RESEARCH ON NEW ANTIMALARIAL DRUGS AND THE USE OF DRUGS IN COMBINATION AT THE BANGKOK HOSPITAL FOR TROPICAL DISEASES

Sornchai Looareesuwan[1], Polrat Wilairatana[1], Watcharee Chokejindachai[2], Parnpen Viriyavejakul[3], Srivicha Krudsood4, and Pratap Singhasivanon[4]

[1]Department of Clinical Tropical Medicine; [2]Department of Tropical Pediatrics; [3]Department of Tropical Pathology; [4]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

Abstract. With the emergence of multidrug resistant falciparum malaria in Thailand, various approaches have been taken. Research on new antimalarial drugs and the use of existing available drugs with modification are urgently needed. New drugs and drugs in combination such as pyronaridine, WR 238605, arteether, dihydroartemisinin, benflumetol atovaquone/proguanil are being evaluated. Drug combinations for the treatment of patients suffering from uncomplicated falciparum malaria include quinine-tetracycline for 7 days, or sequential treatment of artesunate (600 mg given over 5 days) followed by mefloquine (1,250 mg divided into 2 doses 6 hours apart) are recommended. The sequential treatment is highly recommended for those who failed other treatment regimens. Other combinations such as a short course sequential treatment of artesunate (300 mg given over 2.5 days) followed by a single dose of 750 mg mefloquine, or a combination of mefloquine 1,250 mg together with tetracycline 1 g per day or doxycycline 200 mg per day for 7 days are alternative treatment regimens with acceptable cure rates. The simultaneous administration of artesunate and mefloquine, in various doses and duration of treatment, is currently being investigated. Until proven otherwise, the drug combinations are still recommended for all adult patients suffering from acute uncomplicated falciparum malaria contracted in multidrug resistant areas. In severe malaria and malaria in children, the drug combinations need further investigation.

INTRODUCTION

Multidrug resistant falciparum malaria is a serious problem in Thailand and therapeutic failures to all existing antimalarials are well documented (Looareesuwan *et al,* 1992a). New drug development takes years before the drug can be registered for use in man. Therefore studies of currently available drugs with appropriate rationales for use could be beneficial to combat this infection. Two measures have been advocated, modification in dosage (White *et al,* 1983; Chongsuphajaisiddhi *et al,* 1983) and the use of drug combinations (Looareesuwan *et al,* 1992b,c).

RESEARCH ON EXISTING DRUGS WITH MODIFICATION IN DOSAGE

Mefloquine (Lariam®)

Mefloquine, a synthetic 4-quinolinemethanol, was developed by the US Army antimalarial drug development program. It was initially thought to be the ideal antimalarial. Its' action is rapid and it is effective against all species of human malaria parasites, including chloroquine-resistant *P. falciparum.* In an effort to delay development of resistance to mefloquine, a combination of mefloquine and sulfadoxine-pyrimethamine (Fansimef®) was advocated. This drug has been registered for use in Thailand since 1984. The evidence that the combination may delay development of drug resistance came mainly from a mouse model and has not been generally accepted. In patients with acute falciparum malaria, the combination has not prevented the development of resistance to mefloquine. Furthermore, severe adverse effects have resulted (presumably from the sulfadoxine component) when the combination was used for malaria prophylaxis. It now seems unequivocal that mefloquine should be used as a single agent. Treatment failures have been reported in many parts of the world due either to true parasite resistance (intrinsic) or inadequate dosing. In Thailand, Fansimef® with the dosage mefloquine at 15 mg/kg gave a cure rate of over 98% of uncomplicated falciparum malaria in 1983

Correspondence: Professor S Looareesuwan, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Rajvithi Road, Bangkok 10400, Thailand.

to 1986 but subsequently fell to 71% by 1990 (Bunnag and Harinasuta, 1987a). However, a higher dose of mefloqine (25 mg/kg) given in 2 divided doses 6 hours apart, achieved a cure rate of 81% in 1991 (Looareesuwan *et al,* 1992b). In children, mefloquine alone, in doses ranging from 18 to 25 mg/kg, has also shown a decrease in cure rates from 98% in 1986 to 73% in 1990. At present, mefloquine has been used in combinations with artemisinin derivatives or in sequential for treatment of multidrug resistant falciparum malaria. These combinations or sequential treatments proved safe and produced higher efficacy (Looareesuwan *et al,* 1992b, 1993, 1994a, 1997a,b). In severe multidrug resistant areas such as Thailand, the combinations of mefloquine and tetracycline or mefloquine and doxycycline reveals higher cure rates (Looareesuwan *et al,* 1994b,c).

