FURTHER IMPROVEMENT OF ANALYTICAL METHODS FOR THE DETERMINATION OF ARTEMISININ DERIVATIVES (ARTEMETHER, ARTEETHER AND THEIR METABOLITES) IN BIOLOGICAL FLUIDS : APPLICATION TO PHARMACOKINETIC STUDIES

by

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

%	-	Percentage
±	-	Plus and minus
*	-	Time of drug administration
ARE	-	Arteether
ARM	-	Artemether
ARS	-	Artesunate
AUC	-	Area under the curve
bdl	-	Below detection limit
^{o}C	-	Degrees centigrade
CL	-	Clearance
Cmax	-	Maximum plasma drug concentration
CV	-	Coefficient of variation
DQHS	-	Dihydroartemisinin
ECD	-	Electrochemical detector
F	-	Fraction of the administered dose of drug that is absorbed intact
g	-	Acceleration due to gravity. Its value is 9.81 meter/second^2
HPLC	-	High performance liquid chromatography
hr	-	hours
IM	-	Intramuscular
IV	-	Intravenous
kg	-	Kilogrammes
L	-	Litre
MDQ	-	Minimum detectable quantity
MeOH	-	Methanol
mg	-	Milligrammes
min	-	Minutes
ml	-	Millilitre
MS	-	mass spectrometry
n	-	Number of observations
nd	-	Not detectable
ng	-	Nanogrammes
P	-	Level of significance
QC	-	Quality control
QHS	-	Artemisinin
r	-	Correlation coefficient
rpm	-	Revolution per minutes
RT	-	Room temperature
s.d.	-	Standard deviation
s.e.m.	-	Standard error of the mean

SP	-	Sulfadoxine-pyrimethamine
ss	-	Sample spoilt
t1/2	-	Half-life
tmax	-	Time to reach maximum drug concentration
ug	-	Microgrammes
UV	-	Ultraviolet
VBDCP	-	Vector Borne Disease Control Programme
Vd	-	Volume of distribution
vs	-	versus

PEMBAIKAN SELANJUTNYA KAEDAH ANALISIS BAGI PENENTUAN TERBITAN ARTEMISININ (ARTEMETHER, ARTEETHER DAN METABOLITNYA) DI DALAM BENDALIR BIOLOGI: APLIKASI TERHADAP KAJIAN FARMAKOKINETIK

ABSTRAK

Artemisinin (QHS) dan terbitannya adalah sebatian yang paling berpotensi dalam melawan penyakit falciparum malaria yang rintang pelbagai drug. Artemether (ARM) dan arteether (ARE) adalah lebih efektif berbanding sebatian induknya iaitu QHS. Kajian ini bertujuan untuk menghasilkan kaedah analisis yang lebih sensitif bagi pengukuran ARM dan ARE, bersama-sama dengan metabolit utamanya iaitu dihydroartemisinin (DQHS) di dalam plasma serta mengkaji sifat-sifat farmakokinetik ARM di dalam sukarelawan sihat dan juga di dalam pesakit malaria.

Kajian ini telah menggunakan kaedah kromatografi cecair keupayaan tinggi dengan pengesan elektrokimia yang pantas, tepat, boleh diulangi dan spesifik untuk mengukur paras ARM dan DQHS di dalam plasma dengan had kepekaan adalah 2.5 dan 1.25 ng/ml masing-masing. Melalui sedikit pengubahsuaian, kaedah analisis ini telah berjaya dikembangkan untuk menganalisis ARE di dalam plasma pada had kepekaan sebanyak 5.0 ng/ml. Kaedah analisis ini berjaya diuji untuk kajian farmakokinetik ARM di dalam manusia.

Bahagian kedua kajian ini telah mentaksirkan sifat farmakokinetik bagi ARM dan metabolitnya,DQHS di dalam sukarelawan sihat. Selepas pemberian dos tunggal oral ARM, drug ini telah diserap dengan pantas. Ia juga disingkirkan dengan pantas sehinggakan selepas 12 jam drug diberikan, ianya hampir tidak dapat dikesan lagi di dalam plasma. Profil farmakokinetik DQHS diperhatikan menyerupai ARM. Pemberian ARM secara oral didapati mempunyai biokeperolehan relatif sebanyak 24 peratus jika dibandingkan dengan pemberian secara suntikan intraotot. Parameter farmakokinetik didapati tidak menunjukkan sebarang perbezaan statistik yang signifikan di antara sukarelawan sihat di Malaysia dan di negara China. Paras kepekatan plasma ARM dan DQHS untuk pesakit falciparum malaria yang tidak berkomplikasi selepas pemberian dos oral secara berganda dengan kombinasi sediaan ARM dan benflumetol menunjukkan adanya korelasi yang signifikan dengan sukarelawan sihat. Kajian ini menunjukkan ARM dan DQHS disebarkankan dengan cepat di dalam subjek sihat dan juga di dalam pesakit malaria.

ABSTRACT

Artemisinin (QHS) and its derivatives are the most promising compounds against multidrug resistant falciparum malaria. Artemether (ARM) and arteether (ARE) were found to be more effective than their parent compound, QHS. The objectives of the study were to improve the sensitivity of the analytical methods for the quantification of ARM and ARE, together with their major metabolite, dihydroartemisinin (DQHS) in plasma, and to study the pharmacokinetics of ARM in healthy volunteers and in patients with uncomplicated falciparum malaria.

The present study has developed a rapid, selective, reproducible and specific high performance liquid chromatography with electrochemical detector for the determination of ARM and DQHS with the sensitivity limit in plasma of 2.5 and 1.25 ng/ml respectively. Through minor modifications, the analytical procedure was extended to the analysis of ARE in plasma with a sensitivity limit of 5.0 ng/ml. The method was succesfully applied in clinical pharmacological studies of ARM in human.

