CHAPTER I

INTRODUCTION

Malaria continues to be a major endemic infectious disease in tropical countries including Vietnam (Hien *et al.*, 1997), requiring research efforts aimed at its control and eradication. Almost 500 million people are annually infected with the disease and about 2 million die every year (Kondrachine and Trigg, 1997). Of the four species of human malaria, *Plasmodium falciparum* and *Plasmodium vivax* account for the vast majority of cases but *P. falciparum* is the form that causes substantial morbidity and almost all of the mortality. In patients with severe and complicated disease, the mortality rate is between 20 and 50% (White, 1996a). Some 40% of the population in Vietnam lives in malaria endemic areas. As elsewhere, malaria in Vietnam is closely related to the environment and to human behavior (WHO WPRO, 2000). A large proportion of malaria cases and deaths occurred in the central mountainous and forested parts where mosquito vectors are abundant and health services are often inadequate (Annette Erhart *et al.*, 2005). Despite the significant decline in malaria cases and deaths due to malaria in the 1990s, the disease remains an important public health concern. Excessive use of drugs, for treatment or prevention, has led to development of drug resistance. *Plasmodium falciparum* malaria in Vietnam was highly resistant to chloroquine and sulfadoxine/pyrimethamine and there was increasing resistance to alternative antimalarials quinine and mefloquine (MQ) (Hung *et al.*, 1997; Anh *et al.*, 1990; Arnold *et al.*, 1990). The presence of resistance to available antimalarials and compliance in the target population are factors that influence the choice of drugs and regimens (Hien *et al.*, 1997). In response to the increase in resistance of malaria parasites to conventional antimalarial drugs, Vietnam has deployed artemisinin (ARN) and its derivatives with various formulations produced from locally grown *Artemisia annua* plants.

Dihydroartemisinin (DHA) is a semi-synthetic derivative of ARN and has been used in clinical treatment of patients with falciparum malaria in many tropical countries in the world, especially those in the Asian region (WHO, 1995). DHA is

considered as a potent antimalarial drug against *P. falciparum* parasites (de Vries and Dien TK, 1996). The expenditure for production of DHA is cheaper, compared to that of other ARN derivatives (Brossi *et al.*, 1988). This drug is therefore considered to be the most applicable for using in developing countries. ARN derivatives have been used clinically in two approaches, *i.e.*, monotherapy and combination therapy with other antimalarials (Davis *et al*, 2005). For monotherapeutic regimens, DHA has been used in treatment courses of 3 to 5 days. The radical cure rate of monotherapy with artesunate (ARS), artemether, and DHA were reported as high as 90% in some clinical trials (Looareesuwan *et al*, 1992, 1996; Karbwang *et al.*, 1998a; Schwarz *et al.*, 2005), but were as low as 80% or lesser in other reports (Hien and White, 1993; Alin *et al.*, 1995; Alin *et al.*, 1996; Borrmann *et al.*, 2003; Gomez *et al.*, 2003). The ARN derivatives have short half-lives, *e.g.*, 40-60 minutes for DHA (Hien and White, 1993; Benakis *et al.*, 1997; Davis *et al.*, 2005). These drugs are rapidly excreted after administration. In addition, the decline of DHA concentrations in plasma during a 5-day oral treatment course with ARS was reported in patients with acute uncomplicated falciparum malaria (Khanh *et al.*, 1999). Despite their short residence time in the body, continuous reduction in parasite density in malaria patients treated with ARN derivatives was recorded. This may be explained by the "post-antiparasitic" activity of the ARN derivative drugs. In theory, the efficacy of DHA could therefore be enhanced by the increase of treatment dose or by the prolongation of treatment course, or both.

Thus, an open randomized comparative study of the high dose monotherapy of DHA and the combination of high dose DHA with MQ for the treatment of acute uncomplicated falciparum malaria in Vietnamese patients was conducted for answering the above hypothesis.

MQ, a quinoline methanol which is structurally very similar to quinine, is a potent long-acting blood schizontocide effective against all malarial species including *P. falciparum* parasites resistant to 4-aminoquinolines, pyrimethamine-sulfonamide combinations and quinine. The great advantage of MQ is that it can be given as a single dose of 15-25 mg/kg body weight. However, owing to its long elimination half-life and consequent long-lived subtherapeutic concentrations in the blood, the development of resistance is to be expected especially in areas of high transmission.