Halofantrine (Halfan®)

Halofantrine, a phenanthrene methanol, is another product of the US Army antimalarial drug development program. Initial studies showed it to be effective against multidrug resistant *P. falciparum* both in Thailand and Africa. However, subsequent studies in Thailand in 1988 and 1991 have shown that halofantrine (total dose of 1,500 mg given in three doses) cured only 58% and 70% of patients respectively even though this drug had not been widely used (Bunnag *et al,* 1993). Higher doses of halofantrine were tested at different regimens in Thailand and showed increase cure rates (Ter Kuile *et al,* 1993; Looareesuwan *et al,* unpublished data). However, some electrocardiogram changes have been observed in a study in Karen patients living in the Western part of Thailand (Nosten *et al,* 1993). Halofantrine was licensed by the FDA in 1992 for use at a dose of 8 mg/kg given 8 hourly on day 1 and day 7. However, it should not be used in patients with underlying cardiac abnormalities.

Biguanides

Proguanil and chlorproguanil are pro-drugs for the active triazine metabolites cycloguanil and chlorcycloguanil respectively. They are considered the safest of all antimalarials. Their action is based on DHFR inhibition. Proguanil is used for prophylaxis and often in combination with chloroquine. Chlorproguanil is used in combination with dapsone as a treatment of *P. falciparum* infections in Africa. However, the combinations of proguanil or chlorproguanil with dapsone are not sufficiently effective in the treatment of uncomplicated falciparum malaria in Thailand. The antimalarial activities of these compounds are due to their active metabolites which require enzyme cytochrome P450 for their formation. This enzyme shows marked genetic polymorphism. It is estimated that 3% of Africans and Caucasians and 15% of Orientals fail to convert the parent compounds to their active metabolites. The use of high dose of proguanil alone for the treatment of *P. falciparum* in Thailand produced unsatisfactory results (Wilairatana *et al,* 1997). However, the combination of proguanil and hydroxynaphthoquinone (atovaquone) is synergistic, proved safe and produced a 100% cure rate in multidrug resistant falciparum malaria in Thailand (Looareesuwan *et al,* 1996e).

RESEARCH ON NEW DRUGS

Hydroxynaphthoquinones (Atovaquone)

Wellcome Research Laboratories research has been conducted for many years on the development of hydroxynaphthoquinones as potential antimalarial drugs. Atovaquone, one of the derivatives of hydroxynaphthoquinones, has been registered for use in the treatment of *Pneumocystis carinii* infection. It is also effective in the treatment of toxoplasmosis in patients with AIDS. The drug has blood schizontocidal activity but very variable oral bioavailability. The combinations of hydroxynaphthoquinone with tetracycline, doxycycline or proguanil have shown synergistic effects both *in vivo* and *in vitro*. Atovaquone alone has proved safe and produces clinical cure against multidrug-resistant malaria in Thailand in all treated cases. However, one-third of the patients recrudesced. Atovaquone when given together with proguanil was safe and well tolerated and gave a 100% cure rate (Looareesuwan *et al,* 1996e). Recently clinical trials in 7 countries of the combination of atovaquone plus proguanil gave over all cure rate of 98% and the combination is now registered under the name of Malarone® in the United States of America and England (Looareesuwan, unpublished observations).

Pyronaridine

Compounds of the pyronaridine series have been shown to be highly active blood schizontocides, effective against chloroquine-resistant strains of animal and human malaria. They were developed in China. The drug has been used successfully in the treatment of *P. falciparum* malaria in China in combination with sulfadoxine-pyrimethamine. However, a clinical trial in Thailand with oral pyronaridine alone showed a cure rate of 63% and 88% with the total dose of 1,200 mg and 1,800 mg (300 mg given twice in the first day, then 300 mg once daily) given over 3 days and 5 days, respectively (Looareesuwan *et al,* 1996b). Recently new formulation of this drug produced in Malaysia has been studied base on pharmacokinetics and is on clinical trials (Looareesuwan *et al,* 1996d).