The second part of the study has estimated the pharmacokinetic properties of ARM and its metabolite, DQHS in healthy volunteers. Following a single oral dose of ARM, it showed a rapid absorption. Elimination of the drug was also rapid as it was almost undetectable in plasma after 12 hours of drug intake. The pharmacokinetic profiles of DQHS were similar to ARM. The relative bioavailability of ARM after an oral administration was 34% as compared to the intramuscular injection. There was no

statistical difference in pharmacokinetic parameters of ARM between healthy Malaysian and Chinese subjects. The plasma concentrations of ARM and DQHS in malaria patients following multiple oral treatment with a combination of ARM and benflumetol were significantly correlated to those seen in healthy subjects. This study also demonstrated a rapid disposition of both ARM and DQHS in healthy subjects and in malaria patients.

CHAPTER 1

INTRODUCTION

1.1 General

The World Health Organization (WHO) has estimated that 2073 million people (over 40% of the world's population), living in more than 100 countries, are exposed to the risk of malaria (WHO, 1990). Of these, 270 million are infected with malaria parasites and global deaths are estimated at approximately one million a year (WHO,1990). It is the complexity of malaria that has shifted the onus from attempts to eradicate the infection to attempts in controlling the disease (WHO,1984).

Malaria control efforts traditionally have relied on the provision of proven antimalarial drugs, on environmental sanitation and on the application of insecticides. Antimalarial drugs are used to prevent the onset of disease, to treat clinical cases and to prevent disease transmission. However, studies indicated that an unregulated availability of antimalarials bears the risk of promoting an early and rapid occurrence of resistance (Landgraf et al.,1994; Draper et al., 1988), and such resistance has been developed worldwide (Wernsdorfer, 1994, 1991; Bjorkman & Phillips-Howard, 1990; WHO,1984). As such, there is a great need for research of new antimalarials with novel mechanisms of action which prevent or delay the development of antimalarials resistance. The treatment of malaria also has changed in recent years with better definition of the pharmacokinetic and pharmacodynamic properties of the antimalarial drugs which led to revision and optimisation of the treatment regimen, particularly in severe malaria (White,1992).

1.2 Status of malaria in Malaysia

Malaysia is a federation consisting of two regions, West Malaysia (a peninsula stretching out from the mainland of South East Asia) and East Malaysia (a combination of the states of Sabah and Sarawak which occupies the northern part of the island of Borneo). Malaysia officially launched its malaria eradication programme in 1967. Despite the early success of malaria control, complete eradication did not appear imminent although the government had spent millions for the eradication programme. In 1981, the concept of eradication was changed to that of control, and the targets set by the Vector Borne Disease Control Programme (VBDCP) for malaria were to bring the incidence rate below 10 per 10000 in malarious area, keping it below that level in potentially malarious areas as well as, keeping malaria free areas as they are.

In 1987, incidences of malaria in Peninsula Malaysia and Sarawak were 7.4 and 7.3 per 10000 population respectively. Sabah however recorded a higher incidence of 194 per 10000 population. These high incidences in Sabah can be further confined to 48% of the population who live in the areas designated malarious as 95% of the state's cases occur here. According to a recent statistics from January till September of 1993 provided by the VBDCP (personal communication), a majority of the reported malaria cases was due to the P.falciparum (64%). P.vivax recorded 32% and the remaining are caused by mixed infections, with Sabah revealing the highest cases reported.

In Malaysia, chloroquine plus Fansidar combination has long been the first line chemotheraphy for falciparum malaria. However, resistance to chloroquine has been reported as early as 1970's (Andre et al., 1972) and in Sabah, the drug is no longer use in the treatment of falciparum malaria. Drug-resistant strains of malaria parasites to

proguanil (Wilson et al., 1952; Edeson & Field,1950), to pyrimethamine (Wilson & Edeson, 1953) and to Fansidar (Tan & Tan, 1983) have also been reported. With quinine as the only drug for treating resistant cases, there is an urgent need for alternative drugs. Recently, mefloquine has been registered for restricted use against multi resistant parasite.

1.3 The pathology and chemotherapy of malaria

1.3.1 The malaria life-cycle

Since antimalarial drugs affect different particular stages of development of the malaria parasite, a knowledge of its life-cycle is essential for the proper deployment of antimalarials. The malaria parasites belong to the genus Plasmodium of which four species are pathogenic to man. **P.vivax, P.malariae, and P.ovale** rarely produce life-threatening diseases. On the other hand, **P.falciparum** is responsible for severe and often fatal complications in clinical situations.

The life-cycle of all plasmodium species is the same, consisting of an exogenous sexual phase (sprogony) predominantly in the female mosquito of the genus Anopheles and endogenous asexual phase (schizogony) in man. The various stages of the plasmodium's life- cycle are shown in figure 1.1. The asexual phase begins when the sprozoites which reside in the salivary glands of the infected anopheles mosquito enter the human blood stream during the blood meal. The sporozoites then enter the liver parenchyma cells and undergo one cycle of asexual division (pre-erythrocytic schizogony) to form multi-nucleate schizonts. Each schizont contains thousands of "daughter" parasites called merozoites, causing the liver cells to rupture thereby

