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## Anti-malaria drug treatment in Vietnam

## Thesis

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# ANTI-MALARIA DRUG TREATMENT
# IN VIETNAM

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# ABSTRACT

Resistance to antimalarial drugs is increasing nearly everywhere in the tropical world, confounding global attempts to "Roll Back Malaria". Antimalarial treatment with artemisinin, artesunate, or artemether has proved rapidly effective in the treatment of non complicated and complicated malaria and remarkably nontoxic. However there is a number of important questions that still need urgent attention: Should artemisinin be deployed alone or in combination with other antimalarial drugs such mefloquine or other compound (s); which is the best regimen (s); can artemisinin and its derivatives (artesunate or artemether) reduce the mortality in severe malaria and which is the best drug(s); are there important neurotoxic side effects in patients treated with this group of antimalarial drugs.

In order to answer those questions a series of studies were conducted in Vietnam and these form the basis of this thesis. The conclusion is

1/ Dihydroartemisinin-piperaquine is an inexpensive, safe, highly efficacious fixed-dose antimalarial combination treatment that could make an important contribution to the control of multidrug-resistant falciparum malaria for Vietnam and other countries.

2/ Artemether is a satisfactory alternative to quinine for the treatment of severe malaria in adults. The rectal administration of artemisinin would obviously constitute a useful therapeutic advance, in comparing with parenteral drugs such as

artemether and artesunate, particularly in areas where parenteral administration is difficult.

3/ The artemisinin derivatives have an acceptable safety profile.

4/ Viet Nam has shown that it is possible to "Roll Back Malaria" assuming one has access to good drugs and a willingness to implement change.

Those studies have helped to confirm that the qinghaosu (artemisinin) group of drugs is the most important new class of antimalarials developed in the last fifty years.

# ACKNOWLEDGEMENTS

The work described in this thesis would not have been possible without the participation of the malaria patients adults admitted to the Hospital for Tropical Diseases of Ho Chi Minh City; to the Commune Health Station of DaK O in Phuoc Long District Binh Phuoc Province and the Khanh Phu Malaria Station-Khanh Hoa Province. I am indebted to them for their consent to take part in the studies described within this thesis.

But first of all I am enormously grateful to *Professor Nicholas John White*, who since 1990 has taken his own risks to persuade the Wellcome Trust to establish the collaboration with the Hospital for Tropical Diseases of HCM City Vietnam that leads to these joint studies on malaria, for his scientific advice and to *Dr Jeremy Farrar*, my supervisor, who has been a constant source of scientific, logistical and spiritual support. Without them this thesis has never been completed.

A similar magnitude of thanks also goes to Dr Keith Arnold, my teacher at the Medical Training Center (Saigon Medical University), the 1st person, who brought "qinghaosu" from China to Vietnam in 1987, who has persuaded me to conduct studies to assess its efficacy in malaria patients in Vietnam, for the opportunity, guidance and wisdom he has given me.

The following people have also helped with the research described in this thesis

And I also extend my thanks to those who have taken a skeptical view about my work. They have offered me more drive to overcome all difficulties to finish the studies described in this thesis as well as other published ones in evaluating the effectiveness of qinghaosu in treatment of malaria in Vietnam

Finally, I thank Ngoc, my wife, for sharing all sweet and bitter tastes of life especially during the period I perform this thesis

# DECLARATION

Other than the assistance outlined in the acknowledgements, the work described in this thesis is my own work: I participated in designing, implementing, treating and caring for all patients involved in the studies; taking and preparing blood samples (Chapter 3); performing the standard neurological, otoscopic and audiological examinations, BS AER (Chapter 4.1), collecting brain specimens with autopsy from patients who died (Chapter 4.2); conducting the follow-up of the patients (Chapter 5) and managing the complicated malaria cases: performing peritoneal dialysis or haemofiltration (Chapter 6). I have been involving in all the data analysis.

My work described in this thesis has not been submitted for a degree or other qualification to this or any other university.

**Criteria for a new antimalarial drug:**

*............To salivary glands they want*

*And start again; there is no end*

*The moral of this age-old story*

*Is that we can't aspire to glory*

> *Until our principal objective*

> *Will make control much more effective*

> *And faced with chloroquine resistance*

> *We must depend on more assistance*

**And find a new medicament**

**To solve our great predicament**

**A compound that in all event**

**Is active, cheap, polyvalent...............**

> Ballad of the Plasmodium. *Leonard Bruce-Chwatt (1907-1989)*

**And how to build up a new antimalarial drug policy for a country:**

As long as 1937 L.H. Hackett remarked:

*"Clearly governments can trust no formulas devised in Geneva or elsewhere, but must create the simple machinery necessary to define and resolve their own problems, locality by locality" (Malaria in Europe. Oxford University Press, p. 274, 1937)*

His remark is still pertinent today, although the complexity of the disease will not allow simple mechanism for the resolution of the problems.

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# ABBREVIATION

ACT:            Artemisinin based combination therapy

AER:            Auditory evoked response

AEP:            Auditory evoke potential

ARM:            Artemether

ARTS:           Artesunate

ALT:            Alanin animotransferase

AST:            Aspartate aminotransferase

AUC:            Area under the plasma time-concentration curve

BUN:            Blood urea nitrogen

BS:             Brain stem

CYP:            Cytochrome

DHA:            Dihydroartemisinin

FCT:            Fever clearance time

HRP:            Histidine-riche protein

GDP:            Gross Domestic Product

GLURP:          Glutamate-riche protein

GSC:            Glasgow coma scale

ICAM:           Intercellular adhesive molecule

IPL:            Inter peak latency

| IMPE: | Institute of Malarialogy-Parasitology-Entomology (Vietnam) |
|---|---|
| IL: | Interleukine |
| INF: | Interferon |
| MSP | Merozoite surface protein |
| NMCP: | National Malaria Control Programme (Vietnam) |
| PCT: | Parasite clearance time |
| PfEMP1: | Plasmodium falciparum erythrocyte membrane protein 1 |
| PK: | Pharmacokinetic |
| QHS: | Qinghaosu |
| RBC: | Red blood cell |
| RDT: | Rapid diagnosis test |
| SPR: | Smear positive rate |
| SD: | Standard deviation |
| TNF: | Tumor necrosis factor |
| TFG: | Transforming growth factor |
| WHO: | World Health Organization |

# CHAPTER 1

# INTRODUCTION

Malaria is one of humanity's worst diseases and is becoming now a global crisis. One-fifth of the world's population is still at risk of malaria. Nearly five times as many cases of malaria were reported in 2000 as tuberculosis, AIDS, measles and leprosy cases combined. In the time it takes to say the word "malaria" ten children will contract the disease and begin fighting for their lives (WHO, 2000a). Malaria kills over a million people each year. Malaria has an insidious and far-reaching effect on economic and social development. In addition to the direct costs of treatment and prevention, malaria is responsible for significant losses in productivity and it undermines educational achievement. It discourages savings and investment by households, constrains optimal land use and deters foreign investment and tourism. A recent study in Viet Nam has shown that living in malaria-endemic regions places an economic burden on households even if they do not actually suffer an episode of malaria. Households living in endemic malarial regions are less likely to have access to economic opportunities and may have to modify agricultural practices and other household behaviour to adapt to their disease environment. Data from Vietnam demonstrate that reductions in malaria incidence through government-financed malaria control programmes can contribute to higher

household income for all households in endemic areas (R Laxminarayan, 2004). In effect, malaria cripples the economies of malaria endemic countries, and has been shown to be responsible for losses of up to 1.3% of economic growth per year in sub-Saharan Africa. This negative effect has widened disparity in the economic status between malarious and non-malarious regions of the world. In the 1950s the World Health Organization (WHO) optimistically targeted malaria for eradication; since then many countries in the Western world had wiped out the diseases and other countries in Latin America were making progress. Unfortunately, over the past 20 years, efforts to control malaria have met with less and less success. The hope of eradication has disappeared and even control now seems a distant dream. In such a changing world malaria is also exacerbated by altered geographic, ecologic, social factors such as global warming, poverty, political upheavals.

## 1.1. Malaria

### 1.1.1 History of malaria

The mere mention of the word malaria suffices to evoke the devastating effects of this still unconquered disease. Falciparum malaria is a disease largely prevalent in tropical and subtropical regions, but *Plasmodium vivax*, which can survive at lower temperatures in its anopheline mosquito vectors, may also occur in temperate areas.

Until Laveran's identification of the malaria parasite, malaria was synonymous with ague, a debilitating febrile illness associated with intermittent chills or rigors. As early as the 5th century BC Hippocrates was able to diagnose malaria in Asia Minor and to distinguish between different stages of fever. This knowledge became the property of his successors. When malaria occurred in Attica, it was reported by Plato (427- 347 BC) and Aristotle (384-322 BC). Even in the secondary century AD Aretaeus' treatise on the treatment of acute and chronic diseases was based on the findings of Hippocrates, and the

polymath medical author Galen (130-200 AD) also relied upon these writings. The Roman historian Livy (59 BC-17 AD) reported malaria-like diseases in Rome. After the 2$^{nd}$ Punic War whole localities were abandoned by their inhabitants because of the danger of malaria. The Roman scholar Marcus Terentius Varro (116- 27 BC) stated in the his work *Res rusticate* that in humid places there were minute insects which could cause grievous diseases in the bodies of human beings, although these were barely perceptible to the human eye (W Schreiber & FK Mathys, 1987).

Because of its widespread distribution and tremendous infectious potential, malaria was often a more decisive factor than arms in military defeats. The Goth and Norman invaders had to pay a heavy toll of lives. In the Napoleonic wars the English troops landing on the continent in 1809 failed because a large proportion of the soldiers landing on the Netherland's island of Walcheren fell victim to malaria. In 1916 and 1917 the Allied military campaigns in Macedonia were completely paralysed by the disease: among the French alone more than 100,000 out of 150,000 came down with malaria and among the English the figure was 70,000. This occurred in spite of a burgeoning increase in knowledge about the infection. In 1880, the Frenchman Charles Louis Laveran (1845-1922) had succeeded in Constantine, North Africa, in detecting the malaria parasite of Plasmodium in the blood of malaria patients. His findings were confirmed by other workers. In 1897, Ronald Ross (1857-1932) working in Secunderabad, India, had reached the conclusion that mosquitoes inoculated human beings with the disease. Italian scientists soon confirmed the complete cycle of infection in man.(W Schreiber & FK Mathys, 1987) (HM Gilles & DA Warrell, 1993) Doctors waged the war against the disease with the most diverse agents: roots, herbs, and mole's claws, snake heart practices, which were maintained until well into the 19th century.

Extracts of the plant qinghao (*Artemisia annua*), known as **qinghaosu**, have been used in traditional medical practice in China for over two millennia. In AD340 Ge Hong described use of qinghao infusions for the treatment of fever in the famous Handbook of Emergency Treatments. Thereafter qinghao is mentioned frequently in the Chinese materia medica as a treatment for agues. The antimalarial properties of qinghaosu were rediscovered in 1971 when low temperature ethylether extracts of the plant were shown to have activity against experimental rodent malaria.

On the other side of the world another medicinal plant came to medical attention during the reign of the Count of Cinchon as Viceroy of Peru between 1628 and 1629. Legend has it that the Viceroy's wife, the Countess, was afflicted by ague in Lima. She was a well-known and popular figure and news of her illness spread inland. It eventually reached Loja where a Spaniard was in governorship. He knew of a local remedy obtained from the bark of a tree and sent it to the ailing Countess. The therapeutic result was excellent; she improved rapidly, and was so impressed that she ordered the bark in quantity and dispensed it to the poor of Lima who commonly suffered from the dangerous tertian fevers. The pulverised bark became known as "los polvos de la Condeca" or the Countess's powder, and Linnaeus subsequently named the tree from which the bark was obtained 'Cinchona' in honour of the Countess (IA McGregor, 1996) Sadly the detective work of one A.W. Haggis, reported in 1941, has shown that 'the fabulous story of the Countess of Cinchon' is almost certainly a romantic fable. Nevertheless it is likely that the bark was introduced to Europe by the Fathers of the Society of Jesus around the time of the story, or even earlier (c.1630) and was widely promoted in Europe by the Jesuit Cardinal Juan de Hugo. For these reasons it became known as Jesuit's bark. Not everyone was convinced by the new remedy, and when in 1653 Archduke Leopold of Austria relapsed one month after being cured of double quartan fever, his personal physician

Jean-Jacques Chifflet began a bitter polemic on the merits of the bark which was to last for 200 years. Much of the dispute stemmed from the fact that many considered all fevers had the same cause, and clearly not all responded to Jesuit's bark. It was probably Torti in 1712 who first stated that the bark was "specific solely for the ague".

Another source of debate, and one that is still active today was dosage. Sir Robert Talbor (Talbot) was one of the few physicians who was not afraid to give the bark in large and repeated doses, and when he cured the Dauphin (the son of Louis XIV) with his "remede anglais" his fame spread far and wide. He subsequently treated Charles II of England successfully with the same medicament. Others were less enthusiastic. Many Protestants believed the bark to be a poison disseminated by the Jesuits. Lind, who demonstrated clearly that in order to get best results the bark should be given in full doses as soon as the disease was diagnosed, clarified the dose-response question in 1768 (advice that has stood the test of time). In 1820 the French chemists Pierre Pelletier and Joseph Caventou isolated the alkaloid quinine from cinchona bark. Purification of the various cinchona alkaloids allowed standardization of dosage. Adequate doses could now be given in relatively small amounts of pure drug, but by the middle of the nineteenth century enormous doses (up to 100-150 grains over two days) were being prescribed. Toxicity was common and the popularity of the medicine fell. Gradually, however, the diagnosis of agues and the prescription of Cinchona alkaloids became more rational and logical. The new colonial powers recognized the importance of Cinchona, and improved methods of horticulture resulted in better yields of the alkaloids from the cultivated trees. Ledger's identification of high yielding *Cinchona ledgeriana*, and the subsequent rejection by the British authorities of his offer of seeds gave the The Dutch an opportunity, which they gladly accepted. They then took the lead and established vast plantations of high yielding *Cinchona ledgeriana* in the East Indies (principally in Java).

Laveran, having identified haematozoa as the cause of paludism, later concluded that quinine cured the disease by killing the newly discovered parasites. This theory encountered considerable resistance in the years immediately following its publication. In 1880 Bacelli described the intravenous method of administering quinine (although there is evidence that this route had been used for 50 years before that). Laveran considered intravenous injection to be dangerous, giving rise to both local and general complications, and was only justified in 'the most grave and pernicious disease'. He also confirmed the earlier observations of Thomas Willis (1659) that cinchona cured the acute attacks of ague, but did not prevent relapses, and also appeared to have no effect on crescents (gametocytes of *P. falciparum*). The eminent Italian malariologists subsequently showed that quinine prevented asexual blood-stage development but could not stop sporulation of formed segmenters (meronts).

In 1856 William Henry Perkin discovered analine purple (mauve) whilst attempting to synthesize quinine from coal tar products. Thus began the synthetic dye industry. Later in Germany, the antimicrobial properties of those newly discovered aniline dyes were investigated. In 1890, Ehrlich showed that methylene blue had antimalarial activity against *P. cathemerium* in canaries, but the dye proved disappointing in clinical practice, and structural modifications did not lead to compounds with improved activity.

During the Great War (1914-1918) whole armies were immobilized in the Balkans because of malaria, and there were heavy losses in Mesopotamia, East Africa and the Jordan Valley. The British and French armies used quinine extensively, and despite frequent objections to the bitter medicament many lives were saved. The military and strategic importance of antimalarial drugs stimulated much research immediately after the war. In the early 1920s the resurgent German chemical industry again focused its attention on new antimicrobial compounds. The first synthetic antimalarial was

discovered in 1926. This was an aminoquinoline compound, pamaquine, also known as plasmoquine or plasmochin, a precursor of the 8-aminoquinoline primaquine. Pamaquine was followed by the acridine compound mepacrine (quinacrine) in 1932, and the structurally related 4-aminoquinoline, chloroquine, in 1934. Initially chloroquine was rejected as being too toxic for human use, and the research team at Bayer was asked to produce a safer compound. They then produced 3-methylchloroquine (Sontoquine) but, despite clinical studies, these compounds were generally unavailable at the outbreak of the Second World War.

Malaria research has often been tied to warfare. Armies fighting in tropical theatres of war usually lose more men to malaria than bullets. At the outset of the Second World War, the Allies knew their position was precarious in the tropics as most of the world's Cinchona was grown in Java, and this was vulnerable to Japanese invasion. They embarked upon a tremendous combined research effort into the development and evaluation of new antimalarials. In the event Java did fall to the Japanese, but widespread use of mepacrine (quinacrine) prophylaxis by the Allied soldiers proved highly effective (albeit somewhat toxic) and probably saved the day. Information on chloroquine was, in fact, available to the Allied Powers through pre-war reciprocal arrangements between the pharmaceutical companies, Bayer and Winthrop, but lay buried in documents until the defection of two French soldiers in North Africa in 1943. They brought with them a German antimalarial, later identified as Sontoquine. However, chloroquine was not fully evaluated until the end of the war. An entirely separate line of research in the UK led to the discovery in 1945 of the antimalarial biguanides, proguanil and subsequently chlorproguanil. These compounds were later shown to inhibit the plasmodial enzyme dihydrofolate reductase (DHFR). Researchers at the Wellcome Research Laboratories synthesizing purine analogues developed the antimitotic compound 6-mercaptopurine

(and later azathioprine) and in 1952 discovered the antiprotozoal DHFR inhibitor pyrimethamine. This same line of Nobel Prize winning research later developed trimethoprim, which has considerably greater affinity for bacterial DHFR (but also inhibits the plasmodial enzyme), and also allopurinol, acyclovir and zidovudine (AZT).

By the early 1950s the 4-aminoquinolines, chloroquine, and to a much lesser extent amodiaquine, had become the treatment of choice for all malaria throughout the world. Pyrimethamine was used in treatment, and chloroquine, pyrimethamine and proguanil were used for prophylaxis. Primaquine was given to prevent relapses of *P. vivax* and *P. ovale*. The Cinchona alkaloids were little used outside francophone Africa, and with the discontinuation of quinine, blackwater fever became a rarity. This was the heyday of the malaria eradication era, and with the tremendous successes in Europe and many urban areas of the tropics, interest in the development of new antimalarial drugs waned rapidly. But eradication in the tropics failed, and in the 1960s antimalarial drug resistance emerged as a major threat (NJ White, 2003).

Although the cause of the disease, the role of the female Anopheles mosquito in spreading it, and the use of quinine are well known, malaria still defies all efforts to control it in the 20th century as it did in the nineteenth. As late as 1951 the World Health Organisation estimated that 64% of the World's population was living in malaria-infested territories. The major outbreaks in our century were in USSR in 1923, with 5 million patients and 60,000 fatalities; in Ceylon in 1934/1935 with 3 million patients and more than 80,000 fatal cases, and in Ethiopia (1958) with 3 million patients and 150,000 deaths (R Fontaine, *et al.*, 1961). It is estimated that today there are still 500-700 million people affected by this disease and that 1-2 million of them die every year.

## 1.1.2 History of malaria in Vietnam

*If you go to Phu Qui can you come back?*

*This is the green tomb where the wife is mourning for her husband*

Malaria in Vietnam has been associated with water and forest. An early expression for fever-associated malaria was *"nga nuoc"* translated literally as "water falling" disease and *"sot ret rung"*: "cold forest fever". The early concepts of health in Vietnam inform us about associations of knowledge relating to the forest and particular types of water: the term *"rung thieng, nuoc doc"* (spiritual forest, poisoned water). Inhabitants of the plains expressed in these terms their conviction that the hinterland was unwholesome place. Physicians accompanying the French navy during 1875 occupation of the Red River Delta quickly established that soldiers in the uplands were highly susceptible to fever. At the end of the colonial period the "forest spirits" and "poisoned water" was more adequately explained by Pierre Gourou in 1940: "the only propagating agent of malaria is the mosquito; nor the forest spirits nor the poisoned water feared by Vietnamese have the least responsibility. The sunlit terraced rice fields with running water of hill regions are veritable breeding sites for the larvae of dangerous anopheles, in contrast with the stagnant paddies of the plains". The geographical spread of the disease is evident in Simond's map of malaria, which was published in 1907.

Malaria control for civilians was first applied in the context of major projects of public works; but it should be emphasized that the policies were based on economic and not humanitarian calculations. This means they were based on the costs of malaria in terms of the lives and health and loss of productivity of certain categories of people. This is reflected clearly in the railroad project in the Northern provinces and the rubber plantation in the southern provinces. The most ambitious one was the French programme to build a railroad from Hai Phong (Viet Nam) to Yunnan (China). As the work progressed in 1904,

a French medical officer observed that the workers were suffering excessive morbidity with a very high mortality. The difficulty of finding labourers for this project which relied on forced recruitment, led to the recommendation of measures for their protection. In addition to the distribution of quinine, other measures of basic hygiene, such as sanitary house construction and the availability of warm drinks were implemented, but they had little effect on the epidemic. In the early days of the "rubber rush" after its introduction to Indochina in 1910, malaria took a heavy toll among plantation labourers. By the 1920's the high cost of a plantation worker's recruitment, transportation, and maintenance made action against the disease economically essential (A Hardy, 2000). Malaria became immediately and intimately associated with war; the geography of malaria played a crucial role in the conduct of the war of independence. Central to the success of the resistance forces was their ability to survive in an environment of high malaria endemicity.

After 1954 both North and South Vietnam adopted scientific strategies of malaria eradication. In the North, imported insecticides and expertise acquired during the war and from the Soviet Union formed the basis of an extensive and rather impressive programme. In the South, campaigns funded by the USA and the WHO met with less success.

Wars have many times led to the development of new technology but also of new medicaments. The antimalarial drugs are typical examples. Chloroquine resulted from the Second World War. Mefloquine resulted from the Vietnam War at the side of the Americans. Artemisnin also results from the Vietnamese war. When this war was raging in the sixties many Vietnamese soldiers died from malaria, caused by parasites resistant to the currently used medicaments. Ho Chi Minh, the Vietnamese leader, asked for help from his Chinese neighbour. Mao Tse Tung offered very generously his help and. "Project 523 — a secret military outfit that was set up in 1967 to help Vietnamese to deal

with malaria". The biggest enemy for both the Americans and the Vietnameses was malaria. Project 523 was set up to help the Vietnamese military defeat malaria," said Professor Li, who joined 523 at its inception. He spent the next seven years trying to find a cure. Then, on a trip to the mountainous Yunnan province in 1974, he came across a plant called qinghao — which was first mentioned as a cure for malaria in Chinese medicine books dating from 340 BC. The plant extract is called artemisinin, and Professor Li was astonished to see that it could even cure patients with advanced cases of complicated malaria, such as the cerebral version, which are usually fatal. The Vietnamese used the compound for their troops in the final stages of the Vietnam War (Anonymity, 2004) (Li GQ personal communication).

Peace returned in 1975, but - for malaria control - peace did not herald an end to difficulties. Malaria became an important factor of the country's post-war development. It was only during the 1990's that economic growth and support from international agencies ensured a greater availability of resources for malaria control. In addition, the use of artemisinin and its derivatives, which also grows in northern regions of Vietnam, has dramatically reduced the mortality and the morbidity rates of malaria.

### 1.1.3 Current malaria situation on the world

There are 300 to 500 million clinical cases of malaria every year and between one to two million deaths, mostly of children, are attributable to malaria on the world. Although the last century witnessed many successful programmes at country level to eliminate malaria, the world is now facing a rapidly increasing disease burden. This has been attributed to several causes, including population movements into malarious region, changing agricultural practices such as irrigation projects, building damps, weakening of public health systems and speculatively, long-terms climate changes as global warming and El

Nino phenomenon. But of greater importance has been the development of resistance to drugs and insecticides with a rapidly growing population in areas with high malaria transmission, it has been estimated that in the absence of effective measures the number of malaria cases will double over the next 20 years.

Figure 1.1: Malaria situation in the world (Source: WHO 2002)
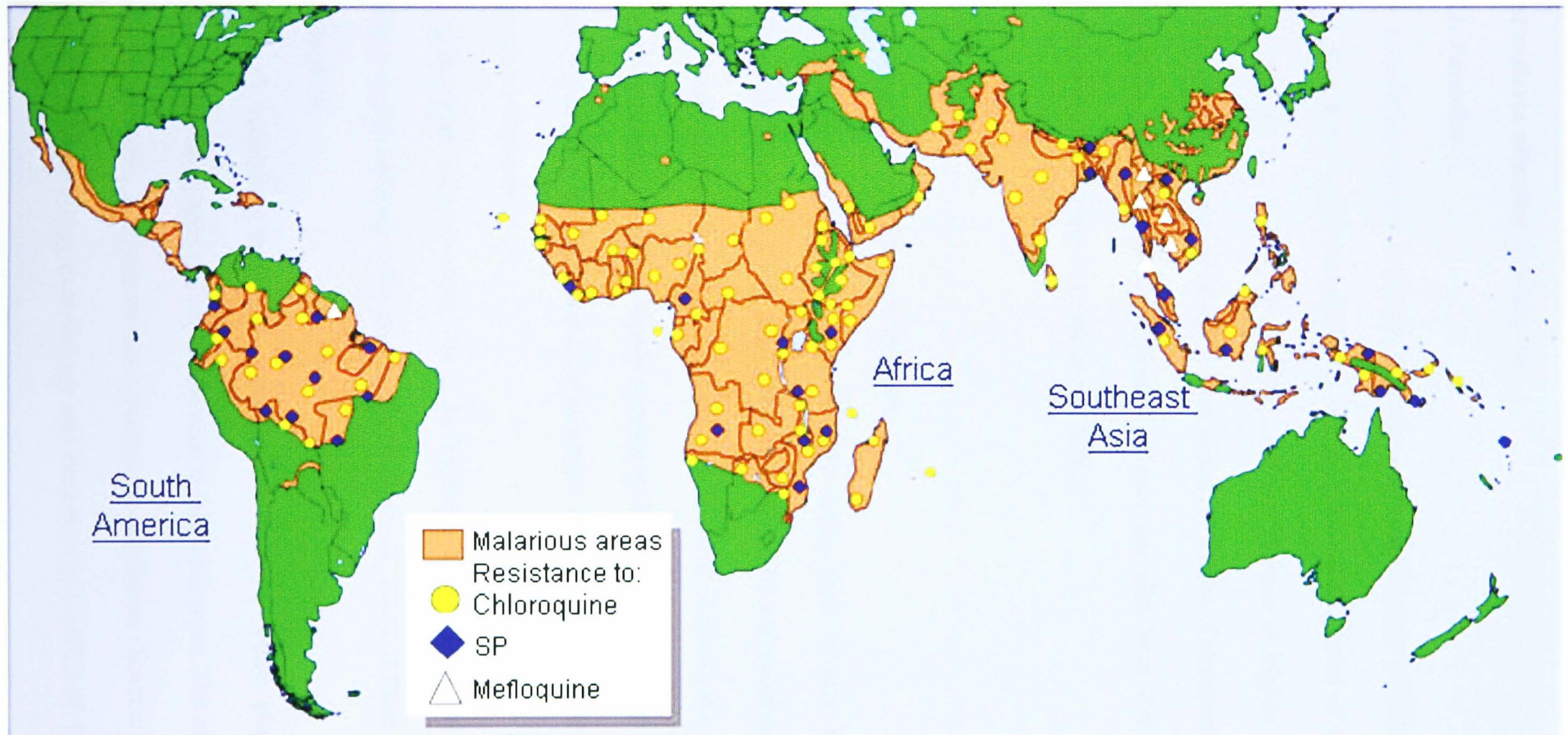
### 1.1.4 Current malaria situation in Vietnam

#### *1.1.4.1. Parasites*

*P.falciparum* is currently the predominant cause of malaria in Vietnam (72%) but *P.vivax* is dominant in Red River and Mekong River deltas. Whereas the level of chloroquine resistance of *P. falciparum* is variable in the North the parasite is highly resistant to chloroquine, and pyrimethamine in southern and central Vietnam. Decreased sensitivity of quinine has been reported and mefloquine resistance has also been detected in the south part of the country (see later chapters in this thesis)

#### *1.1.4.2. Vectors*

There are 3 important malaria vectors in Vietnam:

*A. minimus*: found only in mountainous and hilly areas; their density is relatively independent of rainfall. They are partly zoophilic, partly exophilic and exophagic

*A. dirus*: a highly effective vector, by Asian standards, is associated with the forest; their density is related to rainfall. They are highly anthropophilic, exophilic and exophagic. A. dirus is jointly responsible for the intense transmission in forested areas, particularly in central and southern Vietnam.

*A.sundaicus*: a brackish water breeder, found in Vietnam, largely confined to stagnant water collections in the Mekong delta and on the south coast, south of Phan Thiet. They are mainly endophilic.

There are about 7 secondary vectors: *A. jeyporiensis* in some rubber plantations; *A. subpictus* and *A. vagus* have been considered as vectors in plains areas. The other ones are *A. macculatus, A. aconitus, A. hyrcanus var. sinensis, A.indefinitus* (Source: unpublishes data from Institute of Malariology Entomology and Parasitology [IMPE] of Ho Chi Minh City Vietnam.)

### *1.1.4.3. Epidemiological patterns:*(Source: NMCP of Vietnam)

Transmission is geographically and temporally variable. In the South and the Central malaria transmission tends to be more perennial than in the North and rainfall influences its intensity. On basis of the entomological, ecological and social characteristics it is possible to classify the malaria patterns region by region

+ Hills and mountains of northern provinces:

In lower hill areas *A. minimus* is the main vector where transmission is of low intensity. Malaria risk may be associated with sleeping in huts.

In higher mountains inhabited by different ethnic groups: malaria is stable with significant immunity developing. Migrants have been affected with outbreaks. *A.dirus* is the main vector in these areas

+ Red River and other plains

Malaria is rarely transmitted in this area. Occasionally, *A.minimus* causes minor outbreaks. Above 11°N *A.subpictus* and *A.aconitus* breed in brackish water being associated with minor *P.vivax* outbreaks.

+ The Central and southern highlands:

That is a region with remaining dense, primary forest inhabited by ethnic minority groups who are perennially exposed to intense malaria transmission.

Transmission by *A.minimus* and *A. dirus* is more intense and perennial with peaks occurring at the beginning and at the end of the rainy season.

+ The Mekong delta:

Malaria occurs in foci along the river banks and estuaries with *P.vivax* predominating; and transmitted by *A. vagus*

### *1.1.4.4. Morbidity & mortality*

A malaria eradication programme was implemented in 1958 in North Viet Nam. The smear positive rate in samples of 10% of the population was 5.6%. This had been reduced to 0.28 % in 1964. During the war (1965-1975) the malaria situation fluctuated because of difficulties in the programme but was still maintained below 1%.

In the South after the reunification of the country in 1975 the malaria eradication programme became nationwide. Several surveys which were conducted by the National Programme for Malaria Eradication in different sites by taking blood smears from one million people (about 10% of inhabitants in the malaria region) from the end of 1975 to 1996 revealed a positive rate of 11.7% in 1976 and 2.9% in 1980. But since 1981 malaria was undergoing resurgence. The main contributing factors were the movement of population, serious lack of resources, degradation of the health service network, emergence of drug-resistant strains of *P.falciparum* and the nadir was in 1991 with 4646 fatal cases reported, the highest figure ever recorded.

Malaria situation in Vietnam is presented in Table 1.1.1 and Figure 1.2 compared with the situation in Mekong Sub Region (Figure 1.3)

Table 1.1.1: Malaria situation in Vietnam 1981-2000 (Source IMPE of Ho Chi Minh City)

| Year | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cases x $10^6$ | 654 | 527 | 830 | 875 | 958 | 1,417 | 1,265 | 1,300 | 952 | 873 |
| *P.falciparum*(%) | 67.8 | 68.6 | 65.2 | 61.2 | 60.0 | 47.2 | 49.1 | 59.3 | 74.0 | 73.1 |
| Severe cases | 2,882 | 3,950 | 4,306 | 4,199 | 5,381 | 7,056 | 8,811 | 10,470 | 13,709 | 11,613 |
| Deaths | 1,152 | 1,360 | 1,659 | 1,256 | 1,413 | 1,838 | 2,133 | 2,465 | 3,434 | 2,911 |

| Year | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 2000 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cases x $10^6$ | 1,059 | 1,270 | 1,066 | 861 | 666 | 541 | 455 | 383 | 341 | 268 |
| *P.falciparum* (%) | 76.9 | 76.3 | 71.3 | 71.1 | 71.8 | 72.8 | 73.9 | 76.2 | 77.6 | 77.5 |
| Severe cases | 31,741 | 26,553 | 14,308 | 8,076 | 4222 | 2146 | 1530 | 1447 | 1516 | 1075 |
| Deaths | 4,646 | 2,658 | 1,054 | 604 | 348 | 198 | 152 | 183 | 190 | 139 |

Figure 1.2: Malaria situation in Vietnam 1958- 1999 (Source: NMCP of Vietnam)
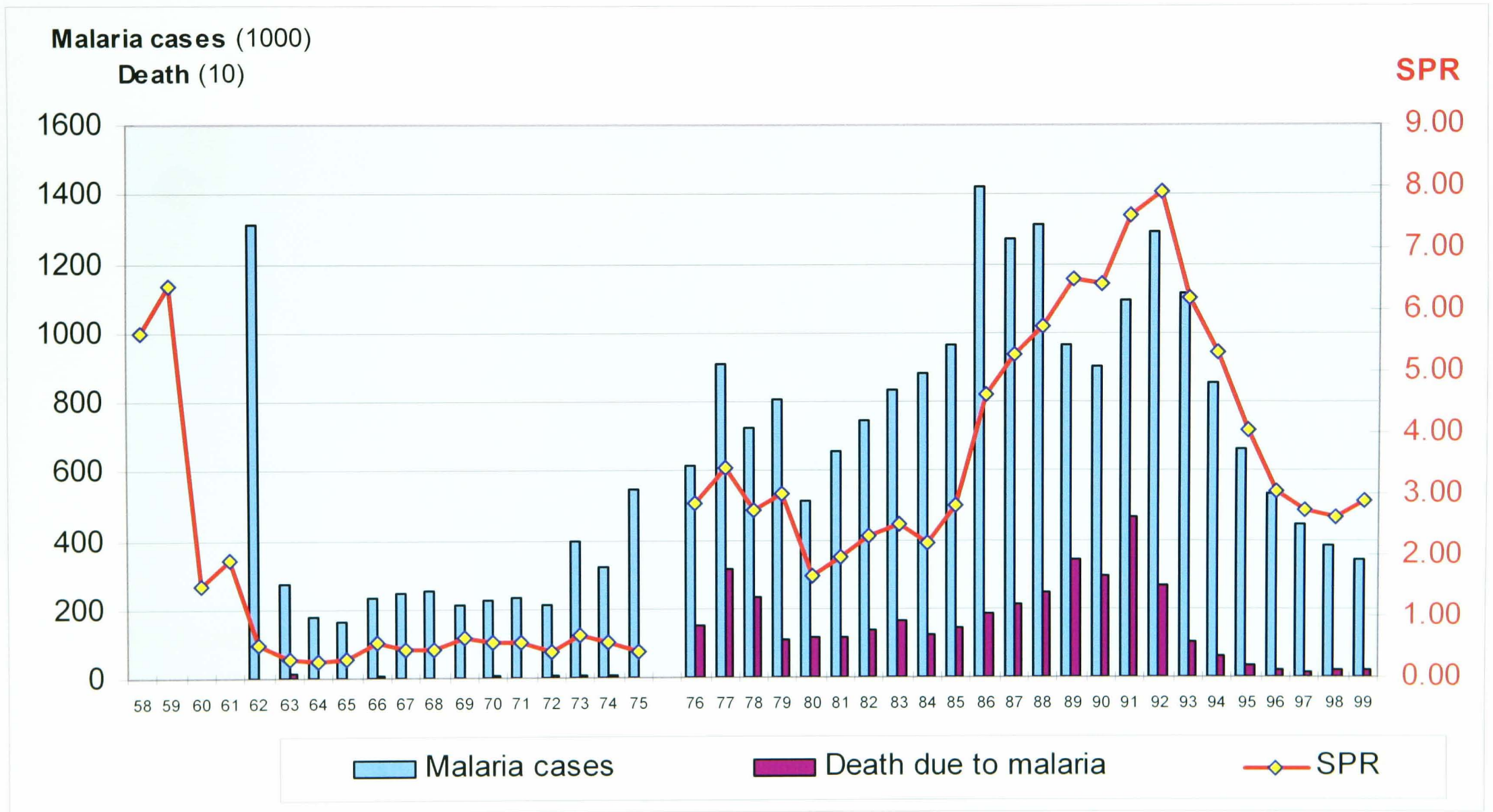
Figure 1.3: Malaria confirmed cases in the Mekong Sub-region 2000
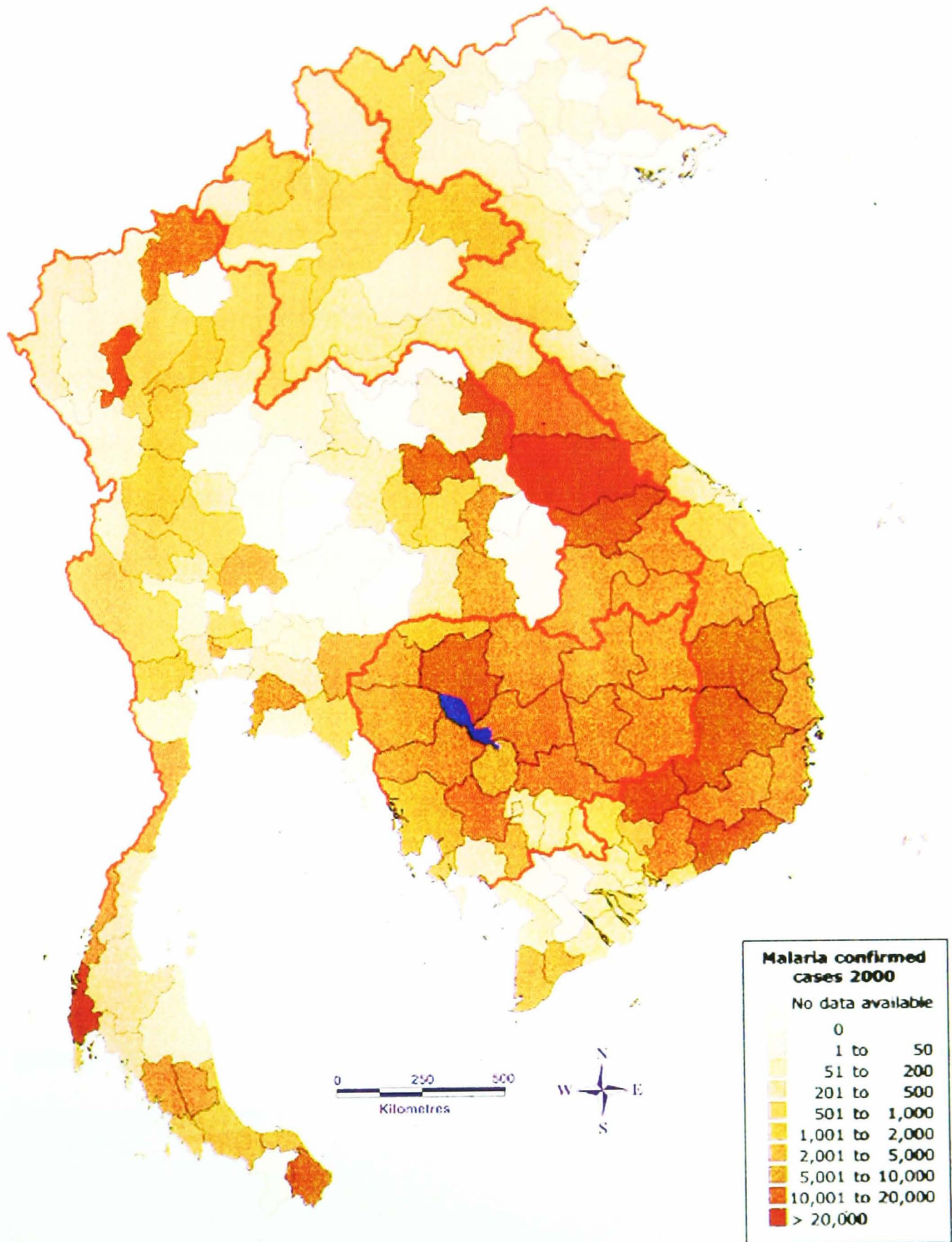


Source: Mekong Malaria Forum 2000

### *1.1.4.5. Anti-malarial drug use*

Viet Nam is a microcosm of the global situation. Resistance rates in vitro to chloroquine, quinine, and mefloquine are 92% (61/66), 18% (11/61) and 6% (4/62) respectively and a recent clinical trial showed that failure rates with mefloquine (15 mg/kg single dose) monotherapy or artemisinin (20 mg/kg x 5 days) monotherapy of up to 60% (TT Hien, *et al.*, 1997).

### *1.1.4.6. National malaria control programme*

A National Committee chaired by a Vice Minister of Health directs the National Malaria Control Programme (NMCP). The Institute of Malariology-Parasitology-Entomology (IMPE) which includes a headquarter in Hanoi and two regional IMPEs - one in the central area (Quy Nhon) and another in the south area (Ho Chi Minh City) is responsible for the day-to-day management of the programme. The IMPE is responsible for technical advice, research, training and data analysis, and assists the peripheral level staff in operations when necessary. In provinces where malaria is not a major concern, the malaria control stations are integrated with the Preventive Medicine Centres; but in provinces where the disease is a major health threat, malaria stations are split as independent institutions under the provincial health service. At the district level, the malarial control programme is conducted by specialised teams integrated into the district medical centre, which provides other medical services such as treatment (district hospital) hygiene, epidemiology.

## 1.2 Malaria parasite

There are four species of parasites causing malaria in humans:

*Plasmodium malariae* (Laveran 1881)

*Plasmodium vivax* (Grassi and Feletti, 1890)

*Plasmodium falciparum* (Welch, 1897)

*Plasmodium ovale* (Stephens, 1922)

*P. falciparum* and *P.vivax* are the main cause of morbidity and mortality of malaria in human being.

The life cycle of all species of human malaria is essentially similar. It includes a sexual phase in the human host with transmission and multiplication in Anopheles mosquitoes (sporogony), of which about 60 are important malaria vectors in nature, and an asexual phase (schizogony) with multiplication in man. The latter phase comprises the phase occurs in the liver parenchyma cells (exo-erythrocytic schizogony) and the development cycle in the red blood cells (erythrocytic schizogony).

### 1.2.1. Parasite life cycle

#### *1.2.1.1 Exo-erythrocytic schizogony*

Following the inoculation of sporozoites by the female mosquitoes into the subcutaneous tissue and less-frequently directly into the bloodstream, many of them are destroyed by phagocytes but some travel to the liver. Evidence indicates that sporozoites pass through several hepatocytes before invasion (M Mota, *et al.*, 2001) median of 8 to 10 sporozoites successfully invade. The thrombospondin domains on the circumsporozoite protein (CSP) and on thrombospondin-related protein (TRAP) are the co-receptors on sporozoites that mediate the invasion. These domains bind specifically to heparan sulphate proteoglycan, a hepatocyte surface molecule in the region in apposition to sinusoidal endothelium and

Kuppfer cells. Each sporozoite develops subsequently into tens of thousands of merozoites, which can each invades and red blood cell on release from the hepatocytes (U Frevert & A Crisanti, 1998).

It was discovered in 1980 that the sporozoites of the relapsing species of human malaria parasites, *P.vivax, P.ovale*, differentiate either into tissue schizonts or into hypnozoites in varying proportions, depending on the strain. The hypnozoites remains dormant in the liver parenchymal cell as uni-nucleated forms, at a predetermined time those hypnozoites begin to grow and undergo exo-erythrocytic shizogony forming a wave of merozoites that are released and then invade the RBCs (W Krotoski, *et al.*, 1982).

### 1.2.1.2 Erythrocytic schizogony

All clinical and pathogenic manifestations associated with malaria infection are caused by the asexual phase of Plasmodium life cycle. Thousands of merozoites are released from mature schizont in the hepatocytes of the liver. These forms of the malaria parasites invade the RBCs where they develop into trophozoites and after full development then begin to multiply within the cells, forming multi-nucleated blood-stage schizonts. These infected RBCs then ruptures, releasing newly formed merozoites into the bloodstream that can invade new RBCs. Parasite ligands binding to specific receptors on the RBC surface is an integral part of the invasion process. The invasion-related antigens of the parasite are located in the apical organelles; namely, rhoptries, micronemes and dense granules. These apical organelles play an important role in the entry of the merozoite into host cell.

The description of the sequence of the invasion of parasites comes from 1975 video recording of merozoites invading RBCs. The first phase of the invasion involves the identification of a potential host cell and the apical attachment: initial attachment of a

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merozoite to an RBC, which can occur at any part of the parasite surface; reorientation of the merozoite so that its apical pole is directed toward the RBC, a process that appears to be facilitated by the adhesive fibrils of the surface coat; tight junction formation between merozoite and RBC. The second phase of the invasion involves a number of molecular events that allow the merozoite to gain physical entry into the RBC and become encapsulated inside a vacuole surrounded by a lipid membrane. Events that occur include (i) movement of the junctional adhesion zone around the merozoite toward its posterior pole, with concomitant entry of the merozoite; (ii) release of the contents of the rhoptries; (iii) localised alteration of the RBC membrane architecture; (iv) invagination of the RBC membrane with the creation of the parasitophorous membrane and a parasitophorous vacuole (PV) and (v) closure and sealing of the RBC and PV membrane. The final phase includes simultaneous closure of the RBC membrane and the PV membrane, which now envelops the merozoite; complete internalisation of the merozoite followed by the extrusion of dense bodies and the parasite's transformation into a young trophozoit

The interaction observed between the merozoite and the RBC during invasion suggests that the contacts are molecularly mediated between specific receptors in the RBC membrane and ligands of the merozoite. Receptors that mediate invasion of RBCs by merozoites are found in micronemes, on the cell surface, and in rhoptries. The location of these receptors within organelles may protect the parasite from antibody-mediated neutralization, as release from apical organelles after contact with RBC may limit their exposure to antibody (JW Barnwell, *et al.*, 1989).