Benflumetol

Benflumetol was synthesized in the 1970s by the Academy of Military Medical Sciences, Beijing, and registered for use in China as an antimalarial drug in 1987. It is formulated for oral administration and used together with artemether for the treatment of *P. falciparum* malaria. Phase III clinical trials have been performed in Thailand and elsewhere (Looareesuwan *et al,* 1997c; van Vugt *et al,* 1997c). Now this combination is on the process of registration.

WR 238605

8-aminoquinolines (Primaquine®)

WR 238605, one of the 8-aminoquinoline derivatives, is being developed by the Walter Reed Army Institute for Research as an alternative to primaquine for radical cure of relapse malaria. Several new 5-phenoxy-compounds (WR 225448, WR 233195, WR 233078, WR 242471) were synthesized and tested in animal malarias. WR 238605 was found to be 5 times more active than primaquine in animal malarias. The clinical trials of WR238605 in vivax patients in Thailand are being evaluated.

Artemisinin derivatives

Artemisinin derivatives ("qinghaosu") are potent antimalarial substances extracted from the plant *Artemisia annua* (Klayman, 1985). Artemisinin, artemether, artesunate and dihydroartemisinin were subsequently developed by Chinese scientists for the treatment of acute uncomplicated and severe falciparum malaria, including cerebral malaria. Oral, parenteral, and rectal preparations of artemisinin derivatives are becoming widely available, particularly in Southeast Asia. The World Health Organization recommends that artemisinin derivatives be limited to the treatment of uncomplicated falciparum malaria, ideally in combination with a long-acting antimalarial drug such as mefloquine in areas of multi-drug resistance, and to complicated malaria cases where quinine resistance is becoming increasingly prevalent (Looareesuwan *et al,* 1990; Pukrittayakamee *et al,* 1994; World Health Organization, 1993; Price *et al,* 1995). The drugs should not be used for chemoprophylaxis.

Artesunate : Artesunate injection formulation is dispensed as a dry powder of artesunic acid. The powder is mixed with sodium bicarbonate and given as an intravenous or intramuscular injection. Artesunate, a water soluble formulation, is the most rapidly acting of the artemisinin compounds probably because it is immediately bioavailable (as dihydroartemisinin) after intravenous injection (Hien and White, 1993; White, 1994a,b). Artesunate oral formulation is also rapidly absorbed. It rapidly clears parasites from the blood with few adverse effects (Bunnag *et al,* 1991a, b, c; Hien *et al,* 1992a, b; Li *et al,* 1994; Looareesuwan *et al,* 1992b,c; Looareesuwan *et al,* 1993; Looareesuwan *et al,* 1994a,c; Looareesuwan *et al,* 1995; Barradell *et al,* 1995; Looareesuwan *et al,* 1997a).

Artemether: Artemether is formulated in peanut oil for intramuscular injection. It is more stable than artesunate intravenous injection. The drug is effective in killing parasites but absorption is slower than artesunate. Artemether intramuscular injection may be a practical alternative to quinine in severe malaria since it is less irritating and less toxic and is given only once a day. Artemether injection has been licensed for use in Thailand. However the oral preparation has not yet approved. Oral artemether is undergoing clinical trials. As with artesunate, the parasiticidal effect is rapid, with clearance of over 90% of parasitemias within 24 hours (Bunnag *et al,* 1991a). Unfortunately, recrudescence rates are also high. The optimum dose of artemether used in sequential with mefloquine revealed a high cure rate to 97%. (Looareesuwan *et al,* 1997b).