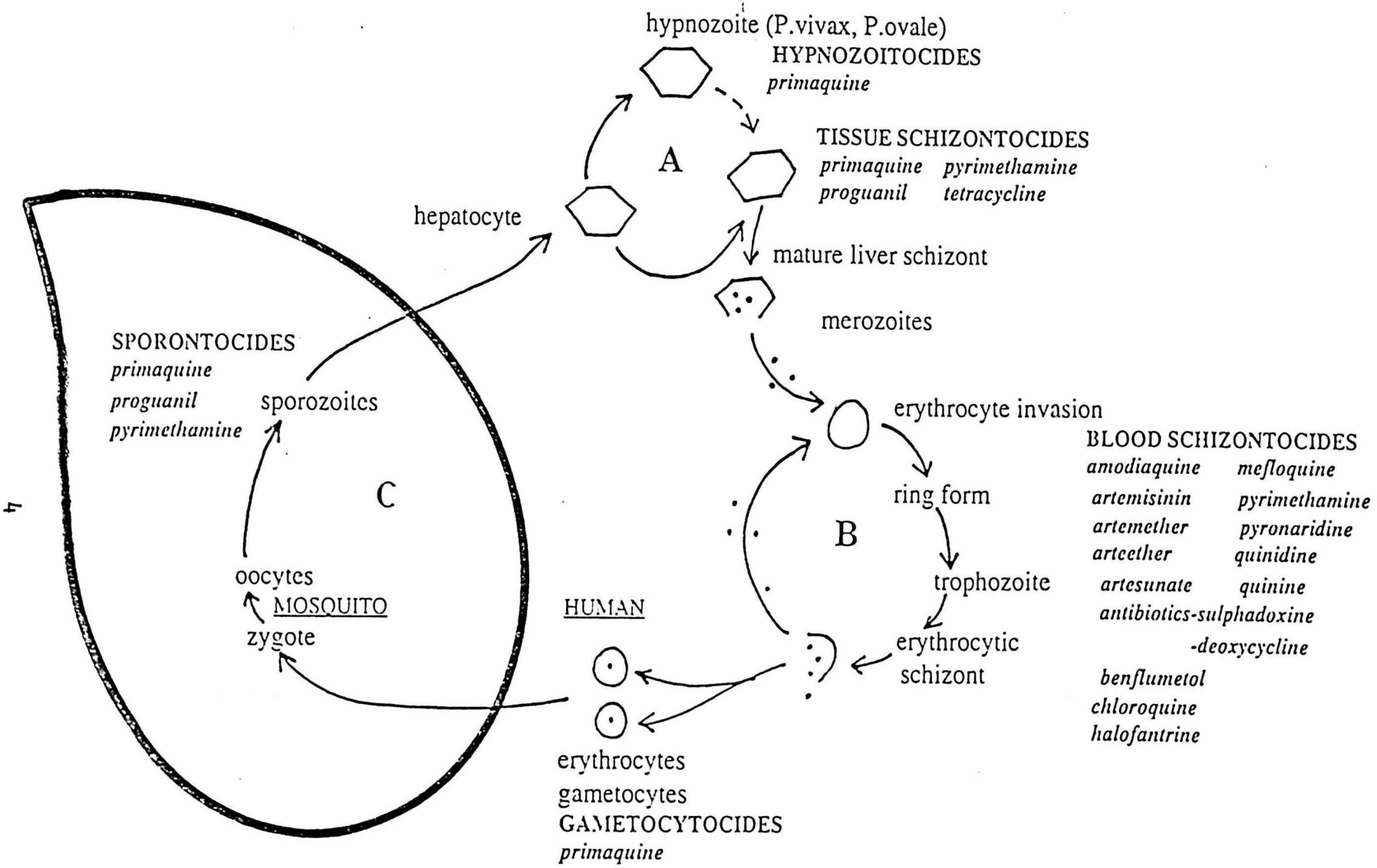

Figure 1.1. The life cycles of the malaria parasite and the sites of action of antimalarial drugs.
A=pre-erythrocytic schizogony phase, B=erythrocytic schizogony phase, C=sporogonic phase.

penetrating erythrocytes and undergoing further cycles of asexual division (erythrocytic schizogony). Inside the erythrocytes, each merozoite matures into a schizont containing new merozoites. At this stage, malaria pigment (haemozoin) is produced within the parasite by breaking down the erythrocyte haemoglobin. The erythrocyte eventually ruptures and releases the merozoites, which are then free to invade additional erythrocytes. The rupturing of erythrocytes is associated with fever and signals the clinical onset of malaria. Some of the merozoites will develop to female and male sexual phases (gametocytes). The gametocytes are incapable of developing further in man. Following another blood meal, the gametocytes combine to form zygotes inside the anopheles stomach. The zygotes develop underneath the outer stomach membrane, divide to produce sporozoites which travel to the salivary glands and await transmission via the next blood meal.

1.3.2 Relapse and recrudescence

Malaria illness may recur months to years after an apparently successful treatment. In patients infected with P.vivax and P.ovale, this phenomenon is known as relapse. Both parasites have dormant liver stages due to secondary exoerythrocytic development or others believed to the hypnozoites (a dormant trophozoites coming from a discrete subpopulations of sprozoites) that resume their developmental cycle and release merozoites into the bloodstream that accounts for relapse (Krotoski, 1985). The recurrence of malaria caused by the nonrelapsing species i.e P.falciparum and P.malariae is due to recrudescence, which is caused by surviving blood-stage parasites from an earlier infection.

1.3.3 The classification of antimalarial drugs

In this thesis, the biological classification of antimalarial drugs as outlined by Bruce-Chwatt et al. (1986) is favoured and drugs are grouped according to their mode of actions on the specific stages of the malaria parasite life cycle (figure 1.1).

1. Tissue schizontocides (causal prophylactic drugs) inhibit the growth of the pre-erythrocytic stages of the parasite in the liver cell. This properties is shared by proguanil, pyrimethamine and the 8-aminoquinoline primaquine. However, primaquine is too toxic at therapeutic doses.