*P.falciparum* and *P. vivax* develop over 48 hours in RBCs, producing about 20 merozoites per mature parasite with each merozoite able to invade other RBCs. A few merozoites, invading the RBCs then differentiate into sexual stage parasites, male and female gametocytes. *P.vivax* develops into gametocytes soon after the release of

merozoites from the hepatocytes; *P.falciparum* gametocytes develop much later. The early treatment of clinical attacks of malaria by anti blood stage drugs for *P.falciparum* also kills the developing gametocytes; but *P.vivax* may transmit before the onset of the clinical stage of the disease. When taken up in the blood meal by feeding female anopheline mosquitoes, gamete formation, fertilisation, and sporozoite development occur and the cycle from mosquito to a new host begins again.

## 1.2.2. Pathophysiology of malaria

The disease malaria results from the blood stage of infection with asexual stages of the parasite; the hepatic stages and sexual stages (gametocytes) do not cause organ dysfunction. Pathophysiological processes in malaria result from the destruction of RBCs, the liberation of parasite and erythrocyte material into the bloodstream, and the host reaction to these events. *P.falciparum* infected RBCs also sequester in the capillaries and venules of vital organs, interfering with microcirculation and host tissues metabolism.

### *1.2.2.1 Parasite multiplication:*

When the hepatic schizonts rupture, they release approximately 105 to 106 merozoites into the circulation. On average, parasites are detectable in the blood by microscopy (20-50 parasites/ µL of blood) on the 11th day after sporozoite inoculation (SF Kitchen, 1949). At this stage the host may still feel well or may complain of vague symptoms such as malaise, headache, myalgia or anorexia. The fever usually begins two days later. At the onset of fever, the adult patient has approximately $10^8$ parasites in the body, which corresponds to 20-200 parasites in each µL of blood. In endemic areas, persons with immunity may tolerate parasitaemias up to 10,000/µL of blood without feeling ill (N White, 2003). The variation of peripheral parasite count resembles a sine wave pattern

with considerable amplitude in synchronous *P.falciparum* infections. In most cases, the parasite multiplication terminates abruptly to limit the infection at a parasitaemia of $10^4$ to $10^5$ /µL. Only *P.falciparum* has the capacity for untrammeled multiplication, and parasite counts may exceed 50% in some cases. Several factors limit the parasite multiplication. The host mobilizes non-specific then specific immune defenses. The parasites are also damaged by high fever, and this could explain the brake on parasite expansion (D Kwiatkowski & M Nowak, 1990). The untreated infection increases exponentially then the rate of expansion decelerates rapidly, parasitaemia fluctuates, then declines and continues for several weeks or months at low levels before finally being eliminated. Although natural infections often contain 2 or more genetically different parasite strains, development of the parasite population tends to be synchronous from the onset. The malaria paroxysm (fever and rigors) is associated with the rupture of blood schizonts. In *P.falciparum* infection, there is usually at least one minor brood or subpopulation cycling 24 hours out of phase with the major brood (GQ Li, *et al.*, 1982a).

### 1.2.2.2 Sequestration

The essential pathological feature of severe falciparum malaria is sequestration of erythrocytes containing mature forms of the parasite in the deep vascular beds of vital organs. Sequestration is not uniformly distributed among the vital organs; it is usually greatest in the brain which may explain why cerebral malaria is such a prominent feature of severe falciparum malaria in man, and least in the skin (GG MacPherson, *et al.*, 1985, E Pongponratn, *et al.*, 1991). Sequestration is also not uniformly distributed at a microvascular level. Some vessels are completely packed with parasitized RBCs, while adjacent vessels are clear (K Silamut, *et al.*, 1999). This may cause more gradually reduction in blood flow perhaps with less hypoxia because of preserved flow in adjacent

capillaries. Two principal mechanisms have been proposed to account for such obstruction, but in both cases the result is capillaries congested with red cells containing parasites.

### *Decreased deformability*

Normal RBCs must undergo considerable deformation in order to traverse the capillary. As the parasite grows inside the red cell, it becomes more rigid and the surface becomes more irregular and covered in small protrusions (HA Cranston, *et al.*, 1984). Several factors are likely to account for reduced deformability of parasitized erythrocytes, notably changes in the cytoskeleton, increased membrane stiffness, increased cytoplasmic viscosity resulting from changes in membrane permeability, reduced surface area to volume ratio (increased sphericity), and principally the rigidity of the parasite itself.

### *Cytoadherence.*

Pathological studies show an intimate apposition of endothelial cell and infected erythrocyte membranes. Cytoadherence, the binding of mature RBCs to endothelial cells in post- capillary venules, is believed to be a key virulence factor. The precise molecular mechanisms underlying this interaction have recently been characterized. Cytoadherence is mediated by receptors on endothelial cells, molecules expressed on the surface of infected RBCs. Uninfected erythrocytes will also bind to the surface of RBCs containing mature forms of the parasite, by a mechanism similar to that of cytoadherence to endothelial cells, causing "rosetting".

Parasites sequester themselves in various organs such as brain, heart, lung, liver, kidney, subcutaneous tissue and placenta. The endothelial cells in those organs express different and various amounts of host receptors. The adhesion phenotype is not homogenous,

different parasites can bind to variable numbers of host receptors. The variability is thought to affect the tissue distribution and pathogenesis of parasites. Molecules on the surface of endothelial cells which bind *P. falciparum* infected RBCs include thrombospondin, CD 36, intercellular adhesive molecule 1 (ICAM 1), vascular adhesive molecules 1 (VCAM 1), E selectin, chondroitine sulfate A (CSA), hyaluronic acid (HA). *P.falciparum* derived molecules on the RBC surface which mediate adherence include Pf EMP 1, sequestrin, modified RBC band 3, rosettins, Pf 332 (N White, 2003) Cytoadherence results in sequestration of infected RBCs in the deep vasculature of organs, and therefore it localizes parasites in sites of low oxygen tension which are ideal for their growth It also prevents infected RBCs being destroyed in the spleen and it is thought to be the cause of organ dysfunction through microcirculation obstruction, endothelial cell activation and damage, cytokine release.

### *Rosetting.*

Rosetting is ability of infected RBCs to adhere to uninfected RBCs. This phenomenon occurs in RBCs containing mature stage of parasite. Rosetting takes place only on the venous side of the microcirculation. The adhesive capacity varies between different *P.falciparum* isolates (J Carlson, *et al.*, 1994). Rosetting is mediated by PfEMP1 and rosettins and involves the complement receptor, heparan sulphate, blood group A antigen, and probably other red cell surface molecule (A Rowe, *et al.*, 1994, A Rowe, *et al.*, 1995). Parasites with broad adhesive capacities might be the ones that are involved in excessive binding and blockade of the microcirculation and the induction of severe malaria. Rosetting has been associated with severe malaria in some studies but not in others (A Rowe, *et al.*, 1995, PA Zimmerman, *et al.*, 2003)

### *1.2.2.3 Cytokines*

For many years, it was thought that parasites secreted toxin(s) at schizont rupture and cause the malaria symptoms. No toxin has been identified but malaria parasites do induce the release of cytokines. Tumor necrosis factor (TNF–$\alpha$), interleukin (IL)-1 and gamma interferon ($\gamma$-IFN) are produced and these induce the release of a cascade of "pro-inflammatory" cytokines including IL-8, IL-12, IL-18. These are balanced by production of the "anti-inflammatory" cytokines IL-6, IL-10 (M Ho, *et al.*, 1998, P Kern, *et al.*, 1989). Plasma concentration of the cytokines are elevated in both vivax and falciparum malaria (GE Grau, *et al.*, 1989, ND Karunaweera, *et al.*, 1992). Acute malaria is associated with high levels of most cytokines, but the balance differs in relation to severity. Il-12 and TGF-$\beta$ 1 (transforming growth factor), which may regulate the balance between pro- and anti- inflammatory cytokines, are higher in uncomplicated than in severe malaria (DJ Perkins, *et al.*, 2000)

Cytokines are responsible for many of the symptoms and signs of malaria particularly fever and malaise. There is no direct evidence that systemic release of TNF or other cytokines causes coma in human. In a recent study on severe malaria in adults, elevated plasma TNF concentrations were associated specifically with acute renal failure and TNF levels were actually lower in patients with pure cerebral malaria than those of other complications (NP Day, *et al.*, 1999). Cytokine are probably involved in placental function, suppression of erythropoiesis and inhibition of gluconeogenesis. Cytokines also up-regulate the endothelial expression of vascular ligands for *P.falciparum* infected red blood cells, notably ICAM-1 and thus promote cytoadherence.

## *1.2.2.4 Cerebral malaria*

Cerebral malaria, a diffuse reversible encephalopathy, is the major lethal complication of the infection. The sequestration of RBCs containing mature forms of *P.falciparum* in the cerebral microvasculature is considered the essential underlying pathological process, although how this cause coma and death remains unanswered. Various theories have been proposed and tested.

Sequestration results from the adherence of parasitized RBCs to vascular endothelium. In vitro studies suggest that infected RBCs begin to cytoadhere at the late ring or early trophoizoite stage of the parasite's cycle. Studies *in vivo* and histopathological observations in fatal *P.falciparum* malaria cases suggest that the RBCs containing more mature parasites are sequestered in the brain and other organs. Post- mortem studies have given conflicting results on the predominant stage of parasite development encountered in the brain. Lemercier et al reported almost exclusively schizonts (G Lemercier, *et al.*, 1966) whereas MacPherson found that both trophozoites and schizonts predominate (GG MacPherson, *et al.*, 1985). Microvascular sequestration was assessed in the brains of 50 Vietnamese and Thai patients who died from severe malaria; the results have shown that within the same brain different vessels had discrete but different populations of parasites indicating that the adhesion characteristics of cerebrovascular endothelium change asynchronously during malaria, and that significant re-circulation of parasitized RBCs is unlikely. Furthermore there were significantly more ring form parasites in the cerebral microcirculation than expected (nine fold)(K Silamut, *et al.*, 1999). The possible role of local mechanical factors causing microvascular obstruction (resulted from parasitized RBC sequestration) and resulting in hypoxia and ischemia can only be inferred and not proved with neuropathological and immunohistochemical or ultrastructural examination (E Pongponratn, *et al.*, 2003). There is no evidence of widespread hypoxic-ischemic

patterns of brain damage. The increased cerebral lactate production reflected in increased jugular venous and cerebrospinal fluid (CSF) concentrations of lactate indicates significant anoxic metabolism, but it is not clear whether these changes in themselves provide sufficient explanation for coma (NJ White & M Ho, 1992).

The role of cerebral oedema in the pathogenesis of coma has been debated for decades. Although this pathological feature has been seen in some post-mortem investigations, it was not common in a MRI study on adults with cerebral malaria in South East Asia. This is consistent with the observations in recent studies on the structural integrity of the blood-brain- barrier and ultrastructure of the brain in fatal cases of *P. falciparum* malaria (H Brown, *et al.*, 1999, IM Medana, *et al.*, 2001, IM Medana, *et al.*, 2002a)

The relative good prognosis of cerebral malaria survivors differs from that following coma in encephalitis, trauma, or vascular diseases. This suggests that coma in cerebral malaria is a metabolic or anaesthetic encephalopathy. This obviously involves neurotransmitter abnormalities and potent inhibitors of neurotransmission could play a role. Recent studies of Kenyan children with cerebral malaria have proposed that the imbalances of excitatory mediators may be implicated in the initiation and maintenance of seizures and neuronal degeneration and might contribute to neurological symptoms in cerebral malaria (M Dobbie, *et al.*, 2000). An imbalance in the production of excitotoxic and neuroprotective tryptophan metabolites produced from the kynurenine (KYN) pathway has also been observed in a murine model of CM in which these pathological processes are prominent. In a retrospective study of 261 Vietnamese adult patients with severe malaria three metabolites, the excitotoxin quinolinic acid (QA); the protective receptor antagonist kynurenic acid (KA); and the proinflammatory mediator picolinic acid (PA) were measured and related to the incidence of neurologic complications, CSF lactate level and the disease outcome. The results have indicated that there was no

difference in the levels of KA between groups; no association between CSF QA concentrations and convulsion or depth of coma, and that the significant elevation of QA, PA levels and QA:KA ratio in patients with poor outcome was only a consequence of impaired renal function in this group (IM Medana, *et al.*, 2002b). In another post-mortem study on 54 Vietnamese patients who died from severe *P.falciparum* malaria the authors have found a marker of potentially reversible axonal damage, namely β-amyloid precursor protein (β-APP) normally transported along the axon, and which accumulates at the site of axonal injury, that is significantly associated with cerebral malaria in adults and that this distinguishes cerebral malaria from non-cerebral malaria. This marker highlights the internal capsule and pons as areas of primary involvement in axonal injuries. Unlike other pathological correlates such as neuronal stress markers, axonal injury does not seem to purely reflect the systemic contribution of severe malaria to the specific neurological syndrome of cerebral malaria (IM Medana, *et al.*, 2002a) and axonal injury may be an important step in understanding the mechanism of cerebral malaria.

### *1.2.2.5 Renal failure*

Renal failure is a common complication of *P. falciparum* infection in adults; approximately half of these patients have evidence of renal impairment (TT Trang, *et al.*, 1992). But renal failure is rare in children with complicated falciparum malaria in Africa and much less common in children than adults in South East Asia. The basic pathology is acute tubular necrosis. Renal impairment may be compounded by hypovolemia, severe aenemia and haemoglobinuria. The pathological processes that result in acute tubular necrosis have not been clarified. Cytoadherence of parasitized red cells in the glomerular capillaries is occasionally seen, but is not as pronounced as in other organs such as the brain (GG MacPherson, *et al.*, 1985). Studies of renal cortical blood flow (V Sitprija, *et*

*al.*, 1977) and radiological studies (angiography and contrast urography) (S Arthachinta, *et al.*, 1974) have shown a reduction in cortical perfusion during the acute stage of disease as in other forms of acute tubular necrosis. The oxygen consumption of the kidneys is reduced in acute renal failure and it is not improved by administration of dopamine which induces arteriolar vasodilatation and consequently increases the renal blood flow; therefore that suggests a fixed injury (NP Day, *et al.*, 2000) The role of local cytokine release and altered regulation of renal microvascular flow is uncertain because vivax malaria does not cause acute renal failure despite the massive cytokine release that occurs during paroxysms.

## 1.2.2.6 Hepatic dysfunction

Impairment of hepatic function is common in severe malaria. Jaundice is significantly more common in adults with severe malaria than in children. The measurable consequences of hepatic dysfunction are coagulation abnormalities resulting from failure to synthesize clotting factors (which may be complicated by the consumption of clotting factors), hypo-albuminaemia, and reduced metabolic clearance of many substances including alanine and lactate, and antimalarial drugs. Hepatic blood flow, measured by indocyanine green clearance, is reduced during acute malaria, and is significantly lower in severe malaria than in uncomplicated malaria. Indocyanine green clearance may reflect not only liver blood flow but also biliary excretion. Galactose clearance, an alternative measure of liver blood flow, and also glycerol clearance have been found to be relatively unperturbed by malaria (S Pukrittayakamee, *et al.*, 1992). These data suggest that sequestration and consequent microcirculatory obstruction in the portal and hepatic circulations, or portal venoconstriction as demonstrated in rhesus monkeys infected with *P. knowlesi* is unlikely to be the sole cause of liver dysfunction. Clearly, some metabolic

processes, such as gluconeogenesis, are more perturbed than others. Liver biopsy usually reveals Kupffer cell hyperplasia and mononuclear cell infiltration. Other studies have shown either no structural changes or slight hepatocyte swelling (S Sherlock, 1975). Centrizonal necrosis has been reported (YK Joshi, *et al.*, 1986). More discriminating measures of liver function are needed to define the importance of hepatic impairment in severe malaria. Recent study investigating the prevalence of hepatitis B virus infection among Vietnamese patients with severe falciparum malaria has revealed that the overall prevalence of HBsAg was higher than that in general catchment population for the study hospital and patients admitted with cerebral malaria had a greater risk of registering positive HbsAg to other manifestation of severe malaria (MJ Barcus, *et al.*, 2002).

### *1.2.2.7 Hypoglycaemia*

Hypoglycaemia is increasingly recognized as an important manifestation of falciparum malaria. There are several possible causes of hypoglycaemia and different patterns of associated biochemical abnormalities.

Quinine/quinidine-induced hyperinsulinaemia: quinine is a most potent in vitro stimulus to islet cell insulin secretion and has been shown to release insulin in vivo in healthy subjects and in patients with falciparum malaria, both in pregnant women with severe or relatively mild disease, and in adults and children with severe disease (TM Davis, *et al.*, 1993b, S Krishna, *et al.*, 1994, S Looareesuwan, *et al.*, 1985b, W Okitolonda, *et al.*, 1987, NJ White, *et al.*, 1983c). Increased glucose consumption: glucose consumption increases in fever and infection. Increased glucose turnover in acute falciparum malaria is caused by both increased glucose consumption of the host and the parasite (TM Davis, *et al.*, 1993a) although the host's requirements are considerably greater. Children with severe malaria have considerably increased glucose requirements compared with adults.

Glycogen depletion or impaired gluconeogenesis: hypoglycaemia in African children with severe malaria, and in adults who have not received previous quinine treatment, is associated with appropriately low plasma concentrations of insulin (TE Taylor, *et al.*, 1988). Hypoglycaemia is considered to result both from variable depletion in hepatic glycogen, an inhibition of hepatic gluconeogenesis, and a 2 - 3 fold increase in glucose turnover (TM Davis, *et al.*, 1993a).

### *1.2.2.8 Lactic acidosis.*

Lactic acidosis is a major cause of death from severe falciparum malaria (NJ White, *et al.*, 1983c),(S Krishna, *et al.*, 1994, TE Taylor, *et al.*, 1988, NJ White, *et al.*, 1987b). Metabolic acidosis is a consistent feature of severe malaria in adults and children (K Marsh, *et al.*, 1995) and in children this is mainly a lactic acidosis, whereas in adults renal failure and lactic acidosis both contribute. Lactate, measured either in venous or arterial blood, or cerebrospinal fluid is an accurate prognostic indicator in severe malaria (NJ White, *et al.*, 1985),(D Waller, *et al.*, 1995). Lactic acidosis results from several processes: the tissue anaerobic glycolysis due to microvascular obstruction, the production of lactate by parasites and the failure of hepatic and renal lactate clearance (NJ White & M Ho, 1992) (MA Pfaller, *et al.*, 1982, DL Vander Jagt, *et al.*, 1990)

### *1.2.2.9 Pulmonary oedema*

The heart function is well preserved in severe malaria despite intense sequestration in the myocardial vessels. Pulmonary oedema in severe malaria results from a sudden increase in pulmonary capillary permeability but the cause of this phenomenon is not known.(P Charoenpan, *et al.*, 1990, MF James, 1985)

## 1.2.3 The clinical aspect of malaria infection

The clinical outcome of malaria infection depends on many parasite, host, geographic and social factors. Those converge in the patient to result in a spectrum, from an asymptomatic infection to severe disease and death.

### *1.2.3.1 Asymptomatic infection*

Clinical aspects of malaria are markedly different in non-immune and semi-immune populations. The term immune is not used because natural infection does not produce true immunity against either reinfection or disease. In non-immune persons such as expatriate travelers, detectable parasistaemias produce significant symptoms, complications or death. In contrast, many infected semi-immune have no symptoms at all.

### *1.2.3.2 Non- complicated malaria*:

The first symptoms of malaria are not specific: loosing the sense of well-being, headache, fatigue, muscle and joint aches, abdominal discomfort, followed by fever, are similar to the symptoms of a minor viral infection. In some instances, a prominence of headache, chest pain, abdominal pain, arthralgia, myalgia, or diarrhea may suggest another diagnosis. Headache may be severe in malaria, but there is no neck stiffness, and neither are Kernig's or Brudzinski signs positive as in meningitis. Myalgia may be prominent as severe as in dengue fever, or as in leptospirosis. Nausea and vomiting are common. The classic malarial paroxysms, in which fever spikes, chills, and rigors occur at regular intervals, only develop after days or weeks of untreated infection. The fever is irregular at the onset of illness. The temperature of non-immune individuals and children often rises above 40°C in conjunction with tachycardia and sometimes delirium. Although childhood febrile convulsions may occur with any of the malarias, generalized seizures are

specifically associated with falciparum malaria and may herald the development of cerebral disease. Many clinical abnormalities have been described in acute malaria, but most patients with uncomplicated infections have few abnormal physical findings other than fever, mild anaemia, and a palpable spleen. Anaemia is quite common among young children living in high transmission. Mild jaundice and slight enlargement of the liver is also commonly seen in adults. Malaria is not usually associated with rash like those seen in patients with measles, viral exanthems or drug reactions; neither are there skin hemorrhages (petechia) as in meningococcal septicemia, or viral hemorrhagic fevers. Uncomplicated falciparum malaria carries a mortality rate of about 0.1%. However, once vital organ dysfunction occurs, mortality rises steeply (NJ White, 2003).

### *1.2.3.3 Cerebral Malaria*:

Cerebral malaria is the most common clinical presentation and cause of death in adult with severe malaria. It manifests as a diffuse symmetric encephalopathy; focal neurologic signs are unusual. Coma is a characteristic and ominous feature of falciparum malaria and, despite treatment, is associated with death rates above 20% (TT Hien, *et al.*, 1996, DA Warrell, 1992). The onset may be gradual or sudden following a convulsion. Lesser degrees of obtundation, delirium, and abnormal behavior may also occur. The eyes may be divergent and a pout reflex is common, but other primitive reflexes are usually absent. The tendon reflexes are variable, and the plantar reflexes may be flexor or extensor. Decortication (flexor) or decerebration (extensor) posturing is also documented. Muscle tone may be either increased or decreased. The corneal reflexes are preserved except in deep coma (NJ White). Approximately 15 - 22% of patients have retinal hemorrhages (S Looareesuwan, *et al.*, 1983, JF Schemann, *et al.*, 2002); with pupillary dilatation and indirect ophthalmoscopy, this figure increases to 30 to 40%. Other abnormalities include

discrete spots of retinal opacification (30 to 60%), papillooedema (8% of children, rare in adults), cotton wool spots (<5%), and decolourization of a retinal vessel or segment of vessel (occasional cases). Convulsions, usually generalized and often repeated, occur in up to 50% of children with cerebral malaria (AA Asindi, *et al.*, 1993). More covert seizure activity is common, particularly in children, and may manifest as repetitive tonic-clonic eye movements (J Crawley, *et al.*, 1996). While adults rarely suffer neurologic sequelae, about 10% of children surviving cerebral malaria particularly those with hypoglycaemia, severe anaemia, repeated seizures, and deep coma have some neurologic sequelae when they regain consciousness; hemiplegia, cerebral palsy, cortical blindness, deafness, and impaired cognition and learning all of varying duration have been reported (ME Molyneux, *et al.*, 1989) (DR Brewster, *et al.*, 1990) (FS Bondi, 1992).

### 1.2.3.4 Renal Impairment:

Renal impairment is common among adults with severe falciparum malaria but rare among children (ME Molyneux, 1990) (XT Cao, *et al.*, 1997a). Clinically and pathologically, this syndrome manifests as acute tubular necrosis; renal cortical necrosis never develops. Without renal replacement therapy the mortality rate may rise up to 75% (TT Trang, *et al.*, 1992). In survivors, urine flow resumes in a median of 4 days, and serum creatinine levels return to normal in a mean of 17 days. Peritoneal dialysis or haemofiltration considerably improves patient's survival.

### 1.2.3.5 Liver Dysfunction:

Mild haemolytic jaundice is common in malaria. Severe jaundice is associated with *P. falciparum* infections, is more common in adults than in children (XT Cao, *et al.*, 1997b). When accompanied by other vital organ dysfunction (often renal impairment), liver

dysfunction carries a poor prognosis. Hepatic dysfunction contributes to hypoglycaemia, lactic acidosis, and impaired drug metabolism.

### *1.2.3.6 Hypoglycaemia*:

An important and common complication of severe malaria, hypoglycaemia is associated with a poor prognosis (NJ White, *et al.*, 1983c) and is particularly problematic in children and pregnant women (S Looareesuwan, *et al.*, 1985b) (NJ White, *et al.*, 1987c) (TE Taylor, *et al.*, 1988). In severe disease, the clinical diagnosis of hypoglycaemia is difficult: the usual physical signs (sweating, gooseflesh, tachycardia) are absent, and the neurologic impairment caused by hypoglycaemia cannot be distinguished from that caused by malaria.

### *1.2.3.7 Lactic Acidosis*:

Lactic acidosis commonly coexists with hypoglycaemia in patients with malaria and that is an important contributor to death from severe malaria (NJ White, *et al.*, 1983c) (NJ White, *et al.*, 1985). In adults, coexisting renal impairment often compounds the acidosis. Acidotic breathing, sometimes called respiratory distress, is a sign of poor prognosis. It is often followed by circulatory failure refractory to volume expansion or inotropic drugs or by respiratory arrest. The plasma concentrations of lactate or bicarbonate are the best biochemical prognostic indicators in severe malaria. The prognosis of lactic acidosis is poor.

### *1.2.3.8 Non-cardiogenic pulmonary oedema*:

Adults with severe falciparum malaria may develop non-cardiogenic pulmonary oedema. (MA von Mach, *et al.*, 2003). The pathogenesis of this form of the adult respiratory distress syndrome is unclear (Y Blanloeil, *et al.*, 1980) (WHO, 2000b). The mortality rate

is >80%. This condition can be aggravated by overloaded administration of intravenous fluid.

### *1.2.3.9 Haematologic abnormalities*:

Anaemia results from accelerated red cell destruction and removal by the spleen in conjunction with ineffective erythropoiesis. In non-immune individuals and in areas with unstable transmission, anaemia can develop rapidly and transfusion is often required (RE Phillips, *et al.*, 1986a) (TT Hien, *et al.*, 1996). In many areas of Africa, children may develop severe anaemia as a result of repeated malarial infections (K Marsh, *et al.*, 1995). Anaemia is a common consequence of antimalarial drug resistance, which results in repeated or continued infection.

Slight coagulation abnormalities are common in falciparum malaria, and mild thrombocytopenia is usual. As mentioned above, fewer than 5% of patients with severe malaria have significant bleeding with evidence of disseminated intravascular coagulation (RE Phillips, *et al.*, 1986a). Haematemesis, presumably from stress ulceration (TT Hien, *et al.*, 1996) or corticosteroid therapy, may also occur (DA Warrell, *et al.*, 1982) (SL Hoffman, *et al.*, 1988).

### *1.2.3.10 Other complications*:

Aspiration pneumonia following convulsions is an important cause of death in cerebral malaria. Chest infections and catheter-induced urinary tract infections are common among patients who are unconscious for >3 days. Septicaemia may complicate severe malaria; in endemic areas, Salmonella bacteremia has been associated specifically with *P. falciparum* infections (WHO, 2000b).

### *1.2.3.11 Malaria in pregnancy:*

In areas with stable malaria (hyper- and holoendemic areas) *P. falciparum* infection in pregnant women (semi-immune), is increased in compared with non-immume pregnant women (living in unstable malaria areas) in terms of frequency and density of parasitaemia; it is also associated with low birth weight (average reduction, ~170 g) and consequently increased infant and childhood mortality (R Menon, 1972) (F Nosten, *et al.*, 1991b) . In general, infected mothers in areas of stable transmission remain asymptomatic despite intense parasitization of the placenta due to sequestration of parasitized erythrocytes in the placental microcirculation (BJ Brabin, 1983) (YJ Garin, *et al.*, 1985) (RW Steketee, *et al.*, 1996). Maternal HIV infection predisposes pregnant women to a higher prevalence of malaria and parasite density and predisposes their newborns to congenital malaria infection and low birth weight. The effects is greatest in women with 1$^{st}$ pregnancy but they do extend to their 2$^{nd}$ or 3$^{rd}$ pregnancy (PB Bloland, *et al.*, 1995) (V Leroy, *et al.*, 1998) (FO ter Kuile, *et al.*, 2004).

In areas with unstable transmission, malaria in pregnant women has devastating effects on both mother and fetus. The most common complications are high parasitaemia with anaemia, hypoglycaemia (S Looareesuwan, *et al.*, 1985b), and acute pulmonary aoedema. Fetal distress, premature labor, and stillbirth or low birth weight are commonly seen. Severe falciparum malaria in those non-immune women is associated with high mortality (20-50%), and is often fatal to the fetus irrespective maternal outcome (TT Hien unpublished data). *P. vivax* malaria in pregnancy is also associated with a reduction in birth weight (average, 100 g), but, in contrast with the situation in falciparum malaria, this effect is greater in multigravid than in primigravid women (F Nosten, *et al.*, 1999). Congenital malaria occurs in fewer than 5% of newborns whose mothers are infected and

is related to the parasite density in maternal blood and in the placenta (TC Quinn, *et al.*, 1982) (TV Hulbert, 1992) (B Balaka, *et al.*, 2000).

### *1.2.3.12 Malaria in Children*:

There is one to 3 million young African children who die of falciparum malaria each year. Convulsions, coma, hypoglycaemia, metabolic acidosis, and severe anaemia are relatively common among children with severe malaria, whereas deep jaundice, acute renal failure, and acute pulmonary oedema are unusual (D Waller, *et al.*, 1995) (K Marsh, *et al.*, 1995) (SJ Allen, *et al.*, 1996) (XT Cao, *et al.*, 1997b). Severely anaemic children may present with labored deep breathing, which in the past has been attributed incorrectly to "anaemic congestive cardiac failure" but is in fact usually caused by metabolic acidosis, often compounded by hypovolaemia (M English, *et al.*, 1996). In general, children tolerate antimalarial drugs well and respond rapidly to treatment.

### *1.2.3.13 Transfusion Malaria*:

Malaria can be transmitted by blood transfusion, needle-stick injury, sharing of needles by infected intravenous drug addicts, or organ transplantation. The incubation period is often short because the pre-erythrocytic stage of development is by-passed. The clinical features and management of these cases are the same as for naturally acquired infections, although falciparum malaria tends to be especially severe in drug addicts (TT Chau, *et al.*, 2002). Radical chemotherapy with primaquine is unnecessary for *P. vivax* and *P. ovale* infections.

### 1.2.3.14 Chronic complications of malaria

### Splenomegaly:

Chronic or repeated malarial infections produce normochromic, normocytic anaemia, hypergammaglobulinemia; and in certain situations, splenomegaly (GG Crane, 1986). (J Torres, *et al.*, 1988), Some residents of malaria-endemic areas in tropical Africa and Asia exhibit an abnormal immunologic response to repeated infections that is characterized by massive splenomegaly, hepatomegaly, marked elevations in serum titers of IgM and malarial antibody, hepatic sinusoidal lymphocytosis, and (in Africa) peripheral B cell lymphocytosis (J Van den Ende, *et al.*, 2000). This syndrome has been associated with the production of cytotoxic IgM antibodies to suppressor (CD8+) lymphocytes, antibodies to CD5+ T cells, and an increase in the ratio of CD4+ T cells to CD8+ T cells. It is believed that these events lead to uninhibited B cell production of IgM and the formation of cryoglobulins (IgM aggregates and immune complexes) (MF Moraes, *et al.*, 2003). This immunologic process stimulates reticuloendothelial hyperplasia and clearance activity and eventually produces splenomegaly. Patients with hyperreactive malarial splenomegaly (HMS) present with an abdominal mass or a dragging sensation in the abdomen and occasional sharp abdominal pains suggesting perisplenitis. In many cases, malarial parasites cannot be found in peripheral blood smears (S Puente, *et al.*, 2000), (JR Torres, *et al.*, 2003). Severe respiratory and skin infections are increased; many patients die of overwhelming sepsis. Persons with HMS who are living in endemic areas should receive antimalarial chemoprophylaxis: the results are usually good. In non-endemic areas, treatment is advised (MI Muniz-Junqueira, *et al.*, 1992), (J Van den Ende, *et al.*, 1994), (F Manenti, *et al.*, 1994)

*Quartan Malarial Nephropathy*:

Long-term and repeated infections with *P. malariae*, and possibly other malarial species, (MB Abdurrahman, *et al.*, 1990) may cause soluble immune-complex injury to the renal glomeruli, resulting in the nephrotic syndrome (RG Hendrickse, 1976) (JW Kibukamusoke, 1986). Other unidentified factors must contribute to this process since only a very small proportion of infected patients develop renal disease. The histologic appearance is that of focal or segmental glomerulonephritis with splitting of the capillary basement membrane. Sub-endothelial dense deposits are seen on electron microscopy, and immunofluorescence reveals deposits of complement and immunoglobulins; in samples of renal tissue from children, *P. malariae* antigens are often visible. A coarse-granular pattern of basement membrane immunofluorescent deposits (predominantly IgG3) with selective proteinuria carries a better prognosis than a fine-granular, predominantly IgG2 pattern with nonselective proteinuria (RH White, 1973). Quartan nephropathy usually responds poorly to treatment with either antimalarial agents or glucocorticoids and cytotoxic drugs (JW Kibukamusoke, 1968), (JA Jefferson & WG Couser, 2003).

*Burkitt's Lymphoma and Epstein-Barr Virus Infection*:

It is possible that malaria-related immunosuppression provokes infection with lymphoma viruses. Burkitt's lymphoma is strongly associated with Epstein-Barr virus. The prevalence of this childhood tumor is high in malarious areas of Africa (G de The, 1993), (LS Young & AB Rickinson, 2004)

## 1.2.4 Diagnosis of malaria

### *1.2.4.1 Conventional methods*

The diagnosis of malaria relies on the demonstration of asexual forms of the parasite in smears of peripheral blood. Following a negative blood smear, repeated smears should be made every 6-8 hours if there is a high degree of suspicion. Giemsa at pH 7.2 is preferred; Wright's, Field's, stain can be used. Both thin and thick blood smears should be examined. The thin blood smear should be dried, fixed in pure methanol, stained, and the red cells in the tail of the film should then be examined under oil immersion. The thick blood film should be of uneven thickness. The smear should be dried thoroughly and stained without fixing. As many layers of erythrocytes overlie one another and are lysed during the staining procedure, the thick film has the advantage of concentrating the parasites (by 20- to 40-fold compared with a thin blood film) and thus increasing diagnostic sensitivity. Both parasites and white cells in each field are counted, and the number of parasites per unit blood volume is calculated from the total leukocyte count, assuming that an average white cell count is of 8000/uL; and a minimum of 200 white cells should be counted. Interpretation of thick films requires some experience because artifacts are common. Before a thick smear is judged to be negative, 100 to 200 fields should be examined under oil immersion. Phagocytosed malarial pigment is sometimes seen inside peripheral blood monocytes or polymorphonuclear leukocytes and may provide a clue to recent infection if malarial parasites are not detectable (NH Phu, *et al.*, 1995). After the clearance of the parasites, malarial pigment is often evident for several days in peripheral blood phagocytes, bone marrow aspirates, or smears of fluid expressed after intradermal puncture. Staining of parasites with the fluorescent dye acridine orange allows more rapid diagnosis of cases in which the level of parasitaemia is low.

The parasitaemia is expressed as the number of parasitized erythrocytes among 1000 red cells, and this figure is converted to the number of parasitized erythrocytes per microliter. The relationship between parasitaemia and prognosis is complex; in general, patients with $>10^5$ parasites per microliter are at increased risk of dying, but non-immune patients may die with much lower counts and semi-immune persons may tolerate parasitaemia levels many times higher with only minor symptoms.

In severe malaria, a poor prognosis is indicated by a predominance of more mature *P. falciparum* parasites (i.e., >20% of parasites with visible pigment), by the presence of circulating schizonts in the peripheral blood film, or by the presence of phagocytosed malarial pigment in >5% of neutrophils (K Silamut & NJ White, 1993),(NH Phu, *et al.*, 1995). Gametocytes may remain evident for several days after treatment has begun; unless trophozoites are also visible on the blood film, their presence does not constitute evidence of drug resistance.

### 1.2.4.2 Rapid diagnosis tests (RDT)

Simple, sensitive, and specific antibody-based diagnostic stick or card tests that detect *P. falciparum*-specific, histidine-rich protein (HRP2) or lactate dehydrogenase (LDH) antigens in finger-prick blood samples have been introduced. Some of these tests carry a second antibody, which allows distinguish falciparum malaria from the less dangerous malarias.

Figure 1.4: Rapid diagnosis test for malaria



Capture antibody specific for *P. falciparum* antigens

*P. vivax* antigen

Capture antibody detecting all *Plasmodium* species

Labelled detection antibody

Control capture antibody specific for detection antibody

Sample origin

Detection lines:

Anti-*P. falciparum* antibody

Anti-malaria antibody (all species)

Control antibody

Absorbent pad

Malaria P.f.

(c)
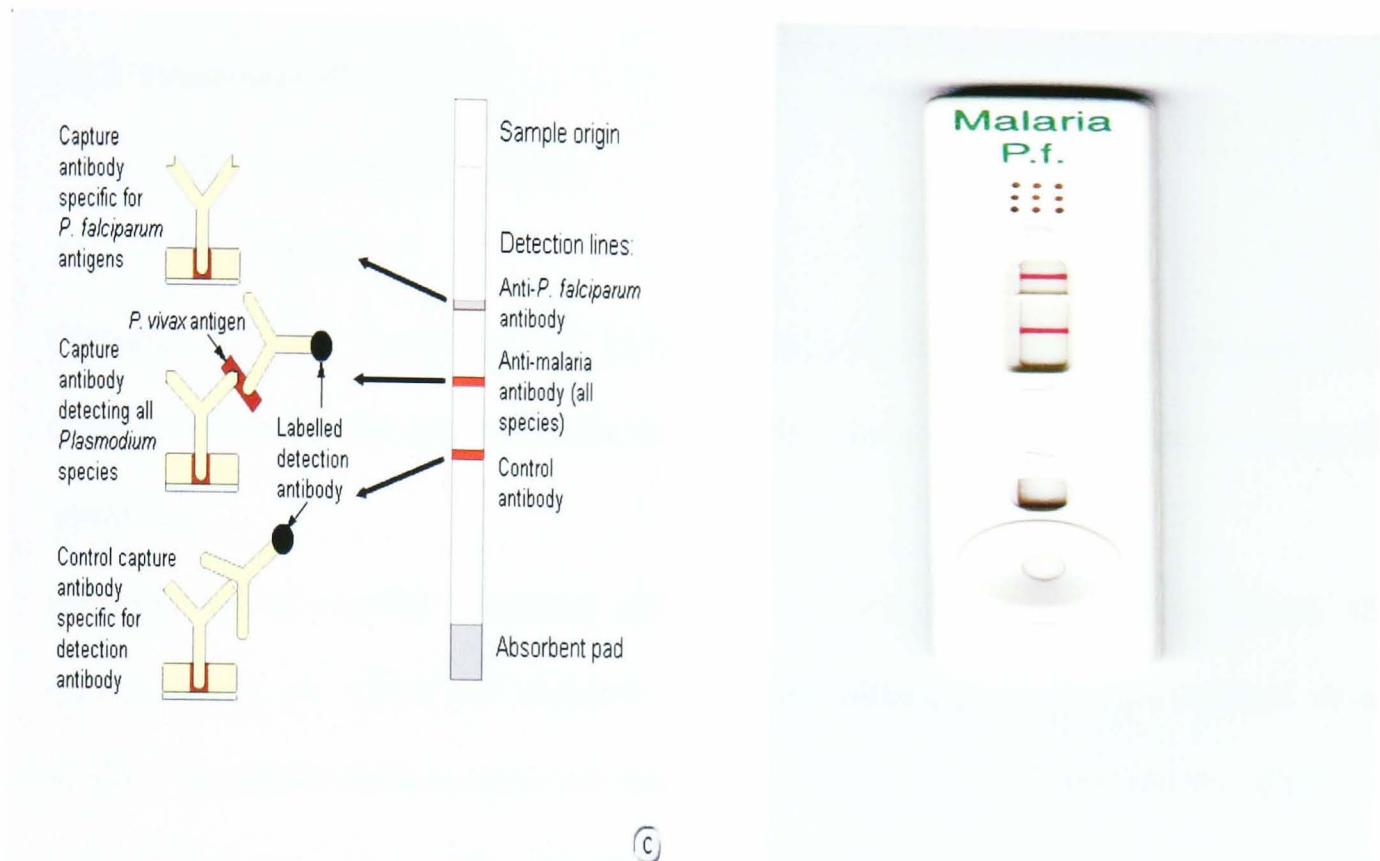
### 1.2.4.3 Laboratory findings:

Normochromic, normocytic anaemia is usually documented. The leukocyte count is generally low to normal, although it may be raised in severe infections. The platelet count is usually reduced to lower than $10^5/\mu L$. Severe infections may be accompanied by prolonged prothrombin and partial thromboplastin times and by severe thrombocytopenia. In uncomplicated malaria, plasma concentrations of electrolytes, blood urea nitrogen, and creatinine are usually normal. Findings in severe malaria may include metabolic acidosis, with low plasma concentrations of glucose, sodium, bicarbonate, calcium, phosphate, and albumin together with elevations in lactate, blood urea nitrogen, creatinine, urate, muscle and liver enzymes, and conjugated and unconjugated bilirubin. In adults and children with cerebral malaria, the mean opening pressure at lumbar puncture is ~160 mm of

cerebrospinal fluid (CSF); the CSF is usually normal or has a slightly elevated total protein level [<1.0 g/L (100 mg/dL)] and cell count (<20/uL).

## 1.2.5 Treatment of malaria

### *1.2.5.1 Antimalarial drugs*

### *Chloroquine*

Chloroquine is the most important of the 4-aminoquinoline compounds. It remains an effective treatment for severe malaria in those few areas where *P. falciparum* retains full sensitivity.

Chloroquine is rapidly absorbed after oral administration in healthy adults (LL Gustafsson, *et al.*, 1983) and children with uncomplicated malaria (SA Adelusi, *et al.*, 1982). In adults with malaria of moderate severity, acute bioavailability relative to parenteral treatment was 70% (NJ White, *et al.*, 1987d) compared with 75% in healthy adults. In children with uncomplicated malaria given an initial treatment dose of 10 mg base/kg as tablets, peak plasma concentrations of approximately 250 g/l were reached in two hours (SA Adelusi, *et al.*, 1982). Time to peak concentrations in severe malaria is usually 20 minutes and can be as short as 5 minutes. This results in transiently high (500 - 3500 g/l), and potentially toxic blood concentrations if doses of 5 mg base/kg or larger are given (NJ White, *et al.*, 1988). Formulations which retard slightly the rate of absorption after intramuscular or subcutaneous administration would presumably have a wider safety margin (S Prakongpan, *et al.*, 1989). Chloroquine administered by rectal suppositories has a bioavailabilty of approximately 30-50%, and a rate of absorption similar to that of oral chloroquine in healthy volunteers (F Minker & J Iran, 1991).

Chloroquine concentrations in red cells are approximately three times higher than in plasma, and there is considerable concentration in granulocytes and platelets. Whole

blood concentrations are 6 to 10 times higher than plasma concentrations (LL Gustafsson, *et al.*, 1983). The enormous apparent volume of distribution results from considerable binding in organs such as the liver, connective tissue and melanin containing tissues such as the skin and retina. Chloroquine is 55% protein bound in plasma (SA Adelusi & LA Salako, 1982, O Walker, *et al.*, 1983). Concentrations in the cerebrospinal fluid are very low with a mean value of 2.7% of corresponding whole blood concentrations(NJ White, 1988). Chloroquine is concentrated slightly in breast milk with milk:plasma AUC ratios of 2.0 to 4.3 (MD Edstein, *et al.*, 1986).

Chloroquine is 51% cleared unchanged by the kidney (LL Gustafsson, *et al.*, 1983). The remainder is biotransformed by the liver, mainly to desethyl and bidose chloroquine. The terminal elimination half-life of chloroquine is approximately 1-2 months but in terms of curative treatment (blood concentration) the real half life is about 6-10 days (M Frisk-Holmberg, *et al.*, 1984). The absorption and disposition of chloroquine in children appears to be similar to that in adults (NJ White, *et al.*, 1988).

Chloroquine is one of the most widely used drugs in the world. It still accounts for over 90% by weight of the global antimalarial consumption. It has undoubtedly saved many patients with severe malaria. Much of the reported toxicity at therapeutic doses is anecdotal and poorly documented. Oral chloroquine is usually well tolerated. Nausea, headache, uneasiness and dysphoria are relatively common but seldom serious. Patients may vomit and may complain of blurred vision. Postural hypotension associated with malaria may be exacerbated and pruritus (NG Osifo, 1984) can be severe especially, though not exclusively, in dark-skinned patients. Parenteral chloroquine is a potent vasodilator. Hypotension is a predictable consequence of the transiently high blood concentrations that follow parenteral administration (S Looareesuwan, *et al.*, 1986, NJ White, *et al.*, 1988)

### Quinine

Quinine is still widely used for severe chloroquine-resistant malaria, although artesunate and artemether are effective and safer alternatives. Although quinine resistance has increased in areas of SE Asia and South America, there is still no high grade resistance in severe malaria that precludes its use. This may change and efficacy in quinine resistant areas needs to be monitored closely (Pukrittayakamee *et al*, 1994).