Arteether : Arteether, a semi-synthetic derivative of artemisinin, has been developed by the UNDP/ World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (TDR) in collaboration with the Walter Reed Army Institute of Research, Washington, DC. In addition, it is supported by the Netherlands Ministry of Development Cooperation, the registration and manufacturing of arteether injection is on the way by a Dutch company; ACF Beheer BV. Because of the investment in development of arteether supported by WHO/TDR and Public Sector Agencies, the cost of arteether is expected to be low and this may benefit malaria-endemic developing countries. The dose used is the same as artemether (9.6 mg/kg total dose in 5-7 days) and the main indication for use of arteether injection will be for the treatment of severe malaria. Comparative clinical trials of arteether versus other antimalarial drugs in severe malaria are ongoing.

Dihydroartemisinin: All artemisinin derivatives are metabolized to a biologically active metabolite dihydroartemisinin once they were absorbed into the body. This compound is simpler and cheaper to manufacture than artesunate or artemether, both of which use dihydroartemisinin as an intermediate in synthesis. *In vitro* it is 2-3 times more active than the derivatives. Dihydroartemisinin (Cotecxin®) manufactured by Beijing Sixth Pharmaceutical Factory, PRC, has proved safe and effective in the treatment of acute uncomplicated falciparum malaria in China and Thailand. A clinical trial of this drug in Thailand showed a 90% cure rate when 480 mg dihydroartemisinin was given over 5 days (Looareesuwan *et al,* 1996a). At present dihydroartemisinin manufactured by various sources such as Vietnam, Thailand, Belgium and the Netherlands are being evaluated.

Suppository preparation: Another development for artemisinin derivativesis the development of a rectal suppository. The suppositories of artesunate (Artesunate rectocap® manufactured by Mepha company, Switzerland) have proved safe and effective in complicated and severe malaria (Looareesuwan *et al,* 1995, Looareesuwan *et al,* 1997a). Since mortality rates in complicated and severe malaria depend upon the availability of medical facilities, the period of delay treatment, and the levels of parasitemia, rectal suppositories could alter 2 of these factors (Field *et al,* 1937; Wilairatana *et al,* 1995). Suppositories would be particularly useful for malaria in children in rural areas where full facilities might not be possible, particularly children. Moreover, the medical care at the village level may be relatively unsophisticated, necessitating simple treatments. At the very least, a rapid reduction in parasitemia might diminish the chances for development of severe malaria and in cases of cerebral malaria, provide additional time to seek advanced medical attention.

Clinical trials of artemisinin derivatives in severe malaria

Trials of artemisinin derivatives in severe and complicated malaria are encouraging. In 1982, 15 of 17 cerebral malaria patients recovered after treatment with 400 mg of intravenous or intramuscular artesunate in 3 divided doses. Comas resolved in a mean time of 12 hours (Li *et al,* 1982). Guo found that 31 of 33 cerebral malaria patients treated with intravenous artesunate recovered and parasites were completely cleared within a mean time of 54 hours (Guo *et al,* 1990). In Vietnam, patients with severe malaria that received intramuscular doses of artesunate cleared parasitemias at the same rate as equivalent doses given intravenously (Hien *et al,* 1992b). Additionally, intravenous and intramuscular artesunate (120 mg initially, then 3 doses of 60 mg over 3 days) were beneficial to patients with hyperparasitemias, coma, jaundice, or renal failure. A recent report from our study described recovery in 62 of 72 (86%) cerebral malaria patients treated with 120 mg of intravenous artesunate, then 60 mg every 12 hours for a total dose of 600 mg. Twelve hours after the last dose of artesunate, 750 mg of mefloquine was administered orally or through a nasogastric tube, followed by 500 mg 6 hours later (Wilairatana *et al,* 1995).

In comparison with quinine, artemether proves to be a good alternative. A preliminary report from Thailand suggested that artemether may be more effective in reducing mortality with fewer side effects than quinine (Karbwang *et al,* 1995). In Vietnam, cerebral malaria patients were treated in sequence with 240 mg of intravenous artesunate (4 doses over 3 days) and 500 mg of mefloquine (Hien *et al,* 1992a). This artesunate regimen cleared parasitemias faster than quinine but the differences in mortality were not significant (16.5% *vs* 26.7% respectively). In Myanmar however, intravenous