Since the late 1980s, resistance of *P. falciparum* to MQ has developed in the shared borders of Thailand, Myanmar, and Cambodia, and more than 50% of patients have recrudescence of parasitemia within 28 days after a dose of 15 mg/kg, and its use as a single antimalarial treatment is no longer recommended in these areas (Fontanet *et al.*, 1993; WHO, 1998; Wongsrichanalai *et al.*, 2002). Combination therapy is now preferable in malaria treatment to prevent the emergence and spread of parasite resistance (Brian *et al.*, 2005). MQ is considered a main counterpart to ARN derivatives in combination regimens. In these combination regimens, MQ has been used at different dosages, *i.e.*, 10 mg/kg (Hung *et al.*, 1997), 15 mg/kg (Na-Bangchang *et al.*, 1999), and 25 mg/kg (Looareesuwan *et al.*, 1994), given initially, or at 2, 6, 8, or 24 h or at 4 days after the first dose of ARN derivative (Looareesuwan *et al.*, 1994; Hung *et al.*, 1997; Na-Bangchang *et al.*, 1999; Simpson *et al.*, 1999; Wang *et al.*, 2001; Hung *et al.*, 2004). The treatment duration of the combination regimens also varies from a single combined dose to a 4 day-combination regimen (Hung *et al.*, 1997; Na-Bangchang *et al.*, 1999; Simpson *et al.*, 1999). One of the reasons for having such different combination regimens is the limitation of information on drug interactions between ARN derivatives and MQ.

To explore the possibility of drug interaction between the two drugs as well as to optimize therapy with these combination regimens, we have conducted a pharmacokinetic study of MQ given in two different regimens, i.e., at 6 h after the first dose of DHA, and at 24 h concurrently with the second dose of DHA, in Vietnamese patients with acute uncomplicated falciparum malaria.

To date, the pharmacokinetic data of ARN and its derivatives have generally been difficult to acquire due to the complexity involved in measuring their concentrations in biological fluids. The analytical methods for these drugs differ from those for the other antimalarials because the sesquiterpene compounds do not contain the chromophore responsible for ultraviolet or fluorescent absorbance (Edwards, 1994). High-performance liquid chromatography (HPLC) with post-column derivatization for measurement of ARS and DHA has been reported in a number of studies. The sensitivity of this technique is limited by its high detection limit (20 - 50 ng/ml) (Batty *et al.*, 1996; Taylor *et al.*, 2000; Newton *et al.*, 2000). The presence of a

peroxide moiety in the chemical structure of ARN and its derivatives permits HPLC with a reductive electrochemical detector (HPLC-ECD) as a sensitive means for determining these drugs in biological fluids (Karbwang *et al.*, 1997; Na-Bangchang *et al.*, 1998; Olliaro *et al.*, 2001a; Teja-Isavadharm *et al.*, 2001). However, it is a rather sophisticated technique that requires a rigorous deoxygenation system, assiduous equipment care, and is generally very time-consuming. Mass spectrometry has now become an indispensable analytical tool used across many disciplines. Indeed, gas chromatography-mass spectrometry has been used for the quantification of artemether and DHA (Mohamed *et al.*, 1999). More recently, liquid chromatography-mass spectrometry (LC-MS) has been utilized for the measurement of ARN derivatives (Karunajeewa *et al.*, 2004; Naik *et al.*, 2005).

We planned to develop and validate a simple, sensitive, and specific LC-MS method for the simultaneous quantification of ARS and DHA in human plasma using liquid-phase extraction, and incorporating ARN as an internal standard. This method will be used for measuring the plasma concentrations of ARS and DHA in our studies.