Quinine sulphate is well absorbed when given by mouth in healthy subjects, and in patients with malaria (G Paintaud, *et al.*, 1993, W Supanaranond, *et al.*, 1991, NJ White, 1987). Peak plasma concentrations are reached in one to three hours (Hall *et al.*, 1973). Use of intramuscular quinine has been controversial for many years, but recent studies (TT Hien, *et al.*, 1996) suggest good bioavailability, safety profile and effectiveness even in very young children (<2 years) with severe malaria (MB van Hensbroek, *et al.*, 1996, D Waller, *et al.*, 1990, NJ White, 1995) Quinine is distributed throughout most of the body fluids. The mean concentration in erythrocytes is approximately one third of that in plasma, rising to one half (presumably because of concentration within malaria parasites) in severe malaria (NJ White, *et al.*, 1983a). Concentrations in salive are one third of those in plasma (LA Salako & A Sowunmi, 1992). Cerebrospinal fluid concentrations are 7 +/- 3% of those in plasma in cerebral malaria (NJ White, *et al.*, 1982). In healthy subjects and patients with malaria, quinine is predominantly (80%) eliminated by hepatic biotransformation (NJ White, 1985) first to 3 and 2 hydroxyquinine, and then to a series of more polar water soluble metabolites. Plasma concentrations of 3-hydroxyquinine are approximately one third to one fifth of those of the parent compound, with a lower ratio in malaria indicating impairment of hepatic biotransformation, but accumulation occurs in renal failure (P Newton, *et al.*, 1999). Approximately 20% of the quinine is eliminated by the kidney, and the remaining 80% by hepatic biotransformation. Total systemic

clearance is reduced in uncomplicated malaria, and further reduced in severe malaria (NJ White, *et al.*, 1982, NJ White, 1987). Renal clearance and hepatic clearance are reduced in parallel. Absorption of quinine is similar in adults and children. The total apparent volume of distribution and the volume of the central compartment is smaller in children(T Chongsuphajaisiddhi, *et al.*, 1981),(AE Greenberg, *et al.*, 1989, CR Newton, *et al.*, 1993, G Pasvol, *et al.*, 1991, D Waller, *et al.*, 1990),(A Schapira, *et al.*, 1993) and pregnant women (RE Phillips, *et al.*, 1986c) than in adults with disease of comparable severity, but elimination is more rapid in both groups. Systemic clearance values are therefore similar. Cord blood concentrations and breast milk concentrations are approximately one third of those in simultaneously sampled maternal plasma (RE Phillips, *et al.*, 1986c).

Minor adverse reactions are common with quinine therapy, but severe life-threatening toxicity is rare. A characteristic symptom complex known as "cinchonism" occurs with plasma concentrations over 5 mg/l (Powell & McNamara, 1966). This consists of tinnitus, high tone deafness (RJ Roche, *et al.*, 1990), nausea, uneasiness, dysphoria and blurring of vision. Vomiting is likely if core temperatures are high. These symptoms of cinchonism are rarely sufficient to warrant stopping treatment.

Hypoglycaemia is a more commonly encountered problem. Quinine is a potent stimulus to pancreatic insulin secretion (NJ White, *et al.*, 1983c) and hyperinsulinaemic hypoglycaemia is particularly likely in pregnant women (S Looareesuwan, *et al.*, 1985b), or patients who remain severely ill for several days. Intravenous injections may cause acute cardiovascular toxicity presumably because transiently toxic blood concentrations occur before adequate distribution (TM Davis, *et al.*, 1988). Postural hypotension is common in acute malaria and this is exacerbated by quinine (W Supanaranond, *et al.*, 1993). Studies of intravenous quinine in severe malaria (S Looareesuwan, *et al.*, 1985b) show no evidence of an oxytocic effect.

## *Quinidine*

Quinidine is the dextrorotatory diastereoisomer of quinine. It may be more effective than quinine in the treatment of chloroquine-resistant falciparum malaria(NJ White, *et al.*, 1981), but is also more toxic. Quinidine is cleared more rapidly with a greater proportion of renal clearance than quinine, and the total apparent volume of distribution is larger. Elimination half times are shorter (NJ White, *et al.*, 1981). Plasma concentrations of quinidine are therefore lower than those with equivalent doses of quinine. Much of these differences can be explained by the increased free fraction in plasma (J Karbwang, *et al.*, 1993); free concentrations are approximately twice those of quinine. Although there is less information, quinidine pharmacokinetic properties are affected in a similar manner to those of quinine in malaria i.e. clearance and volume of distribution are reduced (NJ White, *et al.*, 1981). The terminal elimination half-life in severe malaria was 12.8 hours. The therapeutic range for total plasma quinidine concentrations is estimated to be approximately 5-8mg/L (KD Miller, *et al.*, 1989). There are no data on intramuscular quinidine in malaria.

Minor toxicity with the two diastereomers is similar, although quinidine has a greater tendency to produce diarrhoea and has much less effect on hearing than quinine (J Karbwang, *et al.*, 1993). Quinidine has a fourfold greater effect in prolonging the electrocardiographic QTc interval and has a greater propensity to produce hypotension(NJ White, *et al.*, 1983b),(RE Phillips, *et al.*, 1985). Electrocardiographic monitoring and close attention to circulatory status are therefore essential when parenteral quinidine is given for the treatment of severe malaria (KD Miller, *et al.*, 1989). Quinidine is also equally likely to induce hypoglycaemia (RE Phillips, *et al.*, 1986b). Idiosyncratic and allergic reactions are not common. There are even less data for quinidine than quinine on which to base a recommended therapeutic range.

### *Amodiaquine*

Amodiaquine is also a 4-aminoquinoline with a similar mode of action to chloroquine. It is more active against resistant isolates of *P.falciparum* but it is also more toxic (F Abacassamo, *et al.*, 2004, S Looareesuwan, *et al.*, 1985a, CG Nevill, *et al.*, 1994, AO Talisuna, *et al.*, 2004, WM Watkins, *et al.*, 1984). Amodiaquine may be a useful substitute for chloroquine where oral treatment is required. A parenteral formulation of amodiaquine is not commercially available, but the closely related compound, amopyraquine, is available for intramuscular injection in many francophone countries in Africa. Concern that amodiaquine causes a high prevalence of agranulocytosis (CS Hatton, *et al.*, 1986) and hepatitis (KA Neftel, *et al.*, 1986) has terminated its prophylactic use. Amodiaquine is rapidly and extensively converted to a pharmacologically active metabolite, desethylamodiaquine, following oral administration, and it seems that this metabolite is responsible for most of the antimalarial activity(FC Churchill, *et al.*, 1985),(P Winstanley, *et al.*, 1987). Following intravenous administration, the fall in blood amodiaquine concentration is biphasic. The terminal elimination half life is approximately 10 hours indicating rapid biotransformation to the active metabolite. Mean total volume of distribution was 17.4 l/kg and systemic clearance 5.5 l/kg/hr (NJ White, *et al.*, 1987a).

### *Sulfadoxine-pyrimethamine(SP)*

This is the most widely used of a family of drug combinations which antagonize parasite folic acid synthesis. They act by sequential inhibition of dihydropteroate synthetase (sulphonamides and sulphones) and dihydrofolate reductase (pyrimethamine and biguanides). A formulation for intramuscular injection is available.

Pyrimethamine and sulfadoxine are both well absorbed. In healthy subjects, peak plasma concentrations are reached in 2 to 6 hours (RA Ahmad & HJ Rogers, 1980),(E Weidekamm, *et al.*, 1982). Absorption in uncomplicated malaria is similar to that in healthy subjects(B Sarikabhuti, *et al.*, 1988, PA Winstanley, *et al.*, 1992). The intramuscular formulation is also well absorbed in moderately severe malaria (PA Winstanley, *et al.*, 1992) with an estimated bioavailability that was similar overall to the oral formulation. However, plasma concentrations in the first day after starting intramuscular treatment (the critical period for the treatment of severe malaria) were less than half those following oral administration. In severe malaria, when given together with quinine, pyrimethamine absorption was more rapid, but sulfadoxine was less well absorbed than in uncomplicated malaria (CR Newton, *et al.*, 1993). Pyrimethamine is cleared predominantly by hepatic biotransformation. Systemic clearance is approximately 20 ml/h/kg) (RA Ahmad & HJ Rogers, 1980, E Weidekamm, *et al.*, 1982, E Weidekamm, *et al.*, 1987) and the terminal elimination half-life is approximately 90 hours. Unlike other sulphonamides only 5% of sulfadoxine is N-aceylated and eliminated in the urine in this form. Biotransformation and clearance is approximately 0.5 ml/h/kg and the terminal elimination half-life 180 hours. Both pyrimethamine and sulfadoxine are excreted in breast milk. The amounts likely to be ingested by the baby are unlikely to be effective in treating or preventing malaria but could cause allergic or idiosyncratic reactions.

Intramuscular sulfadoxine-pyrimethamine (S-P) is not painful. Oral S-P is very well tolerated. Minor adverse effects are rare. There is now an extensive literature on the toxicity of these combinations, but almost all refers to continuous prophylactic use in prevention, and not the single dose treatment of malaria. Prolonged use of pyrimethamine may provoke folate deficiency in vulnerable subjects (pregnancy, malnourished patients). Sulphonamides should not be given in late pregnancy or to the newborn because of the

theoretical risk of provoking kernicterus. Prophylactic use of S-P carried an estimated mortality in Americas of between 1:11,000 and 1:25,000(KD Miller, *et al.*, 1986). Severe allergic cutaneous reactions are the most common serious adverse reactions. Agranulocytosis and other blood dyscrasias, hepatitis, pulmonary eosinophilia, and neuropathy have been been reported. It is generally considered that the risks of severe adverse reactions in the treatment of malaria are small and do not preclude use of these drugs for the treatment of uncomplicated drug-sensitive infections.

### Tetracycline

The tetracyclines are relatively slowly acting antimalarials which are used either as prophylactic or adjunctive drugs. They should not be used alone in severe malaria unless no other antimalarial drugs are available. There are extensive reviews of their clinical pharmacology when used as antibacterial drugs. The pharmacokinetic properties of the different tetracyclines vary. Tetracycline has better oral bioavailability than oxy- or chlor-tetracycline. Plateau concentrations following regular dosing of 250 mg six hourly range between 2 and 3 g/ml. Oral bioavailability is increased in the fasting state, and may be considerably reduced by chelation to divalent cations (e.g. calcium in milk, magnesium and aluminium in antacids, iron preparations). Tetracycline has an elimination half-life of approximately 8 hours. Doxycycline has better oral bioavailability than most of the other tetracyclines, peak concentrations of 3.0 g/ml are reached within 2-3 hours. Compared to tetracycline food interferes less with absorption after an oral dose of 200 mg. The elimination half-life is 18-22 hours. All the tetracyclines are excreted by glomerular filtration in the urine (tetracycline 70%, doxycycline 40%, minocycline 5%) and also in the bile. Most of the tetracyclines accumulate in renal failure, but doxycycline, chlortetracycline and probably minocycline do not. Nausea, heartburn, abdominal pain,

vomiting and diarrhoea are not uncommon. Candida superinfections and pseudomembraneous colitis may occur. Hypersensitivity is rare, but photosensitivity is fairly common (particularly with demethylchlortetracycline). These drugs deposit in calcifying areas of bones and teeth and should not be used in children under 8 years or in pregnancy. Tetracyclines may cause nephrotoxicity by five different mechanisms; they may aggravate pre-existing renal failure by their antianabolic effects, very rarely they may cause a hepatorenal syndrome in association with acute fatty liver, outdated degraded tetracyclines may cause tubular damage (Fanconi-like syndrome), demethylchlortetracycline induces nephrogenic diabetes insipidus and on rare occasion an interstitial nephritis may develop. With the exception of doxycycline, these drugs are contraindicated in renal failure. For these reasons, tetracyclines should not be given in the acute phase of severe malaria.

### Antimalarial biguanides

There has been a resurgence of interest in these compounds, which were originally developed over 40 years ago. They were the first of the antimalarial dihydrofolate-reductase inhibitors. Two compounds, proguanil and chlorproguanil, are in current use. There are no parenteral formulations; these drugs should not be used in severe malaria. Both are prodrugs for the biologically active triazine metabolites cycloguanil and chlorcycloguanil respectively. Biotransformation is reduced in pregnancy and with the oral contraceptive (R McGready, *et al.*, 2003). Pharmacokinetic studies have been reported (I Bygbjerg, *et al.*, 1987, WM Watkins, *et al.*, 1987, Y Wattanagoon, *et al.*, 1987). These suggest that the elimination of the parent compounds determine the blood concentration profiles of the active metabolites. For proguanil estimated elimination half-life values (t½ B) range from 11 to 20 hours. Proguanil and the inactive metabolite

parachlorophenylbiguanide, but not cycloguanil, are concentrated in red cells. The combination of chlorproguanil and dapsone is an effective treatment for falciparum malaria (WM Watkins, *et al.*, 1988) and is effective against S-P resistant parasites (T Lang & B Greenwood, 2003, TK Mutabingwa, *et al.*, 2001, P Winstanley, 2001) (provided the Ile164Le mutation is not present) but otherwise these drugs are now used exclusively for prophylaxis.

### *Mefloquine*

Mefloquine is a quinoline methanol compound, which structurally resembles quinine. It is effective against all malaria species including multi-drug resistant *P. falciparum*, although significant resistance has developed in Southeast Asia. There is no parenteral formulation. Mefloquine has no place in the management of severe malaria. Currently available formulations of mefloquine are well absorbed in healthy subjects and patients with uncomplicated falciparum malaria (J Karbwang, *et al.*, 1987b, S Looareesuwan, *et al.*, 1987). Absolute bioavailability is not known because there is no parenteral formulation. The absorption profile in malaria appears to be biphasic (J Karbwang, *et al.*, 1987a) but with the treatment doses of 15 mg/kg plasma or whole blood concentrations exceeding 500 ng/ml are usually reached within 6 hours. Absorption may be dose limited as blood levels following a 25mg/kg dose are significantly higher if the dose is divided compared with single dose administration (R Price, *et al.*, 1999, JA Simpson, *et al.*, 1999, JA Simpson, *et al.*, 2000). Absorption was rapid, but post absorption plasma concentrations were two to three times lower than in studies in healthy subjects or in uncomplicated malaria suggesting reduced bioavailability. In some assays mefloquine concentrations in red cells have been higher than those in plasma (DE Schwartz, *et al.*, 1982, FO Ter Kuile, *et al.*, 1994) whereas in others blood concentrations have been

similar(J Karbwang, *et al.*, 1987a). Mefloquine is highly bound (98%) to plasma proteins. Cerebrospinal fluid concentrations are unmeasurable (<5 ng/ml) (P Chanthavanich, *et al.*, 1985). Mefloquine exhibits a multiexponential decline in blood concentrations with a terminal elimination half-life of approximately three weeks in healthy subjects and two weeks in patients with uncomplicated malaria (J Karbwang & NJ White, 1990, JA Simpson, *et al.*, 1999). Clearance is by hepatic biotransformation. Mefloquine is generally well tolerated, although nausea, abdominal discomfort, and vertigo occur in 10 - 20% of patients. Sinus arrhythmia and sinus bradycardia have been noted in several reports but their significance is unclear. Postural hypotension also occurs. There has been concern that mefloquine and quinine in combination may be cardiotoxic, but there is no evidence of this (W Supanaranond, *et al.*, 1997). If patients develop severe malaria following mefloquine treatment then a full loading dose of quinine should be given, as the most likely explanation is inadequate mefloquine absorption. If a treatment dose of mefloquine has been taken in the 12 hours before severe malaria treatment starts, then electrocardiographic monitoring would be advisable if quinine is used. Acute psychosis or a transient encephalopathy with convulsions are serious occur in 0.1% of patients in Southeast Asia (NT Hoang Mai, *et al.*, 1996), but up to 1% of Caucasian and African patients (PA Phillips-Howard & FO ter Kuile, 1995). The pathological mechanism underlying neurotoxicity is not understood.

### *Halofantrine*

Halofantrine is a phenanthrene-methanol compound, it is more effective than mefloquine against multi-drug resistant falciparum malaria (FO ter Kuile, *et al.*, 1993). A parenteral formulation has been studied (S Krishna, *et al.*, 1993) but this is not commercially available.

The principal problem with current formulations of halofantrine is poor oral bioavailability. Because of erratic and variable absorption, it is currently recommended that the dose of halofantrine be split into three, with six hour dosing intervals. Oral bioavailability is increased up to six- fold if halofantrine is taken with a fatty meal (KA Milton, *et al.*, 1989, GD Shanks, *et al.*, 1992). Mean values for tmax for halofantrine and its principal metabolite desbutylhalofantrine and in malaria are 15-16 hours and 55-56 hours respectively, with corresponding cmax values of 0.9-1.2 and 0.4-0.5 mg/L respectively (J Karbwang, *et al.*, 1991); Karbwang, 1991b (JR Veenendaal, *et al.*, 1991). Halofantrine absorption is reduced by at least 50% in severe malaria (WM Watkins, *et al.*, 1995). The estimated apparent volume of distribution is large (100 to 500 l/kg). Halofantrine is almost entirely eliminated by hepatic biotransformation. The estimated terminal half-life is 3.5-4.5 days (J Karbwang, *et al.*, 1991, JR Veenendaal, *et al.*, 1991). The principal metabolite desbutylhalofantrine has equal biological activity (LK Basco, *et al.*, 1992), and in malaria has a similar elimination half-life. As plasma concentrations of the metabolite usually exceed those of halofantrine by the third day of treatment, they are obviously an important determinant of antimalarial activity and drug efficacy. Halofantrine is extensively bound to low and high density lipoproteins in serum (B Cenni, *et al.*, 1995).

Halofantrine is generally well tolerated. Abdominal pain and diarrhoea occur rarely but are usually mild and self-limited. Pruritus occurred in 13% of Nigerians taking the drug(A Sowunmi, *et al.*, 1998). Halofantrine lacks central nervous system adverse effects and is better tolerated than mefloquine (FO ter Kuile, *et al.*, 1993) but it produces consistent concentration dependent delays in atrioventricular conduction and ventricular repolarisation (F Nosten, *et al.*, 1993). Halofantrine has been associated with sudden death; and this has been attributed to lethal ventricular arrhythmias. Halofantrine should

not be given at doses higher than those currently recommended, to patients with a long electrocardiograph QT interval, to patients taking drugs known to prolong the QT interval i.e. quinine, quinidine, chloroquine, tricyclic antidepressants, neuroleptics, terfenadine, astemizole. or who have received mefloquine within 28 days(NJ White, 1996). If severe malaria develops after halofantrine treatment then it may be safer to use artesunate or artemether rather than quinine treatment.

### *Lumefantrine*

Formerly called benflumetol, lumefantrine was developed by Chinese scientists. It is now available only in a fixed tablet combination with artemether. Each tablet contains artemether 20mg and lumefantrine 120mg. The combination is registered in many tropical countries and in Europe. It is very effective against multi-drug resistant falciparum malaria, and it is remarkably well tolerated, with no evident adverse effects.

Lumefantrine is lipophilic and hydrophobic. Its absorption is considerably augmented by taking the drug together with food (a 16 folds increase with a fatty meal). Absorption is reduced in the acute phase of malaria, but then increases considerably as symptoms resolve and the patient starts to eat (F Ezzet, *et al.*, 1998) The elimination half-life is 3-4 days (F Ezzet, *et al.*, 2000). The pharmacokinetic properties of lumefantrine are similar in adults and children (NJ White, *et al.*, 1999b). The principle pharmacokinetic variable which correlates with therapeutic response is the area under the plasma concentration curve (AUC) (NJ White, *et al.*, 1999b). The plasma level on day 7 after starting treatment is a good surrogate of the AUC; plasma levels of lumefantrine above 500ng/mL are associated with a >90% cure rate. The treatment course which has been most widely evaluated approximates to 1.5/ 9mg/kg (adult dose 4 tablets) given at 0, 8, 24, and 48 hours. This is effective in patients with background immunity, but in non-immune

patients with multi-drug resistant infections cure rates are approximately 80% (M van Vugt, *et al.*, 1998). Increasing the regimen to six doses (i.e. twice daily on each day) results in >95% cure rates (M van Vugt, *et al.*, 2000, MV Vugt, *et al.*, 1999). As children in endemic areas are relatively non-immune the six dose regimen is a preferable regimen for general use. The patient should be encouraged to take the drug with food. There is no experience in pregnancy and therefore the drug should not be used in pregnant women. There is no paediatric formulation. This combination is remarkably free of adverse effects. Concerns about possible cardiotoxicity, have been refuted by careful studies (M van Vugt, *et al.*, 1999). Lumefantrine is not cardiotoxic.

### *Piperaquine*

Also developed in China this bisquinoline compound and its hydroxy-derivative are active against multidrug resistant Plasmodium falciparum (L Chen, 1991). Piperaquine is now available as a fixed combination with dihydroartemisinin (and also sometimes trimethoprim and primaquine). It is relatively inexpensive (adult doses just over US$ 1). These combinations are registered and used in China, Vietnam and Cambodia (MB Denis, *et al.*, 2002, P Wilairatana, *et al.*, 2002). Given as a four-dose (0, 8, 24, 48 hours) regimen comprising a total of 5120mg of piperaquine for an adult, the combination is well tolerated and effective, but more clinical trial information on efficacy and safety is needed before this exciting new drug can be recommended more widely.

### *Pyronaridine*

This naphthyridine derivative was synthesised in China in 1970 (XY Zheng, *et al.*, 1982). It has structural similarities to mepacrine and amodiaquine but unlike both these drugs it is highly active against multi-drug resistant P. falciparum (GE Childs, *et al.*, 1988, S Fu,

*et al.*, 1986). It has been widely used in China for over ten years. A parenteral formulation is available in China but has not been used widely. Originally pyronaridine was deployed as an enteric-coated formulation for monotherapy which had poor oral bioavailability and was given in a 3 day course of 1200 mg or 1800 mg (adult over 5 days). It is active against multidrug resistant *P.falciparum* (S Looareesuwan, *et al.*, 1996, P Ringwald, *et al.*, 1998). Pyronaridine is now being developed as a fixed co-formulation with artesunate

### *Artemisinin and its derivatives*

The artemisinin and its derivatives are the most rapidly effective of the antimalarial drugs and retain efficacy even against multidrug-resistant parasites. Increasing evidence demonstrates that their widespread deployment leads to a reduction in transmission potential, and their early use in an infection may prevent the progression to severe falciparum malaria.

The qinghaosu group includes the prototype artemisinin, a sesquiterpine lactone with an endoperoxide bridge (C-O-O-C) that is unique among antimalarial drugs. Dihydroartemisinin, the reduced lactol derivative of artemisinin, is the common biologically active metabolite of artesunate and artemether. Artesunate and artelinate are the water-soluble derivatives; artemether and arteether are both oil soluble, but water insoluble. These four drugs are the semisynthetic derivatives of the parent artemisinin compound. Artemisinin is not readily available commercially outside China and Vietnam.

## ARTEMISININ

Artemisinin is the natural substance extracted from the leaf of *Artemisia annua L*. It has been formulated as a crystalline powder and can be administered orally as a tablet, or in a capsule, and rectally as a suppository. Initially, in China, it was also given intramuscularly as an oil suspension and as a water suspension (China Cooperative Research Group on Qinghaosu and Its derivatives as Antimalarials Metabolism and pharmacokinetics of qinhaosu and its derivatives, 1982) .

There have been recent pharmacokinetic studies of the artemisinin derivatives using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) and bioassay in healthy volunteers and non-complicated malaria patients. This has shown that the pharmacokinetic parameters of orally administered artemisinin are similar in both groups: artemisinin is rapidly absorbed with the peak concentration recorded at 1-2 hours after administration, but the absorption is incomplete (M Ashton, *et al.*, 1998, A Benakis, *et al.*, 1997, PJ De Vries & TK Dien, 1996, PJ De Vries, *et al.*, 1997, M Hassan Alin, *et al.*, 1996) (Table 1.1). The plasma concentration of the drug exceeded the in vitro mean inhibitory concentration (MIC) of *P.falciparum* (3-30µg/L) for more than 8 hours in all patients studied (LK Basco & J Le Bras, 1993, PJ De Vries, *et al.*, 1997). The elimination of artemisinin is fast with an elimination half-life of 2 to 5 hours (PJ De Vries, *et al.*, 1997, M Hassan Alin, *et al.*, 1996), the AUC and the Cmax values of the 1st day were about 6 times higher than that on the 5th day, indicating considerable autoinduction of metabolic biotransformation (M Ashton, *et al.*, 1998, M Hassan Alin, *et al.*, 1996). Even a single dose induces metabolism. The predominant CYP450 enzyme involved is CYP B6. The metabolites and the potential for drug interactions have not been fully characterised. The rectal bioavailability is 30% relative to the oral dose although there is large inter-individual variation. Because of the similarity in parasite clearance times between oral

and rectal administration, it is assumed that therapeutic concentrations could be achieved with artemisinin suppositories (R Koopmans, *et al.*, 1998, JS Sidhu, *et al.*, 1998).

Early studies in China suggested that an optimum treatment was 3g of artemisinin given over 3-5 days (10-20 mg/kg in the 1st day at (+0 and +4hrs) then 10mg/kg/day in subsequent days) (GQ Li, *et al.*, 1994). However, studies using monotherapy regimens with less than a 7-day treatment course (oral or rectal), and designed with a 28-day follow-up period, confirmed that the true recrudescence rate was unacceptably high (20-50%) (TT Hien, 1994). The combination of artemisinin (20 mg/kg given on one day or 3 day dose) and a long elimination half-life drug such as mefloquine would seem to overcome many of the problems of high recrudescence rates seen with artemisinin monotherapy. Several clinical trials have shown that this is the most effective treatment of multi-drug resistant *P.falciparum* malaria (NN Bich, *et al.*, 1996, TT Hien, *et al.*, 1994, NN Le, *et al.*, 1997).

Artemisinin suppositories remain an effective treatment for *P.falciparum* malaria and offer great promise as a life saving intervention in remote areas where facilities for parenteral drugs maybe limited and of course the diseases has its greatest impact. In the early 1990s artemisinine suppositories were shown to be as effective as parenteral antimalarial drugs in clinical trials for the treatment of severe malaria (XT Cao, *et al.*, 1997a, V Ha, *et al.*, 1997, TT Hien, *et al.*, 1992a)

Table 1.2.1: Pharmacokinetic parameters of artemisinin

| | de Vries PJ et al | | Benakis A | Koopmans R | |
|---|---|---|---|---|---|
| | 500mg po | | 500 mg po | 400mg suppository | 600mg suppository |
| | Healthy volunteers | Patients | Healthy volunteers | Healthy volunteers | Patients |
| Cmax (ng/ml) | 391±147 | 364±250 | 360±90 | 100±102 | 108±60 |
| Tmax (hr) | 1.81±0.73 | 2.88±1.71 | 1.07±0.73 | 7.1±2.1 | 6.5±3.9 |
| AUC(h.µg/L) | 2,054±455 | 2,302±2,023 | 1,190±640 | 747±449 | 1080±678 |
| T ½ el (hr) | 2.8±0.9 | 2.72±1.76 | 4.34±3.46 | NA | NA |

## ARTESUNATE

Artesunate, the water-soluble hemisuccinate derivative of dihydroartemisinin (DHA), was developed as a drug for an intravenous use in the treatment of severe and complicated malaria. It is unstable in neutral solutions and therefore only available in powder form as artesunic acid. This powder requires reconstruction with 5% w/v sodium bicarbonate solution immediately before use. At neutral pH hydrolysis to DHA is rapid. Artesunate is the artemisinin derivative most widely used. It may be administered orally, intravenously, intramuscularly or rectally.

Artesunate is hydrolysed very rapidly after intravenous injection to DHA in 2 minutes (SD Yang, *et al.*, 1985). After oral administration artesunate is also hydrolysed to DHA and relatively little unchanged artesunate is detectable in plasma (KT Batty, *et al.*, 1998a, KT Batty, *et al.*, 1998b). This hydrolysis is accelerated by plasma and red cell esterases. The mean absolute oral bioavailability of the drug in patients with acute malaria was 61%; the absorption and elimination of oral artesunate was rapid with mean elimination half-life of antimalarial activity of 43 minutes (P Newton, *et al.*, 2000b). After repeated oral administration of artesunate a time-dependent decline of artesunate and DHA concentrations in plasma occurs although it remains unclear what the mechanism for this decrease of concentration (NX Khanh, *et al.*, 1999). Artesunate has not been shown to autoinduce metabolism in the same way as artemisinin. Despite rapid clearance of artesunate and DHA in patients with uncomplicated falciparum malaria prompt parasite and fever clearance are achieved and once-daily administration to patients has been shown to be highly effective with similar parasite and fever clearance times to those treated with a twice-daily regimen (F Nosten, *et al.*, 1994)

Several clinical trials in South East Asia have shown that artesunate, given as single oral dose (2-4mg/kg) or for 3 days (4mg/kg for the 1st day then 2mg/kg for 2nd and 3rd day) in combination with mefloquine (15 mg/kg as single dose) was an effective and safe treatment for non-complicated falciparum malaria (V Ha, *et al.*, 1997, F Nosten, *et al.*, 1994, RN Price, *et al.*, 1997). Very large randomized controlled studies in Thailand, Viet Nam, and nine African countries indicate that one dose of artesunate (4mg/kg) given daily for 3 days in combination with mefloquine (25mg/kg), chloroquine, sulfadoxine/pyrimethamine or amodiaquine is better than either a single dose of artesunate with these drugs or the other antimalarials given as monotherapies in terms of

recrudescence rates. These studies assessed efficacy for > 28 days and were supported by PCR genotyping; in addition, treatment with artesunate results in lower gametocyte rates and may thereby reduce transmission rates (JF Doherty, *et al.*, 1999, Lorenz von Seidlein, *et al.*, 2000, RN Price, *et al.*, 1997).

Unfortunately, there are insufficient data on the use of suppositories. Studies in Vietnam, in patients with non complicated falciparum malaria treated with a single rectal dose of 2mg/kg of artesunate (produced in China) confirmed that fever and parasite clearance times obtained were similar to those seen with 20mg/kg artemisinine: FCT 100 (SD): 29(19.1) hrs *vs* 38.6(26.7) hrs; PCT 100(SD): 39.8 (17.4) hrs *vs* 43.1(25.4) hrs (TT Hien, unpublished data). A clinical trial in children with non-complicated falciparum malaria in Gabon using single dose of 50 mg (equivalent 0.8-2 mg/kg) of a new pharmaceutical form of this drug [Plasmotrim® Rectocaps® MEPHA] obtained similar results (B Halpaap, *et al.*, 1998) A trial in Thailand using higher doses of Rectocaps® artesunate (15mg/kg/day for 3 days) was compared with oral artesunate (6mg/kg/day); both in combination with mefloquine (25mg/kg). The fever clearance time (42 *vs* 42 hrs) parasite clearance time (42 *vs* 35 hrs) and cure rates (92% *vs* 100%) were similar in both groups (S Looareesuwan, *et al.*, 1997, A Sabchareon, *et al.*, 1998).

Table 1.2.2: Pharmacokinetic properties of artesunate

| | Batty et al | | Newton et al, 2000 (HPLC-ECD + bioassay) | | |
|---|---|---|---|---|---|
| | IV | Oral (for DHA) | IV | Oral | |
| | | | | Acute | Convalescence |
| Cmax (ng/ml) | 13,670 | 740 | 8,240 | 1,021 | 546 |
| Tmax (hr) | - | - | | 0.75 | 1.00 |
| AUC(h.ug/L) | 2,280 | 870 | 3,013 | 1,738 | 886 |
| T ½ el (hr) | 2.1 min | 39 | 1.01 | 0.71 | 0.84 |

## ARTEMETHER

Artemether is an oil soluble derivative of artemisinin. It is the methyl ether of dihydroartemisinin and has been used widely in China. It is more stable than artesunate.

In healthy volunteers, artemether was absorbed rapidly after oral administration and underwent extensive first-pass metabolism to DHA with the concentration of this metabolite higher than that of the parent compound. The mean Cmax, of 310-406 nmol/L, were reached at 1.2-2.2 hours; the mean elimination half-life was 2.0-2.6 hours(MN Mordi, et al., 1997, P Teja-Isavadharm, et al., 1996). There was no difference in pharmacokinetics between healthy volunteers and patients with uncomplicated malaria (K Na-Bangchang, et al., 1994). In volunteers, intramuscular and intrarectal administration had a biovailability of approximately 25% (i.m) and 35% (i.r) compared to the oral drug. Plasma antimalarial activity following oral administration is significantly greater than following intramuscular administration (P Teja-Isavadharm, et al., 1996) because the first-pass biotransformation is inhibited Recent studies demonstrated that grapefruit juice

significantly increases the oral bioavailability of artemether and acute renal failure significantly modified the pharmacokinetics of intramuscular artemether with better absorption/bioavailability and a reduction of systemic clearance (J Karbwang, *et al.*, 1998). Bioavailability of intramuscular artemether was also shown to be highly variable and may be associated with an inadequate response in children with severe and complicated malaria especially in the presence of respiratory distress (SA Murphy, *et al.*, 1997).

Monotherapy with oral or intramuscular artemether (1-4 mg/kg/day for 3-5 days) results in rapid fever and parasite clearance but with an unacceptable recrudescence rate (25-40%). Artemether has been given in combination with other antimalarial drugs with long half-lives particularly mefloquine. Again this combination has demonstrated cure rates of 95-98% (RN Price, *et al.*, 1995). Since 1992, 2042 patients have been recruited to a series of trials using a new combination of artemether and lumefantrine (benflumetol). Four tablets of artemether and lumefantrine (1 tablets=20 mg of artemisinin+120 mg lumefantril) given at 0, 8, 24, 48 hours, has been shown to be very well tolerated by all age groups with a cure rate of 81% (NJ White, *et al.*, 1999b) . The latest result from a double-blind trial in Thailand in which patients with uncomplicated multidrug resistant falciparum malaria were treated with 6 doses of 4 tablets given at 0, 8, 24, 36, 48, 60 (or 72, 96) hours indicated that the 28 day cure rates were 96.9 - 99.1% (MV Vugt, *et al.*, 1999).

A recent meta-analysis of nine randomised clinical trials comparing artemether and quinine for treatment of severe malaria concluded that these two drugs were equally efficacious in terms of reduction in mortality rate OR (95%CI)=0.76(0.50-1.14). However, pre-specified pooling of data from trials in South East Asia (mainly involving

adults) showed a trend towards a significant reduction of mortality OR (95%CI) = 0.38(0.14-1.02) (MH Pittler & Ernst, 1999)

Table 1.2.3: Pharmacokinetic parameters of artemether

| | Bangchang (94) | Teja-Isavadharm (96) | Mordi (97) | Karbwang (97) | |
|---|---|---|---|---|---|
| | 200 mg po | 5mg.kg −1 | 200 mg po | 300 mg | |
| | Median (range) | Mean (95% CI) | Mean ± SD | po | im |
| Cmax (ng/ml) | 118(112-127) | 406(249-561) | 310±153 | 474 | 540 |
| Tmax (hr) | 3(1-10) | 1.7(1.2-2.2) | 1.88±0.21 | 2.0 | 3.9 |
| AUC (h.µg/L) | 1,100 | | 671±271 | 2,170 | 5,200 |
| T ½ el (hr) | | 2.6(1.8-3.4) | 2.00±0.59 | | |

## ARTEMOTIL (ARTEETHER)

Arteether is the ethyl derivative of dihydroartemisinin and has been selected by the WHO and the US Army for full development (JM Petras, *et al.*, 2000). Clinical trials in India indicated that alpha, and beta-arteether is an effective and rapidly acting antimalarial drug for the treatment of acute non-complicated and severe falciparum malaria (JM Petras, *et al.*, 2000, N Singh, *et al.*, 1998, N Valecha, *et al.*, 1997). There is probably little difference between artemether and arteether

## DIHYDROARTEMISININ (DHA)

Dihydroartemisinin is the active metabolite of the artemisinin derivatives: artesunate artemether and arteether. DHA has been formulated recently as tablets and suppositories.

DHA has a short elimination half-life approximately 40 minutes but little is known of its subsequent metabolism (KT Batty, *et al.*, 1998a).

Pharmacokinetic studies in volunteers indicated that the median (range) values of pooled pharmacokinetics of oral DHA were; Tmax 1.6 (1.1-2.2) hours, Cmax 466 (128-787) ng/ml, AUC 1867 (420-3535) ng.h/ml, inter-individual variation was significant. The Tmax of DHA formulation was comparable with that of DHA metabolite of artemisinin derivatives. Oral dihydroartemisinin was rapidly absorbed and disappeared from the systemic circulation within 8-10 h (NH Le, *et al.*, 1999)

Clinical trials showed oral DHA given at different dose regimens (single dose, 300 mg or multiple doses, 480 mg in 5 days) resulted in similar fever clearance times and parasite clearance times. However, short-course treatments (< 5 days) have higher recrudescence rates (20%) (GQ Li, *et al.*, 1999, K Na-Bangchang, *et al.*, 1999). Combination with mefloquine demonstrated better cure rates (C Xu, *et al.*, 1997).


## ARTELINIC ACID

Artelinic acid (dihydroartemisinin 4-carboxybenzyl ether) is water soluble and said to be more stable then artesunate in solution. It can be given orally or parenterally and is the only preparation that has been studied transdermally (KL Klayman, *et al.*, 1991).


## SAFETY OF THE ARTEMISININ DERIVATIVES

The artemisinin-related compounds have been remarkably well tolerated in clinical evaluations. There has been no documented significant toxicity other than rare type 1 hypersensitivity reactions (incidence approximately 1:3000 treatments). In volunteer studies, a depression of reticulocyte counts has been noted, but increased anaemia has not been observed in clinical studies. In animal studies, they are much less toxic than the

quinoline antimalarials. The principal toxicity in animals has been an unusual dose-related selective pattern of neuronal cell damage affecting certain brain stem nuclei. In animal studies, this has been related to the pharmacokinetic properties of the drug. Neurotoxicity is related to protracted exposure related to sustained blood concentrations, as follows intramuscular administration of the oil based artemether and arteether. It is much less following oral administration or intravenous artesunate because the drugs levels are not sustained. Extensive clinical neurophysiological and to a lesser extent pathology studies have failed to show any evidence of neurotoxicity or cardiotoxicity in clinical use.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 Study site

### 2.1.1 Geography

Vietnam is the eastern part of the Indochina peninsula, which also comprises Laos, Cambodia. It is located between 8° and 23° North latitude and is entirely in tropical region. The country covers an area of 331,114 square Km, 80% of the land area is mountains, high plateaus and forests; the remaining 20% is the two deltas (Red River and Mekong River), and the coastal plains where most of the population live.

### *2.1.1.1 Climate:*

Vietnam has a humid tropical monsoon climate. In the south part, there is only a small variation in temperature between the two seasons: the rainfall (April – November) and the dry season (December – March): the average temperature over the year in range of 26-28°C. In the Northern provinces, the winter is cold (mean temperature in Hanoi is 16.5° C) whereas it is hot and humid in the summer (mean temperature 29°C). Most of the population in the northern highlands areas (the temperatures in the winter sometimes fall down to 10°C) lives at altitudes below 800 m where *P. vivax* transmission is not

interrupted during the winter months but the transmission of *P.falciparum* does for 3-4 months. The average annual rainfall over the country is around 1600 mm and may rises up to 200-300 mm per month in the rainy season; or to 800 mm per months in the Central region. Typhoons are common especially during autumn on the central coast with 10-12 tropical storms per year. Vietnam two main rivers frequently flood the delta areas.

### 2.1.1.2. Population:

The total population in 2004 was 81,000,000 (April 2004 est.) with 33% below 14 years of age; 15-64 years: 62%, 65 years and over: 5% (2000 est.). The population grow rate was 1.49%. Life expectancy at birth in 2000 was 69.27 years, with a crude birth rate 21.62 births/1000 population and crude death rate 6.26 deaths/1000 population. Infant mortality rate was 31.13/1000 live births; under-five mortality was 55.4/1000 and maternal mortality was 16.6/1000.

85% of Vietnam population is of the Kinh group. Apart from the people of Chinese origin (3%), who live in cities, the largest minority group is Kh'mer whose population is about one million mainly living in Mekong delta. Ten percent of Vietnam population belongs to over 50 ethnic groups (Tay, Muong, Nung, Ede, K'hor, S'tieng) who traditionally inhabit in highland areas; they often live in dispersed communities, with houses on stilts, with livestock close to the house. These are the communities, which have been living in conditions of stable malaria for centuries. The H'mong people are an exception as they have lived as nomads above the altitude where the malaria transmission is very unlikely.

Extensive migrations have occurred in Vietnam. It is estimated that a total of 4.3 million people migrated in the country covered by government-sponsored programme; but during the same period, about 530,000 people has migrated spontaneously. Since 1980s more liberal economic policies have led to increased opportunities on plantations, road

building, mining and public services therefore beside ordered programmes, there have been waves of uncontrolled migrations of smaller groups of farmers, shopkeepers, cut and gold and gem miners from plains to highlands. Military personnel are another group of short-term migrants often exposed to malaria risk.

### *2.1.1.3 Environment*

Logging and slash-and-burn agricultural practices contribute to deforestation and soil degradation; water pollution and over-fishing threaten marine life populations; groundwater contamination limits potable water supply; growing urban industrialization and population migration are rapidly degrading environment big cities.

### *2.1.1.4 Economy*

Vietnam is a poor, densely populated country that has had to recover from the ravages of war, the loss of financial support from the old Soviet Bloc, and the rigidities of a centrally planned economy. Substantial progress was achieved from 1986 to 1996 in moving forward from an extremely low starting point (GDP per capita was US$ 213 in 1994) growth averaged around 9% per year from 1993 to 1997. The 1997 Asian financial crisis highlighted the problems existing in the Vietnamese economy. GDP growth of 8.5% in 1997 fell to 4% in 1998 and rose slightly to an estimated 4.8% in 1999. Foreign direct investment has fallen dramatically, from $8.3 billion in 1996 to about $1.6 billion in 1999. Administrative and legal barriers are also causing costly delays for foreign investors.

Although the economy is growing in all provinces there are important and widening gaps. The central and northern highlands where the incidence of malaria is the highest ones are still lagging behind the rest of country in terms of economic output. The ethnic

minority groups, living from traditional agriculture and animal husbandry, in scattered villages with difficult access, are the poorest.

## 2.1.2 Health services

Vietnam is committed to the primary health care approach. A strong public health sector involvement is considered necessary for improving health status particularly in remote rural areas. Vietnam has 30,000 doctors, 44,800 medical assistants and 50,000 nurses and 11,000 midwives. The government has a major responsibility for provision of health care. Health services are delivered through provincial hospital, district hospitals and commune health stations. A commune health station covers about 10,000 people on average. That is the most peripheral unit for provision of health care and is where most patients with malaria have chance to access for diagnosis and receiving treatment.

Private medical practice and pharmacies play an increasing role in cities and in many densely populated rural areas. Private pharmacies sell a wide range of pharmaceutical products; private doctors and medical assistants are also private treatment providers; but in some areas in southern and central provinces shopkeepers also provide drugs including antimalarials. A study, which investigated the use and quality of antimalarial drugs in the growing private sector of Viet Nam was conducted in 1998 by the NIMPE. The practices of drug vendors (called alternative treatment providers [ATPs]) as well as their stocks and the quality of drugs sold by them, and the local production and distribution of antimalarials were investigated. Antimalarials were sold by the vast majority of ATPs, and almost all the common antimalarials being available for sale. The practices and indications for sale, however, varied. Underdosing for malaria was frequent in all three provinces studied, and lack of knowledge of the appropriate regimen for cure was common among the drug-sellers. Samples of antimalarials were collected from ATP

outlets in the three provinces, and the drugs were assessed for their contents and expiry date by the Institute of Drug Quality Control in Hanoi. Of the 218 samples of drugs examined by the Institute, over 96% met the quality requirements. However, a 10% sample of these drugs were independently assessed by WHO and revealed a different picture: 70% of them failed to meet the standard specifications required. There is therefore an urgent need to improve the capability and monitoring procedures of bodies involved in assessing and regulating drugs in Viet Nam (LD Cong, *et al.*, 1998). The wide distribution and extensive use of effective drugs like artesunate and mefloquine have probably contributed to reduction of (severe) malaria, but development of resistance to these drugs is to be feared. Control of drug distribution and of prescription practices is urgently needed (L Bont, *et al.*, 1995).

Most of the preventive services are run as national programmes from institutes that are subordinate to the Department of Preventive Medicine of the Ministry of Health (MOH) such as the National Institute of Hygiene and Epidemiology (NIHE) which are responsible for the Expanded Programme of Immunization, control of diarrhoeal diseases, poliomyelitis. Those programmes operate through provincial Preventive Medicine Centre, which are subordinate to the Provincial Health Service.

## 2.1.3 National Malaria Control Programme (NMCP)

National Malaria Control Programme (NMCP) is directed by the National Steering Committee chaired by a Vice-Minister of Health and responsible for planning, managing, monitoring and evaluating the malaria control programme. The members of the committee represent the southern, central and northern provinces and selected from central institutions. The National Institute of Malariology-Parasitology-Entomology (NIMPE) is responsible for the day-to-day management of the programme. The NIMPE

releases technical advice, conducts research, staff training data analysis and assisting peripheral levels in operations. It also distributes insecticides, drugs and other commodities. The two regional institutes in Qui Nhon city (Qui Nhon IMPE) and Ho Chi Minh City IMPE are responsible for control activities in central and southern provinces of the countries, respectively. The provincial centres for malaria control (where malaria is endemic) or Preventive Medicine Centres (for other provinces) - attached to provincial health services - will conduct all malaria control activities at provincial and district levels. There are about 2000 government employees involving into the national malaria control programme

### 2.1.4 Hospital for Tropical Diseases

The Hospital for Tropical Diseases (HTD) in Ho Chi Minh City acts as tertiary referral centre for patients with infectious diseases, and serves the Ho Chi Minh city area (8 million population) and southern provinces of Vietnam (population around 35 million). The hospital has 500 beds, including 4 separate intensive care units (paediatric, adult, malaria and tetanus) and 15 wards for non-complicated infectious diseases (malaria, Dengue hemorrhagic fever, diphtheria, HIV/AIDS, viral hepatitis, central nervous infections (encephalitis and meningitis), respiratory infections, acute diarrhea. HTD has laboratories for haematology, biochemistry, microbiology, serology and parasitology. The hospital staff has involved in National Committee of Malaria Control, Dengue Hemorrhagic Fever Control, AIDS control, typhoid control, hospital infection and has also involved in WHO Malaria Programme.