artesunate (300 mg in 4 doses over 3 days) combined with oral mefloquine (1,000 mg) did reduce mortality in comparison with the standard intravenous quinine and oral tetracycline regimen in cerebral malaria patients (8.3% *vs* 34.3%) (Win *et al,* 1992). Moreover, none of the artesunate-mefloquine regimen patients recrudesced; in the quinine regimen, 12% of the patients recrudesced. In Malawi, children with cerebral malaria recovered from coma more quickly when treated with artemether than with quinine (Taylor *et al,* 1983). Recently, 2 large studies in adult and paediatric patients with severe *P. falciparum* malaria compared intramuscular artemether with quinine. Artemether accelerated parasite clearance but did not significantly reduce mortality in comparison with quinine (van Hensbroek *et al,* 1996; Hien *et al,* 1996). In addition, recovery from coma in the artemether-treated patients was slightly prolonged. These studies show that artemether is an acceptable alternative to quinine in severe malaria in all age groups. However, the impact of artemisinin drugs on the mortality in severe malaria has to be fully investigated.

Recommended use in severe malaria

In severe malaria in a hospital set up intravenous artesunate is recommended. In a rural area where facilities are limited, the use of intramuscular artesunate or intramuscular artemether or rectal preparations of artesunate are appropriate (Looareesuwan *et al,* 1995, 1997a). In general, it reveals that when artemisinin derivatives are used for severe malaria, higher recrudescent rate will be encountered if other antimalarial therapies such as mefloquine are not added in sequence. This parallels the findings in uncomplicated malaria (Looareesuwan *et al,* 1992b,c, 1993, 1994d).

Toxicity

Experimental studies in animals have shown that artemether, arteether and dihydroartemisinin induce an unusual and selective pattern of damage to the brain stem (Phillips Howard *et al,* 1995; Wesche *et al,* 1994; Brewer *et al,* 1994a,b). However, more than a million patients have been treated with artemisinin compounds without any reports of serious adverse events (Hien and White, 1993; White, 1994a; Looareesuwan, 1994d). Thus, the significance of the animal observations in patient care is not known. In pregnancy, artemisinin is considered safe after first trimester but quinine is still recommended in early pregnancy (World Health Organization, 1993).

DRUGS IN COMBINATION

Until recently when synthetic drugs have been available, a single component has been widely used for treating most infections. The use of a single drug has been generally accepted because the dose can be controlled easily and drug interactions can be avoided. However, with problems of multidrug resistant falciparum malaria, the rationale for using a single compound warrants change. The mechanism of action of different drugs varies, and they can act in different biosynthetic pathways of the plasmodium parasite. Drug combinations can act as additively or in a synergistic way to kill the pathogen. They may also prevent the development of resistance. Some diseases, such as tuberculosis and leprosy, have been successfully treated with drug combinations. In the treatment of malaria, mixtures of active components have been used for centuries. In China, for more than 2,000 years, an oriental remedy extracted from Qinghao plant (*Artemesia anual L*) has been used for treatment of chills and fever, presumed to be malaria infections (Klayman, 1985). In South America, more than 400 years ago, a mixture of cinchona alkaloid extracted from barks of chinchona tree (known as "Peruvian bark extract") was introduced for treating malaria symptoms. During the Second World War, a mixture of cinchona alkaloids was manufactured as a tablet (Totaquine®) containing mainly quinine, quinidine and cinchonidine, and used successfully in the treatment of malaria (Green, 1945). With the deteriorating situation of multidrug resistant falciparum malaria in Thailand (Looareesuwan *et al,* 1992a), attempts have been made to delay the resistance by the use of certain drug combinations. Some suitable combinations have proved effective *in vitro,* in animal malarias and in man.