2. Hypnozoitocides (antirelapse or radically curative drugs) kill the dormant liver stages (hypnozoites) of **P.vivax** and **P.ovale**. 8-aminoquinoline group of drugs is the only agent to be effective in man.

3. Blood schizontocides act on asexual erythrocytic stages of the parasite. The action may be against any phases of the asexual erythrocytic cycle, not only against schizonts as the name would indicate. Such drugs include the cinchona alkaloids (quinine,quinidine), the 4-aminoquinolines (chloroquine), the sesquiterpene lactones (artemisinin, artemether, arteether, artesunate), the quinolimethanols (mefloquine), the phenanthrinemethanols (halofantrine) an the dihydrofolate reductase inhibitors (pyrimethamine, proguanil).

4. Gametocytocides destroy the sexual stages of the parasites in the blood. Primaquine is the only drug effective against gametocytes of falciparum malaria. Most of the blood schizontocides are effective against gametocytes of **P.vivax, P.ovale** and **P.malariae.**

5. Sporontocides prevent or inhibit the formation of oocysts and sporozoites in the mosquito. Drugs with this action include pyrimethamine, primaquine and proguanil.

1.3.4 Malaria vaccine

Vaccination is an attractive strategy for preventing and controlling malaria. Three parasite life cycle stages have attracted attention as potential targets: the infective sporozoite, the blood stage merozoite and gametocytes (McLaren & Terry, 1989). Despite experimental and clinical data supporting the development of effective malaria vaccines, there remain a number of obstacles to vaccine development. At present, SPf66 is the only vaccine that had completed Phase I and Phase II human trial. It is a chemically synthesized 45 amino acid peptide derived from fractions of 4 different proteins of Plasmodium falciparum (83, 55 and 35 kDa and CS, the circumsporozoite protein) (Patarroyo et al., 1988; Lopez et al., 1994). This vaccine has succesfully shown an overall protective effects, induces high levels of specific antibodies, safe, with very few side-effects (Sempertegui et al., 1994), and well tolerated by children from one to five years old (Teuscher et al., 1994). In the next few years, larger trials involving between 15,000 and 150,000 people would be staged in Tanzania, Mozambique, Gambia, Thailand and Latin America. SPf66 could be the first chemically

synthesized vaccine, the first vaccine against parasitic disease and the first long-awaited vaccine against malaria.

1.4 Drug resistance

Resistance of malaria parasites to drugs has been defined as the ability of a parasite strain to survive or to multiply in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication (Bruce-Chwatt et al., 1986). This is usually understood as complete resistance i.e. the ability to withstand maximum doses tolerated by the host. The term of "resistance" in practice, is most commonly referred to as complete resistance of **P.falciparum** to the blood schizontocides.

Chloroquine is the most affected antimalarials towards resistance (WHO,1984). The distribution of chloroquine-resistant population of **P.falciparum** has spread worldwide (WHO, 1984) and almost identical to that of the species (Bjorkman & Phillips-Howard, 1990). Recently, chloroquine-resistant vivax malaria has been reported in Papua New Guinea (Schuurkamp et al.,1992), the South- West Pasific area (Rieckmann et al.,1989) and Indonesia (Schwartz et al.,1991). However, there is no sign that **P.ovale** and **P.malariae** have developed resistance to chloroquine which therefore remains the drug of choice for blood schizontocidal treatment of these plasmodia (Wernsdorfer, 1991).

Sulfadoxine-pyrimethamine (SP) generally became the alternative drug in areas where the therapeutic usefulness of chloroquine had ceased (Wernsdorfer, 1991). However, resistance to this combination has been reported mainly in areas of intense use, particularly in Thailand and Cambodia. Resistance of **P.falciparum** is now

established in Southeast Asia and South America (Bruce- Chwat et al., 1986). Since sulfadoxine alone is a poor drug for vivax malaria, there are often treatment failures when SP is given in areas with pyrimethamine- resistant P.vivax (WHO,1984).

Resistance of P.falciparum to quinine has been known since 1910 in Brazil. Despite its extensive use before the era of synthetic antimalarials, quinine resistance is still rare and is limited mainly to Southeast Asia. Quinine has remained the choice of drug for chloroquine and SP resistant malaria (WHO, 1990). Some highly chloroquine-resistant strains of P.falciparum show cross resistance to quinine, but its administration together with tetracycline has improved its efficacy.

Resistance of P.falciparum to pyrimethamine, proguanil, or both is in existence and the geographical distribution of resistance is widespread with the foci reported to be occurring in the endemic areas where the drugs are being or have been used on a large scale (Bruce-Chwatt et al., 1986). Recently, resistance of P.vivax towards pyrimethamine has been reported. P.malariae resistance to proguanil has been observed in Java and the Province of Taiwan.

Mefloquine has been used particularly in Southeast Asia against multidrug-resistant of P.falciparum. Lately, resistance of P.falciparum to mefloquine has been documented in the Thai-Cambodia border areas and West Africa (Bjorkman & Phillips-Howard, 1990).

1.5 Pharmacology of Artemisinin and its derivatives

Artemisinin (QHS) is the active principle of qinghaosu, an extract of the Chinese medicinal plant, qinghao (**Artemisia annua L.**), used for over a thousand year in China as a herbal remedy for malaria. At present, QHS and its derivatives are undoubtedly the most promising compounds for malaria treatment and are particularly important in the light of the resistance of the malaria parasites to existing antimalaria drugs and even to the more recently introduced compounds.

