ) good

Pupils : diameter.....mm

RTL.....

Reflex : .....

.....

Muscle Power : .....

สรุปการตรวจร่างกาย

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ

แพทย์ผู้ตรวจร่างกาย.....

6. การตรวจทางห้องปฏิบัติการ

6.1 CBC ผล.....

6.2 LFT ผล.....

7. สรุปผลของการตรวจร่างกาย

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ ระบุ.....

ผู้บันทึก.....

VITAE

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Birth Date January 2, 1969

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Biology)	Prince of Songkla University	1991