## 2.1.5 Dak O Commune Health Station

Dak O Commune is in Phuoc Long District, Binh Phuoc Province located 200 km North West of Ho Chi Minh City. The commune consists of 11 hamlets, with about 10,000 people belonging mainly Kinh and S'tieng ethnic group. A minor part of the population is Tay and Nung people who are immigrants from the Northern provinces. The habitations of local people are surrounded by cashew nut or coffee tree gardens. The primitive forests in this area have been cleared and replaced by those trees.

A health station, which includes one doctor assistant, one midwife and 2 microscopists, is responsible for local primary health care. Patients who are more seriously ill will be transferred to the district hospital at Thac Mo Town, the capital of Phuoc Long District, through a dirty path.

The results of cross-sectional surveys conducted in this commune in 1999 and 2000 showed that the positive blood smears rates varied from 20-30%. About 350-500 man-times having fever come to the health station for medical consultation; 75% of those people carrying malaria parasite.

## 2.1.6 Oxford University Clinical Research Unit

The Oxford University Clinical Research Unit opened in 1991, funded by the Wellcome Trust of Great Britain. The OUCRU, located within the HTD, serves as a collaborative centre between HTD and Oxford University and started as an 8-bed ward for the treatment of patients with severe malaria. Over 10 years the Unit has expanded and now performs research on five core areas: malaria, dengue, typhoid, central nervous system infection and tetanus.

## 2.2 Aims, structure of this thesis

While the number of malaria cases in Africa has always overshadowed those in Southeast Asia, the *P. falciparum* in this area is the most abysmal parasite on the world because it has become so difficult to treat. The multi-drug resistant malaria patients can still treated but by 1990s the therapeutic armoury was looking increasingly bare and the shadow of untreatable malaria loomed large. Viet Nam, a SEA country, has had to face to this challenge from the end of the war in 1975. At that moment, in addition to common reasons, a large population movement aggravated the malaria situation and in the 1980s, the control programme was drastically reduced as a result of national financial difficulties and dwindling external support. At the same time technical difficulties in particular multi-drug resistance of *P.falciparum*, the parasite responsible for 70% of malaria cases in the country become apparent and the nadir was in 1993 with 4646 fatal cases the highest figure ever recorded.

The cornerstone of the change in the management of malaria in Viet Nam is the introduction of artemisinin and other derivatives of the Chinese herb "qinghaosu" as first line treatment from 1995. The early laboratory and clinical studies in China provided the basis for clinical trials at the Hospital for Tropical Diseases. The artemisinin used was initially manufactured in China but more recently in Viet Nam from locally grown Artemisia annua plants. Research on *Artemisia annua* was started in early 1980s within the framework of the national malaria control programme: in 1984 samples of this plant were collected in Lang Son Province and 1n 1986 the presence of artemisinin in Vietnam *Artemisia annua* with high yield (0.5-1.2% in dried leaves) was confirmed. Our studies in 1990s have shown that artemisinin and its derivatives (artesunate or artemether) are the best antimalarial drugs available in terms of clearing malaria parasites. They may be given by the oral, parenteral or rectal routes(TT Hien & NJ White, 1993),(XT Cao, *et al.*,

1997a). However, despite of rapid parasite clearance, the recrudescence rate of artemisinin and its derivatives is unacceptably high when these drugs is used in monotherapy less than 7 days (TT Hien, 1994) Therefore, there are a number of important questions that still need urgent attention:

These are:

+ Should artemisinin be deployed alone or in combination with other antimalarial drugs such mefloquine or other coumpound(s)?

If so which is the best regimen(s)

+ Whether artemisinin and its derivatives (artesunate or artemether) can reduce the mortality in severe malaria.

+ Of possibly more relevance is the unanswered question of toxicity and the significant discrepancy between extensive clinical studies in patients where no adverse effects are seen, and animal experiments demonstrating significant brain stem damage.

**Research objectives of this thesis:**

*Primary objectives*

1/ To assess the safety and the efficacy of the artemisinin based combination regimens for uncomplicated multi-drug resistant falciparum malaria

2/ To investigate the efficacy of artemisinin and its derivatives in the treatment of severe malaria

*Specific objectives:*

1/ to describe the pharmacokinetic profiles of artesunate and artemether in severe malaria

2/ to assess the efficacy of artemisinin and its derivative (artesunate, artemether) in the treatment of severe malaria

3/ to compare the efficacy of artesunate + mefloquine with mefloquine monotherapy for acute falciparum malaria

4/ to compare the efficacy of dihydroartemisinin + piperaquine with artesunate + mefloquine for acute falciparum malaria

5/ to assess the pharmacokinetic properties of piperaquine in malaria

6/ to evaluate the potential neurotoxicity of artemisinin

## 2.3 Clinical methods

### 2.3.1 Scientific and ethical approval

HTD scientific and ethical committee approved all study protocols, and informed consent was obtained from each patient or accompanying relative.

### 2.3.2 Patients and treatment

All febrile patients who present to the out-patient department of the hospital at any time are seen by doctors and a peripheral blood smear is performed for complete blood count, differential count and for malaria detection (unless they are transferred from other hospital with laboratory results).

Patients with positive smear for malaria will be transferred to acute or severe malaria ward based on their clinical symptoms and signs (WHO, 2000b).

On admission to the ward, a thorough history will be taken and clinical examination made, and details will be recorded on a medical form specifically designed for infectious diseases. Besides the full blood count blood will also be taken for a routine biochemistry including blood glucose, serum creatinin, blood urea nitrogen, urine analysis. chest X-ray or ultrasound examination. Other laboratory investigations will be also performed for severe patients: blood gases, blood lactate, Na +, K+, Ca++. Anti-malarial drugs (quinine,

chloroquine, primaquine, artesunate) are available and free of charge. Patients will be treated following the national recommendations for malaria. Parasitaemia will be determined daily or every 12 6 hours. Axillary temperature, radial pulse rate, arterial blood pressure, respiratory rate will be recorded daily or every 6-12 hours as prescribed. Specific management for study patients will be described in individual chapters

### *Diagnosis of malaria:*

The diagnosis of malaria in this thesis relies on the demonstration of asexual forms of the parasite in smears of peripheral blood.

- The thin blood smear should be dried, fixed in pure methanol, stained, and the red cells in the tail of the film should then be examined under oil immersion. The thick smear should be dried thoroughly and stained without fixing. Giemsa at pH 7.2 is used; Field's, stain is also used for quick results (within 5-10 minutes)

- Both parasites and white cells in each field are counted, and the number of parasites per unit blood volume is calculated from:

- the thick smear: assuming that an average white blood cells (WBC) count is of 8000/µL; and a minimum of 400 WBC should be counted and the parasiraemia (PC) will be: $PC = number\ of\ parasites/400\ WBC \times 20$

-The thin smear: the number of parasitized red blood cell (RBC) among 1000 RBC should be counted and the parasitaemia will be

$PC = number\ of\ parasite/\ 1000\ RBC \times 125.6 \times patient's\ haematocrit$

Figure 2.1: Blood smear for malaria diagnosis
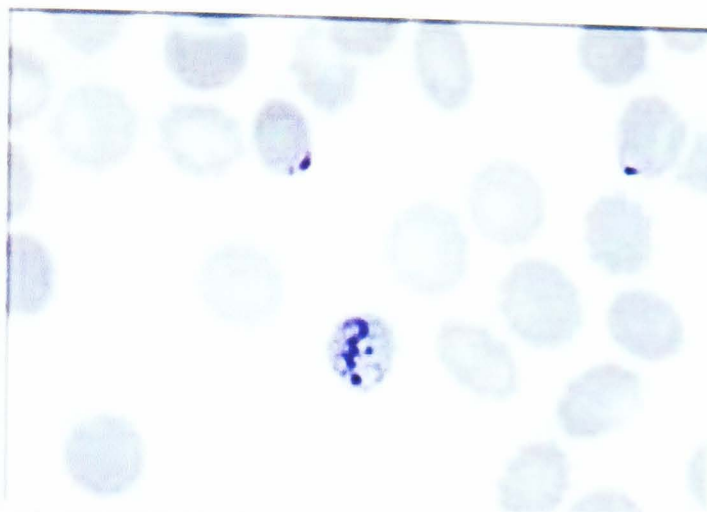


Thin smear *(P.falciparum)*



Thick smear *(P.falciparum)*
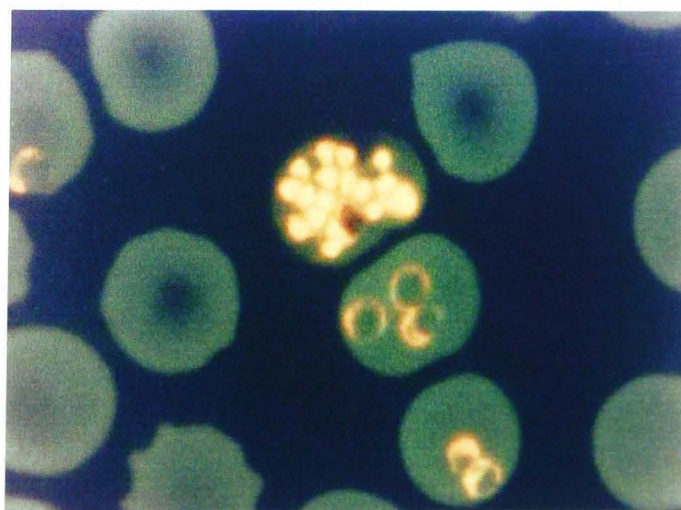


Thin smear *(P.falciparum)* AO stain

## 2.3.3 In vivo assessment:

The efficacy of the antimalarial drugs is assessed by the fever clearance time (FCT), the parasite clearance time (FCT) and the WHO criteria for resistance to the aminoquinoline antimalaria drugs

*Fever clearance time*: the time from starting antimalaria treatment until the patient is afebrile. Fever often fluctuates erratically. The method and the site of measurements should be standardised and the use of antipyretics recorded. One approach is to record when the temperature first falls below 37.5°C and when the temperature falls and remains below 37.5°C for 24 hours.

*Parasite clearance time*: the time between starting antimalarial treatment and the first negative blood smear. The accuracy of the measurement depends on the frequency with which blood slides are taken and the quality of microscopy. The PCT is directly proportional to the admission parasitaemia. The time taken for parasitaemia to fall to half of the admission value (PCT 50) and to fall to 10% of the admission value (PCT 90) are also useful comparative values.

The WHO classification of *in vivo* antimalarial drug sensitivity (1973) was used to assess the antimalarial drug efficacy:

**R1:** initial resolution of the symptoms and parasite clearance but recrudescence of the infection between 7 and 28 days from starting antimalarial treatment.

**R2:** Reduction of parasitaemia by 75% at 48 hours, but failure to clear parasites within 7 days from starting antimalarial treatment.

**R3:** Parasitaemia does not fall by 75% within 48 hours

We are aware that the classification of efficacy outcome for moderate transmission areas was introduced in 2003

**Early treatment failure (ETF):** development of danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; parasitaemia on day 2 is higher than day 0 count irrespective of axillary temperature; parasitaemia on day 3 with axillary temperature > 37.5°C; parasitaemia on day 3 > 25% of count on day 0

**Late treatment failure (LTF):**

- **Late clinical failure (LCF):** development of danger signs or severe malaria after day 3 in the presence of parasitaemia without previously meeting any of criteria of early treatment failure; recurrence of parasitaemia accompanied by fever between 4-28 or 56 days without previously meeting any of criteria of early treatment failure

- **Late parasitological failure (LPF):** recurrence of parasitaemia not accompanied by fever between 4-28 or 56 days, without previously meeting any of criteria of early treatment failure or late clinical failure.

**Adequate clinical and parasitological response (ACPR):** no recurrence of parasite (with or without fever) for duration of monitoring without previously meeting any of criteria of early treatment failure; late clinical or parasitological failure.

In order to keep the consistency of the evaluation the original classification is still in use. The follow-up visits were scheduled for days 2 (+48h), 7, 28, and 56 with standardized clinical examination, peripheral blood smear and filter paper dot. Patients were encouraged to return to the hospital (in hospital-based studies) or commune health station (in community-based studies) at any time they feel sick. Home visits were arranged for patients who did not come back.

The PCR was used to distinguish recrudescence and new infections. The failure cases will be treated with artesunate 3 days + one day mefloquine (25 mg/kg).

Adverse drug reactions (ADR), defined as any symptoms and signs that occurred or became more severe after starting of treatment, were assessed at follow-up visits.

Specific details of clinical assessment for individual study will be described separately in clinical chapters (Chapters 4, 5 and 6).

## 2.4 Laboratory methods

## 2.4.1 Molecular method to differentiate re-infection from recrudescence

### *2.4.1.1 Extraction of DNA from blood infected with P. falciparum*

All samples were collected on admission before the start of treatment. The diagnosis of *Plasmodium falciparum* infection was made by microscopic examination of Field- or Giemsa-stained thin and thick blood smears. During the surveys, most blood samples were collected and stored on filter paper. Two drops of patient's blood were spotted on Whatman (Maidstone, United Kingdom) 3 MM filter paper, air-dried, and stored in sealed plastic bags with silica gel at room temperature. With this method, the quantities of blood collected are small, one drop corresponding to a volume of 30 μl of blood, but collection and storage of blood is cheap and does not require freezers. Alternatively, blood was collected in EDTA-tubes and stored at $-30°C$ until DNA was extracted using a QIAGEN kit.

### *i) DNA extraction by the chelex/ boiling method, from blood spotted on to filter paper*

#### *Principle:*

Saponin is a detergent, which causes lysis of the red blood cell membrane and releases the haemoglobin from the filter paper, which is then washed away. The DNA is bound up in the paper fibers and is released into the supernatant by boiling. Chelex is a chelating ion exchange resin with high preference for copper, iron and other heavy metals, which could be possible inhibitors of the PCR reaction. This method is quick, simple and inexpensive, but it does not yield purified DNA.

#### *Method:*

Blood spots were cut from the filter paper using flamed forceps and scissors. The blood spots were soaked for 10 minutes in a sterile 1.5 ml Eppendorf tube containing 1 ml of

1% Saponin (w/v) (Sigma-Aldrich GmbH, Sternheim, Germany), in phosphate buffered saline solution (PBS; 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4). The tubes were centrifuged for 4 min at 14 000 rpm in a microcentrifuge (Juan, France) and the supernatant was discarded. Filter spots were washed twice by adding 1 ml of PBS to the tubes, centrifugation for 2 min at 14 000 rpm and then the supernatant was discarded. 150 µl of Molecular Biology Grade water and 150 µl of a 20% suspension of Chelex 100 resin (Bio Rad, Hercules, California, USA) were added. The tube was placed in a boiling water bath for 8 minutes and then centrifuged for 2 minutes at 14 000 rpm. The supernatant, which contained the DNA was carefully removed to avoid transfer of the Chelex resin and aliquoted into two Eppendorf tubes. Both tubes were stored at $-20°$ C. When used for PCR, one tube was thawed on ice and after use immediately returned to $-20°$ C storage (S Kyes, *et al.*, 1993).

### ii) Extraction of DNA using the QUIAamp DNA mini kit

400 µl of blood from the stored EDTA-blood tubes were extracted using the QIAamp® DNA minikit (QIAGEN, Germany) according to the manufacturer's instructions. DNA was eluted with 200 µl of buffer AE.

### 2.4.1.2 Genotyping of P. falciparum by Polymerase Chain Reaction using three polymorphic marker genes: Merozoite Surface Protein 1 (MSP1), Merozoite Surface Protein 2 (MSP2) and Glutamate Rich Protein (GLURP).

Polymorphic loci and oligonucleotide primer sequences

Three parasite loci, which are single copy genes (or: exist as single copy genes in the parasite's genome) and exhibit repeat number polymorphisms, were used for the genetic analysis of the parasite population. All three genes were amplified by primary rounds of Polymerase Chain Reaction (PCR), which was followed by a nested round.

**The gene for MSP1** is located on chromosome 9 and can be divided into 17 blocks according to the repetitive and conservative nature of the sequence. The region of amplification surrounds block 2. The primers MSP1 P1: CAC ATG AAA GTT ATC AAG AAC TTG TC and MSP1 P2: GTA CGT CTA ATT CAT TTG CAC G were used for the first round of PCR, which was followed by a nested round of amplification using the PCR primers: MSP1 N1: GCA GTA TTG ACA GGT TAT GG and MSP1 N2: GAT TGA AAG GTA TTT GAC.

**The gene for MSP2** is located on chromosome 2 and can be divided in 4 blocks. The region of amplification surrounds block 2, two pairs of primers ( MSP2 P1: GAA GGT AAT TAA AAC ATT GTC and MSP2 P2: GAG GGA TGT TGC TGC TCC ACA G, MSP2 N1: CTA GAA CCA TGC ATA TGT CC and MSP2 N2: GAG TAT AAG GAG AAG TAT G) are used in consecutive rounds of polymerase chain reaction.

The oligonucleotide primers used for the amplification of MSP1 and MSP2 were described by Lisa Ranford-Cartwright (LC Ranford-Cartwright, *et al.*, 1993).

**The gene for GLURP** is located on chromosome 10 and contains two repetitive regions, R1 and R2. The two pairs of primers used for the PCR reaction, GLURP P1: ACA TGC AAG TGT TGA TCC and GLURP P2: GAT GGT TTG GGA GTA ACG, GLURP N1: TGA ATT CGA AGA TGT TCA CAC TGA AC and GLURP N2: TGT AGG TAC CAC GGG TTC TTG TGG, hybridize to the region surrounding R2. Primers for GLURP were described by RE. Paul and S. Viriyakasol (RE Paul, *et al.*, 1995) (S Viriyakosol, *et al.*, 1995).

*Polymerase Chain Reaction Amplification Conditions*

*PCR Conditions*

Each of the genotyping reactions for the three marker genes was carried out in separate tubes, with one set of primers. Each 25 μL reaction mixture for primary MSP1, MSP2 and

GLURP and nested MSP1, MSP2 and GLURP amplification reactions contained DNA template (5 μL for the first round and 1 μL for the nested), 100 nM of each primer, 2.5 mM MgCl2, PCR buffer (50 mM KCl, 10 Mm Tris-HCl), 200 μM of each deoxynucleoside triphosphate, and 0.6 units of Taq DNA polymerase.

For the primary round of genotyping, the PCR conditions were as follows: step 1, 95°C for 5 min; step 2, denaturation at 95°C for 30 sec; step 3, annealing at 45°C for 30 sec; step 4, extension at 70°C for 1 min; repeat steps 2-4 29 times, followed by step 5, final extension at 70°C for 5 min. One microliter of the primary reaction served as the DNA template of the corresponding secondary reaction. Temperature profiles of the nested genotyping round were similar to those of the primary genotyping round, except that the annealing temperature was lowered to 40°C.

In order to be able to distinguish recrudescence from a new infection with *P. falciparum*, the admission sample of the patient (sample A) and relapse sample (sample B) were analyzed as paired samples under exactly the same PCR conditions and run on the same gel next to each other.

### *Controls*

Positive, negative and water controls were included in each experiment. As a positive control DNA from a field isolate of *Plasmodium falciparum* was used that showed 2 distinctive bands for MSP1, 3 for MSP2 and one for GLURP. DNA from a healthy individual that was not infected with *Plasmodium falciparum* was used as negative control.

*Sensitivity of detection of MSP1, MSP2 and GLURP genes by PCR*

To find the sensitivity of detection, the purified DNA template was amplified by using the optimized PCR condition. The amount of template was varied by serial dilution to correspond to 1000, 500, 100, 50, 25, 10, 5, 2 and 1 parasites/$\mu$L, respectively. Successful nested genotyping PCR reaction was obtained reproducibly from samples containing as little as 1 - 10 *P. falciparum* genomes (ca. 0.00001 % parasitaemia).

*PCR Product Detection*

Five microliters of the amplified PCR product were mixed with 1.5 $\mu$L of loading buffer (30 % glycerol (v/v), 0.4% Orange G (Bio Rad) (w/v)) and electrophoresed on a 2 % agarose gel at 150 mV for two and a half hours. DNA size standards were run on both ends of the gel. After electrophoresis, the gel was stained with ethidium bromide for 15 min so that the DNA fragments could be visualized on an UV-transilluminator and photographed. The size of the amplified DNA was estimated by comparing its mobility to a molecular weight marker (100 bp marker; Life Technologies, U.S.A.).

Table 2.4.1: Sequences of oligonucleotide primers used for genotyping of *Plasmodium falciparum*.

| | | |
|---|---|---|
| MSP1 – primary | MSP1 P1 | 5'- CAC ATG AAA GTT ATC AAG AAC TTG TC – 3' |
| | MSP1 P2 | 5' – GTA CGT CTA ATT CAT TTG CAC G –3' |
| MSP1 – nested | MSP1 N1 | 5' – GCA GTA TTG ACA GGT TAT GG – 3' |
| | MSP1 N2 | 5' – GAT TGA AAG GTA TTT GAC – 3' |
| MSP2 – primary | MSP2 P1 | 5' – GAA GGT AAT TAA AAC ATT GTC – 3' |
| | MSP2 P2 | 5' – GAG GGA TGT TGC TGC TCC ACA G – 3' |
| MSP2 – nested | MSP2 N1: | 5' – CTA GAA CCA TGC ATA TGT CC – 3' |
| | MSP2 N2 | 5' – GAG TAT AAG GAG AAG TAT G – 3' |
| GLURP – primary | GLURP P1 | 5' – ACA TGC AAG TGT TGA TCC – 3' |
| | GLURP P2 | 5' – GAT GGT TTG GGA GTA ACG – 3' |
| GLURP – nested | GLURP N1 | 5' – TGA ATT CGA AGA TGT TCA CAC TGA AC – 3' |
| | GLURP N2 | 5' – TGT AGG TAC CAC GGG TTC TTG TGG –3' |

## 2.4.2 Assay methods for antimalarial drugs

### 2.4.2.1 Analysis of artesunate, dihydroartemisinin and artemether

Fully validated high performance liquid chromatographic (HPLC) or gas chromatographic-mass spectrometric (GC-MS) methods were used for the analysis of ARTS, dihydroartemisinin (DHA) and ARM.

ARTS and DHA in plasma from patients who received i.m. ARTS were assayed by the HPLC method of Batty (KT Batty, *et al.*, 1996). The between-run coefficients of variation (relative standard deviations) were 5% and 6% at 900 nmol/L and 4970 nmol/L respectively for ARTS, and 11% and 9% at 1070 nmol/L and 4730 nmol/L respectively for DHA. Quality control samples were included with each assay batch, with a requirement that the run was acceptable only if the QC was within ± 15% of the nominal value.

ARM and its metabolite DHA in plasma from patients who received i.m. ARM were quantified by the GC-MS method of Mohamed (SS Mohamed, *et al.*, 1999) with minor modifications in the GC-MS conditions. Briefly, separations and quantitations were achieved on a Hewlett Packard 5890 Gas Chromatograph coupled to a Hewlett Packard 5971A Mass Selective Detector operating in selected ion monitoring mode (SIM). A J & W DB-5 30 m x 0.25 mm i.d. GC column coated with a 0.25mm film thickness of 5 % phenyl methyl siloxone (J&W Scientific Products GmbH, Koln, Germany; part # 122-5032) was used. The gas carrier was helium at a flow rate of 1 ml/min, and the injection port temperature was 250°C. The oven temperature was 100°C initially and maintained at this temperature for 2 min, then ramped at 16°C/min to 250°C, held at the latter temperature for 1 min and then ramped to a final temperature of 300°C. Under these conditions, retention times (SIM) were 10.5 min (m/z 152; dwell time = 40 ms), 11 min (m/z 138; dwell time = 40 ms) and 12.4 min (m/z 166; dwell time = 40 ms) for

dihydroartemisinin, ARM and artemisinin (internal standard) respectively. The within-run coefficients of variation for the assay were 3.7% and 7.8% at 35 nmol/L and 352 nmol/L respectively for DHA and 3.8% and 6.1% at 67nmol/L and 671nmol/L respectively for ARM. Similarly, between-run coefficients of variation were 16.0%, 13.5% and 8.7% at 8 nmol/L, 39 nmol/L and 176 nmol/L respectively for DHA and 13.0% and 6.4% at 70 nmol/L and 185 nmol/L respectively for ARM. The limit of quantitation was 40 nmol/L for ARM and 3 nmol/L for DHA. Quality control samples were included with each assay batch, with a requirement that the run was acceptable only if the QC was within ± 15% of the nominal value.

### 2.4.2.2 Piperaquine

Plasma piperaquine concentration assay

Piperaquine concentrations were determined using solid phase extraction (SPE) and liquid chromatography (LC) (N Lindegardh, *et al.*, 2003). Plasma (0.5 mL) was precipitated with 1 mL acidic acetonitrile (acetonitrile-acetic acid 1%, 85:15 v/v) and vortex mixed for about 10 seconds. Five hundred μL phosphate buffer (pH 4 0.005M) containing internal standard (1000 nmole/L) was added and the tubes were vortex mixed again for about 10 seconds and left at room temperature for 10 minutes. The microtubes were centrifuged at 13 000 g and the supernatant was transferred to a polypropylene tube and loaded onto a PRS SPE column (1mL, 100 mg, Argonaut Ltd, Hengoed, Glamorgan, UK). The SPE column had prior to loading been activated with methanol and conditioned with acidic acetonitrile (acetonitrile-acetic acid 1%-water, 42.5:7.5:50 v/v). The SPE columns were washed with 0.5 mL water and 2 mL methanol-phosphate buffer (pH 2.5 0.1M) (80:20, v/v) before eluted with 2 mL methanol-triethylamine (98:2, v/v). The SPE eluates were evaporated, reconstituted and injected into a LC-system using a Chromolith Performance

(VWR International, Darmstadt, Germany) and a mobile phase containing acetonitrile-phosphate buffer pH 2.5 0.1M (8:92, v/v). The total precision for all quality controls during the analysis was 8.4% and 5.4% at 230 nmole/L and 1350 nmole/L respectively.

### 2.4.3 Pharmacokinetic analysis

#### *2.4.3.1 Uncomplicated malaria*

Pharmacokinetic parameters were determined by non-compartmental analysis using STATA (release 7; Stata corporation 2001, Texas). The $AUC_{0\to24}$ was calculated using the trapezoid method in which missing drug concentrations were interpolated from the neighbouring points using the straight line. Maximum observed concentration Cmax and observed time to Cmax, Tmax were determined from observed data. Terminal elimination half-life $t_{1/2elim}$ was determined from the log linear fit to the final serial plasma concentrations. Compartmental models were fitted by weighted iterative least squares regression. The fundamental parameters derived were: absorption rate constant (Ka), apparent clearance (CL/F) and apparent volume of distribution (V/F), where F is the fraction of drug absorbed. In addition to the conventional compartmental and non compartmental analyses a population pharmacokinetic analysis was also conducted for piperquine data.

In the population approach, inter-subject variability in the pharmacokinetic parameters were modelled with log-normal error models, i.e $(CL/F_i)=(CL/F) \exp ( \eta_i^{CL/F})$, where $CL/F_i$ is the pharmacokinetic parameter for the $i^{th}$ individual, CL/F is the population mean, $\eta_i^{CL/F}$ is the random effect with zero mean and variance $\sigma_{CL/F}$, which represents the inter-subject variability for the parameter.

To explain inter-subject variability, a number of covariates were investigated: presence of parasitaemia at day 3, weight, and gestational age. Weight and gestational age were

centred around their median values so that the population estimates would represent those of an average patient.

The log of likelihood function, Akaike information criterion, and Schwartz criterion were used to determine the models that best fitted the data, firstly between different pharmacokinetic models and then between models with different covariates. The latter were compared using the backward elimination procedure. The goodness of fit of each model was also assessed by the examination of the scatter plots of residuals versus predicted drug levels. The actual time of the sampling was used in the analysis. All compartmental analysis was performed using the S plus programme (SPLUS 6.0 for Windows, Mathsoft, Inc).

### 2.4.3.2 Severe malaria studies

Data were plotted graphically and analysed using WinNonlin® 3.1 (Pharsight, Mountain View, CA, USA). Where possible, one- or two-compartment open models were fitted to the plasma concentration-time profiles. The final choice of model was based on the Akaike Information Criterion, and on the distribution of residuals. Conventional pharmacokinetic parameters (area under the plasma concentration time curve [AUC] from time to infinity [AUC $_{0-\infty}$I; with extrapolation to $\infty$ $C_{last}/\lambda z$, where $C_{last}$ is the final measured concentration and $\lambda z$ is the terminal elimination rate constant], $t_{1/2}$, apparent clearance after intramuscular administration [CL or $Cl_{i.m.}/f$], volume of distribution at pseudo-distribution equilibrium (Vd), maximum concentration in plasma [Cmax] and the time [Tmax] to Cmax were determined from the plasma concentration-time data by both compartmental and noncompartmental analysis. The estimates of pharmacokinetic parameters for DHA assumed complete conversion of ARTS to DHA as reported previously (TM Davis, *et al.*, 2001).

## 2.4.4 Statistical Analysis

The pharmascokinetic variables were analysed using the computer package SPSS for Windows (version 10.0; SPSS Inc., Chicago, Ill.). Data are presented as means ± standard deviation (SD) or geometric means (SD range). Two-sample comparisons were by Student's t-test or Chi-squared test as appropriate. A two-tailed level of significance of 0.05 was used throughout.

# CHAPTER 3

# PHARMACOKINETICS

In order to optimise treatment the pharmacokinetic properties of a drug need to be characterised. For the immediate future the artemisinin compounds are our most important compounds. Artemisinin derivatives have an excellent safety profile in humans documented in a large number of randomised controlled trials and more extensive community use. Dihydroartemisinin (DHA) is the active metabolite of artesunate and artemether, and is manufactured as an oral antimalarial drug in China. Artesunate is more widely used. When taken orally artesunate is almost entirely hydrolysed to DHA, and so has equivalent therapeutic efficacy. There are very few data on the pharmacokinetic properties of the artemisinin derivatives in severe malaria, where there are different concerns to uncomplicated malaria. In severe malaria it is critical that therapeutic concentrations are achieved rapidly and so absorption parameters are critical. This is particularly relevant to intramuscular or rectal administration. As artemther is an oil-based intramusculary administered drug it was necessary to study particularly its absorption profile.

Dihydroartemisinin-piperaquine is an artemisinin-containing fixed combination antimalarial drug developed in China. It is being used increasingly in South East Asia.

and is now part of first line treatment in Vietnam. It has tremendous potential for worldwide use. Piperaquine, an orally active bisquinoline discovered by Rhône-Poulenc in the early 1960s was developed for clinical use in 1973. It is structurally related to chloroquine with a similar mechanism of action through the chemical inhibition of parasite haem detoxification (K Raynes, *et al.*, 1995). Piperaquine phosphate (PQP) replaced chloroquine as the recommended treatment for falciparum malaria in China in 1978 and was used extensively for mass prophylaxis and treatment. Reported side-effects are generally similar to those observed with chloroquine although pruritus is uncommon (MB Denis, *et al.*, 2002)

## 3.1 Pharmacokinetics of dihydroartemisinin-piperaquine (DHA-PQP)

### 3.1.1 Introduction

The pharmacokinetic properties of the oral artemisinin derivatives are now well established. All compounds are rapidly absorbed and rapidly eliminated. Although metabolic biotransformation of artemether to dihydroartemisinin via CYP 3A4 maybe affected by malaria this does not have a significant effect on bioactivity. Artesunate is readily and spontaneously hydrolysed to DHA. The DHA is then inactivated by glucuronidation. For piperaquine there are few data; the estimated terminal elimination half-life in human adults has been estimated only once and was reported as at 17 days, but precise pharmacokinetic data in man are not yet available (TY Hung, *et al.*, 2003).

### 3.1.2 Patients and Methods

#### 3.1.2.1 Patients

We recruited patients who were admitted to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, and had microscopically confirmed uncomplicated falciparum

malaria. On admission, a full clinical examination was performed and blood samples were taken for full blood count, biochemistry, parasite count, and baseline antimalarial drug assay.

Patients were randomly allocated either

    i)   regimen 1: DHA-PIP (Tonghe Pharmaceutical, Yuzhong Chongqing, People's Republic of China) four tablets at 0 h then 2 tablets at 24 and 48 h

   OR

    ii)  regimen 2: two tablets at 0 h, 6 h, 24 h, and 48 h.

DHA-PIP contains 32 mg dihydroartemisinin, and 320 mg of piperaquine per tablet.

Drugs were kept in identically numbered opaque envelopes, and drug administration was directly observed in all cases.

Blood samples were taken at 0, 2, 4, 6, 24, 26, 48, 50, 72, 96, 120, 144 hour and then on day 28 and 56. Vital signs were assessed 6 hourly and before taking blood samples. All blood samples were centrifuged within 10 minutes and separated plasma stored at $-20^{\circ}$C until sending to Sweden for assay.

The study was approved by the Ethical and Scientific Committee of the Hospital for Tropical Diseases. We obtained written informed consent from patients or their guardians.

### 3.1.2.2 Methods

Pharmacokinetic parameters of piperaquine were determined by non-compartmental analysis using STATA. The $AUC_{0\to\infty}$ was calculated from the composite of area under the concentration-time curve (AUC) from 0 hours (time of first dose) to last drug measurement using cubic spline and from extrapolating the log-linear fit to the last 3 points on the curve. The $AUC_{0\to 48}$ was calculated using the cubic spline for concentrations measured between time 0 and 48 hours. AUCs and other pharmacokinetic

parameters were summarised using median and 90% range and were compared between the two treatment regimens using the nonparametric Mann-Whitney test.

Piperaquine pharmacokinetic characteristics were also examined using compartmental analysis in which the population approach was employed. Two compartment and three compartment models with first order absorption and first order elimination were considered.

To explain inter-subject variability, a number of covariates were investigated: temperature, parasitaemia, weight, and age, measured at the start of the treatment (day 0). All covariates were centred around their median values so that the population estimates would represent those of an average patient.

The log of likelihood function, Akaike information criterion, and Schwartz criterion were used to determine the models that best fitted the data, firstly between different pharmacokinetic models and then between models with different covariates. The latter were compared using the backward elimination procedure. The goodness of fit of each model was also assessed by the examination of the scatter plots of residuals versus predicted drug levels.

The actual time of the sampling was used in the analysis. All compartmental analysis was performed using the S plus programme (SPLUS 2000 for Windows, Mathsoft, Inc), while the population analysis used the NLME (non-linear mixed effect) procedure

### 3.1.3 Results

A total of 25 patients with uncomplicated falciparum malaria were recruited into the study. Two of them were excluded because of their interruption of the scheduled follow-up. Details of admission characteristics are summarized by group of treatment in table 3.1.1. All of the recruited patients had a negative blood smear at the last visit on day 56

Table 3.1.1: Baseline clinical characteristics of patients including in piperaquine PK study.

| Parameters | | Group 1 (n = 10) | Group 2 (n = 13) | p value |
|---|---|---|---|---|
| Age (years) | mean | 29.4 | 30.7 | 0.83 |
| | 95% CI | 22.1-36.7 | 22.5-38.9 | |
| Sex | male/female | 10/0 | 11/2 | 0.48[†] |
| Weight (kgs) | mean | 54.8 | 46.9 | 0.19 |
| | 95% CI | 45.5-64.1 | 44.1-49.6 | |
| Temperature ($^{o}$C) | mean | 38.5 | 38.7 | 0.48 |
| | 95% CI | 37.8-39.1 | 38.3-39.1 | |
| Hct (%) | mean | 35.9 | 35.0 | 0.83 |
| | 95% CI | 32.5-39.2 | 30.4-39.6 | |
| White blood cell count $10^6$/L | mean | 6,544 | 5,571 | 0.74 |
| | 95% CI | 3,868-9,221 | 4,317-6,826 | |
| BUN (mmol/L) | mean | 4.28 | 5.77 | 0.002 |
| | 95% CI | 3.77-4.79 | 4.91-6.62 | |
| Creatinine ($\mu$mol/L) | mean | 83.0 | 73.2 | 0.93 |
| | 95% CI | 67.2-98.8 | 71.6-89.3 | |
| Glucose (mmol/L) | mean | 6.2 | 6.6 | 0.48 |
| | 95% CI | 5.16-7.29 | 5.56-7.64 | |
| Parasite count on admission (/$\mu$L) | geometric mean | 10,836 | 16,358.3 | 0.88 |
| | 95% CI | 14,270-102,150 | 20,092-70,267 | |

Mann Whitney U test used in all tests except where specified; † Fisher's exact test

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Table 3.1.2: Clinical outcomes and cure rates of patients by groups of treatment piperaquine PK study.

| Outcomes | | Group 1 (n = 10) | Group 2 (n = 13) | P value |
|---|---|---|---|---|
| Fever clearance time (hrs) | Mean | 15.6 | 19.4 | 0.32 |
| | 95% CI | 5.7-25.6 | 11.8-27.0 | |
| Parasite clearance time (hrs) | Mean | 43.2 | 48 | 0.52 |
| | 95% CI | 23.7-62.7 | 33.5-62.5 | |
| Cure rate (/ observed 56 day follow-up) | | 9/9 | 9/9 | $1.0^{\dagger}$ |

Mann Whitney U test used in all tests except where specified; † Fisher's exact test

There were 23 patients who completed the pharmacokinetic sampling, 10 of them received regimen 1 (3 doses: 1280, 640, 640mg of piperaquine phosphate at 0, 24,48h) and 13 received regimen 2 (4 doses of 640 mg of piperaquine phosphate, at 0, 4, 24, 48h). In total, there were 299 drug level measurements, median (range) of 14 (7-15) measurements per patient. The times of sampling were for each patient at: 0, 2, 4, 6, 24, 26, 48, 50, 72, 96, 120, 144, and then in intervals: 284-362h for 21 patients, 640-832h for 8 patients and 1309- 1536h for 16 patients.

The plasma piperaquine concentration-time profile is showed separately by regimens group in Figure 3.1.1.

Figure 3.1: The plasma piperaquine concentration-time profile is showed

separately for the two regimens
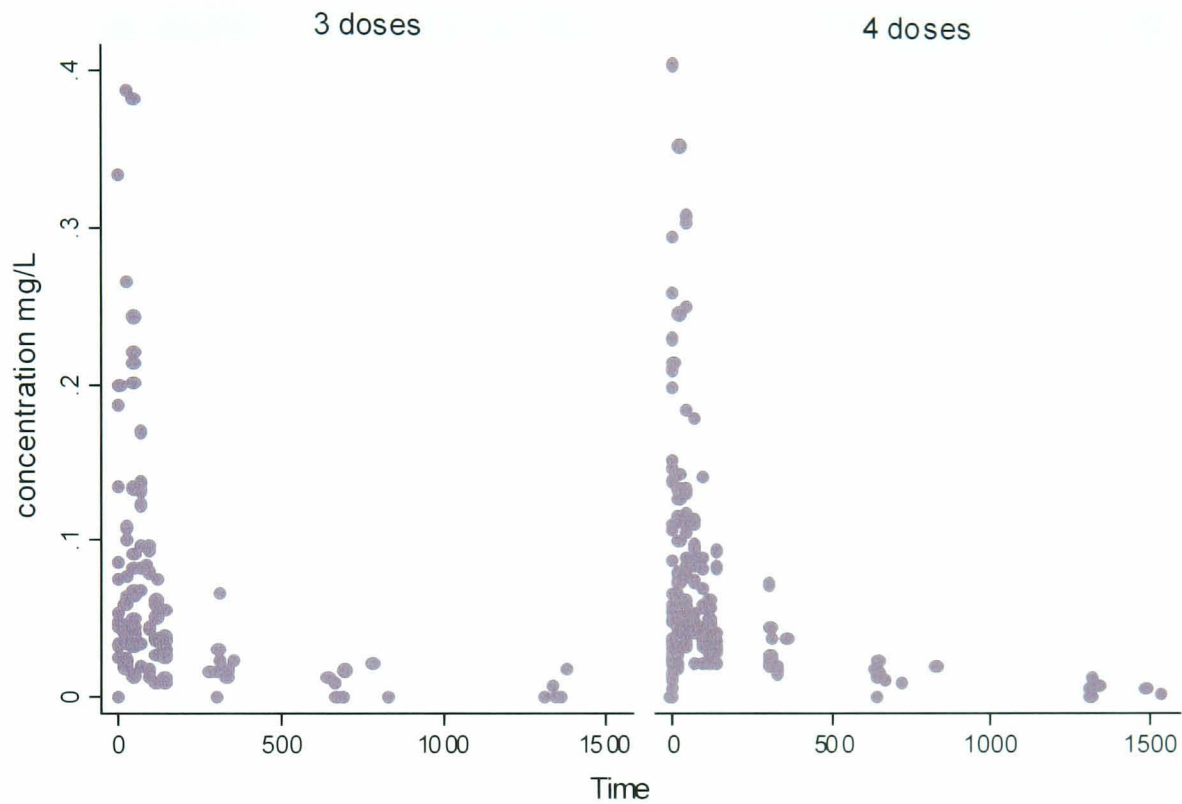


Drug levels were measured in nmole/L and were converted to µg/L by multiplying by

factor 0.53551 (535.51 is the molar weight of piperaquine base).   Dose of x µg of

piperaquine phosphate corresponds to the 0.58x µg of the piperaquine base (0.58 =

535.51/927.42 = molar weight of piperaquine base/ molar weight of piperaquine

phosphate).

Table 3.1.3: Pharmacokinetic parameters derived from the non-compartmental analysis of two regimen groups in piperaquine PK study.

| | Group 1 (n=10) | Group 2 (n=13) | p-value* |
| --- | --- | --- | --- |
| | Median (90% range) | Median (90% range) | |
| T1/2 elim (days) | 14.5 (1-67) | 24 (3-30) | 0.121 |
| Ke (h$^{-1}$) | 0.002 (0.0004- 0.024) | 0.001 (0.00095-0.009) | 0.121 |
| Cmax (mg/L) | 174 (77-387) | 215 (45-406) | 0.577 |
| Tmax (h) | 50 (26-50) | 6 (4-50) | 0.035 |
| AUC 0→∞ (µg h/L) | 22611 (4395-81018) | 39468 (11697-62331) | 0.063 |
| AUC 0-48 (µg h/L) | 3263 (1386-15386) | 5007 (1982-17333) | 0.133 |

* Mann-Whitney test

t½ elim: half life, Ke(h-1): rate constant of elimination, Cmax: peak plasma concentration, Tmax: time of peak concentration, Vd: total apparent volume of distribution, k10: first order elimination rate constant, AUC: area under the plasma concentration-time curve.

## Population approach

Two and the three compartment models were examined. Although it looks that data may support the three compartment model, there is not enough data to fit such a model. Especially the second phase of elimination (around 200 hours and 500 hours) is not measured in this dataset. For these reasons, the three compartment model has been abandoned.

A two-compartment model with first order absorption and first order elimination was selected as the kinetic model. The fundamental parameters used to characterize the two-compartment model were: absorption rate constant (Ka), apparent clearance (CL1) from the central compartment, apparent clearance from the peripheral compartment (CL2), volume of the "central" compartment (V1), volume of the "peripheral" compartment (V2). Absorption rate constant Ka was only considered as a fixed effect because of limited individual data to characterize this phase. Random effects were considered for the log of the CL1, CL2, V1 and V2 and a number of explanatory variables were examined in order to explain the random-effect variation. So in general, the model was:

$$Log(CL1)ij = (\beta1 + b1i) + \beta2\ X1 + \beta3\ X2 + \beta4\ X3 + \beta5\ X4$$

$$Log(CL2)ij = (\gamma1 + b1i) + \gamma2\ X1 + \gamma3\ X2 + \gamma4\ X3 + \gamma5\ X4$$

$$Log(V1)ij = (\delta1 + b1i) + \delta2\ X1 + \delta3\ X2 + \delta4\ X3 + \delta5\ X4$$

$$Log(V2)ij = (\eta1 + b1i) + \eta2\ X1 + \eta3\ X2 + \eta4\ X3 + \eta5\ X4$$

where X1-X4 are the explanatory variables (temperature, parasitaemia, weight, and age, measured at the start of the treatment ).

When the standard model was fitted assuming the within-group errors to be $\varepsilon \sim N\ (0, \sigma2I)$, the pattern was observed in residuals plot. Therefore heteroscedacity (within-group

variance) was modelled proportionally to the fitted values, first assuming the linear relationship, and then using the best fitting power function.

The best fit to the data was obtained with a two-compartment model with only log (V1) fitted as random effects and the variance increasing linearly with the fitted values (Table 3.1.2). None of the measured covariates explained any significant proportion of the variation in the random effects. However, the random effects were very small, a reason for which may be a sparse nature of the data.

Table 3.1.4: Estimates of piperaquine population pharmacokinetic parameters

| Parameter | Estimate (95% CI) |
| --- | --- |
| Ka (h$^{-1}$) | 1.51 (0-4.009) |
| CL (L/h/kg) | 0.760 (0.574-1.007) |
| CLD (L/h/kg) | 2.272 (1.707-3.022) |
| V1 (L/kg) | 153.449 (105.758-222.680) |
| V2(L/kg) | 299.692 (204.043-440.171) |
| $\omega$V1 | 0.81 |
| $\sigma$ residual | 143 |
| AUC ($\mu$g h/L) | 41892 |
| T1/2 abs(h) | 0.46 |
| Ke (h$^{-1}$) | 0.0017 |
| T$_{1/2}$ elim (days) | 17 |

Ka – absorption constant, CL - total clearance, CLD - inter-compartmental clearance, V1- volume of the central compartment, V2 – volume of the peripheral compartment, V=V1+V2 – total volume, AUC=Dose/CL – area under the time –concentration curve, T1/2 abs = 0.693/Ka – half life of absorption, Ke = CL/V – elimination constant, T1/2 elim = 0.693/Ke – half-life of elimination.