1.5.1 Chemistry of QHS and its derivatives

In 1972, Chinese scientists characterized the structure of QHS (figure 1.2) as a sesquiterpene lactone with an internal peroxide linkage, and its 1,2,4-trioxane ring is unique in nature. QHS is labile to acid or basic treatment, but is stable in neutral solvents at temperature up to 150^{o}C (Webster & Lehnert, 1994). It is soluble in most aprotic solvents but poorly soluble in oil and water, thus poor efficacy was experienced with QHS due to its relative insolubility in water (Webster & Lehnert, 1994). Since its activity and solubility could be improved by chemical modifications as long as the peroxide linkage was preserved (WHO, 1986), various QHS derivatives have been synthesized.

Using the major and active metabolite of QHS, dihydroartemisinin (DQHS) as the precursor, various esters, ethers and succinyl derivatives were developed. Of these various derivatives prepared, artemether (ARM), the oil-soluble methyl ether, and sodium-artesunate, the water-soluble sodium-succinyl ester, have greatly improved the efficacy of QHS and facilitated their use (Webster & Lehnert, 1994). Another derivative, arteether (ARE), a beta-ethyl ether, is a crystalline, stable, insoluble in water

and highly lipophilic compound, has been recommended for development by WHO, for use in high-risk malaria patients including those with cerebral malaria (WHO, 1986). This decision was based on the assumption that ARE with an ethyl ether group instead of the similarity oriented methyl ether group in ARM would be more lipophilic, a possible advantage for its accumulation in brain tissue (Brossi et al., 1988). At Walter Reed Army Institute of Research, USA, two other water-soluble derivatives, sodium artelinate and artelinic acid have been used as transdermal formulations due to their solubility in water (Klayman, 1992; Klayman et al., 1991). The structures of QHS derivatives are presented in figure 1.2.

1.5.2 Preclinical development

1.5.2.1 Efficacy against malaria parasites in animal models

QHS was shown to be active and was comparable to chloroquine in the erythrocytic-induced P.berghei/ mouse model (China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials, 1982). The activity is retained against rodent malaria model which is resistant to primaquine, cycloguanil, pyrimethamine, sulphonamides, mefloquine and menoctone (Peters et al., 1986). Another study showed that strains of P.falciparum/mouse model that have been developed to be resistant to chloroquine, did not exhibit any cross resistance to QHS (Ye et al., 1987).

In the P.yoelii/mice model resistant to mefloquine, quinine and chloroquine were found to be completely susceptible to ARE (5 mg/kg x 3 days, IM), however QHS (50 mg/kg x 7days, IM) was ineffective against this strain (Dutta et al., 1989a). Further

study in blood- induced P.cynomolgi/rhesus monkey model showed that QHS needed much higher dose regimen (10 mg/kg x 7 days or 20 mg/kg x 3 days) than ARE (5 mg/kg x 3 days) to give similar curative effects (Dutta et al., 1989b). Another study by Brossi et al. (1988), showed that ARE was significantly more active than QHS in the P.berghei/mice models resistant to chloroquine, mefloquine, halofantrine, quinine, pyronaridine, primaquine, cycloguanil, pyrimethamine, sulfaphenazole, menoctone and QHS. Conversely, there are no significant differences in the activities of ARE and ARM in mice infected with P.berghei, and in Aotus lemurinus infected with chloroquine resistant P.falciparum (Shmuklarsky et al., 1993).

Experiments in mice revealed that P.berghei in reticulocytes were less sensitive to ARM than those in mature erythrocytes (Waki et al., 1987). Another study in blood-induced P.vinckei/mouse model, ARE was susceptible to rings and young trophozoites but had no effect on merozoites and little effect on mid-term trophozoites which is the stage most sensitive to chloroquine (Caillard et al., 1992). DQHS, the active metabolite of QHS and derivatives, causes the pigment of P.berghei to clump, but in a different fashion from the pigment changes induced by chloroquine or quinine, reflecting a different mode of action of the sesquiterpenes from that of other antimalarials (Peters et al., 1986).

In sporozoite-induced P.cynomolgi/rhesus monkey model, QHS and its derivatives were not effective as a causal prophylactic or radical curative agent (Dutta et al., 1989b). Brossi et al. (1988) also showed that QHS (300 mg/kg) and ARE (30 mg/kg) have no causal prophylactic activity in sporozoite-induced P.yoelii nigeriensis/mice model. Another study revealed that doses of 10 mg/kg and 50 mg/kg

QHS did not affect the sporogenic development of P.berghei/mice model as sporozoites were appeared in the mosquitoes haemolymph and salivary glands 8 days post-feed on mice (Coleman et al., 1988).

1.5.2.2 Toxicology

At therapeutic dosages, QHS and its derivatives exhibited low toxicity in animal studies (Klayman, 1985). Acute toxicity studies indicated that QHS and derivatives have higher LD50s and chemotherapeutic indices than chloroquine (WHO, 1990). At high doses, these compounds have been associated with haemopoietic, cardiac and nervous system toxicity in dogs and rats (Brewer et al., 1994) and rhesus monkeys (Klayman, 1985).

Dogs given ARE at 10 mg/kg day and 20 mg/kg/day for 8 days, showed a progressive syndrome of clinical neurological defects with progressive cardiorespiratory collapse and death in 5 of 6 animals from each treatment (Brewer et al., 1994). Further studies in Sprague-Dawley rats using IM administration of ARE and ARM at a dose of 12.5-50 mg/kg/day for 28 days confirmed the onset of a clinical neurological syndrome (Brewer et al., 1994). QHS administered intramuscularly as an oil suspension to rhesus monkeys at a dose of 192 mg/kg/day for 14 consecutive days caused the death of 75 percent of the animals within 3 days after the final dose. Cardiac and blood abnormalities (including inhibition of hemopoietic function) were also observed (Klayman, 1985). No mutagenic activity of QHS and derivatives has been observed, but teratogenic studies in mice and rats indicated that all these drugs exhibited fetal toxicity even at 1/200-1/400 of the LD50 (WHO, 1990).