Combination of para-aminobenzoic acid (PABA) antagonist and dihydrofolic acid reductase (DHFR) inhibitor together with other compounds

The principle use of the two combinations are blocking the formation of nucleoproteins of *Plasmodium* at two different stages levels by inhi-

bition of syntheses of dihydrofolic acid (PABA antagonists) and inhibition of reductase of dihydrofolic acid (DHFR inhibitors). PABA antagonists include sulfonamide and sulfone groups; one example of a DHFR inhibitor is pyrimethamine. Drug combinations in this group has been manufactured and marketed in a single tablet and used extensively in the treatment of malaria. Those commonly in use are Fansidar® (sulfadoxine +pyrimethamine), Metakelfin® (sulfalene +pyrimethamine) and Maloprim® (dapsone+ pyrimethamine). These drugs have been used successfully in the treatment of malaria, including chloroquine-resistant falciparum malaria (Harinasuta *et al,* 1967). Over the years, resistance has developed in many parts of the world. In some areas of Thailand, these drug combinations are now ineffective (Bunnag *et al,* 1987a; Chongsuphajaisiddhi and Sabchareon, 1981a; World Health Organization, 1984).

The demonstration that a combination of sulfadoxine-pyrimethamine and chloroquine delays the development of resistance to the individual compounds, in *Plasmodium berghei* infection in mice, can be explained by their synergistic effect. Peters (Peters, 1974) advocated the extensive use of chloroquine together with Fansidar® for chemoprophylaxis in areas where multidrug resistant falciparum malaria exists. Although this combination could not prevent drug resistance, it has some beneficial effect because of the additional protection against coincident *P. vivax* infection. Another combination which was tested in Thailand is sulfadoxine-pyrimethamine plus tetracycline. This combination was used on an empirical basis. In the treatment of uncomplicated falciparum malaria, the cure rates in 1981-1982 for Fansidar® 3 tablets plus tetracycline 1g per day, for 7 days, in adult patients admitted in the Bangkok Hospital for Tropical Diseases was 75% (Bunnag *et al,* 1987a) and patients treated in a field study was 82% while Fansidar® 3 tablets cured only 22% of patients admitted to the hospital in the same period. Unfortunately, with high grade multidrug resistant falciparum malaria at the present time, the combination has limited use.

Combination of chloroquine or amodiaquine with antibiotics (tetracycline or erythromycin)

The use of antibiotic combinations in the treatment of malaria is derived from *in vitro* and animal malaria studies (Kaddu *et al,* 1974), demonstrated that minocycline, a tetracycline derivative, has some action against both chloroquine-sensitive and chloroquine-resistant *P. berghei* in mice. Tetracycline alone is a weak antimalarial (Puri and Duta, 1982). However, it has an additive effect when used with chloroquine. In the Bangkok Hospital for Tropical Diseases, the cure rate of chloroquine 1,500 mg base given over 3 days was 0% in 1981 and it has not been used as a single drug for treatment since then. The cure rate increased to 75% in the same year when tetracycline 1g per day given for 7 days was added to the chloroquine (Bunnag *et al,* 1987a). This combination gave even higher cure rate of 90%, in 1981, when used in the field. However, a study in eastern Thailand in 1983 revealed that this combinations appeared ineffective with 2 out of the first 5 patients studied had RIII resistance with clinical deterioration (Phillips *et al,* 1984).

Erythromycin has been known to have antiplasmodial activity since 1912 (Warhurst *et al,* 1976). It is active *in vitro* against both animal and human malarias (Warhurst *et al,* 1983; Geary and Jensen, 1983). Erythromycin alone is not very effective against *P. berghei* infections in mice, but in combination with chloroquine it has a synergistic effect against chloroquine-resistant strains (Warhurst *et al,* 1976; Gershon *et al,* 1984). The use of erythromycin with chloroquine was tested in the hope that this combination might prove effective against chloroquine-resistant strains of *P. falciparum,* particularly in children and pregnant women in whom tetracycline is contraindicated. However, a study in eastern Thailand in 1983 revealed that this combination produced only a 19% cure rate in uncomplicated malaria (Phillips *et al,* 1984; Pang *et al,* 1985).

Amodiaquine was introduced into clinical use nearly 40 years ago. In recent years, resistance to the drug has developed in parallel to the distribution of chloroquine resistance. However, its' cure rates improve when used together with tetracycline. In the Bangkok Hospital for Tropical Diseases in 1983, the combination of amodiaquine 1,500 mg base given over 3 days, together with tetracycline 1g per day for 7 days, cured 86% of patients suffering from uncomplicated falciparum malaria (Bunnag *et al,* 1987a). Another study in Central Thailand, in 1981, reported a cure rate of 96% (Noeypatimanond *et al,* 1983). The combination of amodiaquine and

erythromycin has an additive effect *in vitro,* but in patients suffering from uncomplicated falciparum malaria this combination proved ineffective (Looareesuwan *et al,* 1985).