Figure 3.2: Individual predicted concentration levels from the model (triangles) and the observed levels of piperaquine (circles) for concentrations measured in the first 100 hour
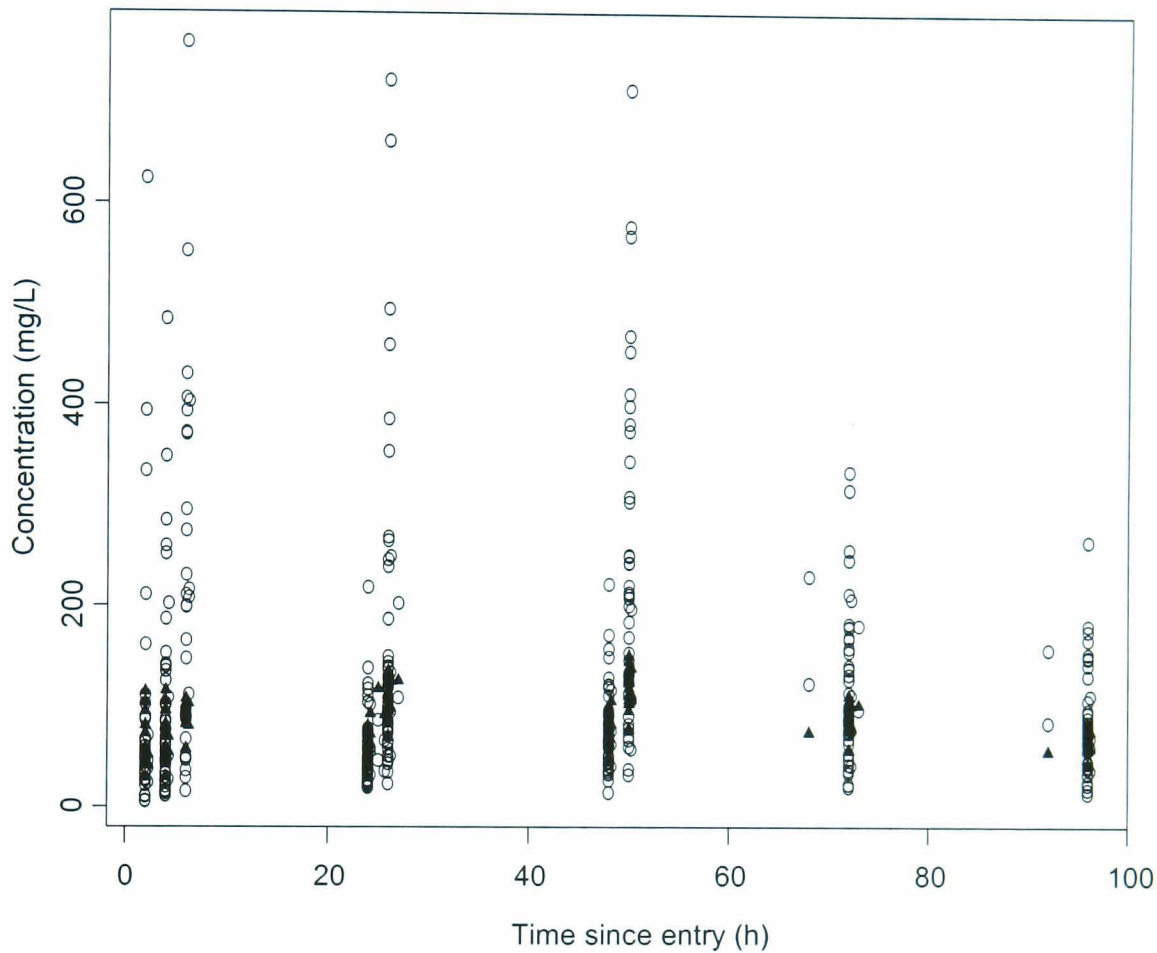


## Final elimination stage

Later points in the concentration-time curve, after the absorption and distribution phase finished, describe the elimination process. A straight line may be fitted to the log-transformed data and the slope of this line estimates the elimination rate constant.

For four patients final measurements were negative so in the analysis (table 3) they were excluded. When these samples were included in the analysis as very low levels (0.05) the median (95%CI) elimination rate did not change much: 0.001525 (0.0011021 - 0.0041917). From this, we conclude that median elimination rate is Ke=0.001, which corresponds to the estimated elimination half-life of 29 days.

Table 3.1.5: Slope of the final curve corresponding to the final elimination stage; calculated assuming first order elimination process starting at different time points (a) 200 hours (b) 400 hours in piperaquine study.

| Patient ID | (a) Time>200 | (b) Time>400 |
|---|---|---|
| 4 | 0.00075 | |
| 8 | 0.00043 | 0.00026 |
| 11 | 0.00199 | 0.00088 |
| 12 | 0.00116 | 0.00064 |
| 13 | 0.00200 | 0.00122 |
| 14 | 0.00095 | 0.00071 |
| 15 | 0.00146 | |
| 16 | 0.00127 | 0.00272 |
| 17 | 0.00026 | |
| 19 | 0.00159 | 0.00087 |
| 20 | 0.00121 | 0.00092 |
| 21 | 0.00177 | 0.00131 |
| 22 | 0.00100 | 0.00070 |
| 24 | 0.00165 | |
| 25 | 0.00104 | 0.00090 |
| Median. | 0.00121 | 0.00088 |
| (95%CI) | 0.0009589 - 0.0016393 | 0.0006828 - 0.0012459 |
| 90% range | 0.00026 - 0.002 | 0.00026 - 0.00272 |

Figure 3.3: The standardised residuals from population model.
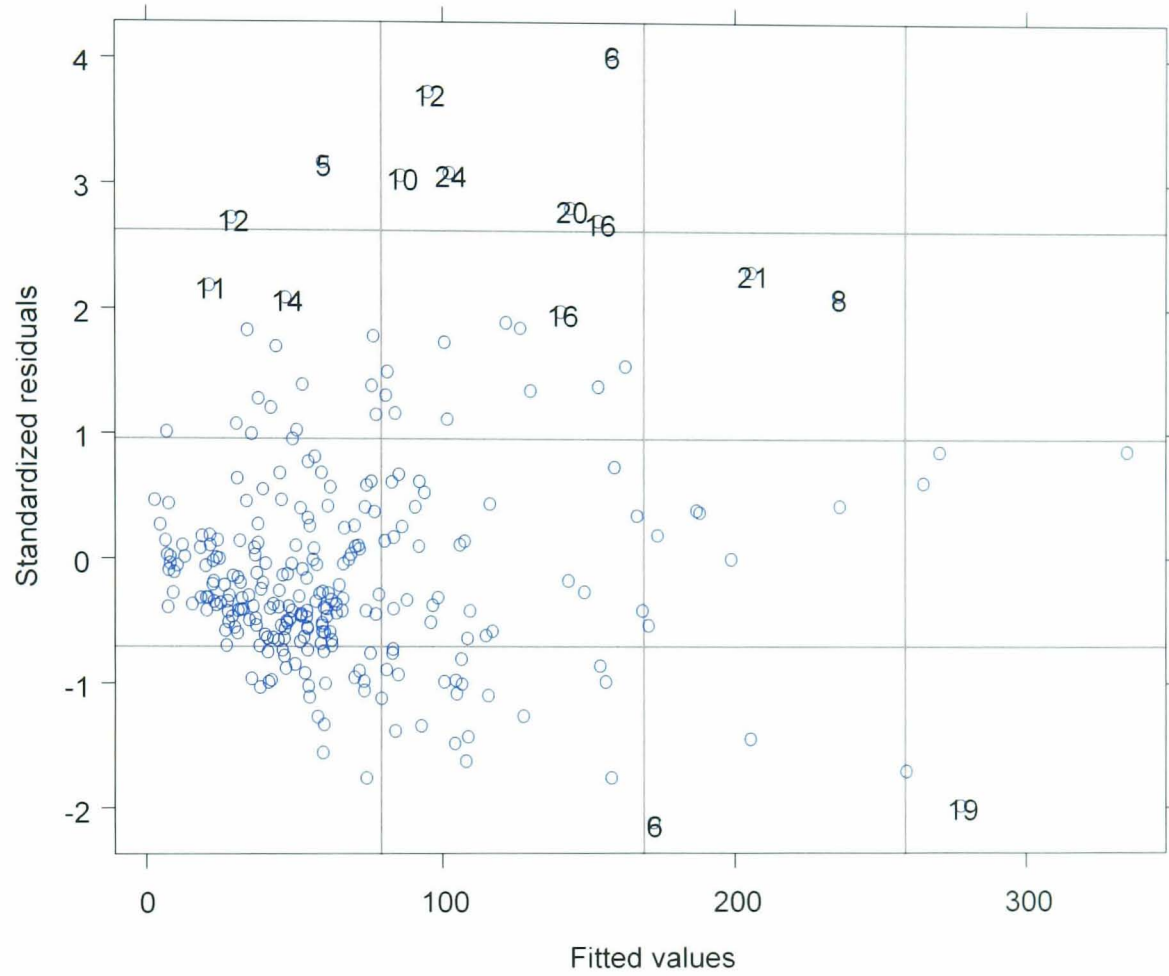


Numerical label: patient ID for whom the absolute value of residual was greater than 2

## 3.1.4 Discussion

The pharmacokinetic properties of piperaquine are very similar to those of chloroquine, as might have been predicted from their structural similarity. There is considerable inter-individual variation in peak concentrations which presumably reflects the relatively small relative volume of the central compartment, and thus the dependence of peak concentration on rate of absorption. But, this is probably of no clinical relevance as no toxicity has been demonstrated. At this stage the main antimalarial effect is mediated by DHA. The terminal elimination phase is critical for antimalarial effect. The estimated elimination half-life of approximately one month may still prove to be an underestimate with further assay refinement. The longer half-life in these studies than reported previously almost certainly reflects greater assay sensitivity and thus better characterization of the terminal elimination phase of a multi-exponential decline. As might be expected this phase is unaffected by severity of malaria, as it takes place mainly after recovery. With drugs such as chloroquine it may not be possible to characterize adequately in a multi compartmental model each distinct phase of distribution and elimination. But unless there are major toxicity concerns, it is of greatest clinical relevance to concentrate on the elimination phase, as this is the phase where piperaquine must eradicate the individual infection, suppresses reinfections, but may also select for resistance. The very long elimination phase may well explain why those few recrudescences which do occur arise more than six weeks after treatment. More work is now needed to determine the in-vivo MIC and thus the duration of post treatment prophylaxis that might be expected in a high transmission setting.

## 3.2 Comparative Pharmacokinetics of IM Artesunate and Artemether in Severe Falciparum Malaria

### 3.2.1 Introduction

Severe malaria kills between one and two million people each year. The qinghaosu derivatives artesunate (ARTS) and artemether (ARM) are being used increasingly for the parenteral treatment of severe falciparum malaria (NJ White, 1996, NJ White, 1998). They are intrinsically more potent as antimalarials, have a broader stage specificity of action against the malaria parasite(F ter Kuile, *et al.*, 1993) and are simpler to administer and safer than parenteral quinine (Artemether-Quinine-Meta-analysis-Study-Group, 2001, TT Hien, *et al.*, 1996, MB van Hensbroek, *et al.*, 1996). Artemether has been more intensively evaluated, although artesunate is more widely used. ARM is formulated in an oil base because of its poor water solubility and can only be injected by the intramuscular (i.m.) route.   There is evidence that i.m. ARM may not be absorbed adequately in severely ill children with falciparum malaria who are hypotensive or acidotic (MN Mordi, *et al.*, 1997) . Nevertheless, i.m. ARM has proved as effective as parenteral quinine in the largest randomized trials ever conducted in severe malaria (TT Hien, *et al.*, 1996, MB van Hensbroek, *et al.*, 1996), and in adult patients with multi-organ dysfunction ARM was therapeutically superior (Artemether-Quinine-Meta-analysis-Study-Group, 2001). ARTS is a hemisuccinate artemisinin derivative formed by the addition of sodium bicarbonate to lyophilised artesunic acid. ARTS is water-soluble, and can be given by either intravenous (i.v.) or i.m. injection.   Both these routes of administration give rapid therapeutic responses in severe malaria (TT Hien, *et al.*, 1992b). Data from patients with uncomplicated malaria suggest that i.m. ARTS is well absorbed (KF Ilett, *et al.*, 2002) but there have been no detailed pharmacokinetic studies in severely ill patients, and, despite

extensive use, there have been no large clinical trials comparing the efficacy of ARTS with other antimalarial drugs in severely ill patients.

In areas of the rural tropics where health care facilities are basic, i.m. injection of an artemisinin derivative may represent the best therapeutic option for severe malaria. We have, therefore, investigated the pharmacokinetic properties of ARM and ARTS given by intramuscular injection in severely ill Vietnamese adults with falciparum malaria.

## 3.2.2 Patients and methods

### *3.2.2.1 Patients*

We studied 19 adult patients between June 1998 and September 1999. All were admitted to the Malaria Ward at the Centre for Tropical Diseases, Ho Chi Minh City with microscopically confirmed severe falciparum malaria. The diagnosis of severe malaria was based on modifications of World Health Organisation criteria reported previously (WHO, 1990) (TT Hien, *et al.*, 1996). Briefly, severe malaria was defined as one or more of the following a) Glasgow Coma Score (GCS) <11, b) jaundice (serum bilirubin >50μmol/litre; serum aspartate aminotransferase (SGOT) more than twice the upper limit of the reference range), c) acute renal failure (serum creatinine >250 μmol/L), d) anemia (venous hematocrit <15%), e) hyperparasitemia (>250,000 asexual forms/μl whole blood, f) plasma lactate >4.0 mmol/L. Subjects who had been treated with an artemisinin derivative within 24 h were excluded, as were pregnant women and children under the age of 14 years. All patients or their attendant relatives gave informed consent to the study which was approved by the Ethical and Scientific Committee of the Hospital for Tropical Diseases and the Health Services of Ho Chi Minh City, Viet Nam.

### 3.2.2.2 Methods

### Clinical procedures

On admission, a full clinical examination was performed and blood samples were taken for full blood count, biochemistry, blood culture, parasite count, and baseline antimalarial drug assay. An indwelling intravenous catheter was inserted into the antecubital vein of the arm. Patency was maintained by flushing with small volumes of heparinised saline. Patients were randomised to receive either

i) ARTS (Guilin No 2 factory, Guangxi, PRC) 2.4 mg/kg i.m. stat followed by 1.2 mg/kg i.m. daily (corresponding to 6.25 and 3.13 μmol/kg respectively). The injection volumes were all 5mL.

or

ii) ARM (Kunming Pharmaceutical factory, Kunming, PRC) 3.2 mg/kg i.m. stat (10.7 μmol/kg; injection volume 0.04mL/kg) followed by 1.6 mg/kg i.m. daily (5.4 μmol/kg respectively; injection volume 0.02mL/kg) .

All injections were given to the anterior thigh. After treatment was initiated, vital signs and parasitemia were monitored four hourly (or more frequently if indicated clinically) for 24 h, and then six hourly. Complications were managed as described previously (TT Hien, *et al.*, 1996)

Blood samples were taken at 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 min then at 2.5, 3, 3.5, 4, 5, 6, 8 and 10, or 10 and 12 h following drug administration. In patients randomised to ARM, initial plasma concentration profiles from the first 5 patients (last sample 10 h in two patients and at 12 h in three patients) showed that further samples were needed to characterise the terminal elimination phase, and hence sampling in this group was extended mid-way through the study to include 12, 18, 20 and 24 h. At each

time point, 4 mL of whole blood was collected in lithium heparin tubes (L.I.P Northampton UK). These samples were centrifuged immediately at 1500rpm for 5 minutes at 4°C and aliquots of separated plasma and frozen at −80°C in Corning 1.8ml cryotubes (Corning BV Life Sciences Netherlands) until analysed.

Thick blood films were stained with Giemsa. The number of asexual parasites per microlitre of blood was determined by counting the white blood cells (WBC) in a high powered field containing a total of 500 parasites in which the ratio of parasites/WBC was less than 1. The parasitemia was calculated as the product of the parasite/WBC ratio and the WBC count. The time to a 50% reduction in the original parasite count was determined by linear interpolation of the parasite count-time data. Fever clearance time was taken to be the time of the first two axillary temperature readings <37.5°C.

*Analysis of ARTS, DHA, and ARM:* as described in chapter 2

### 3.2.3 Results

Of the total of 19 patients studied, 9 were allocated to ARTS and 10 to ARM. There were no adverse reactions to either drug. One patient received peritoneal dialysis in the ARM group. Two patients died, both from the ARM group. The first, a 34 year old male was admitted obtunded (GCS 7), jaundiced, acidotic (plasma lactate 11 mmol/L), in acute renal failure, and in shock. He died 39 h after admission. The parasitemia (proportion of red cells parasitised) was 46% on admission, rose to a peak of 61% at 4 hours, and was still above the admission value at 24 hours. Plasma ARM levels remained below 100 nmol/L during the first 10 h following admission. The second, a 42 year old male, was admitted jaundiced, comatose (GCS 6) and in shock, and died after 30 hours post

admission. The admission parasitemia was 47%, falling to 2% at 24 hours. Plasma ARM levels remained below 20 nmol/l for the first 10 h, and did not rise above 50 nmol/L during the first day. The other 17 patients made an uneventful recovery. The median (range) times to fever and parasite clearance time were similar in the two groups: 34 (15-60) h and 48 (21-60) h respectively in the ARTS group and 35 (15-60) h and 48 (30-60) h in the ARM group respectively. The baseline characteristics of the patients in the two groups are summarized in table 3.2.1

### *3.2.3.1 Pharmacokinetics of intramuscular artesunate*

ARTS was absorbed very rapidly with peak concentrations measured in the first sample (5 min) in 5 of the 9 patients. A one-compartment model was fitted satisfactorily to the plasma concentration-time data for both ARTS and DHA (Figure 3.2.1). No significant improvements in fit were obtained using a two- compartment model, with mean Akaike information criteria for one and two compartmental models 101 and 95 respectively (95% CI for the difference -4.3 to 16.8).

Table 3.2.1: Patient characteristics on admission artesunate –artemether PK study

| Parameter | ARM | ARTS | p |
|---|---|---|---|
| Number of patients (male) | 10 (6) | 9 (7) | |
| Mean age (years) | 36.7 | 31.6 | 0.47 |
| Range | 24 - 60 | 17 - 62 | |
| Mean temperature on admission ($^{\circ}$C) [SD] | 38.9 [1.1] | 38.7 [1.0] | 0.73 |
| Mean hematocrit (%) [SD] | 33.0 [8.9] | 33.8 [9.6] | 0.83 |
| Mean white blood cell count ($10^9$/L) [SD] | 8.4 [3.5] | 7.4 [2.4] | 0.52 |
| GCS <8 | 1 | 0 | 1.0[b] |
| GCS 8-11 | 2 | 0 | 0.4[b] |
| GCS 12-15 | 7 | 9 | 0.2[b] |
| Serum creatinine (μmol/L) [SD] | 106.0 [36.3] | 185.6 [238.6] | 0.32 |
| Blood lactate (mmol/L) [SD] | 5.1 [4.2] | 6.4 [6.7] | 0.67 |
| Total plasma bilirubin (μmol/L) [SD] | 147.0 [119.7] | 63.2 [76.9] | 0.09 |
| Parasitemia on admission /μL[a] (range) | 225,944 (30,144 – 1,775,230) | 57,747 (360 – 816,750) | 0.32 |
| Fever clearance time (hours) [SD] | 56 [34.7] | 65 [67.9] | 0.72 |
| Parasite clearance time (hours) [SD] | 48 [11.5] | 38.2 [13.5] | 0.14 |
| Mortality | 2 (20%) | 0 (0%) | 0.4[b] |

[a] = geometric mean [b] = Fisher 2-tailed results

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Table 3.2.2: Artesunate and dihydroartemisinin pharmacokinetics following intramuscular artesunate injection in severe malaria

| ARTS | | DHA | |
|---|---|---|---|
| Parameter | median (range) | Parameter | median (range) |
| Cmax (nmol/L) | 5,710  (1,362-8,388) | Cmax (nmol/L) | 3,060 (1,718-7,080) |
| Vd (L/kg)[1] | 1.09  (0.47-4.58) | Vd/f (L/kg)[1] | 1.79 (0.63-3.53) |
| k10 (/h) [1] | 1.39  (0.62-120) | Tmax (min) | 35  (10-86) |
| t½ (min) [1] | 30  (67-3.5) | $k_{01}$ (/h)[1] | 2.34  (0.70-17.9) |
| MRT (min) | 43  (5-97) | $k_{10}$ (h)[1] | 0.79  (0.60-1.59) |
| AUC (nmol.h) /L)[2] | 2,228  (533-4,672) | Elimination t½ (min) | 52.7  (69.3-26.2) |
| CLi.m (L/ h /kg) | 2.84  (1.33-11.73) | AUC (nmol.h/L)[2] | 5,262  (3,812-7,436) |
|  |  | CL/f (L/ h /kg/) | 1.18 (1.64-0.84) |

Cmax: peak plasma concentration, Vd: total apparent volume of distribution, k10: first order elimination rate constant, t½: half life, MRT: mean residence time, AUC: area under the plasma concentration-time curve, CL: clearance, Tmax: time of peak concentration, k01: first order formation rate constant, f: fraction of parent drug converted to DHA (assumed to be 100%).

[1]: Estimated from one compartment modeling (DHA values assume 100% bioavailability)

[2]: None of the artesunate AUC was extrapolated (0-10% of the DHA AUC was extrapolated)

Derived pharmacokinetic parameters are summarized in Table 3.3.2. There was a wide variation between individuals in peak plasma ARTS concentrations (range 1,362 to 8,388 nmol/L). Elimination rates were rapid and varied relatively little between patients (c.v. 11.6%). ARTS was converted rapidly to DHA. By the first sample (5 min) DHA levels were a median (range) of 21% (5 to 122%) of the parent compound concentration.The molar AUC values for dihydroartemisinin were almost double those for artesunate (mean (95% CI) difference 727 (321 to 1132) nmol/L.h). DHA was eliminated significantly more slowly (p =0.018). The median (range) estimated elimination half-life for dihydroartemisinin was 52 (26-69) minutes compared with 30 (3-67) minutes for artesunate. These two values were significantly correlated (r = 0.79; p = 0.012)

### 3.2.3.2 Pharmacokinetics of artemether

Plasma concentrations of ARM and DHA fluctuated widely following i.m. injection of ARM (see Figures 2 and 3). As a result, compartmental modeling was not possible. In general the absorption profile was biphasic with a relatively rapid initial absorption phase during the first 30 min, followed by a more gradual rise to a peak concentration at a median of 10 hours (see upper panel, Figure 2). Thereafter, mean ARM concentrations tended to decline slowly, but the levels at 24 h were still higher than those during the first 4 h of sampling. In individual patients (lower panel, Figure 2), peak plasma ARM concentrations ranged between 67 and 1631nmol/L (median 574 nmol/L) and occurred at a median (range) of 10 (1.5 – 24) h. The mean plasma DHA concentration-time profile followed the same pattern as ARM (upper panel, Figure 3) but at much lower concentrations. The mean ratio of parent drug to metabolite varied between 10 and 30 throughout the 24-hour sampling period, consistent with relatively little metabolism of

ARM to DHA. In only 2 patients did peak DHA concentrations exceed 25 nmol/L and in
6 patients, the concentration did not rise above 5 nmol/L.
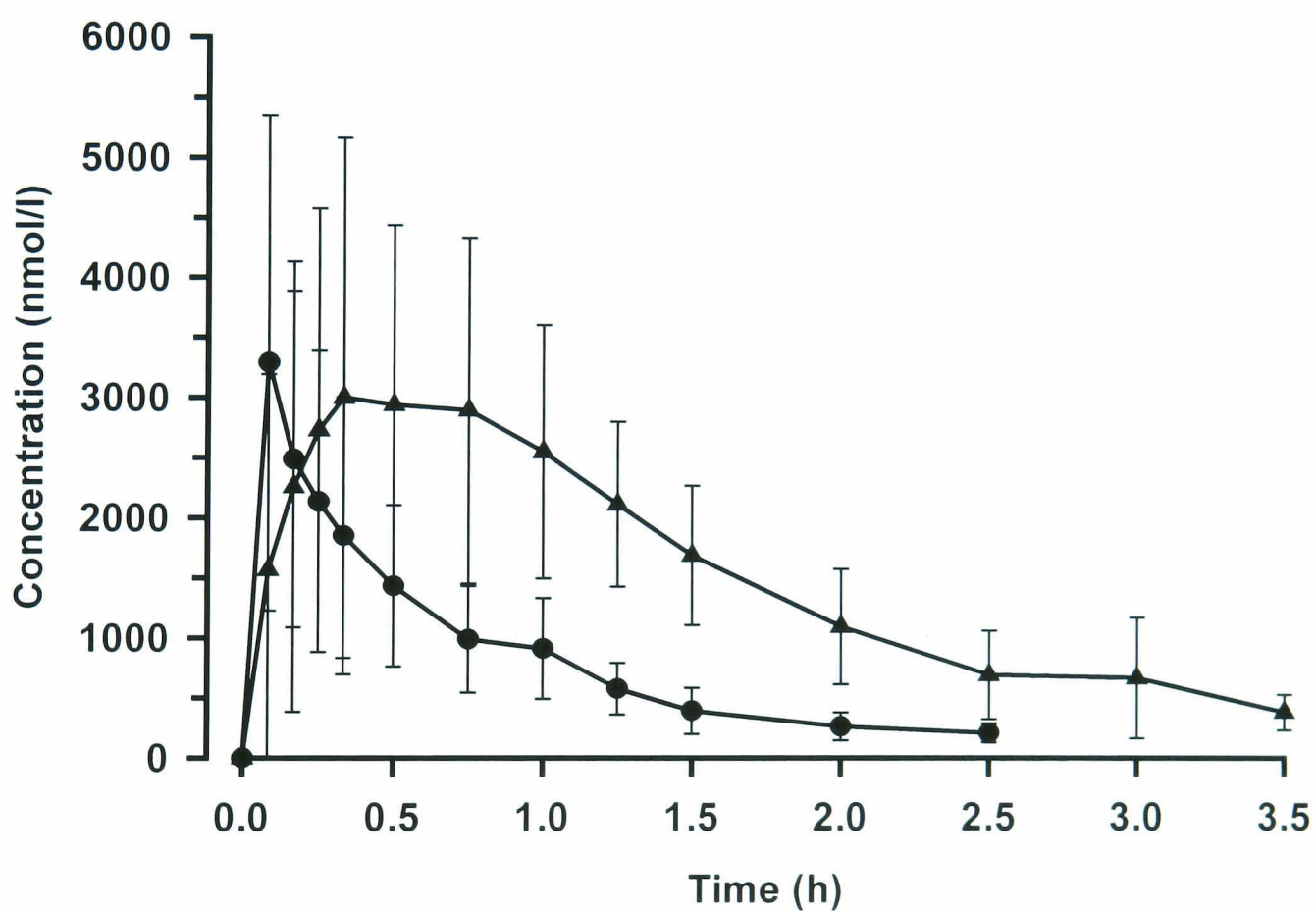


Figure 3.4:  Concentration-time profile for ARTS (•) and its principal biologically active
metabolite DHA (▲) following the first i.m. dose of 6.25 µmol (2.4 mg)/kg of ARTS to 9
patients.  Data are summarized as mean ± SD
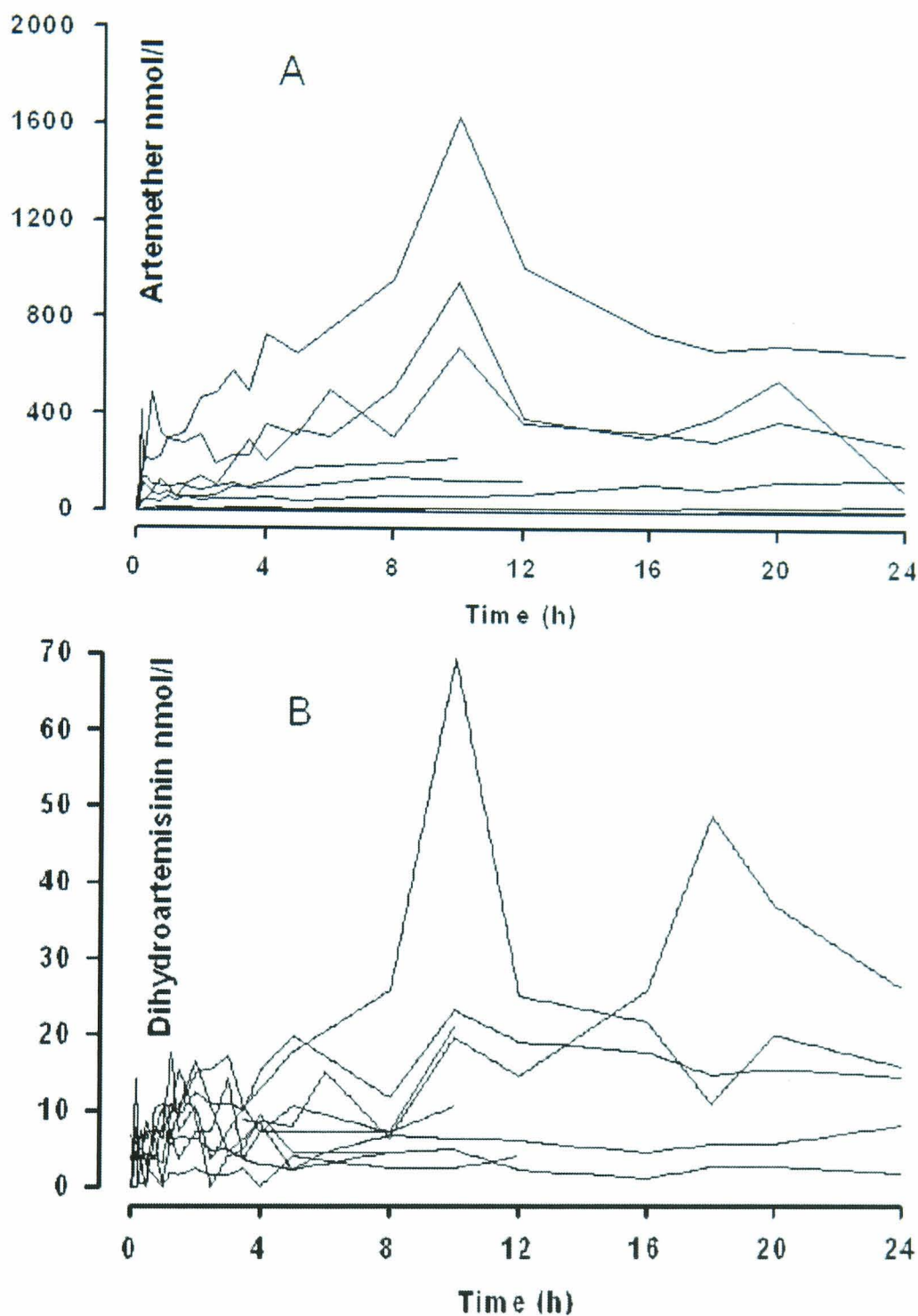
Figure 3.5: Individual concentration-time profiles for ARM (A) and its metabolite DHA (B) following the first i.m. dose of 10.7 μmol (3.2 mg)/kg of ARM to 10 patients with severe *P falciparum* infection. For ARM, two patients had samples collected to 10 h, three to 12 h and five to 24 h. For DHA, two patients had samples collected to 10 h, three to 12 h and five to 24h. In 5 of the profiles some of the concentration-time points shown were undetected (zero), or below the assay limit of quantitation.

## 3.2.4 Discussion

Severe malaria carries a high mortality which can only be reduced by effective antimalarial therapy (WHO, 1990) (NJ White, 1998). It is therefore essential that parasiticidal concentrations of antimalarial drugs are achieved as soon as safely possible following admission to hospital or health clinic. ARM is the most widely assessed of the parenteral artemisinin derivatives. Although it is an oil-based formulation that can be given only by the i.m. route, it has proved at least as effective as i.m. quinine in the treatment of severe malaria in large randomized trials which have enrolled collectively nearly 2000 patients both in Asia and Africa (Artemether-Quinine-Meta-analysis-Study-Group, 2001). The dose regimens recommended for both ARM and ARTS, and used in these studies, were developed empirically before valid analytical techniques were available that allow detailed pharmacokinetic-pharmacodynamic evaluation.

Despite the generally good clinical results with ARM, our data suggest that the absorption of intramuscular ARM is erratic and unpredictable. Indeed the plasma concentration profiles were so variable that we were unable to perform pharmacokinetic modelling. In 2 of the 10 ARM-treated patients studied, one of whom died, plasma concentrations of ARM did not exceed 50 nmol/L within 6 h of dosing. Furthermore, conversion to the more active principal metabolite DHA was minimal, and thus antimalarial efficacy depended almost entirely on the parent ARM.

Although malaria parasites are exquisitely sensitive to artemisinin derivatives, these low levels are very close to the highest reported 50% inhibitory concentrations (IC50) for *Plasmodium falciparum* isolates taken from patients with primary or recrudescent infections on the north-western border of Thailand (up to 60 nmol/L for ARTS, 58 nmol/L for ARM and 29 nmol/L for DHA (A Brockman, *et al.*, 2000), and therefore below the concentrations required for maximum parasiticidal effect. In general in-vitro

minimum inhibitory concentrations are lower than those extrapolated in vivo partly because of differences in protein binding, and because drug concentrations are constant in vitro whereas they fluctuate in vivo. Poor absorption was documented previously in a study in African children with cerebral malaria (TM Davis, *et al.*, 2001), in which the combined plasma levels of ARM and DHA peaked at less than 100 µg/L (336 nmol/L). Taken together these observations give rise to the concern that some severely ill patients may not absorb intramuscular ARM adequately for maximum parasite killing in the critical early hours following the start of treatment. The superior intrinsic antimalarial potency of ARM compared with quinine, therefore, may be counter-balanced by its erratic absorption in the most severely ill patients.

There are few other reports of ARM pharmacokinetics after i.m. injection, mostly in healthy subjects. In one, 8 volunteers received a dose of 300 mg, with a geometric mean Cmax of 540 µg/L (1812 nmol/L) and mean half-lives of absorption and elimination of 2 and 6.9 h respectively (J Karbwang, *et al.*, 1997). The corresponding mean peak for DHA was greater than that of ARM (646 µg/L or 2275 nmol/L) but the elimination t1/2 was of similar magnitude (5.1 h). In another, 8 subjects received 5 mg/kg by i.m. injection and mean peak concentrations were 588 µg/L (1973 nmol/L) and 142 µg/L (500 nmol/L) for ARM and DHA respectively (Y Suputtamongkol, *et al.*, 2001). The erratic absorption of ARM prevented accurate estimation of elimination half-lives (Y Suputtamongkol, *et al.*, 2001), as in the present investigation. It is difficult to know why the results of these two published studies differ so markedly, as the same dose of the same formulation of ARM was given and the same assay methodology (HPLC with electrochemical detection) was used.

A third study involving 17 Thai adults with severe malaria, carried out by the same group as the first volunteer study described above (J Karbwang, *et al.*, 1997), reported that the dose-adjusted AUC of ARM was increased in severe malaria, especially in patients with renal impairment (J Karbwang, *et al.*, 1998). All subjects had plasma concentration profiles of both ARM and DHA that exhibited the same pattern as observed in healthy subjects (J Karbwang, *et al.*, 1997), with $t_{max}$ values all close to 4 h and plasma concentrations that fell progressively thereafter to levels that were below, or close to, the limit of detection at 24h (J Karbwang, *et al.*, 1997). These findings are in marked contrast to those in our patients and the other reported studies with artemether (MN Mordi, *et al.*, 1997); (P Teja-Isavadharm, *et al.*, 1996) and the closely related compound arteether (S Looareesuwan, *et al.*, 1999) which suggest that the absorption of the oil based drugs from an i.m. depot is prolonged and erratic, and biotransformation to DHA much reduced compared with that after oral dosing.

Comparison of these results with studies of oral administration(Y Suputtamongkol, *et al.*, 2001), suggests that extensive first-pass metabolism of ARM occurs when the drug is given by mouth compared to parenteral routes. Following oral administration the AUC of the metabolite considerably exceeds that of the parent compound, whereas the reverse is seen following intramuscular administration. The plasma DHA concentrations measured in our patients with severe malaria were, after adjusting for the the lower dose used (3.2 vs 5 mg/kg), still considerably lower than those measured in healthy volunteers following intramuscular administration (Y Suputtamongkol, *et al.*, 2001), suggesting that hepatic metabolism of ARM, mainly by CYP3A4, but also 2B6 and 3A5 (JM Grace, *et al.*, 1998, SA Murphy, *et al.*, 1997) is reduced by malaria infection. Acute malaria reduces the hepatic biotransformation of many drugs (P Newton, *et al.*, 2000a). The metabolism of

quinine (also largely via CYP3A4) has been shown to be reduced in proportion to the severity of malaria (P Teja-Isavadharm, *et al.*, 1996). The effect of severe malaria on ARM pharmacokinetics may be even more pronounced, as both absorption from the intramuscular injection site and hepatic conversion to the active metabolite are reduced.

In contrast to ARM, the absorption of ARTS was rapid and reliable. The plasma concentrations for ARTS and DHA in our patients (median peak values: 5707 and 3223 nmol/L respectively) were generally similar to those reported previously in uncomplicated malaria (KT Batty, *et al.*, 1996, KF Ilett, *et al.*, 2002, SA Murphy, *et al.*, 1997). Peak concentrations of ARTS and DHA are 50-100 times higher than those required for full activity against the parasite. Both are eliminated rapidly, even in severe malaria, yet this brief exposure to the infecting parasite population (several hours) is sufficient for maximum antiparasitic effect in all cases. There are also important pharmacokinetic differences between the two artemisinin derivatives follwing oral administration in malaria. In a recent cross over study in uncomplicated malaria in which oral ARTS was compared with oral ARM, the relative oral bioavailability of oral ARM was 58% compared to that of ARTS (S Pukrittayakamee, *et al.*, 1997).

Unfortunately, there is still only one manufacturer of parenteral ARTS, and this formulation is not yet prepared to an internationally recognised Good Manufacturing Practices standard. Parenteral ARTS has considerable pharmacokinetic advantages over ARM in the treatment of severe falciparum malaria. Since these drugs have a similar cost and are both widely available in the tropics, ARTS is the preferred choice for the treatment of severe falciparum malaria, particularly in those patients who are most seriously ill and in whom absorption from an i.m. depot may be compromised. These findings should be formally assessed by a randomised clinical trial.

# CHAPTER 4

# SAFETY OF

# ARTEMISININ AND DERIVATIVES

The Qinghaosu (artemisinin) group of drugs, first isolated by Chinese scientists from the plant Artemesia annua in 1972, are now in widespread use throughout South East Asia and are used increasingly elsewhere in the tropical world for the treatment of both uncomplicated and severe malaria (China Cooperative Research Group on qinghaosu and its derivatives as antimalarials, 1982), (D Klayman, 1985, P Newton & NJ White, 1999). Several different compounds are available including the parent compound artemisinin, and the derivatives artesunate, artemether and arteether. Several million patients have now been treated with this group of drugs and, to date, no unequivocal drug-attributable toxicity has been reported. Recent large comparative studies of patients with severe falciparum malaria have confirmed artemether to be an acceptable, well-tolerated alternative to quinine. No significant adverse effects were found. In a double blind trial of 560 Vietnamese adults treated with artemether, which included thorough neurological evaluation and double blind audiometry, no evidence for iatrogenic neurotoxicity was found. In large studies of uncomplicated malaria in Thailand again no evidence of

significant toxicity was found (TT Hien, *et al.*, 1996, P Newton, *et al.*, 1999, MB van Hensbroek, *et al.*, 1996). Two recently completed meta-analyses of the use of artemisinin derivatives in uncomplicated and severe malaria conducted under the auspices of the Cochrane group and the World Health Organisation have provided no evidence of neurotoxicity (H McIntosh & P Olliaro, 1998a),(H McIntosh & P Olliaro, 1998b) . The excellent safety profile of the qinghaosu compounds contrasts with the relatively frequent minor toxicity reported with the quinoline, antimalarials, quinine, chloroquine and mefloquine(PA Phillips-Howard & FO ter Kuile, 1995). However, concerns have been raised by in vitro and animal studies. In laboratory experiments using the oil-based parenteral artemisinin derivatives arteether and artemether, selective neurotoxicity was noted in the brain stem nuclei with a dose-related site-specific necrosis primarily in the pons and medulla of rats, dogs and monkeys (TG Brewer, *et al.*, 1994a, TG Brewer, *et al.*, 1994b, RF Genovese, *et al.*, 1998, J Petras, *et al.*, 1997). Use of the water-soluble qinghaosu compound, artesunate, and oral administration of all the compounds produced considerably less neurotoxicity in animal experiments.

Despite the lack of clinical reports of neurotoxicity, these data from animals is a cause for concern. In order to rule out neurotoxicity in man there is a need of investigations at the bedside and in more sophisticated facilities.

## 4.1 Clinical and neurophysiological study of the effect of multiple doses of artemisinin on brain-staim function in Vietnamese patients

### 4.1.1 Introduction

In order to evaluate the possibility of brainstem neuropathology in patients who have received multiple treatment courses of the qinghaosu compounds, we assessed the

auditory pathway of the brainstem with studies of brain stem auditory evoked potentials (BSAEP) together with thorough audiometric and neurological examinations.

The BSAEPs occur in the brainstem during the first 10 ms after a transient auditory stimulus. The potentials are recorded as a characteristic series of waveforms. These are detected by scalp electrodes and correspond to anatomic structures in the auditory pathway of the brainstem. Specifically, the auditory nerve and the cochlear nucleus are the generators of peaks I and II, the superior olivary complex generates peak III, the lateral lemniscus generates peak IV, and the inferior colliculus generates peak V (Figure 4.1) (A Moller, 1994). Together, the series of waveforms encompass these nuclei and the relays between them. BSAEPs are used to demonstrate the integrity of the neuronal pathway from the cochlea, via the auditory nerve to the brain stem, and they allow localization of dysfunction within this pathway. The neuropathological damage reported in experimental animals treated with artemisinin is highly localized to the auditory, vestibular nuclei, and trapezoid body. If there was significant iatrogenic damage to these centres then measurement of BSAEPs in patients treated with repeated doses of artemisinin or its derivatives would be expected to be abnormal.

The purpose of this study was to determine whether there was clinical or neurophysiological evidence of neurotoxicity in humans exposed to multiple doses of the qinghaosu compounds.

### 4.1.2 Materials and methods

#### 4.1.2.1 Study Site

The study was carried out at the Khanh Phu Medical Station between May and November 1997. Khanh Phu is in the Central Highlands of Vietnam 450kms north of Ho Chi Minh City. Malaria transmission is very high and Khanh Phu village has the

highest incidence of malaria reported in Vietnam. The entomological inoculation rate (EIR) is approximately 100 per year; 70% of infections are *P.falciparum*, 25% *P.vivax*, and 5% *P.malariae*. Sporozoite rates are 3-4%. The artemisinin drugs became available in the village from 1995 onwards, and they are supplied free from the health clinic. Every patient attending the clinic with fever has a blood smear examined, and is treated with antimalarial drugs at the clinic if the blood smear is positive and there are also clinical features of malaria. Asymptomatic parasite positive patients are not treated. If the patient lives far from the clinic they are given a course of treatment to take at home, if they live close they return daily for supervised administration of drugs. All attendances at the clinic are recorded along with basic clinical information, result of smear, and drugs dispensed. All the treatment episodes from 1995 until May 1997 had been recorded. There is little incentive in this extremely poor village to seek external medical help or to purchase drugs elsewhere. Thus the details of all malaria episodes from the introduction of artemisinin in 1995 are likely to include all antimalarial treatments.

### 4.1.2.2 Drug Usage

Both artesunate (Guilin No 1 factory, Guangxi, PRC) and artemisinin (Factory 19, Ho Chi Minh City) were used. The treatment regimen for artesunate in use by the Health Clinic since 1995 has been: less than 1 year of age 150mg total dose; 1-5 years 300mg; 5-13 years 550mg; more than 13 year 600mg. For artemisinin the total doses used have been: less than 1 year of age 750mg total dose; 1-5 years 1500mg; 5-13 years 2750mg; more than 13 year 3000mg. Both were given as five day regimens. 500 mg/kg artemisinin as the total cumulative dose of drug received was selected (a priori) to look at the very heavily exposed in an analysis suited to a binary outcome.

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Mefloquine was given as antimalarial treatment between July and December 1995 at a time when artemisinin derivatives were not available. This was as 15mg/kg monotherapy. Mefloquine was not used at any stage as a combination therapy with an artemisinin derivative. Using the dates of drug administration and present age of the subject, the age of each patient at the time of receiving drug was back calculated. Using these ages and a standardized height and weight growth chart (for Vietnamese children) for ages ranging from birth to 18 years the total weight adjusted dosage was calculated for all subjects who had previously received anti-malarial treatment. Demographic details of the subjects and controls are shown in Table 4.1.1 A

For the subjects aged eighteen years or younger and whom received antimalarial therapy the mean difference in the estimated weight (from height and weight growth charts) and the weight recorded was 1.88 kg (95% normal range:- -5.69 up to 9.45 kg). The magnitude of the difference between the measured and estimated weight was significantly positively correlated with age (Spearman Rank correlation coefficient = 0.524, p-value<0.001). Table 4.1.1B shows the mean difference in estimated weight (from height and weight growth charts) and the weight measured during the study.