1.5.3 Clinical aspects

All of the studies have shown QHS and its derivatives to be therapeutically effective against both chloroquine- resistant and chloroquine-sensitive P.falciparum with initial cure rates approaching 100 percent; however, the recrudescence rates were as high as 100%. To date, there has been no significant toxicity reported to the available QHS and its derivatives preparations in human.

1.5.3.1 Tolerance

At therapeutic dosages, all preparations of QHS and its derivatives (ARM, Artesunate (ARS), ARE, DQHS) have been well tolerated (Mishra et al., 1995; Li et al., 1994; Looareesuwan, 1994; Nosten, 1994; Salako et al., 1994; WHO 1990). Side effects were transient, self limiting and needed no curative action. They include skin rash, fever and a low reticulocyte count for oral and parenteral administrations (Li et al., 1994). As for suppository preparation, side effects of tenesmus, abdominal pain and diarrhoea have been noted (Li et al, 1994). However, based from recent studies in animals, the possibility of subclinical neurotoxicity is of particular concern especially when drugs are used in high doses or for prolonged periods of time (Brewer et al., 1994). As such, more detail studies are needed to define the therapeutic ratio of these drugs in clinical practice (White, 1994a).

1.5.3.2 Efficacy

QHS and its derivatives have been used extensively in China, Viet Nam and Thailand in the treatment of both severe and uncomplicated falciparum malaria and also in vivax malaria. Overall, when compared with other antimalarial drugs, they are

associated with a shortening of the time to fever clearance (14-35 hours) and of the time to parasite clearance (32-64 hours) (Hien, 1994; Li et al., 1994; Looareesuwan et al.,1994; WHO, 1990).

In Thailand where P.falciparum is highly resistant to chloroquine and sulfadoxine/pyrimethamine and increasingly resistant to the alternative antimalarials, quinine and mefloquine, clinical trials of ARS and ARM have been conducted since March 1988 (Looareesuwan et al., 1994). A single oral dose of ARS (200 mg) administered alone or together with chloroquine or Fansidar gave no curative effects (Bunnag et al., 1991a). Modification of the dosage regimens have improved cure rates in uncomplicated falciparum malaria (72-100%) at total doses ranging 600-1200 mg when oral ARS was given over a duration of 5-7 days. (Bunnag et al., 1991a; Bunnag et al., 1991b; Bunnag et al., 1991c).

ARM given intramuscularly in uncomplicated malaria patients recorded cure rates of 84% and 92% after total doses of 480 mg and 600 mg respectively, administered over a period of 5 days (Bunnag et al., 1992). With respect to severe malaria patients, the cure rates were 65% and 76% after total ARM doses of 480 mg and 600 mg were given intramuscularly over a period of 5 days respectively (Bunnag et al., 1992). Another study in acute uncomplicated malaria patients showed that cure rates were 74% and 97% following total ARM doses of 500 mg and 700 mg respectively (Karbwang et al., 1992). Therefore, Looareesuwan (1994) has suggested that cure rates were dependent on the severity of disease. The more severe the disease was, the lower would the cure rates be, eventhough the same dose and the same duration of treatment were used. The results also showed that higher ARM doses gave better cure rates in both uncomplicated and severe malaria.

In Nigerian children with moderate falciparum malaria, ARM (3.2 mg/kg/day for 3 days,IM) was well tolerated and gave a cure rate of 100% (Salako et al., 1994). With respect to children with severe malaria, intramuscular ARM was also well tolerated and as effective as intravenous quinine (Salako et al., 1994). No adverse clinical reaction was noted in children (Salako et al., 1994).

The first clinical efficacy study with ARE in falciparum malaria patients has been reported by Mishra et al. (1995). ARE was effective and did not result in any side effects or toxicity when given as an injectable dose of 150 mg daily for 3 consecutive days. Complete parasite clearance from the peripheral blood was observed in 80% and 98% of the patients at 48 and 72 hours respectively. The mean fever clearance time was 52.04 ± 27.09 hours (Mishra et al., 1995).

Recrudescences are very common during antimalarial treatments using QHS and its derivative compounds. Recrudescence rates were as high as 100% depending on the dose and duration of treatment (Li et al., 1994). Clinical trials with uncomplicated malaria patients in China showed that the recrudescence rates were approximately 50% with 3 days of treatment, 5-10% with 5 days of treatment and 2-6% with 7 days of treatment when QHS and its derivatives were given alone (total doses of 240, 360, 480 and 2800 mg) although a single dose of 500 mg or 1000 mg is enough to clear over 90% of parasitaemia within 24 hours (Li et al., 1994). Recrudescence rates which were dependent on the duration of dose and treatment have also been experienced in Thailand (Looareesuwan, 1994) and Viet Nam (Sy et al., 1993). The high recrudescence rates and sub-optimum cure rates are partly due to dose regimens and routes of administration used which have not yet been optimized (Looareesuwan, 1994).

Modification of drug schedule or combining QHS and its derivatives with other synergistic drugs might produce better cure rates and minimize recrudescence rates. A combination between one of QHS group of compounds with mefloquine has been extensively studied and found to be very effective (Shwe et al., 1988; Shwe et al., 1989). In China a dose regimen of ARM plus benflumetol is now used for the treatment of falciparum malaria (WHO, 1990). Cure rates of 100 % after a total oral dose of 600 mg ARS given in a period of 5 days, followed by mefloquine 25 mg/kg divided into 2 doses were reported in Thai patients with acute uncomplicated malaria (Looareesuwan et al., 1992a; Looareesuwan et al., 1992b). A `double blind' comparative trial of ARM-mefloquine combinations in acute uncomplicated falciparum malaria patients showed that a single oral dose of ARM (300 mg) on the first day, followed by mefloquine 750 mg at 24 hours and 500 mg at 30 hours gave cure rates of 97%. In addition, the limited duration of treatment time (30 hours) might encourage compliance to malaria patients (Bunnag et al., 1995).