After oral administration, ARS is rapidly hydrolyzed to DHA and has relative bioavailability of 82 - 85%, comparable to that observed for intravenous ARS administration (Batty *et al.*, 1998). DHA has a broad spectrum of activity throughout the phases of the asexual intra-erythrocytic schizogonic cycle, and also acts on young gametocytes (Olliaro *et al.*, 2001b). It can be taken orally in treating malaria, with fewer side effects (Xu *et al.*, 1997). DHA is typically administered as an initial oral dose of 120 mg (approximately 2.0 - 2.5 mg/kg body weight), followed by 60 mg daily for 4 - 6 days, for the treatment of acute uncomplicated falciparum malaria (Looareesuwan *et al.*, 1996; Xu *et al.*, 1997). The time-dependent pharmacokinetics of ARN has been reported in both healthy volunteers (Ashton *et al.*, 1998c) and in malaria patients (Ashton *et al.*, 1996, 1998a; Alin *et al.*, 1996a, 1996b). The auto-induction of ARN on its metabolism is thought to be the main cause for the reduction of drug bioavailability during treatment. The decline in concentrations of DHA in plasma during 5-day treatment with oral ARS for falciparum malaria has also been reported by Khanh *et al.*, 1999. It appears that this auto-induction phenomenon may be

a feature that is common to several endoperoxide antimalarial compounds. The liver is primarily responsible for DHA metabolism (Lee and Hufford, 1990). Malaria disease impairs vital organ functions, especially those for the liver and kidney. Consequently, these organs have a reduced capacity to metabolize and eliminate xenobiotics such as antimalarial drugs. A compromised clearance of quinine and MQ was noted in malaria patients when compared to those in healthy subjects (White *et al.*, 1982; Warrell *et al.*, 1990; Karbwang and White, 1990). In an *in vitro* study assessing the effect of malaria infection on hepatic clearance of DHA, it was shown that DHA has a high hepatic extraction ratio and the bioavailability of DHA was lower in controls than in malaria infected livers, indicating an impaired hepatic clearance of DHA in malaria (Kevin *et al.*, 1998). Since there was a significant decrease in the hepatic clearance of DHA during the acute phase of malaria, it was likely that the recovery of hepatic enzyme activity, the normalization of the apparent volume of distribution, and the increase presystemic drug metabolism could result in the reduction of plasma DHA concentrations during the convalescent phase of the illness.

To examine the changes in DHA pharmacokinetics during a 5-day oral treatment for uncomplicated falciparum malaria, the pharmacokinetics of DHA in the acute (the first day of treatment) and convalescent (the final day of treatment) phases of malaria was investigated.

ARS was derived from ARN and had more potent blood schizontocidal activity than the parent compound (Hien and White, 1993; Li *et al.*, 1994; World Health Organization 1994; Skinner *et al.*, 1996). ARN, a new, highly-effective antimalarial compound, was an endoperoxide sesquiterpen lactone found in the wormwood *Artemisia annua*. The pharmacokinetics of ARN and its derivatives has been investigated in many studies. The time-dependent pharmacokinetics of ARN was reported in healthy volunteers (Ashton *et al.*, 1998b, 1998c) and in malaria patients (Ashton *et al.*, 1996, 1998a; Alin *et al.*, 1996a, 1996b). The auto-induction of ARN on its metabolism was thought to be the main cause for the reduction of drug bioavailability during treatment. The decline in plasma DHA concentrations during the 5-day treatment with oral ARS for falciparum malaria was reported by Khanh *et al.*, 1999. It seemed that the auto-induction characteristic was usual to several

endoperoxide sesquiterpen antimalarials. However, there was no evidence of time-dependent pharmacokinetics for oral DHA in patients with uncomplicated falciparum malaria in our previous study (Diem Thuy *et al.*, submitted, 2007). The same result was reported in a study of Zhang *et al.*, 2001, in which it was mentioned that DHA did not alter the elimination of ARN, but DHA elimination was inhibited by ARN. The difference in drug metabolism between ARN and its derivatives was put forward as a hypothesis.

To verify the time dependency in pharmacokinetics of ARS, the present study was conducted in healthy volunteers with the administration of oral ARS repeated in five consecutive days.

The increase of resistance to antimalarial drugs has been continued in many regions of the world, with the resultant effect on morbidity and mortality. It is therefore essential to ensure rational deployment of the few remaining effective drugs, to maximize their useful therapeutic life while still ensuring that safe, effective and affordable treatment is accessible to those at risk. This requirement has resulted in the multiple-drug therapies which are used to exploit the synergistic or additive potentials of individual drugs. The World Health Organization has recommended artemisinin-based combination thepapies as first-line treatment for multidrug-resistant falciparum malaria, and judgements of efficacy and optimal administration should be based on the pharmacokinetic properties as well as the drug interactions of the combination components.