### 4.1.2.3 Study Design

Fully informed consent was obtained from the subjects and their parents for the neuro-otological evaluation. Subjects were selected randomly from the village population of 1764 people, the controls were the total number of people in the village who had received no artemisisin derivative. All subjects were assessed clinically using a standard neurological examination. Particular emphasis was placed on identifying abnormalities in hearing, vestibular and cerebellar function, and the control of voluntary movement. The presence of nystagmus or tremor was noted, and abnormalities in auditory localisation,

finger-nose movement, Romberg test, gait, repetitive alternating movements, and fine motor control were assessed. Otoscopic examination of the external auditory canal was performed, with particular reference to the presence of scarring, perforation, or pus. Audiological examinations were performed on both ears on those over 4 years old (Kamplex Screening Audiometer AS7 Japan). The initial screen was done at 40dB with a frequency of 250Hz, subsequently increasing to 500Hz, 750Hz, 1000Hz, 1500Hz, 2000Hz, 3000Hz, 6000Hz, and 8000Hz. All the data were recorded together with the patients' clinical history and exposure to all drugs. The neurological, otoscopic, and audiological assessments were performed blinded to the treatment histories of the subjects.

The BSAEP measurements were performed throughout by a single investigator (EK) using a Bio-Logic Traveller® Express E Auditory Evoked Potential Machine according to manufacturer's instructions. Briefly, surface electrodes were applied to the vertex and to both mastoids, and the subject asked to lie down. Electrical impedance was checked and kept below 10kOhm for all recordings. The 100µs click stimulus was delivered to each ear in turn via the headphones at a rate of 11.1 per second with an intensity of 80dB. The contralateral ear was masked with noise delivered to this ear at 40dB. The bipolar BSAEPs of 1024 clicks were amplified, averaged and recorded. At least two recordings were made on each ear to ensure reproducibility. The wave forms were identified subsequently and marked by a single investigator (LK), blind to information regarding drug exposure. All the wave forms and markings were subsequently checked, again blind to all subject details, by a single experienced neurophysiologist (CM) and these values accepted. Interpeak wave latencies were calculated and the wave forms assessed for other abnormalities. Subsequently all BSAEP measurements were checked (again blind to all subject details) by the neurophysiologist (CM). The accuracy of the Bio-Logic

Traveller® Express E Auditory Evoked Potential Machine is 0.1ms; variation below this level was considered not significant. The interpeak latencies between waves III and V were considered prospectively to be most likely to be affected if the same pattern of neurological toxicity observed in experimental animals was found in the subjects. The analysis concentrated on the I to V and III to V latencies.

### *4.1.2.4 Statistical Analyses*

Data were analyzed using SPSS for Windows (SPSS Inc., Release 7.5.1). Categorical data were compared using the $\chi2$ test. Normally distributed continuous data were compared by the Student's t test. Data not conforming to a normal distribution were compared by the Mann Whitney U test. A p-value of <0.05 was considered statistically significant. In comparisons with published normative data a value of the mean plus 2.5SD was regarded as within the normal range.

Figure 4.1: The brain-stem auditory evoked potentials generated from a single patient
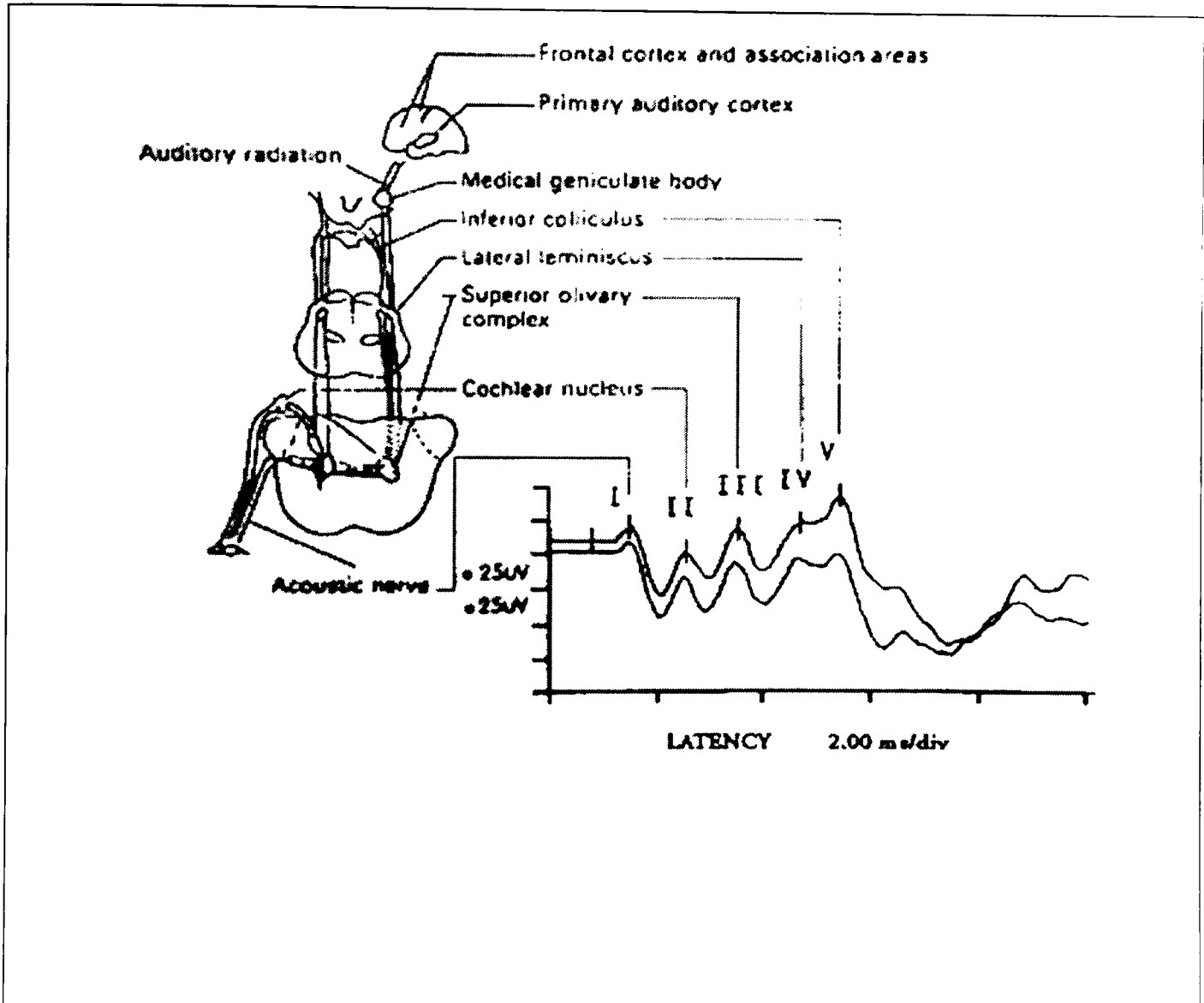
## 4.1.3 Results

Neurological and otoscopic examinations, auditory localization, audiometry, and BSAEPs were assessed in 242 subjects who had received previous antimalarial treatment with artemisinin or artesunate alone (QHS), 98 who had received both artemisinin and mefloquine (QM), and 10 who had been treated with mefloquine alone (M), and 108 controls from the same location who had never received these drugs before. The controls were older (median[range] age 13.5 [4 to 65] years) compared with the other groups: QHS 10 [1 to 57], QM 8 [4 to 48], and M 10 [7 to 17] years: $p<0.001$, and they had a larger body mass index (BMI) (median[range] 16.0 [11.75 to 34]) compared with the other groups: QHS 15.63 [6.85 to 25.95], QM 14.72 [11.34 to 21.37], and M 15.12 [13.42 to 17.85]. The controls also had a lower body temperature (mean [sd] °C: 36.9[0.4]°C) compared with the other groups; QHS 37.0 [0.5], QM 37.0 [0.4],and M 36.9 [0.2]. The groups were otherwise comparable (Table 1A).

### *4.3.1.1 Intensity of Drug Exposure*

The qinghaosu compounds dispensed by the Health Station were either artemisinin or artesunate depending on availability. The median (range) number of treated episodes in individual patients over the previous two years was 2 (1-21) separate courses of artemisinin. Artemisinin is approximately five times less potent in terms of antimalarial activity compared with artesunate. The weight adjusted (milligram per kilogram) dosing regime for each treatment course of artemisinin was therefore five times that for artesunate, and artesunate dosage was multiplied by five in order to express all treatment in terms of artemisinin. The median (range) of treatment dose was 168 mg/kg [11,2705]. Overall 20% of subjects received total doses of artemisinin (or the adjusted equivalent of artesunate) exceeding 500 mg/kg (see Table 4.1.2 and Figure 4.1.2).

Figure 4.2: Percentage of subjects receiving a total dose of QHS of either <200, 201-

500,501-1000, or >1000 mg/kg
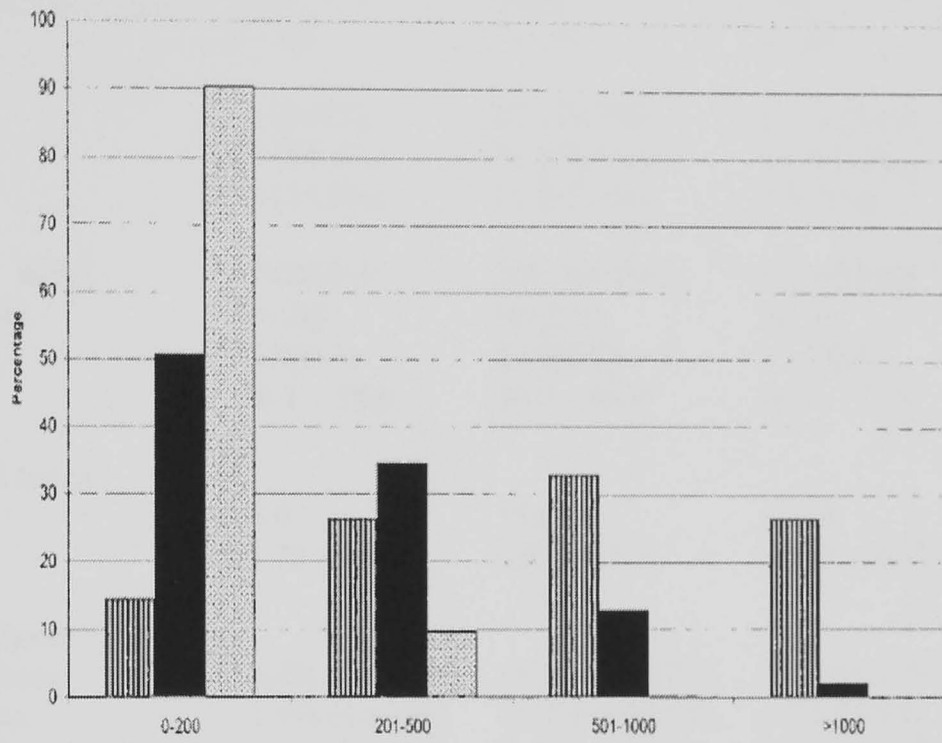
Table 4.1.1 A: Demographic details BS AEP study

| | Controls<br><br>(n=108) | Qinghaosu compound alone (n=242) | Qinghaosu compound + mefloquine (n=98) | Mefloquine alone<br><br>(n=10) |
|---|---|---|---|---|
| Median age | 13.5 | 10.0 | 8.0 | 10 |
| (range) | 4 – 65 | 1 – 57 | 4 –48 | 7 – 17 |
| n(%) | | | | |
| 2-5yrs | 2 (1.9%) | 67 (27.7%) | 31 (31.6%) | 0 |
| 6-15yrs | 70 (64.8%) | 94 (38.8%) | 58 (59.2%) | 9 (90%) |
| 16+ | 36 (33.3%) | 81 (33.5%) | 9 (9.2%) | 1 (10%) |
| | | | | |
| Male sex    n(%) | 50 (46.3%) | 108 (44.6%) | 45 (45.9%) | 5 (50%) |
| Temperature | N=105 | N=222 | N=79 | N=9 |
| Mean(SD) | 36.9(0.4) | 37.0(0.5) | 37.0(0.4) | 36.9(0.2) |
| Range | 36.1 – 38.0 | 36.0 – 46.0 | 36.1 – 37.8 | 36.5 – 37.1 |
| | | | | |
| Body Mass Index | | | | |
| Median | 16.0 | 15.63 | 14.72 | 15.12 |
| Range | 11.75 – 34.0 | 6.85 – 25.95 | 11.34 – 21.37 | 13.42 – 17.85 |
| | | | | |
| Otoscope Exam | | | | |
| Dry perforation | 10.4% (11/106) | 19.7% (45/229) | 19.4% (18/93) | 12.5% (1/8) |
| Otitis Media | 13.4% (14/106) | 11.4% (26/229) | 8.6% (8/93) | 12.5% (1/8) |
| Otitis&/or perforation | 23.6% (25/106) | 31.0% (71/229) | 28.0% (26/93) | 25.0% (2/8) |
| | | | | |
| Audiometry | N=102 | N=153 | N=60 | N=9 |
| Right 4000 Hz | | | | |
| ≤30    dB | 89(87%) | 123(80%) | 47(78%) | 7(78%) |
| 35-45  dB | 11 | 28 | 12 | 2 |
| 50-55  dB | 1 | 0 | 1 | 0 |
| ≥ 60 | 1 | 2 | 0 | 0 |
| Left 4000  Hz | | | | |
| ≤30    dB | 81(79%) | 124(81%) | 53(88%) | 8(89%) |
| 35-45  dB | 18 | 20 | 5 | 1 |
| 50-55  dB | 3 | 6 | 2 | 0 |
| ≥60    dB | 0 | 3 | 0 | 0 |

### 4.1.3.2 Neurological Exam Results

A detailed neurological examination was conducted in 375 (81.5%) of the study subjects. This total was limited by ability to follow commands. There was one subject with nystagmus in the control group. Otherwise no abnormalities were found on neurological examination in any of the treatment groups or controls.

Table 4.1.1 B: Mean difference for estimated weight (from height and growth charts) minus recorded weight (95% normal range) BS AEP study.

| Age group | Mean difference, kgs (95% normal range) |
| --- | --- |
| 1 – 4 years | 0.08 (-2.43, 2.59) |
| 5 – 9 | 1.61 (-2.33, 5.55) |
| 10 – 14 | 2.36 (-5.97, 10.69) |
| 15 - 18 | 4.28 (-8.73, 17.29) |

### 4.1.3.3 Otoscopic Exam Results

Overall, there was a high prevalence of middle ear infection. Of the 436 otoscopic exams 124 (28%) were found to have pus and/or perforation. Consistent with the natural history of otitis media, the rates of otitis as determined by pus in the external canal and at the tympanic membrane were much higher in younger children. 16% (15 of 92) in the 2 to 5 year age group, 14% (31 of 221) in the 6 to 15 year old age group, and 2.4% in those over 16 years. As there were no controls in the youngest age group, comparisons cannot be made between rates of infection in control versus treatment groups in the very young. For children over 5 years of age, the rates of dry perforation were as follows, 10.6% (11/104) in controls, 17.9% (30/168) in the QHS group, 14.1% (9/64) of the QM group and 12.5% (1/8) of the M group (p-value = 0.43). The rates of otitis were 11.5% (12/104) of controls,

9.5% (16/168) of the QHS group, 7.8% (5/64) of the QM group and 12.5% (1/8) of the M group (p-value = 0.87).

Audiometry Results

There were no differences between the groups (Table 4.1.1 A).

### 4.1.3.4 Auditory Evoked Potentials

Analysis of the inter-peak latencies (IPLs) was performed separately for each ear. (Table 3). No differences in the IPLs of the left ear were observed between the qinghaosu compounds and the left ear of the controls. On univariate analysis the IPLs I-V and I-III of the right ear were significantly longer for those patients in the QM group compared to the controls. Patients in the QHS group had a significantly longer I-V IPL of the right ear compared to right ear of the controls. There were no differences for the left ear.

As age is related to IPLs, and there were only two subjects less than 5 years of age in the control group (see Table 1A), a sub-analysis was performed in which IPLs of the treatment groups were compared with those of the control group in those study subjects greater than five years old (see Table 4). This revealed very small but statistically significant prolongation for the right IPLs I-III (2.03 vs 1.98 (p=0.027)) and I-V (3.82 vs 3.74 (p=0.007)) in the QM group compared to the control group. In each case, the difference from controls was less than the accepted 0.1ms measurement accuracy range of the equipment. Again there were no differences for the left ear.

In those individuals less than 4 years of age (for whom there were no controls in the population studied) the IPLs were also compared with published normative age matched data (normal values for IPL I-V 1 year old <5.01, 2 year old <4.76, 3 year old <4.75; for IPL III-V 1 year old <2.9, 2 year old <2.688, 3 year old <2.664). One patient had an IPL

longer than 2.5SD from the mean IPL for their age (2 year old IPL III-V 2.78). This child also had pus in the external auditory canal obstructing the tympanic membrane.

Throughout the analysis there were no differences between the right and left ear within the treated groups, the apparent prolongation of the right IPL I-III and I-V is due to the shorter IPL in the right ear of the controls (Table 3 ).

Table 4.1.2: Frequency of qinghaosu total doses (weight adjusted) given to subjects

| | All Qinghaosu compounds N=337* | Qinghaosu compound alone N=240 | Qinghaosu compound + MFQ n=97 |
|---|---|---|---|
| Weight adjusted total dose§ | | | |
| Median [range] | 168 [11,2705] | 130 [11,2705] | 237 [19,2646] |
| 0 – 200  mg/kg | 188 (55.8%) | 148 (61.7%) | 40 (41.2%) |
| 201 – 500 | 82 (24.3%) | 47 (19.6%) | 35 (36.1%) |
| 501 – 1000 | 44 (13.1%) | 29 (12.1%) | 15 (15.5%) |
| >1000 | 23 (6.8%) | 16 (6.7%) | 7 (7.2%) |

MQF: mefloquine

§:The artesunate dose multiplied by 5

*: 3 subjects without weight measured

### 4.1.3.5 Effect of total drug exposure on IPL

A comparison was made of the effects on cumulative quantity of drug received (less than or equal to 500 mg/kg or >500 mg/kg of artemisinin or artesunate dose times five). There was no significant difference in the IPLs between these two groups except for IPLs I-III (2.07 ms in the greater than 500 mg/kg group versus 2.00 ms in the less than or equal to 500 mg/kg group [p=0.014]) and I-V (3.89 ms versus 3.79 ms [p=0.014]) of the right ear. However, because of the effect of age on IPL, a sub-analysis was undertaken (see Table

5A and 5B). We compared the IPLs between those who received less-than or equal to 500

mg/kg with those who received more

Table 4.1.3: Descriptives of inter-peak latencies (msec) BS AEP study

| Inter-peak latencies a | Controls | All Qinghaosu compounds | Qinghaosu compound alone | Qinghaosu compound + mefloquine |
|---|---|---|---|---|
| Right I-III | 1.98 (0.15) | 2.02 (0.17) | 2.01 (0.17) | 2.03 (0.17) |
| | 1.66 – 2.44 | 1.52 – 2.60 | 1.56 – 2.52 | 1.52 – 2.60 |
| | | p* = 0.035 | p* = 0.075 | p*=0.029 |
| Right I-V | 3.74 (0.18) | 3.81 (0.22) | 3.81 (0.23) | 3.81 (0.19) |
| | 3.20 – 4.12 | 3.20 – 4.74 | 3.20 – 4.74 | 3.43 – 4.40 |
| | | p* = 0.006 | p* = 0.011 | p* = 0.011 |
| Right III-V | 1.78 (0.17) | 1.79 (0.17) | 1.80 (0.18) | 1.78 (0.14) |
| | 1.28 – 2.28 | 1.40 – 2.78 | 1.40 – 2.78 | 1.44 – 2.32 |
| | | p* = 0.324 | p* = 0.254 | p*=0.758 |
| | | | | |
| Left I-III | 2.00 (0.16) | 2.02 (0.17) | 2.02 (0.18) | 2.04 (0.16) |
| | 1.64 – 2.64 | 1.52 – 2.76 | 1.52 – 2.76 | 1.54 – 2.48 |
| | | p* = 0.289 | p* = 0.507 | p*=0.108 |
| Left I-V | 3.78 (0.20) | 3.81 (0.21) | 3.80 (0.21) | 3.83 (0.22) |
| | 3.40 – 4.24 | 3.16 – 4.72 | 3.16 – 4.72 | 3.32 – 4.56 |
| | | p* = 0.219 | p* = 0.364 | p*=0.122 |
| Left III-V | 1.78 (0.16) | 1.79 (0.18) | 1.80 (0.18) | 1.78 (0.18) |
| | 1.44 – 2.30 | 1.32 – 2.54 | 1.36 – 2.54 | 1.32 – 2.24 |
| | | p* = 0.690 | p* = 0.552 | p*=0.900 |

a – mean (sd), range

*- treatment group vs controls, unpaired t-test

than 500 mg/kg in those older than five years of age. The same comparison was made

separately for those younger than or equal to five years old. In each case, when

correcting for age there was no significant differences in IPLs between the group

receiving higher cumulative dosages and the group receiving the lower cumulative

dosage.

Table 4.1.4: Descriptives of inter-peak latencies for subjects > 5 years of age

| Inter-peak latencies a | Controls N=106 | All Qinghaosu compounds N=241 | Qinghaosu compound alone N=174 | Qinghaosu compound + mefloquine N=67 |
|---|---|---|---|---|
| Right I-III | 1.98 (0.15) | 2.00 (0.16) | 1.99 (0.16) | 2.03 (0.15) |
| | 1.66 – 2.44 | 1.56 – 2.48 | 1.56 – 2.48 | 1.72 – 2.40 |
| | | p* =0.180 | p* = 0.511 | p*=0.027 |
| Right I-V | 3.74 (0.18) | 3.80 (0.21) | 3.79 (0.22) | 3.82 (0.20) |
| | 3.20 – 4.12 | 3.20 – 4.64 | 3.20 – 4.64 | 3.43 – 4.40 |
| | | p* = 0.018 | p* =0.069 | p* = 0.007 |
| Right III-V | 1.78 (0.17) | 1.80 (0.17) | 1.81 (0.17) | 1.80 (0.15) |
| | 1.28 – 2.28 | 1.40 – 2.40 | 1.40 – 2.40 | 1.44 – 2.32 |
| | | p* = 0.178 | p* = 0.178 | p*=0.420 |
| | | | | |
| Left I-III | 2.00 (0.17) | 2.02 (0.17) | 2.01 (0.17) | 2.03 (0.16) |
| | 1.64 – 2.64 | 1.52 – 2.44 | 1.52 – 2.44 | 1.54 – 2.44 |
| | | p* = 0.409 | p* = 0.551 | p*=0.321 |
| Left I-V | 3.78 (0.20) | 3.81 (0.20) | 3.80 (0.20) | 3.84 (0.22) |
| | 3.40 – 4.24 | 3.16 – 4.56 | 3.16 – 4.52 | 3.48 – 4.56 |
| | | p* = 0.225 | p* = 0.515 | p*=0.063 |
| Left III-V | 1.78 (0.16) | 1.79 (0.17) | 1.79 (0.17) | 1.80 (0.18) |
| | 1.44 – 2.30 | 1.36 – 2.44 | 1.36 – 2.44 | 1.48 – 2.24 |
| | | p* = 0.624 | p* = 0.699 | p*=0.595 |

a – mean (sd), range

*- treatment group vs controls, unpaired t-test

Table 4.1.5 A: Mean (SD) inter-peak latency (msec) for subjects in the artemisinin group ≤5 years of age

| | Drug dosage | | |
|---|---|---|---|
| | ≤ 500 mg/kg N=30 | > 500 mg/kg N=36 | p-value |
| Right I-III | 2.03 (0.18) | 2.08 (0.18) | 0.258 |
| I-V | 3.79 (0.19) | 3.89 (0.29) | 0.125 |
| III-V | 1.76 (0.15) | 1.80 (0.24) | 0.436 |
| | | | |
| Left I-III | 1.99 (0.17) | 2.07 (0.20) | 0.105 |
| I-V | 3.80 (0.20) | 3.85 (0.27) | 0.456 |
| III-V | 1.81 (0.22) | 1.79 (0.18) | 0.764 |

Table 4.1.5 B: Mean (SD) inter-peak latency (msec) for subjects in the artemisinin group > 5 yrs of age

| | Drug Dosage | | | |
|---|---|---|---|---|
| | Controls N=106 | ≤ 500 mg/kg N=165 | > 500 mg/kg N=8 | p-value |
| Right I-III | 1.98 (0.15) | 1.99 (0.16) | 2.01 (0.21) | 0.782 |
| I-V | 3.74 (0.18) | 3.79 (0.22) | 3.88 (0.15) | 0.113 |
| III-V | 1.78 (0.17) | 1.80 (0.18) | 1.85 (0.17) | 0.308 |
| | | | | |
| Left I-III | 2.00 (0.17) | 2.01 (0.17) | 2.07 (0.15) | 0.627 |
| I-V | 3.78 (0.20) | 3.80 (0.20) | 3.79 (0.22) | 0.789 |
| III-V | 1.78 (0.16) | 1.80 (0.17) | 1.73 (0.15) | 0.476 |

## 4.1.4 Discussion

The artemisinin (Qinghaosu) group of compounds are now first line antimalarial drugs in South East Asia, and they are being used increasingly in Africa and South America. Their use has increased because of their ease of drug delivery, lack of adverse effects and rapid therapeutic responses even against multidrug resistant falciparum malaria (S Looareesuwan, *et al.*, 1998). The demonstration, in experimental animals, of a reproducible pattern of selective damage to the brain stem following large doses of artemisinin derivatives has been a major concern (S Meshnick, 1996). Neurotoxicity in rats, dogs, and rhesus monkeys, has resulted from the administration of large intramuscular doses of arteether (AE), or artemether (AM), two lipophilic derivatives of artemisinin. Following administration of 10-50mg/kg/day AE for eight to twenty eight days, a dose dependent, region specific pattern of damage was noted in the pons and medulla of beagle dogs and Sprague-Dawley rats (TG Brewer, *et al.*, 1994a, TG Brewer, *et al.*, 1994b, J Petras, *et al.*, 1997). In rhesus monkeys no pathological effects could be demonstrated after 7 days treatment with arteether (8-24mg/kg/day), but dose dependent neuropathological lesions were noted in the reticular, vestibular and auditory nuclei after 14 days of treatment. Foci of neurotoxicity were also found in the brainstem nuclei of the reticular formation, the vestibular system, and the auditory system (including the superior olivary nuclear complex and most consistently, the trapezoid nuclear complex) (J Petras, *et al.*, 1997). A marked species variation was noted with a ranking of susceptibility of nervous system the species examined; dog>rat>rhesus monkey. In cell culture, toxicity has been demonstrated against neuronal cell types (J Fishwick, *et al.*, 1995, DL Wesche, *et al.*, 1994). It has also been noted that considerably higher doses of oral artemether or arteether, or the water soluble artemisinin derivatives given by any route, are required to produce neuropathological lesions. This suggests that the development of neurotoxicity

with parenteral AE and AM results from slow release of drug following intramuscular injection and thus sustained exposure of the central nervous system.

Khanh Phu village, the site for this study is a remote community in a region of high transmission for malaria with an entomological innoculation rate of 100 per year and sporozoite rates of 3-4%. The population may have had a higher cumulative dose of artemisinin or artesunate than any other group of people in the world. The subjects had received up to 21 separate treatment courses. This provided an unusual opportunity to assess possible neurotoxicity. The treated group was younger than the control group, but there were no other significant differences in demographic details, or in the results of otoscopic examination and audiometry. The clinical examination concentrated on the assessment of hearing, vestibular function, cerebellar and control of voluntary movement, as these were considered a priori to be most likely to be affected by neurotoxicity. No significant differences were found between cases and controls.

Brain stem auditory evoked potentials (BSAEP) are a sensitive non-invasive technique to assess the integrity of the auditory pathways from the cochlea to the mid-brain. The interpeak latencies (IPLS) are the least variable and most independent of subject, stimulus and recording parameters compared with other measures derived from the BSAEP, and were therefore used throughout this study (J Jacobsen & J Hall, 1994, D Schwartz, *et al.*, 1994) .The accuracy of the recording system in this study was 0.1ms; variation below this level cannot be assessed accurately. In this study a very small, but statistically significant differences in IPLS were found in recordings from the right side of subjects over four years old who had received artemisinin or artesunate compared to those versus those who had not. No differences were found on the left side. The differences were below the accepted sensitivity of the recording device. As there is no reason to

believe neurotoxicity should occur preferentially on one side of the brain, these findings are not considered of pathological significance.

In this study there were no untreated controls under 5 years of age and therefore, for those subjects less than 5 years old, IPL values were compared with published normative data (D Schwartz, *et al.*, 1994). Using a conservative definition of the upper limit of normal as 2.5SD greater than the mean, only one subject in the treatment group had an IPL outside the accepted normal range for their age. This was a two year old child with pus in the external auditory canal (EAC) such that the ear drums could not be visualized, and in whom the rest of the clinical neurological assessment was normal. Whilst we cannot rule out absolutely a neuropathological process in this individual, severe inflammation in the unilateral EAC in a two year old would be an acceptable clinical cause of a prolonged IPL 1-V. The contralateral ear was normal and had normal IPLs. All other subjects had IPL within the normal range for their age.

If artemisinin and its derivatives are neurotoxic with cumulative exposure, there should be some relationship between total dose, and effect. There was no evidence for dose-dependency in IPLS. This provides further support for lack of neurotoxicity.

The artemisinin derivatives have been deployed mainly in China and South East Asia, where the main at-risk populations live in areas of low malaria transmission and infrequent infection. The situation in Khanh Phu is unusual in its transmission intensity, and thus exposure of the population to the artemisinin derivatives. This study aimed at careful neurological evaluation of the population exposed to multiple treatments with the artemisinin derivatives. As a field study in a small rural community it could not meet the rigorous criteria of a drug-safety study. However, the lack of any convincing clinical or neurophysiological evidence of brain stem neurotoxicity, despite thorough examination in subjects receiving up to twenty-one treatment episodes in a two year period, is reassuring.

It is possible that the combination of detailed clinical assessment plus brain stem neurophysiology used in this study is not sensitive enough to detect covert neuropathology. The BSAEP measures neuronal activity from the acoustic nerve through the cochlear nucleus, the superior olivary complex, the lateral lemniscus, the inferior colliculus and finally via the medial geniculate body to the primary auditory cortex. This pathway is directly adjacent to the structures damaged by arteether in male rhesus monkeys; the reticular formation (pontine nuclei, medullary gigantocellular nuclei), the vestibular system, and the auditory system (superior olivary and trapezoid nuclear complex) (J Petras, *et al.*, 1997) . These nuclei and their projections are intimately connected to the neuronal pathway assessed by the BSAEP. If the combined clinical and neurophysiological assessments conducted in this study are not sensitive enough to detect a potential lesion, then these defects would have to be very subtle indeed. This study provides further reassurance, in addition to the recent Cochrane Group meta-analysis (H McIntosh & P Olliaro, 1998b), that there is no evidence of neurotoxicity from studies in man that would lead one to restrict the use of appropriate antimalarial treatment regimens with these drugs. The safety of these drugs in continuous use (e.g. prophylaxis) has not been established and cannot be inferred from these data.

## 4.2 Neuropathological assessement of artemether-treated severe malaria

### 4.2.1 Introduction:

Artemisinin and its derivatives are the most rapidly acting antimalarial drugs. They are reliably effective against multidrug-resistant falciparum malaria and are used increasingly throughout the tropical world. In a large randomised trial, intramuscular artemether (an oil-based artemisinin derivative) proved very well tolerated and effective in people with severe falciparum malaria compared with parenteral quinine (Artemether-Quinine-Meta-analysis-Study-Group, 2001). In Southeast Asian adults, mortality was lower in artemether recipients (TT Hien, *et al.*, 1996). Despite excellent tolerability, a main concern has been that, in studies in animals, high doses of intramuscular artemether—and the closely related artemotil—produce an unusual selective pattern of damage to certain brainstem nuclei, especially those implicated in hearing and balance (J Petras, *et al.*, 1997). In a randomised double-blind comparison of intramuscular quinine and artemether (TT Hien, *et al.*, 1996), full autopsies were done on patients who died, if informed consent was obtained from attendant relatives. After the concerns over possible neurotoxic effects emerged from studies in animals, we decided to preserve the entire brains of these patients who died specifically with the aim to ascertain whether similar neurotoxic findings were present.

### 4.2.2 Methods and material

This study was done at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. Patients received either intramuscular quinine dihydrochloride (20 mg salt/kg followed by 10 mg/kg every 8 h) or artemether (4 mg/kg followed by 2 mg/kg every 8 h). The doses used in this trial were more than double those generally recommended. Protocols for autopsy and specimen collection were approved by the Scientific and Ethics

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committee of the Hospital for Tropical Diseases. We fixed brains in 10% buffered formalin saline until sectioning. We removed brainstems at the midbrain level and sectioned them, beginning at the pontomedullary junction. Serial transverse sections were taken at 5-mm intervals both rostrally and caudally. We then prepared standard formalin-fixed wax-embedded sections for histopathological examination, with haematoxylin and eosin, solochrome-cyanin, and periodic acid-Schiff staining. We examined sections with a light microscope, while masked to clinical details. Pathological changes associated with toxic effects of the drug were sought specifically. We particularly examined the neurons in the nuclei in which neuropathological changes had been noted in animals (J Petras, *et al.*, 1997). These included the trapezoid nucleus, red nucleus, superior and inferior olivary nuclei, cochlear and vestibular nuclei, and the cerebellar dentate nuclei. Neuronal changes, which were qualitatively assessed, included frank neuronal necrosis, apoptosis, Nissl substance swelling, nuclear chromatin changes, size and placement of the nucleolus within the nucleus, intracellular neuronal oedema, and presence of perineuronal microglial cell satellitosis (neuronophagic nodules). Parenchymal changes assessed included a generalised increase in number or shape of activated microglia, foci of axonal swelling, and focal white-matter necrosis. In a subset of patients, we did immunohistochemical staining for glial fibrillary acidic protein and CD68 to examine astrocyte and microglial responses (IM Medana, *et al.*, 2001),4 and for β-amyloid precursor protein (β-APP) to assess axonal injury. For neuronal chromatolysis, results were subdivided by brainstem nuclei:

    i) oculomotor nucleus;

    ii) motor nucleus of Vth nerve;

    iii) vestibular nucleus;

    iv) reticular nucleus and raphe nuclei; and

v) other nuclei (lateral cuneate, trigeminal nuclei, and $X^{th}$ nerve).

Axonal injury was expressed as the proportion of the section area showing $\beta$-APP staining. Pathological features were reviewed by four neuropathologists (JC, AD, RW, and WB) before unmasking drug treatments. Comparisons of variables between treatment groups were done with Student's t test, Kruskall-Wallis test, or Fisher's exact test as appropriate.

### 4.2.3 Results

Clinically, the percentages of the patients with cerebral malaria and other complications were comparable in two treatment groups. Time from admission to death in Artemether group was 76.6 h (median) and Quinine was 3.1 h (median) (p-value = 0.17) (table 4.2.1). No evidence was recorded of neurotoxic effects of artemether despite receipt of intramuscular doses up to 44 mg/kg (table 4.2.2).

Table 4.2 1: Baseline characteristics by drug treatment group

| Variable | Artemether (n=6) | Quinine (n=15) | p |
|---|---|---|---|
| Age (years, mean [SD]) | 37 (21) | 34 (12) | 0·72 |
| Male/female | 4/2 | 11/4 | 0·61 |
| Admission GCS (median [IQR]) | 11 (8–15) | 10·5 (7–14) | 0·51 |
| Coma on admission | 3 (50%) | 7 (50%) | 0·99 |
| Jaundice at any time | 5 (83%) | 12 (86%) | 0·99 |
| Acute renal failure | 4 (67%) | 11 (79%) | 0·61 |
| Hypoglycaemia | 2 (33%) | 8 (57%) | 0·63 |
| Haemodynamic shock | 5 (83%) | 13 (93%) | 0·52 |
| Severe anaemia | 3 (50%) | 9 (64%) | 0·64 |
| Admission parasite count /$\mu$L (geometric mean [95% CI]) | 148 000 (42 000–517 000) | 120 000 (33 000–441 000) | 0·83 |
| Time to death from admission (h, median [IQR]) | 76·5 (8·0–331) | 31·1 (8·6–63) 0·17 | |

Data are number of patients (%) or median (IQR), unless otherwise stated.

GCS=Glasgow coma scale.

Table 4.2.2.: Neuropathological findings

|  | Artemether (n=6) | Quinine (n=15) | p |
| --- | --- | --- | --- |
| Frank neuronal necrosis | 0 | 1 (7%) | 0·99 |
| Glial response | 6 (100%) | 13 (88%) | 0·99 |
| Demyelination | 1 (17%) | 6 (40%) | 0·61 |
| Axonal injury | 0·01 (0–0·3) | 0·02 (0–4) | 0·62 |
| Neuronal chromatolysis |  |  |  |
| 1 | 1 (17%) | 3 (20%) | 0·67 |
| 2 | 2 (33%) | 1 (7%) | 0·18 |
| 3 | 1 (17%) | 6 (40%) | 0·61 |
| 4 | 2 (33%) | 12 (80%) | 0·12 |
| 5 | 2 (33%) | 2 (13%) | 0·54 |

Data are number of patients (%) or median (range).

## 4.2.4 Discussion

In animals, high doses of intramuscular artemether or artemotil are much more neurotoxic than are comparable doses administered orally, or when water-soluble artesunate is given by any route (A Nontprasert, *et al.*, 2000). Neurotoxic effects result from sustained exposure of the CNS to artemether, and its principal metabolite dihydroartemisinin, which follows slow absorption of intramuscular injections. In primates, daily doses of artemotil of 8 mg/kg or greater for 14 days were neurotoxic, whereas 8 mg/kg for 7 days was not (J

Petras, *et al.*, 1997). Neuropathological abnormalities in animals associated with artemether and artemotil neurotoxic effects were reported as frank neuronal necrosis, associated astroglial responses, pallor of myelin staining, axonal damage, and chromatolysis (J Petras, *et al.*, 1997) . In this series, chromatolysis was evident in all patients, axonal injury and astroglial activation were frequent, and focal pallor of myelin staining was noted in some cases, but these findings were similar in recipients of artemether and quinine. Widespread neuronal stress responses and axonal injury have been reported previously (IM Medana, *et al.*, 2001). No evidence was recorded for either irreversible neuronal injury or the selective distribution of neuropathological abnormalities confined to certain brainstem nuclei, which was such a consistent feature in animals (J Petras, *et al.*, 1997). These findings suggest that the neuropathological abnormalities resulted from severe malaria itself, and not artemether treatment. This series is small, and the quality of neuropathological assessment that is possible—even from rapid autopsy—will always be inferior to that of perfusion-fixation in animals. However, complete absence of neuronal death in artemether recipients and the similarity of the other neuropathological findings between the two different treatments, together with normal neurological and audiometric findings in survivors (TT Hien, *et al.*, 1996), all suggest that no relevant neurotoxic effects are associated with intramuscular artemether in severe falciparum malaria. These data provide reassurance that therapeutic doses of these important antimalarial drugs do not damage the nervous system.

# CHAPTER 5

# TREATMENT OF

# UNCOMPLICATED FALCIPARUM

# MALARIA IN VIETNAM

## 5.1 Mefloquine vs single day artesunate+mefloquine *vs* 3 day artesunate + mefloquine

### 5.1.1 Introduction

In Vietnam although the malaria situation has improved compared to the 1980's and the early 1900's, hundreds of thousands of people still suffer from this debilitating infection each year and the resistance to the available antimalarial drugs is particular problematic. Since 1991 because of the increased resistance of conventional drugs as chloloquine, sulfadoxine-pyrimethamine and quinine, mefloquine (15mg/kg) was introduced as a first line antimalarial drug in areas of multi-drug resistant *P.falciparum*. Until 1994 the cure rate of mefloquine (MSP) was still 100% (TT Hien, *et al.*, 1994). However in other countries in South East Asia, especially in Thailand, at the same time, the resistance to mefloquine was evident on the western and eastern borders and this has increased steadily

despite the dose of mefloquine was raised from 15 to 25 mg/kg (NJ White, 1994b). In addition, results from 2 other studies conducted in 1997 and 2001 in Vietnam have also shown that the cure rates of single dose of mefloquine (500 mg) or combination mefloquine – artesunate were critically low (NN Le, *et al.*, 1997, TN Trung, *et al.*, 2001) . In order to be able to assess the response of multi-drug resistant *P.falciparum* to mefloquine and mefloquine in combination with artesunate in this region we conducted this study at the Hospital for Tropical Diseases by comparing 3 regimens recommended by the national malaria control project in Viet Nam in 1997: mefloquine monotherapy (15 mg/kg), combined therapy including one day of artesunate (AM) and 3 days of artesunate + mefloquine (15 mg/kg) (A3M)

### 5.1.2 Materials and methods

#### 5.1.2.1 Objective

This study, conducted at the Hospital for Tropical Diseases of Ho Chi Minh City in Vietnam, was designed to detect a difference in cure rate of 20 % with 95% confidence and 80% power. The cure rate of artemisinin (comparable to artesunate) single day + mefloquine (15mg/kg) was 95% in the study carried out in 1994 (TT Hien, *et al.*, 1994) at the same hospital.

#### 5.1.2.2 Patients

Consecutive patients with non-complicated malaria admitted to the malaria ward D were recruited in the study if they were aged at least 12 years old and < 60 years old, having the typical symptoms and signs of acute uncomplicated malaria (>37o5C) and/or at least one constitutional malaria symptom; having asexual forms of P. Falciparum in the peripheral blood smear on the admission. Patient were excluded if they received

antimalarials during the last 48 hours; having mixed infections (with *P. vivax*) concomitant chronic infections. Fully informed consent was obtained from patients before entry into the study. The study was approved by the Scientific and Ethical Committee of the hospital.

Patients were assigned to receive one of 3 regimens: M: a single dose of oral mefloquine 750 (Lariam® Hoffmann – La Roche, Basel Switzerland), or AM, a single dose of 200 mg oral artesunate (Artesunate tablet, Guilin No 1 Factory, Guangxi, China) and mefloquine 750 mg, or A3M, artesunate (200 mg for the 1st day then 100mg the 2nd and 3rd day) and mefloquine 750 mg in the 3$^{rd}$ day of treatment). The drug for each patient was contained in opaque, sealed and coded envelopes which were randomised in block of 6. Once a patient was enrolled in the study the envelope was opened. The treating physicians and the technicians who performed the patient's parasitaemias were blinded to the treatment.

### 5.1.2.3 Clinical procedure

After randomisation, a detailed history was taken from the patient and full clinical examination conducted. Venous blood was taken for a pretreatment investigation included blood smear, full blood count, blood glucose, serum creatinin, blood urea nitrogen and mefloquine level. Patients were reassessed every 6 hours for vital signs as axillary temperature, pulse, blood pressure during febrile period then daily until discharge. The peripheral blood smear should be taken and the number of asexual forms per 400 WBC on thick films or per 1000 RBC on thin films are to be counted, and the results recorded in terms of number of parasites per microlitre of blood. Parasitological checks are to be performed at baseline (hour 0) and every 12 hours after treatment, if possible also on days 14, 21, 28, 35, 42, 49 and 56. Efficacy will be expressed in time to clearance (time until

first negative slide) and as parasitological cure rate. Patients whose parasitemia cleared within 7 days but who became smear positive again during the follow up period (days 7-56) with the same genotype of either *P. falciparum*, or a mixed infection including the same genotype of *P. falciparum*, were defined as treatment failures. Treatment failures were retreated with artesunate 4 mg/kg/day once daily for 3 days, plus mefloquine 25 mg/kg given in divided doses on day 3.

### 5.1.2.4 *PCR for reinfections*:

The study primary end point was the incidence of reappearance of parasites within 56 days in the three treatment groups. Blood samples on filter paper were taken on admission to the study and on the day of reappearance of parasites. Recrudescence was distinguished from re-infection by parasite genotyping of three polymorphic loci (MSP1, MSP2 and GLURP) using the polymerase chain reaction (PCR). Genotyping was performed blind to the clinical details of the patients.

### 5.1.2.5 *Statistical analysis*:

Analysis of variance was used for normally distributed data, and the Mann-Whitney U test was used to compare data that were not normally distributed. The Chi-square test with Yate's correction or the Fisher's exact test was used to compare proportions. Fever and parasite clearance time was assessed by survival analysis and by the log rank test.

**5.1.3 Results**

Between November 1999 and June 2000, 216 patients were enrolled (70 received M, 72 received AM, and 73 received A3M) all of them are non- immune to malaria. No patient was taking malaria prophylaxis. There were 48 patient excluded (13 received M, 16 received AM, and 19 received A3M) because of mixed infection with *P.vivax*, or incomplete treatment course, or unable to be followed. Finally, 167 patients were analysed (57 received M, 56 received AM, and 54 received A3M). Baseline characteristics are similar in 3 groups (Table 5.1.1).

The initial responses to the three treatment groups were different. Mean fever clearance times were similar in all three groups 25.2 (3.1) hours in the group M, 21.5 (2.5) hours in AM and 22.6 (2.1) hours in A3M. But the parasite clearance times were significantly different, 64.6 (4.4) hours in M, 48.2 (3.8) hours in AM and 44.5 (3.4) hours in A3M. There are 4 patients in the M group whose peripheral parasiteamia were not cleared at the seventh day of the treatment course (R2). There were 23 patients in the M group, 16 in the AM group and 6 in the A3M group had fever and parasitaemia confirmed by microscopy during the 28-day follow up period. In addition, 3 in M group, 9 in AM group and 4 in the A3M group had also fever and positive blood smear during the time elapsed between the 28th and 56th day of the follow up. Of those 5 patients in the group M, 2 patients in the AM group and 2 in the A3M group had a recrudescence of the original infecting parasite, confirmed by PCR-genotyping representing a treatment failure. Therefore the failure rates at 56th day adjusted for re-infections are 36.8%, 44% and 14.8% for the M, AM and A3M group respectively. The recrudescence of the original genotype occurred on days 35, 35, and 28. The new infections were seen on days 28, 29, 34.