1.5.3.3 Therapeutic dosage regimen

The minimum inhibitory concentration of QHS and its derivatives remains uncertain. Therapeutic regimens currently recommended are largely empirical. There is no concensus on which is the best drug or the best route of administration because of the limited pharmacokinetic data and the little understanding of pharmacodynamics to support rational prescribing recommendations (White, 1994b). However in Thailand, due to therapeutic failures with all available antimalarial drugs, ARS (oral and IV preparations) and ARM (IM preparation) are now licensed for use in the treatment of falciparum malaria (Looareesuwan, 1994). The dosage regimens of the drugs were

derived mainly from clinical trials which had confirmed the previous Chinese findings (Looareesuwan, 1994). In China, since 1979, several different formulations of QHS and derivatives i.e QHS (suppositories), ARS (tablets and injections), ARM (injections) and DQHS (tablets) have been used (Li et al., 1994).

1.5.4 Analytical methods

As a rule of thumb, the method employed in any pharmaco- kinetic studies should be sufficiently selective, sensitive and reproducible. However, the development of analytical methods for the quantitation of QHS and its derivatives in biological fluids faces challenging problems due to their chemical properties. The compounds are thermally labile, lacking ultraviolet (UV) absorbance and fluorescent chromophores and also do not posses any functional groups for derivatization (Edwards, 1994).

Analytical techniques that have been used for quantifications of QHS and its derivatives include infrared and nuclear magnetic resonance spectrometry, optical rotary dispersion, gas chromatography with mass detector, high performance liquid chromatography (HPLC) employing UV, electrochemical and mass detectors, and immunoassays. The latter are handy, rapid, relatively sensitive and do not require extraction, however handicapped with cross reactivity with metabolites (Teja-Isavadharm et al., 1992). Mass spectrometry attached to HPLC or GC is sensitive (Hufford et al., 1990; Theoharides et al., 1988); however is too expensive by most laboratories standard, and is not cost-effective for routine work. The various published analytical methods to analyse QHS and its derivatives are documented in table 1.1.

1.5.5 Pharmacokinetics

To date, limited clinical pharmacokinetic informations on QHS and its derivatives have been reported. Only several studies involving small number of subjects were carried out and are presented in table 1.2.

1.5.5.1 Absorption

Oral absorption of QHS and its derivatives are rapid, reaching maximum concentrations at 0.75-3 hours (Na Bangchang et al., 1994; Boxtel et al., 1994; Titulaer et al., 1990). The absorption of ARM does not appear to be changed in acute uncomplicated falciparum malaria as the time to maximum concentration of 1-3 hours in patients is comparable to those derived in healthy subjects (Na Bangchang et al., 1994). An incomplete absorption of QHS after an oral administration to healthy subjects, with a relative oral bioavailability to the IM administration of 32% has been observed (Titulaer etal., 1990). This value is in agreement with the observed QHS bioavailability of 37% in rats (Niu Xinyi et al., 1985).

A high first pass clearance may partially contribute to the incomplete absorption of QHS; since in vitro experiments have demonstrated a high metabolite clearance occured in rat liver slices but not in stomach and ileum preparations (Niu Xinyi et al., 1985). The first pass effect has also been suggested by the observation of higher metabolite concentrations (DQHS) in plasma relative to the parent drug (Na Bangchang et al., 1994).

1.5.5.2 Disposition

QHS and its derivatives are highly bound to plasma protein. Wanwimolruk et al. (1992) suggested that approximately 75% of plasma ARE was bound to protein especially albumin and alpha-1 acid glycoprotein with the latter binding some 20-fold greater than the former. Another study showed that approximately 20% of the added QHS was covalently bound to albumin within 24 hours (Yang et al., 1993). The binding of drug to plasma protein is known to be important, as only the unbound drug concentration is responsible for the antiparasitic and toxic actions of the drug.

QHS and its derivatives are biotransformed rapidly to the biologically active metabolite, DQHS and can be detected in the blood soon after an oral administration. A series of other metabolites have been characterised, but DQHS appears to be the most important (Lee & Hufford, 1990).

Following oral ARM administration, clearance of the parent drug is rapid with terminal half-life (t1/2) of 1-10 hours in healthy subjects (Na Bangchang et al., 1994; Boxtel et al., 1994; Titulaer et al., 1990). The plasma clearance of DQHS is very similar to the parent drug as the t1/2 of 2-12 hours has been noted (Na Bangchang et al., 1994). Exclusively for ARS, it has an average elimination t1/2 of 23 minutes which is shorter than DQHS (45 minutes) (WHO, 1990).

Following IM administration of QHS and its derivatives, a slightly longer elimination half-life has been observed (Titulaer et al., 1990). The range of terminal half-life of QHS after an IM administration was 4-16 hours which can be explained by the slow release characteristics rates and rapid elimination rates that occur in IM depot. As such, the apparent elimination rates will be longer after an IM administration than after an oral administration (Titulaer et al., 1990).