Combination of quinine and other compounds

The total extracts of cinchona alkaloids have been used in the treatment of malaria for several centuries. The mixture, Totaquine®, containing varying proportions of each of the cinchona alkaloids has been used widely in many endemic regions (Green, 1945). More recently, individual alkaloids have been purified and a single active component is advocated. The first case of quinine resistance was reported from Brazil (Nocht *et al,* 1910), where 25.5 gm base given over 21 days failed to cure the infection. Increased resistance has already appeared in Thailand and adjacent areas such as Kampuchea and Vietnam (Chongsuphajaisiddhi *et al,* 1981a,b; Looareesuwan *et al,* 1990). In the Bangkok Hospital for Tropical Diseases in 1979, only 86% of patients treated with quinine for 7 days were completely cured (Bunnag *et al,* 1987a). In an attempt to improve the cure rate, many combinations of quinine and other compounds were tested.

A combination of quinine, quinidine and cinchonine in equal ratio (1:1:1) has proved superior to quinine in treating *P. berghei* infection in mice, and it was 4 times more effective *in vitro* than the individual compounds. There was no evidence of cross resistance between the three components of the combination (Druilhe *et al,* 1988). The combination has proved to be at least as effective as quinine alone in one open and two double-blind trials in acute uncomplicated falciparum malaria (Bunnag *et al,* 1987b; Bunnag *et al,* 1989).

In 1972, studies in Thailand showed that cure rates of short courses of quinine (1-3 days) were improved by adding tetracycline (3-10 days) (Colwell *et al,* 1972, 1973). These findings could be explained by *in vitro* and pharmacokinetic studies. It has been shown that additive effects are obtained when tetracycline or erythromycin is added to quinine (Gershon *et al,* 1984; Rahman *et al,* 1985). Quinine levels were significantly higher in patients treated with quinine-tetracycline than those treated with quinine alone (Karbwang *et al,* 1990). Since 1980, quinine given for 7 days, combined with tetracycline 1g/day for 7 days, has become standard treatment in the hospitals for adults suffering from uncomplicated falciparum malaria in Thailand. This combination gave cure rates approaching 100% when it was first introduced (Bunnag *et al,* 1987a; Reacher *et al,* 1981). However, the combination has given a cure rate of only 90% in adult patients admitted to the Bangkok Hospital for Tropical Diseases in 1980-1991 (Looareesuwan *et al,* 1992d). In one study in eastern Thailand, the most serious area for multidrug resistant falciparum malaria, a combination of quinine with erythromycin failed to improve the cure rate of quinine alone (Pang *et al,* 1985).

Sequential treatment of quinine with other antimalarials were also tested. In one study, quinine given for 2-3 days followed by mefloquine 1,000 mg, divided into two doses given 6 to 24 hours apart, cured all 35 patients (Hall *et al,* 1977). However, this sequential combination has not been widely accepted because of anxiety concerning the cardiotoxicity of the two drugs, which are both quinoline-methanols. Quinine 1,800 mg per day given for 7 days followed by sulfadoxine-pyri methamine (Fansidar®) 3 tablets cured only 76% of adult patients admitted to the Bangkok Hospital for Tropical Diseases (Bunnag *et al,* 1987a).

Combination of mefloquine and other compounds

Both *in vitro* studies and animal models of malaria have revealed that resistance develops rapidly to mefloquine when used alone (Merkli *et al,* 1980a). The addition of sulfadoxine plus pyrimethamine to mefloquine delayed mefloquine resistance. In addition, it was demonstrated that the three components showed an additive effect (Merkli *et al,* 1980b). In an effort to delay development of resistance to mefloquine, the World Health Organization and Hoffman La Roche Co advocated the combination of mefloquine and sulfadoxine-pyrimethamine (Fansimef®). In 1983-1986 a single dose of Fansimef® 3 tablets (contained a total of mefloquine 750 mg and sulfadoxine 1,500 mg and pyrimethamine 75 mg) cured 98% adult patients admitted in the Bangkok Hospital for Tropical Diseases (Harinasuta *et al,* 1987) and 98% adult patients in the western part of Thailand (Nosten *et al,* 1987). The adding of sulfadoxine-pyrimethamine to mefloquine did not improve cure rate significantly. In 1987-1988, mefloquine 750 mg in combination with sulfadoxine 1,500 mg and pyrimethamine 75