The most common side effect was nausea. All side effects in both groups were self limiting and in no patient was it necessary to repeat the dose of the tablets. 6 (16%)

patients in the A3M group had significant adverse events possibly related to their

treatment regimen. Four patients (10%) had vomiting, 3 patients (8%) had dizziness

and 1 patient had a transient neurological syndrome of ataxia, dizziness, and slurred

speech lasting 24 hours. All of these reactions were short lived and settled spontaneously.

There were no significant differences in the liver function tests between the two groups

carried out on all patients on day 3, 7, and 28.

Table 5.1.1: Patients characteristics on admission by drug treatment groups (M- AM- A3M)

| Characteristic | | Treatment | | | P value |
|---|---|---|---|---|---|
| | | M (n=57) | AM (n=56) | A3M (n=54) | |
| Age (yr) | mean (95% C.I) | 31.07(27.8-34.3) | 32.30(28.9-35.7) | 32.10(28.4-35.8) | 0.866† |
| Gender | Male/Female | 47/10 | 47/9 | 43/11 | 0.798‡ |
| Weight (kg) | mean(95% C.I) | 50.3(47.9-52.7) | 51.0(48.7-53.4) | 51.3(48.0-54.5) | 0.788† |
| Temperature ($^{o}$C) | mean(95% C.I) | 38.7(38.5-39.0) | 38.7(38.4-38.9) | 38.7(38.4-39.0) | 0.915† |
| Haematocrit (%) | mean(95% C.I) | 36.2(34.0-38.4) | 35.2(33-37.4) | 37.0(34.9-39.1) | 0.531† |
| White blood cell $10^{6}$/L | mean(95% C.I) | 6034(5534-6535) | 5638(5177-6100) | 5703(5262-6146) | 0.679† |
| Blood glucose (mmol/L) | mean(95% C.I) | 5.9(5.4-6.3) | 6.0(5.6-6.4) | 5.8(5.2-6.4) | 0.415† |
| Serum creatinine (μmol/L) | mean(95% C.I) | 94.5(89.2-99.8) | 93.7(88.4-98.1) | 90.1(86.6-94.5) | 0.689† |
| BUN (mmol/L) | mean(95% C.I) | 4.9(4.5-5.3) | 5.3(4.7-5.8) | 4.4(4.0-4.8) | 0.094† |
| Parasite count on admission / μL | Geometric mean | 14,693 | 16,766 | 13,755 | 0.753† |

† Kruskal Wallis Test ‡ Chi-Square test ⸶ Fisher exact test

Table 5.1.2: Patient outcomes by drug treatment groups (M- AM- A3M)

| Patients | M* | AM* | A3M* |
|---|---|---|---|
| Patients recruited | 70 | 72 | 73 |
| Excluded £ | 13 | 16 | 19 |
| Fever clearance times (h) † | 25.2 (18.9-31.6) | 21.6 (16.5-26.6) | 22.6 (18.4-26.8) |
| Parasite clearance time (h) ‡ | 64.7 (55.8-73.5) | 48.2 (40.6-55.9) | 44.5 (37.7-51.4) |
| Failure of parasite clearance within 7 days ( R 2) | 4 / 57 (7.0%) | 0/56 (0%) | 0/54 (0%) |
| Failure rates at 28 day–follow-up | 23 / 57 (40.3%) | 16 / 56 (28.5%) | 6 / 54 (11.1%) |
| Failure rates at 56 day–follow-up | 3 / 57 (5.2%) | 9 / 56 (16.0%) | 4 / 54 (7.4%) |
| Total failure cases | 26 / 57 | 25 / 56 | 10 / 54 |
| Re-infection (by PCR) | 5 | 2 | 2 |
| Real failure rates § | 21 / 57 (36.8%) | 23 / 56 (41.0%) | 8/ 54 (14.8%) |

* AM = artesunate 1 day (4mg/kg) + mefloquine (15 mg/kg) single dose;

   A3M = artesunate 3 days (4mg/kg then 2 mg/kg) + mefloquine (15 mg/kg) single dose;

   M = mefloquine 15 mg/kg single dose.

£ Mis-diagnosis (*P.vivax*) or discontinue the treatment, or unable to follow- up

† Kruskal Wallis test p=0.834

‡ Kruskal Wallis test p=0.001

§ Chi-Square test      p=0.003

**5.1.4 Discussion:**

Mefloquine and combination artemisinin/artesunate + mefloquine have been used in southern provinces of Viet Nam since 1991. Those drugs were introduced into the country in early 1990s with high cure rate > 95% (KA Trinh, *et al.*, 1990). But since 1991, reports from Thailand have shown that *P.falciparum* has developed resistance to mefloquine rapidly, despite the addition of sulphadoxine and pyrimethamine but there is no information on the resistance of the parasite to mefloquine in Vietnam, particularly in vivo studies. This is a first randomized comparative trial conducted in Vietnam assessing the effectiveness of mefloquine and the combination mefloquine + artesunate since 1995. Although there were 2 studies conducted between 1997 and 2000 but the dose of mefloquine and artemisinin used was rather low (500 mg) than the ones recommended by the national malaria control programme.

This study revealed that there is a dramatic decrease in mefloquine efficacy in southern provinces of Viet Nam since it was first introduced as one of drugs of choice in treatment of uncomplicated malaria in 1991. The cure rate has declined from 100% in 1990 (KA Trinh, *et al.*, 1990) to 45.5% in 2000 with 4 R2 response cases. While the main source of pressure for development of resistance in Thailand was thought coming from the use of mefloquine (F Nosten, *et al.*, 1991a), the frequency of exposure of *P.falciparum* to this drug was rather low in Vietnam (approximately 300,000 doses of mefloquine was distributed each year since 1992 but it was used in combination with artemisinin or artesunate) (Annual report of NIMPE). Worryingly, the efficacy of the combination of mefloquine (15 mg/kg) + one day artesunate (AM) which was proved very effective in 1995 (TT Hien, *et al.*, 1994) has also declined to a similar level. This result indicated that the dosage of components of the artemisinin based combination therapy (ACT) should be

revised and optimised. So far, the combination of mefloquine (25 mg/kg) + artesunate (4 mg/kg x 3 days) is still the gold standard regimen (with nearly 100% cure rate) for patients whose initial treatment failed with lower dose of mefloquine (Hien TT, unpublished data). The level of immunity of patients involved in this study is probably different from previous ones: all patients recruited were non-immune people it may contribute to the higher failure rates of the 3 regimens of treatment.

In order to measure the mefloquine levels at different timing during the treatment and the follow-up, a 5 ml of blood specimen were taken on admission, at 4th and 8th week from admission. Unfortunately, because of different reasons, results of those specimens were not available at the time of data analysis. It should be emphasized that mefloquine used in this study is Lariam (Roche) which is considered to have the best bioavailability amongst the different formulations.

In contrast with artemisinin group, mefloquine did not reduce rapidly parasitaemia (table 5.1.2) and that may put patients with falciparum malaria at risks of lethal complications. We have ruled out false RII due to delayed parasite clearance with mefloquine described elsewhere (F Nosten, *et al.*, 1987) by monitoring those patients for further 3-5 days before re-treatments

This study has also confirmed that the duration of follow up is an important factor being considered when assessing antimalarial drug efficacy (NJ White, 2003). Our result has indicated that the cure rate would decrease by 0.2 –2 fold (table 5.2) if we double the duration of follow up from 28 days to 56 days. Thanks to the PCR we can easily rule - out re-infection even as patients going back home in malaria areas.

This study confirms *P.falciparum* in Southern provinces in Vietnam has developed resistance to mefloquine rapidly. The results support the suggestion that mefloquine should no longer be used alone but only in combination with appropriate doses of

artemisinin or derivatives. There is also an urgent need to investigate newer combination with artemisinin.

## 5.2 Assessment of the efficacy of the combination Dihydroartemisinin-piperaquine

## 5.2.1 Introduction

Resistance to antimalarial drugs is increasing nearly everywhere in the tropical world, confounding global attempts to "Roll Back Malaria"(F Nosten & P Brasseur, 2002) Southeast Asia has the most resistant malaria parasites in the world, which has limited treatment options in this region. To combat the further spread of resistance, it is generally accepted that combinations of antimalarial drugs that include an artemisinin derivative should be used, and, if possible, that preparations should be formulated in a single tablet(NJ White, *et al.*, 1999a, WHO, 2001). There is only one combination available that meets these criteria: artemether-lumefantrine (MV Vugt, *et al.*, 1999).

In 1978, piperaquine—a bisquinoline antimalarial— replaced chloroquine as the antimalarial recommended by the national control programme of the People's Republic of China. Over the next 14 years, 214000 kg of piperaquine phosphate—ie, about 140 million adult doses—were used for mass prophylaxis and treatment. In 1990, a group of Chinese scientists began development of fixed-dose artemisinin-based combinations with piperaquine. The prototype, CV4, included four drugs: dihydroartemisinin, trimethoprim, piperaquine, and primaquine. These combinations were tested in small non-randomised clinical trials in China, Vietnam, and Cambodia. Modification of the doses of the individual components led to a new version (CV8), which was subsequently tested, marketed, and manufactured. CV8 was introduced to the Vietnamese National Malaria Control Programme in 1998. Concerns about the risk/benefit ratio of primaquine in the combination led to its removal and to the use of the triple combination. This preparation

contained dihydroartemisinin (32 mg/tablet), trimethoprim (90 mg/tablet), and piperaquine (320 mg/tablet). Uncertainties about the contribution of the trimethoprim component and dose then led to an increase in the dose of dihydroartemisinin to 40 mg/tablet, and removal of the trimethoprim. This new two-drug fixed combination antimalarial is quite inexpensive; in 2003 it cost about US$1 for an adult treatment.

Other effective treatments against multidrug-resistant Plasmodium falciparum malaria are much more expensive ($\geq$$2·40 per treatment) than widely used, but increasingly ineffective, drugs such as chloroquine and sulphadoxine-pyrimethamine ($\leq$$0·20). Cost is a major factor deterring countries and international donors from providing artemisinin-based combinations to combat the worsening global malaria situation. (F Nosten & P Brasseur, 2002, NJ White, *et al.*, 1999a) (WHO, 2001). There is an urgent need for alternative combination antimalarial drugs in fixed doses that are effective, safe, and affordable.

To assess the safety and efficacy of dihydroartemisinin-piperaquine combinations, we did two randomised trials in Vietnam. The first, a pilot study of dihydroartemisinin-trimethoprim-piperaquine (DHA-TP) versus the standard recommended regimen of 3 days' artesunate and a split dose of mefloquine (A3M) was based in a tertiary referral hospital in Ho Chi Minh City. The second study was a large open community-based randomised trial of DHA-TP, dihydroartemisinin-piperaquine (DP), and A3M in a provincial health station in Binh Phuoc Province.

## 5.2.2. Methods

### 5.2.2.1 Patients and randomisation

*Pilot study*

We recruited patients who were admitted to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, and had microscopically confirmed uncomplicated falciparum malaria. Patients were randomly allocated either DHA-TP (Tonghe Pharmaceutical, Yuzhong Chongqing, People's Republic of China) two tablets at 0 h, 6 h, 24 h, and 48 h (one tablet if the patient was younger than 15 years), or A3M (artesunate, Guilin Pharmaceutical Factory No 1, Guilin, China; mefloquine, Hoffman-La Roche, Basel, Switzerland) in a ratio of 2 (DHA-TP) to 1(A3M). DHA-TP contains 32 mg dihydroartemisinin, 90 mg of trimethoprim, and 320 mg of piperaquine per tablet. Patients allocated A3M received artesunate 4 mg/kg/day once daily for 3 days, plus mefloquine 25 mg base/kg given as two split doses, 6 h apart on day 3. Randomisation was in blocks of six.

Drugs were kept in identically numbered opaque envelopes, and drug administration was directly observed in all cases. We excluded women who were pregnant or breastfeeding, and children younger than 2 years.

The pilot study was approved by the Ethical and Scientific Committee of the Hospital for Tropical Diseases. We obtained written informed consent from patients or their guardians.

*Community based study*

Patients in the community based study were seen at the Dac O Health Station, Phuoc Long District, Binh Phuoc Province. The Dac O commune is in the southwestern region of Vietnam on the border with Cambodia, about 200 km from Ho Chi Minh City. There are roughly 9000 people living in the community served by the health station.

Transmission of malaria in this region is low and seasonal. Nearly all falciparum malaria infections there are symptomatic.

Adults and children older than 2 years presenting to the Dac O Health Station with microscopically confirmed uncomplicated falciparum malaria were eligible for the study. We excluded patients who had any evidence of organ dysfunction (impaired consciousness, severe anaemia [packed cell volume <0·20], oliguria, shock, convulsions, hypoglycaemia, respiratory distress, or tachypnoea [>50 per min]). Patients were also excluded if they were known to be pregnant, unable to tolerate oral medication, or were unable to return for follow-up. To control for the contribution of immunity to the treatment response, we did not include patients who had been resident in Dac O for more than 2 years.

Patients were randomly allocated one of three treatments: DHA-TP (two tablets at 0 h, 6 h, 24 h, and 48 h [one tablet if the patient was younger than 15 years]), or dihydroartemisinin-piperaquine (Holleykin Pharmaceutical, Guangzhou, People's Republic of China) (two tablets at 0 h, 6 h, 24 h, and 48 h [one tablet for children younger than 15 years]), or artesunate plus mefloquine in a ratio of 2 (DHA-TP):2 (DP):1(A3M). Patients allocated A3M received artesunate 4 mg/kg/day once daily for 3 days, plus mefloquine 25 mg/kg given as two split doses, 6 h apart on day 3. Drugs were kept in identically numbered opaque envelopes and drug administration with water was observed in all cases throughout the treatment regimen. Patients were monitored for 6 h after taking the drugs. A case report form was used to record the exact time that every dose was given. The contents of the randomisation envelopes were concealed from investigators and patients.

The Scientific and Ethical Committee of the Hospital for Tropical Diseases, and the Health Services of Binh Phuoc Province approved the study. Patients, or their guardians, gave written informed consent.

### 5.2.2.2 Procedures

*Pilot study and community based study*

At enrolment (day 0), patients had a full medical examination and we recorded all information on a standard case form. Blood was taken for quantitative parasite counts and routine haematological and biochemical analyses. In the pilot study, blood smears were taken every 12 h until patients were free of parasites, and they were then followed up every week until day 56. At each visit, a blood smear was taken and a questionnaire about symptoms and adverse effects was completed. Parasite counts were done from Giemsa-stained thick and thin blood films. If the count on the thick film exceeded 500 parasites per 500 white blood cells, we recorded the thin film result (expressed as the number of infected erythrocytes per 1000 red blood cells). In the community based study, blood smears were taken on admission (day 0), at the completion of treatment (day 2), and at day 7. All patients were followed up until day 56.

The primary endpoint for both studies was the reappearance of parasites within 56 days (ie, parasitological failure). Secondary endpoints were the parasite clearance time, fever clearance times, and the occurrence of side-effects. As reinfection during the follow-up period was possible, we took blood samples on filter paper at admission to the study and on the day that parasites reappeared. Recrudescences were distinguished from reinfections by PCR genotyping of three *Plasmodium falciparum* polymorphic loci (MSP1, MSP2, and GLURP) by standard methods (A Brockman, *et al.*, 2000) For patients whose parasitaemia cleared within 7 days but who had a recurrent infection at 56 days with the

same genotype of either P falciparum, or a mixed infection including the same genotype of P falciparum, treatment was deemed to have failed. People whose treatment had failed were retreated with artesunate 4 mg/kg/day once daily for 3 days, plus mefloquine 25 mg/kg given in divided doses on day 3.

### 5.2.2.3 Statistical analyses

In the pilot study, we calculated that a sample size of 114 patients allowed a difference between 75% efficacy (DTP) and 95% efficacy (A3M) to be detected with 80% power and 95% confidence, assuming a 10% dropout rate.

In the second community based study, randomisation was done in accordance with the ratio 2 DHA-TP:2 DP:1 A3M. A sample size of 160 patients in the DHA-TP and DP groups allowed for a cure rate of 95% in either arm to be estimated with 5% precision, and also to show efficacy equivalence with a maximum allowable difference in cure rates between groups of 10% in either direction with 80% power and 95% confidence, taking into account a drop-out rate of up to 30%. We used the Wilson procedure with a continuity correction to calculate 95% CI for efficacy differences (RG Newcombe, 1998). ANOVA was used for normally distributed data, and the Mann-Whitney U test was used to compare data that were not normally distributed. We used X2 test with Yates' correction or Fisher's exact test to compare proportions. Fever and parasite clearance time were assessed by survival analysis and by the log rank test. Analysis was by intention to treat.

### 5.2.3 Results

#### 5.2.3.1 Pilot study

Between February and July, 2001, 114 patients were enrolled. Of these, 107 (94%) lived outside malaria endemic areas and contracted their malaria when travelling into an

endemic region. No patient was taking malaria prophylaxis. The average dose (based on the number of tablets taken and bodyweight) of dihydroartemisinin was 2·4 mg/kg on the first day and 1·2 mg/kg on subsequent days, the average dose of piperaquine was 24 mg/kg on the first day and 12 mg/kg on subsequent days, and the average dose of trimethoprim used was 6·8 mg/kg on the first day followed by 3·4 mg/kg on subsequent days.

At admission, all patients were febrile (temperature >37·5°C), and the median (95% CI) fever clearance times were similar in both groups: 24 h (12–50) in A3M, and 24 h (2–49) in DHA-TP. Parasite clearance times (95% CI) were not significantly different at 72 h (23–97) in A3M and 48 h (22–98) in DHA-TP.

No patient developed severe malaria, none was lost to follow-up, and no patients were excluded. Five patients in the DHA-TP group had a reappearance of parasites within the 56-day follow-up period: two were recrudescences; and in three patients, a new genotype was confirmed by PCR, thus indicating reinfection. After adjustment for reinfections, the 56-day cure rate was 97·4% (74/76) after treatment with DHA-TP. The two recrudescences arose on days 29 and 30. New infections were noted on days 28, 34, and 35. There was no reappearance of parasites in the A3M group–ie, cure rate was 100% (38/38).

DHA-TP was well tolerated and no adverse drug reactions were noted or reported, whereas six (16%) patients in the A3M group had important adverse events that we judged to be related to treatment (p=0·01). Four patients (10%) had vomiting, three (8%) complained of dizziness, and one had a transient neurological syndrome of ataxia, dizziness, and slurred speech lasting 24 h. All of these reactions were short lived and settled spontaneously. There were no differences between the results of liver function tests done on days 3, 7, and 28.

Table 5.2.1: Admission characteristics of patients in A3M-DTP pilot study.

|  | A3 M | DTP |
|---|---|---|
| Number of patients (Female) | 38 (6) | 76 (11) |
| Mean age (years) ; range | 31.7( 8 – 56) | 32.9(5 – 67) |
| Mean admission temperature ($^{\circ}$C)[a] | 39.0 (38.6 – 39.3) | 39.0 (38.7-39.3) |
| Mean haematocrit (%)[a] | 38.0 (36.0 – 40.0) | 36.3 (34.3-38.3) |
| Mean white blood cell count (109/L) [a] | 5.161 (4080-5656) | 4.481 (4082-4815) |
| Blood urea nitrogen (mmol/L) [a] | 4.7 (4.2-5.2) | 5.4 (4.9-5.9) |
| Serum creatinine (μmol/L) [a] | 99.1 (83.9-97.2) | 95.4 (90.1-99.0) |
| Blood glucose (mmol/L) [a] | 5.8 (5.3-6.4) | 5.6 (5.1-6.0) |
| AST (aspartate aminotransferase) (U/L) | 23.1 (15.8-30.4) | 28.2 (16.0-40.3) |
| ALT (alanine aminotransferase) (U/L) | 6.4 (4.6-8.1) | 6.2 (4.9- 7.4) |
| Admission geometric mean parasitaemia (/μL) | 24,747 | 19,127 |
| Fever clearance time (hours) [a] | 24 (12 - 50) | 24 (2 - 48) |
| Parasite clearance time (hours) [a] | 72 (23 - 97) | 48 (22 –98) |
| Result of treatment assessed at D56 (cure) [b] | 38/ 38(100%) | 74 / 76 (97.4%) |

Data are median (95% CI) unless otherwise stated

AM = artesunate (4mg/kg/day ) at 0, 24, 48 hours + mefloquine (25 mg/kg split in two doses) at 48 and 56 hours, DTP=dihydroartemisinin 32 mg + piperaquine phosphate 320 mg + trimethoprim 90 mg; for adult : 4 tablets at 0, 8, 24, 48 hours; for children: age adjusted; a: Median (95% Confidence Interval), b: Fishers 2 – tailed test, all characteristics of 2 treatment groups are comparable unless otherwise stated

### 5.2.3.2 Community based study

Between November, 2001, and March, 2002, 1204 patients attended the health station in Dac O with fever and a positive blood smear for *P falciparum*. Of these, 30 were excluded because they were pregnant, and 774 were not enrolled because they were not able to return for follow-up, or they had been resident in Dac O for more than 2 years. Thus, we enrolled 400 patients in this community-based study, including 51 who were younger than 15 years. Of these children, 19 received DHA-TP, 21 DP, and 11 A3M. No patient was taking antimalarials for the present illness. Patients' baseline characteristics and outcomes were similar in all treatment groups (Table 5.2.2). In the DHA-TP group the average dose of dihydroartemisinin was 2·66 mg/kg on the first day and 1·33 mg/kg on subsequent days; the average dose of piperaquine was 26 mg/kg on the first day and 13 mg/kg on subsequent days; the average dose of trimethoprim was 7·5 mg/kg on the first day followed by 3·8 mg/kg on subsequent days. In the DP group, average dose of dihydroartemisinin was 3·4 mg/kg on the first day and 1·7 mg/kg on subsequent days and the average dose of piperaquine was 27 mg/kg on the first day and 13·5 mg/kg on subsequent days.

The initial responses to the three treatment groups were similar. On admission, all patients were febrile (temperature >37·5°C), but by day 2 (at the completion of treatment) fever had resolved in all cases. 17 (22%) in the A3M group, 50 (32%) in the DHA-TP group, and 45 (27%) of the DP group still had positive blood smears but with low parasitaemia at 48 h (Table 5.2.2). All patients were smear negative at day 7.

No patient developed severe malaria, none was lost to follow-up, and no patients were excluded. There were seven patients in the A3M group, 13 in the DHA-TP group, and 16 in the DP group who had fever and parasitaemia confirmed by microscopy during

Table 5.2.2: Admission characteristics of patients in A3M-DTP community based study:

| | DPT | DP | A3M |
|---|---|---|---|
| Number of patients (Male) | 157(124) | 166(121) | 77(53) |
| Mean age (years) | 26 (24.6-27.7) | 26.7(24.9-28.5) | 27.1(24.4-29.9) |
| Range | (7 – 60) | (10 - 65) | (8 – 52) |
| Mean admission temperature (°C) | 38.6 (38.7-38.8) | 38.7(38.5-38.9) | 38.5 (38.3-38.8) |
| Serum creatinine (μmol/L) [a] | 70.4 (17.6-114.4) | 79.2 (17.6-149.6) | 70.4 (17.6-123.2) |
| AST (Aspartate aminotransferase) (U/L) | 26.1 (19.9-32.3) | 19.2(13.8-24.6) | 31.0(21.6-40.3) |
| ALT (Alanine aminotransferase) (U/L) | 18.1 (7.6-28.6) | 12 (7.6-16.3) | 14 (7.6-20.3) |
| Geometric mean parasitaemia on admission (/μL) | 7789 | 6544 | 6272 |
| Mean temperature at 48 hours (°C) [a] | 37 | 37 | 37 |
| Geometric mean parasitaemia at 48 hours /μL) | 100 | 358 | 62 |
| Result of treatment (cure) [b] Assessed at 56 days follow-up | 153 (97.4%) | 164 (98.7%) | 76 (98.7%) |

Data are median (95% CI) unless otherwise stated

AM = artesunate (4mg/kg/day) at 0, 24, 48 hours + mefloquine (25 mg/kg split in two doses) at 48

and 56 hours; DTP =dihydroartemisinin 32 mg + piperaquine phosphate 320 mg + trimethoprim 90 mg.

DP = dihydroartemisinin 40 mg + piperaquine phosphate 320 mg

For adults: 4 tablets at 0, 8, 24, 48 hours; for children: age adjusted

a: Median (95% Confidence Interval) b: Fisher's 2 – tailed test all characteristics of 2 treatment groups are comparable unless otherwise stated

the 56-day follow-up period. Of these, one patient in the A3M group, four in the DHA-TP group, and two in the DP group had a recrudescence of the original infecting parasite confirmed by PCR-genotyping. Therefore, the 56-day cure rates, adjusted for re-infections, were 97·4% for DTP (153/157), 98·7% for DP (164/166), and 98·7% for A3M (76/77); P=1. The recrudescences and the new infections all occurred between days 28 and 35. The difference in efficacy between A3M and DHA-TP treatments was 1·2%

(95%CI –5·7 to 5·7%) and between A3M and DP treatments was –0·1% (–6·9 to 3·6%). Both CIs lie entirely within the equivalence range.

DHA-TP and DP were well tolerated. three (2%) and five (3%) of the DHA-TP and DP group, respectively, had minor adverse effects, mostly transient nausea. All side-effects in both groups were self limiting and resolved with the abatement of fever. No patient needed to repeat the dose of the tablets. In the A3M group, 12 (16%) patients had significant adverse events that might have been related to treatment (p=0·002). Ten patients (13%) had vomiting, and two (3%) had dizziness. All of these reactions were short lived and settled spontaneously. There were no significant differences between the three groups in the results of liver function tests done on all patients on days 3, 7, and 28.

### 5.2.4 Discussion

Our hospital and community based studies have shown that a combination of dihydroartemisinin and piperaquine, is an effective and safe treatment for multi-drug resistant falciparum malaria in Vietnam. In this country, P falciparum is resistant to chloroquine and antifolates and there has been a decline in sensitivity to mefloquine (A Brockman, et al., 2000); (NM Huong, et al., 2001). The use of the artemisinin derivatives has been central to successful malaria control efforts in Vietnam (F Nosten, et al., 1998); (TT Hien, 1994). The dihydroartemisinin-piperaquine combinations used in these studies were very well tolerated by adults and children. This reassuring safety profile accords with experience with the individual components. Dihydroartemisinin, both as a drug itself, and as the main biologically active metabolite of artesunate and artemether, and piperaquine have been used in millions of patients (TT Hien, 1994); (K Raynes, 1999). The bisquinoline piperaquine has a similar mode of action to other quinoline antimalarials (K Raynes, 1999). Some resistance did develop in China when piperaquine was used

widely as a monotherapy, and in mass prophylaxis, against already chloroquine-resistant *P falciparum* (X Guo & L Fu, 1989); (L Changxiong, *et al.*, 1989); (C Lin, *et al.*, 1982). However, piperaquine is clearly making an important contribution to controlling multidrug-resistant *P. falciparum* in Vietnam, since 3-day regimens of artemisinin monotherapy in Vietnam are associated with failure rates of more than 40% (TT Hien, *et al.*, 1994), and elsewhere 3-day regimens of artesunate were associated with 80% failure rates, compared with only 4% seen in this study. Combination with an artemisinin derivative would be expected to delay the emergence of resistance. The efficacy of dihydroartemisinin-piperaquine was equivalent to that of artesunate-mefloquine, the current treatment of choice for multidrug-resistant falciparum malaria in Southeast Asia. This combination has retained excellent cure rates (>95%) on the northwestern border of Thailand over a 10-year period, despite being introduced when mefloquine monotherapy failure rates had reached 50%. Dihydroartemisinin-piperaquine has several advantages over artesunate-mefloquine. It is a fixed-dose coformulation, which improves adherence, it is better tolerated, and it is around three times less expensive. In Vietnam, this combination is available for about $1 for an adult treatment course. This cost is generally regarded as the absolute upper limit of affordability for an antimalarial treatment in most countries affected by malaria. The cost of artesunate-mefloquine in Vietnam is $6, and the discounted price for artemether-lumefantrine after approval by WHO is $2·40.

Much remains to be learnt about dihydroartemisinin-piperaquine. Although there has been extensive clinical experience with the individual drug components, detailed large-scale adverse effect reporting (phase IV assessment) will be needed to confirm that rare adverse effects do not arise with the new combination. There are few pharmacokinetic data for piperaquine in human beings, although indirect evidence would suggest that it has a long terminal elimination half-life. The potential for resistance has already been shown when

used as a monotherapy, and a long terminal elimination half-life is a predisposing factor for the emergence of resistance. The dose of dihydroartemisinin is still low by comparison with other artemisinin-based regimens. Despite an increase in the amount of dihydroartemisinin per tablet in DP, the adult treatment dose still provides only of 3·4 mg/kg of dihydroartemisinin on the first day and 1·7 mg/kg on subsequent days. This amount may still be too low. The average dose of piperaquine used in this study was 27 mg/kg on the first day and 13·5 mg/kg on subsequent days. Increasing the dose of piperaquine may be limited by patients' nausea and vomiting (K Raynes, 1999). The late recrudescences confirm earlier suggestions that piperaquine has a long terminal elimination half-life. Clearly, the trimethoprim component did not make any contribution to the combination therapy, as would be expected from the high prevalence of antifolate resistance in this region, and is not necessary. Thus, further dose adjustments might still be necessary before the optimum combination of this antimalarial drug is found. Nevertheless, our results provide promise of a generally affordable treatment for multidrug-resistant falciparum malaria and lend support to the findings from a non-randomised trial in Cambodia (MB Denis, et al., 2002).

Quite small investments are needed to facilitate further development of this and the other already available artemisinin-based combinations such that they can become generally available. If used widely, this inexpensive, fixed-dose artemisinin-based combination antimalarial could make important contributions to "rolling back malaria".

# CHAPTER 6

# TREATMENT OF SEVERE MALARIA

## 6.1 A double blinded controlled comparative trial of artemether or quinine in Vietnamese adults with severe falciparum malaria.

### 6.1.1 Introduction

Since the cinchona alkaloids were introduced as a specific treatment for agues 350 years ago, the treatment of severe malaria has changed little. Quinine and quinidine remain the drugs of choice for severe chloroquine-resistant malaria due to Plasmodium falciparum, and with the spread of these resistant parasites, the usage of these drugs is increasing (WHO, 1990). In 1972 scientists in China discovered the antimalarial properties of a group of sesquiterpene lactone peroxides derived from the qinghao plant (*Artemisia annua*) (Qinghaosu Antimalaria Coordinating Research Group, 1979). The principal component, qinghaosu (artemisinin), and two derivatives — the water-soluble hemisuccinate artesunate and the oil-soluble artemether — are the most rapidly acting and potent of all antimalarial drugs (TT Hien & NJ White, 1993, Qinghaosu Antimalaria Coordinating Research Group, 1979, NJ White, 1994a); (GQ Li, *et al.*, 1994); (TT Hien, 1994, S Looareesuwan, 1994);(GQ Li, *et al.*, 1982b). These compounds retain activity

against all malarial parasites, including multidrug-resistant strains of *P. falciparum*. Over 2 million people have received antimalarial treatment with artemisinin, artesunate, or artemether (N White, 1994a). These drugs have proved rapidly effective in the treatment of severe malaria and remarkably nontoxic (GQ Li, *et al.*, 1994);(TT Hien, *et al.*, 1994); (GQ Li, *et al.*, 1982b); (J Karbwang, *et al.*, 1992b); (J Karbwang, *et al.*, 1995); (K Win, *et al.*, 1992b). Artemether has been reported to reduce the mortality rate for cerebral malaria caused by chloroquine-resistant *P. falciparum* by a factor of 3 (WHO, 1994).

### 6.1.2 Materials and methods

This study was conducted in a special research ward at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam, and was approved by the center's ethics and scientific-review committee. A full-time study team of specially trained physicians and nurses provided medical care. All laboratory measurements made during the acute phase of illness were conducted on site. A three-month pilot study was conducted before the main study to familiarize staff members with the clinical and laboratory procedures.

#### *6.1.2.1 Objectives*

The study was designed to detect a difference in mortality of 33 percent with 95 percent confidence and 80 percent power. The mortality rate for severe malaria was approximately 30 percent before the study. Except where indicated the secondary endpoints were defined prospectively.

#### *6.1.2.2. Drugs*

Patients were randomly assigned to receive artemether (50 mg per milliliter) or quinine dihydrochloride (250 mg per milliliter) for a minimum of 72 hours. The drugs were issued

in packs of 10 identical 3-ml ampules by the Kunming Pharmaceutical Company (Kunming, People's Republic of China). Randomly selected ampules were checked for sterility and potency. The initial dose of both drugs was 0.08 ml per kilogram of body weight (4 mg of artemether per kilogram and 20 mg of quinine salt per kilogram), half of which was injected into the anterior thigh of each leg. The maintenance dose was 0.04 ml per kilogram for each drug (2 mg of artemether per kilogram and 10 mg of quinine salt per kilogram) and was given every eight hours.

When the patient could take oral medication reliably, a second independent randomization was performed. Patients received either a single oral dose of 15 mg of mefloquine per kilogram (Lariam, Hoffmann–LaRoche, Basel, Switzerland) or oral quinine sulfate (Government Pharmaceutical Organization of Thailand, Bangkok) at a dose of 10 mg per kilogram three times daily for up to four days (i.e., for a total of seven days of antimalarial treatment). Treatment with quinine was followed by two tablets of pyrimethamine–sulfadoxine (Fansidar, Hoffmann–LaRoche).

### 6.1.2.3 Randomization and Blinding

The drugs for each patient were placed in a coded sealed envelope, and the envelopes were randomized in blocks of 20. Once a patient was enrolled in the study the envelope was opened. Subsequent analysis of efficacy was on an intention-to-treat basis. Although the ampules, drug volumes, and administration schedules of artemether and quinine were identical, the viscosity and color of the two drugs were slightly different. To maintain blinding, a separate team of nurses, who were not otherwise involved with the care of the study patients, drew up and gave the injections. The drugs were kept in an opaque packet in a locked cabinet during the study.

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### *6.1.2.4 Clinical Procedures*

Patients were included in the study if they (or an accompanying relative) gave informed consent, had asexual forms of P. falciparum on a peripheral-blood smear, were older than 14 years, were not in the first trimester of pregnancy, were not intravenous drug users, had received less than 3 g of quinine or two doses of artemisinin or a derivative in the previous 48 hours, and had one or more of the following: a score on the Glasgow Coma Scale of less than 11 (indicating cerebral malaria); anemia (hematocrit, <20 percent), with a parasite count exceeding 100,000 per cubic millimeter on a peripheral-blood smear; jaundice (serum bilirubin, >2.5 mg per deciliter [50 μmol per liter]), with a parasite count of more than 100,000 per cubic millimeter on a peripheral-blood smear; renal impairment (urine output, <400 ml per 24 hours; and serum creatinine, >3 mg per deciliter [250 μmol per liter]); hypoglycemia (blood glucose, <40 mg per deciliter [2.2 mmol per liter]); hyperparasitemia (>10 percent parasitemia); and systolic blood pressure below 80 mm Hg with cool extremities (indicating shock).

Each patient underwent a full clinical examination that included a detailed neurologic assessment. A complete blood count, count and estimation of the life-cycle stage of the parasites, biochemical analyses, measurements of plasma glucose and lactate, and blood cultures were done, and plasma was stored for measurement of cytokines, plasma quinine, and coagulation indexes. Arterial-blood gases and pH were measured on admission beginning in April 1992 (when 234 patients had been enrolled). Blood was obtained by a finger-prick for hematocrit measurements and blood smears every 4 hours for the first 24 hours and every 6 hours until three consecutive smears were negative for asexual stages of P. falciparum. The degree of parasitemia was determined on the basis of the number of parasitized red cells per 1000 red cells (thin film) or the number of parasites per 400 leukocytes (thick film).

### 6.1.2.5 Management and Clinical Monitoring

Patients were cared for according to standard recommendations (WHO, 1990). All patients were given isotonic saline to restore fluid balance, and fluid balance was maintained with saline or 5 percent dextrose in water. When necessary, a central venous catheter was inserted and the central venous pressure maintained at 5 cm of water. Blood was transfused if the hematocrit fell below 20 percent. Hypoglycemia was corrected with a bolus injection of 50 ml of 30 percent dextrose in water and a subsequent maintenance infusion of 5 to 10 percent dextrose in water. Detailed clinical and nursing observations were recorded a minimum of every 4 hours for the first 24 hours and every 6 hours thereafter.

In all patients blood glucose, lactate, and cytokine levels were measured 4, 8, 12, and 24 hours after admission. Beginning in June 1992 (after the enrollment of 259 patients) an electrocardiogram with a rhythm strip (paper speed, 50 mm per second) was obtained before treatment, 12 hours after treatment was begun, 4 hours after the last parenteral dose of antimalarial agent, and at discharge. Standard intervals were recorded, and the QT interval was corrected with the use of Bazett's formula ($QT/\sqrt{RR}$). A diagnostic lumbar puncture was performed if the score on the Glasgow Coma Scale was below 14. Opening cerebrospinal fluid pressures were measured, the cerebrospinal fluid was analyzed microscopically, and levels of protein, glucose, and lactate were determined. Peritoneal dialysis was started in patients with established renal failure. Beginning in September 1993 (after the enrollment of 427 patients) hemofiltration was also available. There were no facilities for ventilation. Acetaminophen was given for a temperature above 39°C, and intravenous diazepam, intramuscular phenobarbital, and if necessary, intravenous phenytoin were given for convulsions. Antibiotics with no clinical antimalarial activity

(i.e., not tetracyclines, macrolides, trimethoprim–sulfamethoxazole, or chloramphenicol) were prescribed for suspected cases of bacterial sepsis.

On discharge a detailed neurologic examination, electrocardiography, and beginning in November 1993 (after the enrollment of 441 patients), audiography (frequency range, 250 to 8000 Hz) were performed. A full autopsy was performed on patients who died of malaria if permission could be obtained from relatives. All information was recorded in the patients' records and then triple-entered in a computer data base.

### 6.1.2.6 Statistical Analysis

The study was reviewed continuously by an outside monitor. Categorical data were analyzed by Fisher's exact test, and continuous data by the Kruskal–Wallis test with the statistical programs Statview v.4.1 (Abacus Concepts, Berkeley, Calif.) and Stata v.4 (Stata, College Station, Tex.). The lengths of time to recovery were compared in the two groups by survival analysis with the Peto and Peto modification of the Wilcoxon test (R Peto & J Peto, 1972). Cox regression analysis was used to determine the contribution of different variables to recovery.

### 6.1.3 Results

Between May 1991 and January 1996, 561 patients were enrolled in the study. Only one patient was excluded from the analysis, because a review of the admission blood smear did not confirm the presence of malarial parasites. He died of an intracranial hemorrhage. There was a steady decline in the number of patients recruited during the study that paralleled the overall decline in the incidence of malaria in Vietnam. After reviewing the results of an interim analysis conducted in October 1994 (after 500 patients had been recruited), we decided to continue the study for one more year.

# Table 6.1.1 Characteristics of the artemether quinine patients on admission.

CHARACTERISTICS OF THE PATIENTS ON ADMISSION, ACCORDING TO TREATMENT GROUP.

| CHARACTERISTIC* | ARTEMETHER (N = 284) | QUININE (N = 276) | P VALUE | CHARACTERISTIC* | ARTEMETHER (N = 284) | QUININE (N = 276) | P VALUE |
|---|---|---|---|---|---|---|---|
| Age — yr | | | 0.99 | Shock — no. of patients (%) | 18 (6) | 25 (9) | 0.27 |
| Median | 30 | 30 | | Hypoglycemia — no. of | 23 (8) | 18 (7) | 0.52 |
| Range | 15–79 | 15–78 | | patients (%) | | | |
| Interquartile range | 22–40 | 22–41 | | Hematocrit — % | | | 0.18 |
| Sex — M/F | 212/72 | 213/63 | 0.49 | Median | 30 | 31 | |
| Previous treatment with quinine for | 155 (55) | 149 (54) | 0.93 | Range | 10–60 | 6–53 | |
| current episode of malaria — | | | | Interquartile range | 24–35 | 25–37 | |
| no. of patients (%) | | | | Parasite count — ×10⁻³/mm³ | | | 0.71 |
| Previous treatment with artemisinin or | 30 (11) | 22 (8) | 0.46 | Median | 90.4 | 86.9 | |
| derivative for current episode of | | | | Range | 0.02–1680 | 0.04–3690 | |
| malaria — no. of patients (%) | | | | Interquartile range | 16.8–339 | 19.3–343 | |
| Convulsions — no. of patients (%) | 30 (11) | 33 (12) | 0.59 | White-cell count — ×10⁻³/mm³ | | | 0.06 |
| Temperature — °C | | | 0.3 | Median | 8.35 | 9.08 | |
| Median | 38.2 | 38.5 | | Range | 1.9–35 | 3.5–38.5 | |
| Range | 36.3–41 | 36.5–41 | | Interquartile range | 6.2–11.9 | 6.5–12.6 | |
| Interquartile range | 37.5–39 | 37.5–39.2 | | Serum creatinine — mg/dl | | | 0.25 |
| Pulse rate/min | | | 0.87 | Median | 2.0 | 2.0 | |
| Median | 102 | 105 | | Range | 0.3–11.8 | 0.5–13.8 | |
| Range | 70–160 | 60–188 | | Interquartile range | 1.5–3.7 | 1.4–3.2 | |
| Interquartile range | 96–120 | 95–120 | | Total bilirubin — mg/dl | | | 0.71 |
| Respiratory rate/min | | | 0.59 | Median | 3.6 | 3.3 | |
| Median | 28 | 28 | | Range | 0.5–20 | 0.5–19 | |
| Range | 16–64 | 16–48 | | Interquartile range | 1.8–7.9 | 1.6–8.6 | |
| Interquartile range | 24–32 | 24–32 | | Aspartate aminotransferase — | | | 0.78 |
| Glasgow Coma score | | | 0.14 | μmol/hr/dl | | | |
| Median | 10 | 10 | | Median | 153 | 160 | |
| Range | 3–15 | 3–15 | | Range | 20–630 | 30–830 | |
| Interquartile range | 8–15 | 8–15 | | Interquartile range | 95–248 | 100–235 | |
| Glasgow Coma score <11 — | 142 (50) | 148 (54) | 0.4 | Plasma lactate — mmol/liter | | | 0.94 |
| no. of patients (%) | | | | Median | 3.2 | 3.4 | |
| Opening pressure of cerebrospinal | | | 0.45 | Range | 0.4–19.3 | 0.4–18.3 | |
| fluid — mm of water | | | | Interquartile range | 2–5.5 | 2.1–4.7 | |
| Median | 160 | 165 | | | | | |
| Range | 50–340 | 40–510 | | | | | |
| Interquartile range | 125–190 | 130–200 | | | | | |

*To convert values for creatinine to micromoles per liter, multiply by 88.4; to convert values for total bilirubin to micromoles per liter, multiply by 17.1.

The characteristics of the patients on admission are shown in Table 1. There were no significant differences in any of the major variables between the two treatment groups. The median total dose of antimalarial agent given parenterally, including that given before the study, was 120 mg of quinine per kilogram (range, 20 to 246) and 20 mg of artemether per kilogram (range, 4 to 44).

### 6.1.3.1 Outcome

The overall mortality rate was 15 percent (83 of 560 patients). The difference in the mortality rate between the artemether group and the quinine group was not significant (13 percent vs. 17 percent, P = 0.16) (Table 6.1.2). Four patients had neurologic sequelae (three in the artemether group and one in the quinine group), and thus there was also no significant difference in the combined number of deaths and cases of neurologic sequelae between the groups. In a multiple logistic-regression model that incorporated factors identified at the outset as associated with outcome, treatment with artemether was associated with a lower mortality (P=0.028) (Table 6.1.3). Among patients with cerebral malaria (score on the Glasgow Coma Scale, <11), the overall mortality rate was 16 percent (45 of 290 patients): 15 percent in the artemether group (21 of 142) and 16 percent in the quinine group (24 of 148, P = 0.75).

### 6.1.3.2 Causes of Death

The cause of death was often multifactorial. Of the 83 patients who died, 59 had acute renal failure (dialysis had been started in 30 of these patients), 56 had intractable shock, 35 had a terminal respiratory arrest with continued pulse, 25 had serious gastrointestinal bleeding, and 12 had pulmonary edema. A full autopsy was performed on 50 of the

patients who died. Serial measurements of arterial-blood gas were begun in 1992 and

showed that 42 of the 50 (84 percent) had metabolic acidosis

Table 6.1.2: Assessement of outcome after treatment with artemether or quinine

| Variable | Artemether (N = 284) | Quinine (N = 276) | Relative Risk (95% CI)* | P Value |
|---|---|---|---|---|
| . Assessment of Outcome after Treatment with Artemether or Quinine. | | | | |
| | no. of patients/total (%) | | | |
| Mortality rate | | | | |
| Overall | 36 (13) | 47 (17) | 0.74 (0.5–1.11) | 0.16 |
| Patients previously treated with quinine or an artemisinin derivative† | 18/187 (10) | 30/178 (17) | | 0.045 |
| Patients with no previous treatment† | 18/97 (19) | 17/98 (17) | | 0.85 |
| Convulsions | 36 (13) | 27 (10) | 1.3 (0.8–2.1) | 0.29 |
| Need for blood transfusion | 70 (25) | 70 (25) | 0.97 (0.73–1.3) | 0.85 |
| Need for dialysis | 34 (12) | 42 (15) | 0.8 (0.5–1.2) | 0.27 |
| Hypoglycemia | 31 (11) | 69 (25) | 0.44 (0.30–0.64) | <0.001 |
| Decrease in Glasgow Coma score after admission | 124 (44) | 107 (39) | 1.1 (0.9–1.4) | 0.27 |
| Gastrointestinal bleeding | 34 (12) | 42 (15) | 0.8 (0.5–1.2) | 0.27 |
| Chest infection | 64 (23) | 56 (20) | 1.1 (0.8–1.5) | 0.54 |
| Culture-positive urinary tract infection | 30 (11) | 18 (7) | 1.6 (0.9–2.8) | 0.1 |
| Culture-negative pyuria | 20 (7) | 8 (3) | 2.4 (1.1–5.4) | 0.03 |
| Thigh abscess | 1 (0.4) | 5 (2) | 0.18 (0.02–1.5) | 0.1 |
| Leg discomfort causing difficulty walking | 5 (2) | 7 (3) | 0.45 (0.14–1.5) | 0.56 |
| No parasite clearance within 7 days | 5 (2) | 6 (2) | 0.81 (0.25–2.6) | 0.77 |

*The relative risk is for the artemether group. The groups were compared by Fisher's exact test for proportions. CI denotes confidence interval.