The effect of malaria infection on ARM disposition has been demonstrated by Na Bangchang et al. (1994). They compared the pharmacokinetics of ARM together with its metabolite, DQHS in six healthy Thai volunteers and eight Thai patients with acute uncomplicated malaria. Pharmacokinetic parameters of ARM and DQHS were similar for both groups except for the Cmax which was significantly higher in patients ranging from 116 to 411ng/ml within patients vs 112 to 127 ng/ml within healthy subjects for ARM and from 483 to 729ng/ml within patients vs 162 to 702 ng/ml within healthy subjects for DQHS). The study suggests that the pharmacokinetic parameters of ARM together with DQHS are not altered in malaria.

1.5.5 Mode of action

QHS and its derivatives appear to be a potent blood schizontocide against malaria parasites. They act early in the asexual parasite development cycle by destroying the very young small ring forms (Li et al., 1994). The specific mechanism of antimalarial action of QHS and its derivatives is still unclear. Recently, Meshnick (1994) proposed two sequential steps of action. The first step is activation, comprises the iron-mediated cleavage of the endoperoxide bridge to generate an unstable organic free radical and/or other electrophilic species. This is followed with the alkylation step, which involves the formation of covalent adducts between the drug and malarial proteins.

Table 1.2 Pharmacokinetics of artemisinin and its derivatives

Reference	Drug	Route	Method of assay	No. of subjects	Absorption t1/2 (hr)	Cmax (ng/ml)	tmax (hr)	CL (ml/kg/min)	Vd (L/kg)	Elimination t1/2 (hr)	AUC (ughr/ml)
Na Bangchang et al. (1994)	ARM 200mg(tablet)	oral	LC-UV	6 healthy Thais	-	112-127	1-10	-	-	1.0-9.6	0.3-4.4
	200mg(tablet)	oral		6 Thai patients	-	162-702	2-12	-	-	4.7-19.2	0.8-38.7
Zhao et al. (1988)	ARM 3.2mg/kg	IM	RIA	4 healthy Chinese	-	1200	4.1	8.3	-	7.1	-
	6.0mg/kg	IM		4 healthy Chinese	-	900	9.0	11.7	-	10.9	-
	10.0mg/kg	IM		4 healthy Chinese	-	800	7.3	11.7	-	13.2	-
Zhou et al. (1988)	ARM 6mg/kg	IM	LC-ECD	6 healthy Chinese	2.6	145	5.2	38.5	-	7.7	-
	10mg/kg	IM		6 healthy Chinese	2.0	224	6.3	32.3	-	11.1	-
Boxtel et al. (1994)	QHS 500mg	oral	LC-ECD	not available	0.58±0.54	391±147	1.81±0.73	-	19.4±6.9	0.58±0.54	-
Titulaer et al. (1990)	QHS 400mg	oral	LC-UV	10 healthy Caucasian	0.14-0.93	159-440	0.75-2	-	-	1.0-2.9	0.57-1.02

Table 1.2 , Continued.

Reference	Drug	Route	Method of assay	No. of subjects	Absorption t1/2 (hr)	Cmax (ng/ml)	tmax (hr)	CL (ml/kg/min)	Vd (L/kg)	Elimination t1/2 (hr)	AUC (ughr/ml)
Titulaer et al. (1990)	QHS 400mg	IM	LC-UV	10 healthy Caucasian	0.76-2.47	91-331	0.75-7	-	-	4.16-15.96	0.99-3.95
Zhou et al. (1988)	QHS sup. 10mg/kg	PR	LC-ECD	9 healthy Chinese	179	9.6	-	-	-	4.0	-
Shen et al. (1989)	QHS sup. 10mg/kg	PR	LC-ECD	6 healthy Chinese	110	6.7	-	-	-	4.4	-
Yang et al. (1986)	ARS 3.3-4.4mg/kg	IV	LC-ECD	4 healthy Chinese	-	3088	-	35.3	1.5	0.5	-
Zhao et al. (1986)	ARS 2.0mg/kg	IV	RIA	3 healthy Chinese	-	33000	-	15	0.4	1.4	-
	3.8mg/kg	IV		3 healthy Chinese	-	16000	-	5	0.3	0.8	-
Kager (1994)	ARE 3.6mg/kg	IM	LC-ECD	1 healthy Caucasian	0.61	-	-	1 (L/min)	2000(L)	23.1	-

IM=intramuscular, PR=per-rectum, LC=high performance liquid chromatography, UV=ultraviolet detection, ECD=electrochemical detection, RIA=radioimmunoassay, QHS sup.=QHS suppository

Table 1.2 , Continued.

Reference	Drug	Route	Method of assay	No. of subjects	Absorption t1/2 (hr)	Cmax (ng/ml)	tmax (hr)	CL (ml/kg/min)	Vd (L/kg)	Elimination t1/2 (hr)	AUC (ughr/ml)
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Zhou et al. (1988)	QHS sup. 10mg/kg	PR	LC-ECD	9 healthy Chinese	179	9.6	-	-	-	4.0	-
Shen et al. (1989)	QHS sup. 10mg/kg	PR	LC-ECD	6 healthy Chinese	110	6.7	-	-	-	4.4	-
Yang et al. (1986)	ARS 3.3-4.4mg/kg	IV	LC-ECD	4 healthy Chinese	-	3088	-	35.3	1.5	0.5	-
Zhao et al. (1986)	ARS 2.0mg/kg	IV	RIA	3 healthy Chinese	-	33000	-	15	0.4	1.4	-
	3.8mg/kg	IV		3 healthy Chinese	-	16000	-	5	0.3	0.8	-
Kager (1994)	ARE 3.6mg/kg	IM	LC-ECD	1 healthy Caucasian	0.61	-	-	1 (L/min)	2000(L)	23.1	-

IM=intramuscular, PR=per-rectum, LC=high performance liquid chromatography, UV=ultraviolet detection, ECD=electrochemical detection, RIA=radioimmunoassay, QHS sup.=QHS suppository