†This comparison was not specified in the protocol's original analytic plan.

Table 6.1.3 The factors associated with death from severe falciparum malaria

MULTIVARIATE LOGISTIC-REGRESSION
ANALYSIS OF THE FACTORS ASSOCIATED WITH DEATH
FROM SEVERE FALCIPARUM MALARIA.

| VARIABLE | ADJUSTED ODDS RATIO (95% CI)* | P VALUE |
|---|---|---|
| Quinine treatment | 1.85 (1.07–3.19) | 0.028 |
| Year of study (1993–1996 vs. 1991–1992) | 1.47 (0.82–2.66) | 0.193 |
| Cerebral malaria† | 1.56 (0.89–2.8) | 0.12 |
| Shock on admission | 4.5 (2.03–9.9) | <0.001 |
| Jaundice on admission | 1.66 (0.90–3.1) | 0.11 |
| Elevated plasma lactate concentration on admission | 2.64 (1.72–4.1) | <0.001 |
| Elevated plasma creatinine concentration on admission | 4.0 (2.45–6.6) | <0.001 |
| Elevated hematocrit on admission | 1.0 (0.33–1.65) | 0.24 |

*In each case, the odds ratio was adjusted for the other variables listed. CI denotes confidence interval.

†Cerebral malaria was defined as a score of less than 11 on the Glasgow Coma Scale.

### 6.1.3.3 Recovery

Artemether treatment was associated with quicker clearance of parasites from the peripheral blood but slower resolution of fever, slower recovery from coma, and longer hospitalizations (P<0.005 for all comparisons) (Figure 6.1 and Table 6.1.4). We used a Cox proportional-hazards model to assess the contribution of different variables evaluated at admission (score on the Glasgow Coma Scale, creatinine and lactate values, log parasite count, and the presence of jaundice, hypoglycemia, and shock) to the time to recovery from coma (defined as the time to reach a score of 15 on the Glasgow Coma

Scale). In patients with cerebral malaria, quinine treatment remained significantly associated with a more rapid recovery than artemether treatment (hazard ratio, 1.39;

Figure 6.1: Kaplan Meier plots of overall survival (A), time to recovery from coma (B), parasite clearance time (C) and fever clearance time (D).



Kaplan–Meier Plots of Overall Survival (Panel A), Time to Recovery from Coma in Patients with Cerebral Malaria (Panel B), Time to Clearance of Parasites from the Peripheral Blood (Panel C), and Time to Resolution of Fever (Panel D).
In Panel C the four patients in whom clearance of parasites took longer than 250 hours (two in the artemether group and two in the quinine group) are not shown.
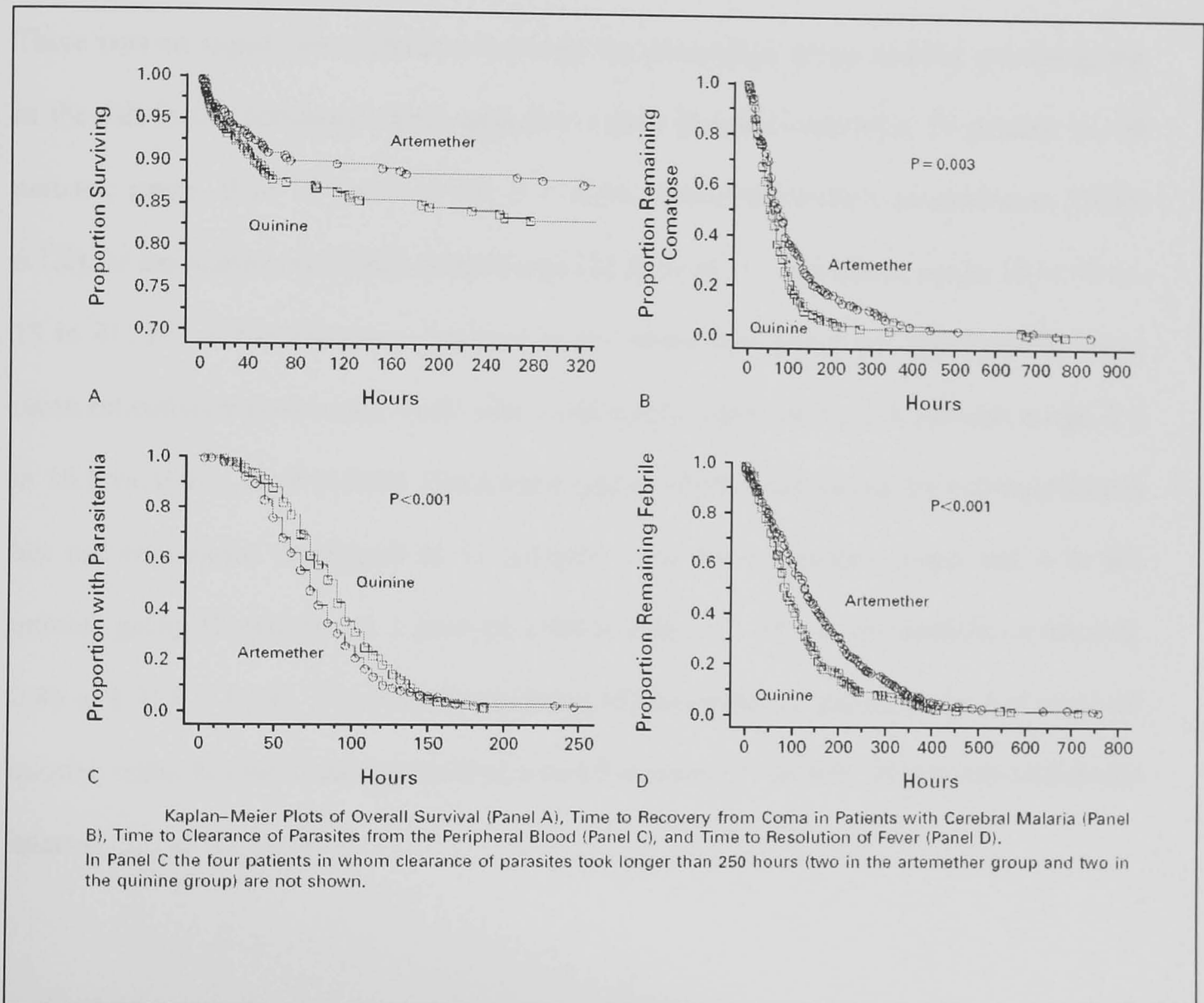
95 percent confidence interval, 1.06 to 1.81; P = 0.017), and this difference became evident after 48 hours (Figure 6.1 A, B, C, and D). The markers of disease severity at admission in patients with impaired consciousness that lasted more than 48 hours were

similar in the two groups. In a similar model, the differences in the time to resolution of fever between the two groups could not be explained by the occurrence of supervening bacterial infections.

### 6.1.3.4 Hematologic Recovery

There was no significant difference between the artemether group and the quinine group in the fall in the hematocrit from base-line values (median reduction, 27 percent vs. 30 percent; range, 0 to 71 vs. 0 to 68; P = 0.48), blood-transfusion requirements (Table 6.1.2), or the hematocrit values at discharge (22 percent vs. 24 percent; range, 10 to 40 vs. 13 to 41; P = 0.18). However, patients in the artemether group had significantly lower mean reticulocyte counts one week after treatment (2.3 percent vs. 5.6 percent; range, 0.1 to 16.1 vs. 0.0 to 28; P<0.001). Blackwater (red or black urine caused by hemoglobinuria but not hematuria) developed in 11 patients: 7 in the artemether group and 4 in the quinine group (2 percent vs. 1 percent; relative risk, 2.7; 95 percent confidence interval, 0.86 to 8.3; P = 0.11). The overall incidence of blackwater in patients who had received quinine either before or during the trial was 4.9 percent (21 of 431; 95 percent confidence interval, 3.0 to 7.4 percent).

## Table 6.1.4: Assessement of recovery after treatment of artemether or quinine

| | ASSESSMENT OF RECOVERY AFTER TREATMENT WITH ARTEMETHER OR QUININE. | | |
|---|---|---|---|
| **VARIABLE** | **ARTEMETHER** (N = 284) | **QUININE** (N = 276) | **P VALUE\*** |
| | hours | | |
| Duration of parenteral antimalarial treatment | | | 0.21 |
| Median | 82 | 80 | |
| Range | 56–180 | 40–168 | |
| Interquartile range | 64–108 | 64–96 | |
| Time for plasma lactate level to fall below 2.5 mmol/liter† | | | 0.97 |
| Median | 48 | 48 | |
| Range | 4–168 | 4–168 | |
| Interquartile range | 12–72 | 12–72 | |
| Time to parasite clearance | | | |
| Decrease to 50% of admission value | | | 0.084 |
| Median | 10.3 | 11.5 | |
| Range | 2–74 | 2–189 | |
| Interquartile range | 5–16 | 6–19.5 | |
| Decrease to 10% of admission value | | | <0.001 |
| Median | 19 | 23.5 | |
| Range | 3.5–182 | 3.5–235 | |
| Interquartile range | 13–28 | 16–34 | |
| Total clearance | | | <0.001 |
| Median | 72 | 90 | |
| Range | 4–330 | 16–414 | |
| Interquartile range | 54–102 | 66–108 | |
| Resolution of fever | | | <0.001 |
| Median | 127 | 90 | |
| Range | 0–756 | 0–714 | |
| Interquartile range | 60–216 | 54–144 | |
| Time to Glasgow Coma score of 8‡ | | | 0.039 |
| Median | 57 | 42 | |
| Range | 4–270 | 4–258 | |
| Interquartile range | 24–90 | 12–78 | |
| Time to Glasgow Coma score of 11§ | | | 0.023 |
| Median | 48 | 30 | |
| Range | 4–468 | 4–480 | |
| Interquartile range | 16–84 | 12–66 | |
| Time to Glasgow Coma score of 15¶ | | | 0.003 |
| Median | 66 | 48 | |
| Range | 0–828 | 0–768 | |
| Interquartile range | 30–132 | 20–84 | |

CONTINUED.

| Variable | Artemether (N = 284) | Quinine (N = 276) | P Value* |
|---|---|---|---|
| | hours | | |
| Time before patient able to drink | | | 0.35 |
| Median | 24 | 29 | |
| Range | 0–760 | 0–539 | |
| Interquartile range | 4–78 | 8–65 | |
| Time before patient able to eat | | | 0.82 |
| Median | 48 | 45 | |
| Range | 0–760 | 0–592 | |
| Interquartile range | 12–95 | 20–85 | |
| Time before patient able to sit | | | 0.70 |
| Median | 82 | 78 | |
| Range | 0–1068 | 0–1452 | |
| Interquartile range | 42–140 | 48–120 | |
| Time before patient able to stand | | | 0.52 |
| Median | 108 | 96 | |
| Range | 0–1200 | 0–1608 | |
| Interquartile range | 60–168 | 60–144 | |
| Time before patient able to walk | | | 0.38 |
| Median | 126 | 114 | |
| Range | 0–1200 | 0–1608 | |
| Interquartile range | 72–200 | 72–168 | |
| Time before patient able to leave hospital | | | 0.005 |
| Median | 288 | 240 | |
| Range | 96–1248 | 98–1608 | |
| Interquartile range | 216–432 | 192–336 | |

*The groups were compared by survival analysis with use of the Peto and Peto modification of the Wilcoxon test.[13]

†Only data from patients who survived and whose venous plasma lactate concentrations were above 2.5 mmol per liter on or after admission (171 in the artemether group and 173 in the quinine group) were analyzed.

‡Only data from patients whose scores on the Glasgow Coma Scale were below 8 at any time (110 in the artemether group and 116 in the quinine group) were analyzed.

§Only data from patients whose scores on the Glasgow Coma Scale were below 11 at any time (139 in the artemether group and 140 in the quinine group) were analyzed.

¶Only data from patients whose scores on the Glasgow Coma Scale were below 15 at any time (175 in the artemether group and 174 in the quinine group) were analyzed.

### 6.1.3.5 Adverse Effects

Quinine was associated with hypoglycemia: hypoglycemia developed in 25 percent of the quinine-treated patients as compared with 11 percent of the artemether recipients (relative risk, 2.3; 95 percent confidence interval, 1.6 to 3.4; P<0.001). There was an increased

incidence of culture-negative pyuria in the artemether group (P = 0.03), but the incidence of urinary tract infections was not significantly different between groups. There were no other definite systemic adverse effects. There were no significant electrocardiographic abnormalities in the patients in whom recordings were made, although in 60 of the quinine recipients (45 percent), as compared with 38 of the artemether recipients (25 percent), the corrected QT interval was prolonged by more than 0.5 second (relative risk, 1.8; 95 percent confidence interval, 1.3 to 2.5; P = 0.001). Only 12 patients in the quinine group (9 percent) and 11 in the artemether group (7 percent) had a corrected QT interval that was prolonged by more than 25 percent (P = 0.67). Prolongation of the corrected QT interval was not associated with any other clinical finding, including the development of shock or the duration of coma. There were no differences between the two treatment groups in auditory acuity on discharge from the hospital.

### 6.1.3.6 Oral Treatment

Overall, 247 patients were randomly assigned to receive oral mefloquine and 224 to receive oral quinine; 89 patients did not receive oral antimalarial agents because they had either died (74) or had received seven days of parenteral treatment (15). Nine patients died after receiving oral antimalarial agents (four given mefloquine and five given quinine). The oral antimalarial agents had no effect on recovery times or parasite clearance as assessed by Cox regression analysis.

### 6.1.4 Discussion

Our results show that artemether is a safe and effective treatment for severe falciparum malaria in Vietnamese adults. The mortality rate among artemether-treated patients was 26 percent lower than that among quinine recipients, but the 95 percent confidence

interval ranged from a 50 percent reduction to an 11 percent increase in mortality. However, in our prospectively designed multivariate analysis, which took into account other factors that contribute to outcome, treatment with artemether was associated with a significantly lower mortality rate than treatment with quinine. This suggests that artemether is at least as good as, and may be better than, quinine for severe chloroquine-resistant malaria. In a setting of relative quinine resistance (S Pukrittayakamee, *et al.*, 1994), similar to that in Vietnam (K Arnold, *et al.*, 1990), artemether proved considerably superior to quinine in smaller, open trials of severe malaria conducted in Burma and Thailand (J Karbwang, *et al.*, 1992a, K Win, *et al.*, 1992a) (J Karbwang, *et al.*, 1995). The large differences in mortality observed in these studies were not substantiated in our large and detailed double-blinded, randomised trial. This cannot be ascribed to differences in clinical severity or drug administration, since the clinical and laboratory features of severe malaria in our trial were similar to those reported previously and the doses of artemether were higher than those usually recommended.

Although treatment with artemether resulted in a more rapid reduction in the level of parasitemia as reported previously (TT Hien & NJ White, 1993), the overall times to defervescence, recovery of consciousness in patients with cerebral malaria, and discharge from the hospital were longer in artemether-treated patients. Survival analysis indicated a significant divergence in the times to recovery from coma among patients who remained unconscious for more than two days. In these patients, treatment with artemether was associated with slower recovery. There are three possible explanations for this unexpected finding: the play of chance, neurotoxicity, or a consequence of the beneficial effect of artemether — patients who would have died if they had received quinine survived because they received artemether and took longer to recover. Although there was no other evidence of neurotoxicity (TG Brewer, *et al.*, 1994a, TG Brewer, *et al.*, 1994b), an acute

reversible drug effect cannot be excluded, particularly since the difference in the times to recovery from coma became evident after 48 hours, after a total of 14 mg of artemether per kilogram had been given. In support of the third explanation, the times to recovery from coma were strongly associated with other measures of disease severity, and these measures were similar in all patients whose recovery from coma took longer than 48 hours.

In uncomplicated malaria, the artemisinin derivatives consistently shorten all aspects of recovery, whereas in severe malaria, in which the processes that cause organ dysfunction and death may already be largely irreversible (WHO, 1990), they do not. Pharmacokinetic factors may contribute to this difference. Parenteral artemether is dissolved in groundnut oil and administered by intramuscular injection. As compared with orally administered artemether, intramuscularly administered artemether is absorbed slowly and incompletely. This delay in achieving therapeutic blood levels may offset any intrinsic pharmacodynamic advantage of artemether. In general, the water-soluble artesunate has resulted in the most rapid therapeutic responses. Artesunate is given intravenously and is also rapidly bioavailable after intramuscular or oral administration (TT Hien & NJ White, 1993, N White, 1994b). Artesunate may be more effective than artemether in severe malaria. By contrast, absorption of intramuscular quinine in severe malaria is regular, and its plasma-concentration profiles are similar to those seen after intravenous administration(D Waller, *et al.*, 1990) (G Pasvol, *et al.*, 1991).

The intramuscular administration of quinine is painful and causes local tissue damage, which sometimes results in sterile abscesses (W Fletcher & S Visuvalingam, 1923, R Ross, 1914) and, occasionally, tetanus (LM Yen, *et al.*, 1994). In this study, both treatment regimens were well tolerated and only nine quinine-treated patients (3.2 percent) had abscess formation, difficulty walking because of local pain, or both, despite

the use of a concentrated solution (250 mg per milliliter, with a pH of 2). Thus, the risks of serious local reactions from intramuscular quinine are low, provided a scrupulous aseptic technique of injection is used. Quinine causes hypotension if given too quickly by intravenous injection, and it also prolongs ventricular repolarization, but substantial (>25 percent) prolongation of the corrected QT interval occurred in only 12 patients (9 percent), and no serious toxic effects were associated with the use of a loading dose of quinine despite the fact that the majority of patients had been treated previously with quinine (K Silamut, *et al.*, 1995). Indeed, there were no serious cardiovascular or nervous system effects with either drug.

Quinine is a potent stimulator of the secretion of insulin by pancreatic beta cells (NJ White, *et al.*, 1983c) and was associated with an increased risk of hypoglycemia. Overall, our results confirm that intramuscular administration of quinine is an acceptable alternative to intravenous administration and that the principal adverse effect of quinine in severe malaria is hypoglycemia (NJ White, *et al.*, 1983c) . In rodents, dogs, and primates, artemether (and the related compound, arteether) induced an unusual and selective pattern of damage to certain brain-stem nuclei (TG Brewer, *et al.*, 1994a, TG Brewer, *et al.*, 1994b); This has been the main concern overlying the further development of these compounds. With the possible exception of prolonged recovery from coma in patients given artemether, we did not detect any evidence of permanent damage to the central nervous system despite the use of maintenance doses that were three times higher than those now usually recommended. The auditory nuclei are among the most sensitive to damage by these compounds, but there was no evidence of residual hearing impairment.

Artemether is an effective alternative to quinine for severe malaria. It is simple to administer, equivalent in overall cost to quinine, and has no apparent local or serious systemic adverse effects. It is one of a family of new antimalarial agents that are active

against quinine-resistant parasites. These new drugs should not be used in an uncontrolled or unregulated way, or resistance to them will develop.

## 6.2 Assessement of IR artemisinin, IV IM artesunate and IM artemether in the treatment of severe and complicated malaria in Vietnam

### 6.2.1 Introduction

In recent years, artemisinin and its derivatives have been used widely in Vietnam and other developing countries to treat uncomplicated malaria caused by *Plasmodium falciparum*. They have also been used in severe and complicated malaria with good results (NJ White, 1994a). Recent studies have compared individual preparations of artemisinin with quinine, but direct comparisons between all the artemisinin derivatives have not been made. Consequently, it is not known which preparation and route of administration are preferable. In 1992, we reported a study comparing artemisinin suppositories with intravenous (i.v) artesunate and iv quinine in the treatment of cerebral malaria. However there were few cases in the group treated with artemisinin suppositories, owing to discontinuation of the supply from manufacturer(TT Hien, *et al.*, 1992a). We also showed that intramuscular(im) artesunate and iv artesunate were equally efficoacious (TT Hien, *et al.*, 1992b). We conducted the present study to compare the efficacy of different regimens of artemisinin and its derivatives in the treatment of *P. falciparum* severe and complicated malaria in a rural hospital setting.

### 6.2.2 Patients and methods

The study was an open randomized comparative study, conducted in Tan Phu regional hospital, which is responsible for health care in 2 districts, Tan Phu and Dinh Quan, in the

rural area of Dong Nai province, southern Vietnam. In this region, malaria is endemic throughout the year and the major employment is rice farming and forestry.

### 6.2.2.1 Patients

The patients were recruited into the study if 15 years of age or older, of either sex, with clinical symptoms and signs of malaria and the presence of asexual form of P. falciparum in their peripheral blood. In addition, they must have had at least one of the following signs: i) unarousable coma (Glasgow Coma Score (GCS) <11, ii) hypoglycaemia (blood glucose <2.2 mmol/L [40 mg%], iii) acute renal failure (plasma creatinine >265.2 µmol/L) [3 mg%] with or without oliguria, iv) jaundice (total bilirubin >51.3 µmol/litre [3 mg%] with parasitaemia > 100,000/µL or with plasma creatinin > 1.5 mg %, v) anemia (hematocrit <20% with parasitaemia. 100,000/ uL), vi) shock ( systolic arterial blood pressure < 80 mm Hg with a thready pulse and cold clammy extremities, and vii) hyperparasitemia (>250,000 asexual forms/µl whole blood (Hien et all 1996).

Patients were excluded from the study if prior treatment with more than 3 g of quinine or two doses of artemisinin or a derivative had benn recorded by the peripheral health care worker. Pregnant women in the first trimester and patients with concomitant diseases (active tuberculosis, bacterial meningitis, ect.) or mixed infections with P.vivax, were also excluded from the study.

Informed consent for participation was obtained from the patients or their relatives (in the case of comatose patients). The study was approved by the scientific and ethical committee of the Hospital for Tropical Diseases of Ho Chi Minh City Vietnam

### 6.2.2.2 Methods

*Clinical procedures*

On admission, a thorough history was taken and full clinical examination made, and the details were recorded on a standard form. Peripheral blood film was prepared for parasite counts every 6 hours, until 3 consecutive films had failed to reveil parasites. Blood was also taken a complete blood count and routine biochemistry (blood glucose, serum creatinine, serum bilirubine). The parasitaemia was determined as the number of parasitized red blood cells per 1000 red blood cells (thin film) or the number of parasites per 400 leucocytes (thick film). Axillary temperature, pulse, arterial blood pressure, respiratory rate and Glasgow coma scale were recorded every 6 hours.

*Treatment*

When a patient fulfilled the enrolment criteria, a sealed envelope containing the code for the treatment regimen was opened to allocate him/her to one of the following 4 treatment groups:

i) Artemether (Kunming Pharmaceutical factory, Kunming, People's Republic of China - PRC) 200 mg i.m initially then 100mg at 24, 48, 72.

ii) Artemisinin suppositories (Vietnam Industrial Development of Pharmaceutics, Ho Chi Minh City, Vietnam) 1200 mg initially, then 400 mg at 4, 24, 48, 72. The same dosage of artemisinin was re-administered if the suppositories were expelled within 2 hours of insertion

iii) Artesunate (Guilin No 2 factory, Guangxi, PRC) 120 i.m. initially, then 60 mg at 4, 24, 48, and 72 hours.

iv) Artesunate (Guilin No 2 factory, Guangxi, PRC) 120 i.v. initially, then 60 mg at 4, 24, 48, and 72 hours.

All patients received 750 mg mefloquin (Lariam®, Roche) as a single dose after regaining consciousness or at 72th hour. Fluid and electrolyte replacement, antipyretics, and other ancillary treatment were given when needed, as guided by the World Health Organisation(WHO, 1990). Patients with acute renal failure requiring dialysis were transferred to the Hospital for Tropical Diseases in Ho Chi Minh City for peritoneal dialysis.

We allocated the first 120 cases randomly to the treatment groups. When 30 patients had been recruited to the i.v. artesunate group, we stopped recruitment of patients into this group and randomly allocated the next 60 to the remaining 3 groups

*Assessment of results*

We assess fever clearance time (FCT) – time for axcillary temperature to fall to, and remain for > 24 hours at, 37.5 °C or lower, parasite clearance time, time to regain full consciousness(in comatose patients) and fatality rate.

*Statistical analysis*

The Kruskal-Wallis test was used to compare continuous variables, the $\chi 2$ test for categorical variables, the Kaplan-Meier procedure with log rank tests for survival analysis of fever clearance time, parasite clearance time, and time to recovery of consciousness. Epi Info version 6.0 and SPSS for Windows version 6.0 packages were used. All values of p < 0.05 were considered significant.

## 6.2.3 Results

Overall 180 patients were enrolled. Five patients were excluded from the analysis (one each in the im and iv artesunate groups, 2 in the im artemether group, and one in the

artemisinin suppository group) because a review of admission blood film showed that their parasite counts were below 500,000/ μL and there was no additional criterion of severity. Therefore, 175 patients, 51 in the artemisinin suppositories group, 45 in the i.m. artemether group, 49 in the i.m artesunate group and 30 in the i.v. artesunate group, were included in the analysis. There was no significant difference in any of the major admission clinical and laboratory characteristics of patients between the 4 treatment groups (Table 6.2.1)

### 6.2.3.1 Mortality

The overall mortality rate was 13.7 % (24 cases) and it was similar in each groups: 10.2% (5 cases) in the i.m. artesunate group, 11.5% in the i.m. artemether group, 16.6% ( 5 cases) in the i.v artesunate group, 17.6% in those receiving artemisinin suppositories ($\chi 2$ = 1.66 p = 0.64). Post mortem examination was unavailableand so the exact cause of death could not be established.

### 6.2.3.2 Recovery

The feverclearance time, parasite clearance time, and the time to regain full consciousness (in comatose cases) are presented in Table 6.2.2. The differences between the 4 groups were not significant. There was no neurological sequel among those who recovered from comatose state.

Table 6.2.1: Admission clinical and laboratory characteristics of the 4 artemisinin - treatment groups

| | Artemether intramuscular | Artemisinin suppositories | Artesunate intramuscular | Artesunate intravenous | P[a] |
|---|---|---|---|---|---|
| No of patients | 45 | 51 | 49 | 30 | |
| Gender (female/male) | 17/28 | 16/35 | 18/31 | 7/23 | |
| Age (year) [b] | 28 (16-65) [22-37] | 28 (16-62) [21-41] | 24 (15-66) [18-39] | 30 (15-60) [22-37] | 0.7 |
| Admission temperature($^{\circ}$C) [b] | 38 (37-41.5) [37.5-39] | 38 (37-40.6) [37-39] | 38 (37-41) [37.5-38.5] | 38 (37-41) [36.5-40] | 0.9 |
| Haematocrite(%) [b] | 34 (11-45) [25-38] | 31.5 (10-57) [26-38] | 30.5 (15-48) [24.5-36] | 32 (16-42) [26-36] | 0.8 |
| Leucocytes ( x $10^9$/L)[b] | 7500 (4200-19000) [700-9000] | 7500 (5000-25300) [6700-8200] | 7500 (2000-14400) [6500-8550] | 7000 ( 4100-10500) [6800-8000] | 0.8 |
| Parasitaemia (/$\mu$L)[c] | 20178 (140-1044000) [2000-151976] | 34538 (40-1065000) [18212-286368] | 33174 (60-1751000) [3893-244794] | 17159 (20-1217000) [1520-364742] | 0.5 |
| Cerebral [d] | 34 | 35 | 36 | 24 | |
| Anaemia [d] | 7 | 10 | 9 | 5 | |
| Jaundice [d] | 7 | 6 | 10 | 1 | |
| Acute renal failure [d] | 3 | 2 | 3 | 1 | |
| Hypoglycaemia [d] | 1 | 0 | 4 | 1 | |

a: Kruskal-Wallis test

b: Median values (range in parenthesis) [interquartile range in brackets]

c: Geometric mean (range in parenthesis) [interquartile range in brackets]

d: Some patients have more than one complication

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Table 6.2.2. Results of 4 artemisinin treatment groups

|  | Artemether intramuscular | Artemisinin suppositories | Artesunate intramuscular | Artesunate intravenous | P b |
|---|---|---|---|---|---|
| Fever clearance time (h)a | 48 (38-58) | 42(36-48) | 36 (30-42) | 30 (18-42) | 0.13 |
| Parasite clearance time (h) a | 30 (26-34) | 30 (24-36) | 24 (15-33) | 24 (15-33) | 0.3 |
| Conciousness recovery time (h) a | 47 (31-63) | 24 (18-30) | 30 (18-42) | 24 (4-44) | 0.18 |
| Fatality rate (%) | 11.1 | 17.6 | 10.2 | 16.6 | 0.64 |

a:All values are medians (95% confidence intervals in parenthesis) except fro fatality

b: Log rank test, except for fatality ($\chi 2$)

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## 6.2.4 Discussion

Since 1979, research in China has shown good results after the treatment of uncomplicated and severe and complicated malaria with artemisinin and its derivatives (GQ Li, *et al.*, 1994). Following these initial results, many other comparative studies (including double blind studies) have shown that both artesunate and artemether can lower the fatality rate in severe and complicated malaria (TT Hien, *et al.*, 1992a, J Karbwang, *et al.*, 1995, K Win, *et al.*, 1992a). However, recent large randomized studies have not shown significant differences in mortality and there is no consensus about the best drug and the best route of administration(N White, 1994a). We believe that is the first randomized study directly to compare artemisinin (in suppositories) with its derivatives, artesunate and artemether, in the treatment of severe and complicated malaria in a rural hospital.

The difference in mortality rates between the 4 groups was not significant. Nevertheless, any conclusion about the mortality rates should be circumspect because the power to detect a difference between these case mortality rates was quite low (8.4-11%) due to relative small number of patients. However the rates were in general, similar to those reported in Myanmar (14% for i.m. artemether and 8.3% for i.v. artesunate)(K Win, *et al.*, 1992a) and in Thailand (12.8% for i.m. artemether (J Karbwang, *et al.*, 1995).

The longest time to recovery of consciousness in our trial was in the artemether group (although the difference was not statistically significant) and this is concordant with the result of a recent study in which a prolonged coma period was also reported in patients treated with i.m artemether (median 66 hours compared with 48 hours in patients receiving quinine (TT Hien, *et al.*, 1996). The reason for this prolonged coma in patients treated with artemether is unknown, but an acute reversible drug effect cannot be

excluded (TT Hien, *et al.*, 1996). A large study is needed to compare i.m artesunate with i.m artemether.

The results of the present study substantiate our previous report that artemisinin suppositories can be used to treat severe and complicated malaria with efficacy equal to that of i.v. artesunate (TT Hien, *et al.*, 1992a). Artemisinin suppositories, unlike artesunate or artemether given by injection, are simple to administer, easy to store, Artemisinin suppositories have been used successfully to treat uncomplicated falciparum malaria in adult (K Arnold, *et al.*, 1990) and children (TT Hien, *et al.*, 1991) and have also been shown to reduce the mortality of cerebral malaria (TT Hien, *et al.*, 1992a). For the best outcome, treatment of severe and complicated malaria should be started as soon as possible. In Vietnam, the district hospitals serve as an intermediate level of medical care between communes health centres and provincial or tertiary hospitals; they received patients transferred from commune health centres or directly from neighboring households. Therefore, the district hospitals are the ideal setting for early treatment of severe and complicated malaria. Artemisinin suppositories may, therefore, be useful at the peripheral level of the health care system in many tropical areas where malaria is endemic and resources are limited.

# CHAPTER 7

# DISCUSSION

## 7.1 Artemisinins and the treatment of acute falciparum malaria in Vietnam

The problem of drug resistance in falciparum malaria increased throughout the 70's and 80's in Viet Nam and contributed to the worsening malaria situation in the country during those decades. The need for alternative antimalarial drugs was obvious and also that any treatment should be effective, safe, inexpensive, well tolerated, and readily complied with. It was also obvious that we need to develop a research programme to address these issues and provide solutions.

The cornerstone of the change in the management of malaria in Viet Nam was the introduction of artemisinin and other derivatives of the Chinese herb "qinghaosu" as first line treatment from 1995. The earlier laboratory and clinical studies in China provided the basis for clinical trials at the Hospital for Tropical Diseases. The artemisinin used was initially manufactured in China but more recently this manufacture has moved to Viet Nam from locally grown *Artemisia annua* plants. Research on *Artemisia annua* was started in early 1980s within the framework of the national malaria control programme: in 1984 samples of this plant were collected in Lang Son Province and in 1986 the presence of artemisinin in Vietnam *Artemisia annua* with high yield (0.5-1.2% in dried leaves) was

confirmed. The first randomised controlled comparative studies to assess the efficacy of artemisinins - outside of China - conducted at our hospital in 1991, demonstrated the value of artemisinin suppositories in adults and children with uncomplicated falciparum malaria (K Arnold, *et al.*, 1990) (TT Hien, *et al.*, 1991); during the 1990s we showed that artemisinin and its derivatives (artesunate or artemether) are the best antimalarial drugs available in terms of clearing malaria parasites. They may be given by the oral, parenteral or rectal routes (TT Hien & NJ White, 1993) (XT Cao, *et al.*, 1997a). However, we realised soon after these drugs were deployed (despite the rapid reduction in parasitaemia they caused), that when given alone for less than 7 days, there was a high recrudescence rate (50%) (K Arnold, *et al.*, 1990) (TT Hien, *et al.*, 1991). At that time, it was not clear whether the high failure rates reflected a "real" resistance (ie resistance despite using the maximum dose) or whether it was a failure secondary to insufficient dosage. Therefore, attempts were made to lower recrudescence rates in those malaria patients. There are two approaches to deal with high failure rate of antimalarial drugs: to increase the dose to the highest level at which the patient can tolerate or to combine with other drugs to exploit synergy. The former was the case of Thailand in 1991 when the mefloquine dose was increased from 15 mg/kg to 25 mg/kg (FO ter Kuile, *et al.*, 1992) and subsequently in Vietnam (TT Hien, *et al.*, 1994) and Thailand (F Nosten, *et al.*, 1994). It should be emphasized that i) while in Thailand artesunate was used to protect mefloquine, in Vietnam we added mefloquine to increase the cure rate of artemisin and its derivatives. ii) The objective of the Vietnam national malaria programme were effective prevention of death due to malaria at an affordable cost and a clear need to avoid reliance on foreign aids for antimalarial drugs such as mefloquine. Vietnam made a crucial decision, backed up by a strong political commitment, to ensure availability of artemisinin and artesunate sourced from the local production of these drugs. Therefore, artemisinin was initially

provided as monotherapy (5 - day regimen) without mefloquine this choice was influenced by limited availability, costs and need to rely on outside asssistance). However because of poor compliance to this regimen the combination with mefloquine was introduced to increase the adherence of the patients to treatment courses with an effective, simple regimen: single day treatment with artemisinin + mefloquine (TT Hien, *et al.*, 1994). The efficacy of this combination, supported by results of a similar trial (C Luxemburger, *et al.*, 1994), has been one of the regimens recommended by NIMPE since 1995.

Manufacturers in China based on their unpublished data recommended the doses of artemisinin and its derivatives. Our patients were treated with artemisinin 10 - 20 mg/kg or artesunate 1 - 2 mg/kg. The PCTs were satisfactory < 48 hours (TT Hien, *et al.*, 1994). Results from a more recent study imply that there is no reduction in PCTs with the use of single oral doses of artesunate higher than 2 mg/kg and that is the minimum to produce maximum effects in an average patient (BJ Angus, *et al.*, 2002). That study also supported the reliability of the earlier pharmacodynamic studies in China

The efficacy of this combination had progressively declined and in 2000, the cure rate was reduced by 50% as presented in Chapter 5. It suggested that the single day treatment of artesunate (artemisinin) despite combination with mefloquine could not protect itself. The effects of antimalarial drugs will be determined by an array of factors: absorption, distribution, elimination, which also varies between individuals. In addition, one needs to also factor in the susceptibility profile of the causal parasite. Our *in vitro* testing results indicate that there has been no remarkable change in the susceptibility of P. *falciparum* since 1996 (Hien, unpublished data). These data suggest that in planning artemisinin combination therapy (ACT) regimens the artemisinins should be part of the regimen for longer than one day.

Since 1994 the combined regimen artesunate + mefloquine has been the treatment of choice for multidrug resistant *P.falciparum* in Vietnam. While the provision of artemisinin /artesunate was from local production, the supply of mefloquine relied on a financial support from AusAid - a non-refunded aid from the Australian Government - which ended after 5 years. In order to move towards self–sufficiency for primary commodity of the malaria control programme, studies were done to assess newer combinations. From this Viet Nam started to assess "CV" drugs, standing for China and Vietnam. These were to be produced locally and were designed to replace artemisinin - mefloquine. Among the prototypes, CV4, included four drugs: dihydroartemisinin, trimethoprim, piperaquine, and primaquine. These combinations were tested in small non-randomised clinical trials in China, Vietnam, and Cambodia. Modification of the doses of the individual components led to a new version (CV8), which was subsequently tested, marketed, and manufactured. CV8 was introduced to the Vietnamese National Malaria Control Programme in 1998. Concerns about the risk/benefit ratio of primaquine in the combination led to its removal and to the use of the triple combination. This preparation contained dihydroartemisinin (32 mg/tablet), trimethoprim (90 mg/tablet), and piperaquine (320 mg/tablet). Uncertainties about the contribution of the trimethoprim component and dose then led to an increase in the dose of dihydroartemisinin to 40 mg/tablet, and removal of the trimethoprim. This new two-drug fixed combination antimalarial is quite inexpensive; in 2003 it cost approximately US$1 for an adult treatment compared with $6 of artesunate – mefloquine and $2.4 of Coartem (artemether-lumefantrine). Dihydroartemisinin-piperaquine has several advantages over artesunate-mefloquine and artemether-lumefantrine (Coartem). It is a fixed-dose co-formulation, which improves adherence and avoid monotherapy that could accelerate the development of resistance of *P.falciparum* to either drug these drugs; In addition, unlike artemether-

lumefantrine, of which the absorption is very dependent on co-administration with fat, (NJ White, *et al.*, 1999b) which may limit its effectiveness, the bio-availability of dihydroartemisinin-piperaquine may be independent with the administration of food; it is better tolerated, and it is around three times less expensive. In Vietnam, this combination is available for about $1 for an adult treatment course. This cost is generally regarded as the absolute upper limit of affordability for any antimalarial treatment in most countries affected by malaria has become the first choice treatment for *P.falciparum* malaria in Vietnam since 2003 and another study attempting to recruite over 4,000 patients, is ongoing in the same areas to assess the side effects of this new combination. In summary, among three available ACTs, dihydroartemisinin + piperaquine seems the most effective, safest, simplest administer and inexpensive combination for acute multi-drug resistant *P. falciparum* malaria in Vietnam and maybe for other malaria endemic countries.

## 7.2 Artemisinins and severe falciparum malaria

For decades, the mortality of severe and complicated falciparum malaria had been between 30-40% in hospitals in Vietnam (TT Hien, 1994). Many attempts had been made to reduce the case fatality. Thanks to the introduction of "qinghaosu" the death toll has been hugely reduced from almost 5000 people dying from malaria in Viet Nam in 1994 to 50 in 2003 ( NMCP report in 2004). Unfortunately, despite conducting the largest ever double blind, controlled trial in malaria comparing i.m artemether and i.m quinine (Chapter 6) we failed to show any significant difference in mortality. Why that new and powerful antimalarial drug could not reduce the case fatality rates in hospitalised patients? These patients were severely ill, they may have been treated unsuccessfully elsewhere with different drugs and they may have developed complications before they or their families decided to go to the central hospital. At this late stage, the antimalarial

treatment seems to be no longer the most important measure. The failure of treatment in patients who die may be due to multiple pathological processes which are irreversible by the time these patients are seen in the tertiary hospitals. However the lowest ever mortality reported in severe falciparum malaria in Vietnam in this trial indicated the impact of better and appropriate ancillary treatment such as renal replacement treatment and nursing care. Concerning the choice between artemether and artesunate, the pharmacokinetic study (Chapter 3) indicated that artesunate is the preferred choice for the treatment of severe falciparum malaria, particularly in those patients who are most seriously ill and in whom absorption from an i.m. lipid-soluble depot may be compromised. These findings have been assessed by a recent randomised clinical trial at our hospital and the findings of which will be available soon.

The number of deaths from severe malaria in Vietnam, however, continues to fall from 4646 cases in 1991 to 198 in 1996 and there were only 75,144 malaria patients with 50 deaths in 2003 (Report from NMCP 2004). It is likely that artemisinin and its derivatives have contributed to the decline in malaria mortality rate, not by being more effective in treating the lethal form of the disease, but by reducing the number of severe cases. By rapidly clearing the parasitaemia in addition to a possible anti-cytoadherence effect (R Udomsangpetch, *et al.*, 1996), artemisinin and derivatives may help to prevent the development of complications when these drugs are given early to malaria patients, particularly at peripheral level of health care system. Another possible advantage of qinghaosu which has just been demonstrated elsewhere (RN Price, *et al.*, 1996) is a reduction of the transmission potential of falciparum malaria with artemisinin treatment. The deployment of these compounds may prevent the spread of multi-drug resistant disease. These factors may explain the improvement of malaria situation in China and in Viet Nam.

In the early 1990s artemisinin suppositories were shown to be as effective as parenteral antimalarial drugs in clinical trials for the treatment of severe malaria(XT Cao, *et al.*, 1997a, V Ha, *et al.*, 1997, TT Hien, *et al.*, 1992a) The rectal bioavailability is 30% relative to the oral dose although there is large inter-individual variation. Because of the similarity in parasite clearance times between oral and rectal administration, it is assumed that therapeutic concentrations could be achieved with artemisinin suppositories(R Koopmans, *et al.*, 1998, JS Sidhu, *et al.*, 1998). Following these resuls, the WHO has developed artesunate suppositories (Rectocaps) and is now under large-scale evaluation (MI Awad, *et al.*, 2003, KI Barnes, *et al.*, 2004, HA Karunajeewa, *et al.*, 2004)·(HA Karunajeewa, *et al.*, 2003, S Krishna, *et al.*, 2001). All results from those studies indicated that artesunate suppositories are safe, effective initial treatment for uncomplicated and severe falciparum malaria in Africa and in Asia.

Today intrarectal qinghaosu (artemisinin and artesunate) is becoming an effective treatment for *P.falciparum* malaria and offers great promise as a life saving intervention in remote areas where facilities for parenteral drugs maybe limited and of course the diseases has its greatest impact.

## 7.3 Neurotoxicity

Because of concern about the possible risk of neurotoxicity of artemisinine, particularly artemether, detected by experimental studies at the Walter Reed Army Institute of Research in the USA, studies were conducted to rule out that adverse effect in man. The first study aiming at careful neurological evaluation of the population exposed to multiple treatments with the artemisinin derivatives could not detect any convincing clinical or neurophysiological evidence of brain stem neurotoxicity, despite thorough examination in subjects receiving up to twenty-one treatment episodes in a two-year period. Although as

a field study in a small rural community it could not meet the rigorous criteria of a drug-safety study, the lack of evidence of neurotoxicity is reassuring. Those evidences consolidated the results from another study in Thailand in which 79 patients who received more than 2 treatments of artemether or artesunate and 79 age- and location- matched controls were assessed with a similar protocol (M Van Vugt, *et al.*, 2000)

In the second study, the results of a pathological examination of brain tissue from patients with severe falciparum malaria treated artemether or quinine indicated that there were no neuronal death in artemether recipients and other neuropathological findings between the two different treatments were similar. These data provide reassurance that therapeutic doses of these important antimalarial drugs do not damage the human nervous system. However surveillance programmes will be needed to identify a variety of adverse events caused by artemisinin and its derivatives as well as other partner drugs of artemisinin based combinations such as mefloquine, lumefantrine or piperaquine

## 7.4 Conclusion

In summary the objectives set out in this thesis have been largely met. Through the production and use of artemisinin and its derivatives Vietnam has successful implemented a Malaria control programme that would otherwise have been impossible. The implementation has been underpinned by clinical and operational research. The research described in this thesis addresses both the treatment of severe and uncomplicated malaria with artemisinin and its derivatives, and artemisinin based combination treatment. The pharmacological properties relevant to treatment, safety and efficacy have all been characterised. These new drugs have been remarkably safe and effective.

Since 1990 our studies on artemisinin and it derivatives, both oral and parenteral preparation, and especially in combination with mefloquine (K Arnold, *et al.*, 1990) (TT

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Hien, *et al.*, 1991) (TT Hien, *et al.*, 1992a) (TT Hien, *et al.*, 1994) (XT Cao, *et al.*, 1997b) have made an impact on the recommendations for the clinical management of patients with malaria by the National Malaria Control Programme of Vietnam. This has contributed directly to the success of the malaria control programme of the country and we also believe that our studies have also exerted considerable influence over the drug policy for malaria at the World Health Organisation. The results from the studies in this thesis are still influencing the recommendations issued by the NMCP. In 2003 combination of dihydroartemisinin and piperaquine (CV8 and Artekin) was the first line antimalarial drug for falciparum malaria in Vietnam and the production of artekin will start in 2005. That move will allow the country to deal with the increasing failure rate of the original regimen (A3M), to minimise the expense for imported antimalarial drugs (mefloquine), and help the government's policy of being self-sufficiency in antimalarial drug. At an international level, these results maybe useful for the development of new antimalarial drugs to replace the old drugs that are rapidly loosing their effectiveness. They could and should contribute to "Rolling Back Malaria" throughout the malaria endemic world.

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