mg (Fansimef®) and mefloquine 750 mg alone (Lariam®) were studied in a randomised trial in the western part of Thailand and the cure rates were 96% and 93% respectively (Thimasarn *et al,* 1990). There are some doubts on the use of these combinations in areas where sulfadoxine-pyrimethamine resistance has already become established. The risk and benefit of the combination was open to criticism. Above all the pharmacokinetic properties of the three components do not match ideally. It now seems reasonable to use mefloquine alone as a single component in areas where widespread resistance to sulfadoxine-pyrimethamine exists.

In 1985, Rahman and Warhurst demonstrated that an additive effect of mefloquine plus tetracycline was obtained on *P. falciparum* isolates studied *in vitro*. This information encouraged us to test the combination in the hope that it may increase cure rates. We have treated adults patients suffering from uncomplicated falciparum malaria with mefloquine 1,250 mg divided into 2 doses 6 hours apart, together with tetracycline 1g per day for 7 days, and compared it with a standard regimen of quinine- tetracycline for 7 days. The cure rate of mefloquine-tetracycline was as effective as quinine-tetracycline (94% *vs* 98% respectively) with very few side effects (Looareesuwan *et al,* 1994b). A further study in 1992, using mefloquine 1,250 mg together with doxycycline 200 mg per day for 7 days, reported a cure rate of 96% (Looareesuwan *et al,* 1994c). Therefore, the combination of mefloquine with either tetracycline or doxycycline is an alternative regimen for treating multidrug resistant falciparum malaria where severe multidrug resistant occurs.

Combination of artemisinin derivatives and other compounds

Artemisinin derivatives which are commonly used include artesunate and artemether. Their action on killing parasites is rapid, with more than 90% parasitemias cleared during the first 24 hours of administration. Recrudescence rates are high, ranging from 10 to 100%, depending on doses and duration of treatment (Bunnag *et al,* 1991a,b,c). Even with the recommended dose (600 mg total dose of artesunate given over 5 days), a cure rate of only 88% was obtained in a very recent study (Looareesuwan *et al,* 1992b). To overcome such high recrudescence rates, sequential treatment with mefloquine, or combination with antibiotics such as tetracycline or doxycycline have been tried. In recent studies, the sequential treatment of artesunate (600 mg given over 5 days) followed by mefloquine (1,250 mg given in 2 doses 6 hours apart) gave 100% cure rates both in acute and recrudescent falciparum malaria (Looareesuwan *et al,* 1992b,c). This sequential treatment is safe and is recommended for acute uncomplicated falciparum malaria and for those who fail with other regimens. The rationale for this sequential treatment is that artesunate clears the parasitemia, whilst mefloquine prevents recrudescence, but it avoids the potential double toxicity of the two components. Because of the excellent results of the sequential treatment, a half-dose regimen (artesunate 300 mg given over two and a half days followed by 750 mg mefloquine) was tested subsequently and produced a cure rate of 90% (Looareesuwan *et al,* 1993). This is acceptable when compared to other commonly available drugs for treating acute uncomplicated falciparum malaria in Thailand. A randomised trial of short course therapy of artesunate followed by mefloquine in 2 days compared with a high dose of mefloquine (25 mg/kg divided into 2 doses 6 hours apart) revealed cure rates of 92% *vs* 74% respectively (Looareesuwan *et al,* 1994a). Studies, both *in vitro* and in animal malaria, have demonstrated a synergistic effect with the combination of artemisinin derivatives and mefloquine and the combination of artemisinin derivatives and doxycycline (Chawira and Warhurst, 1987a; Chawira *et al,* 1987b) clinical trials using these combination are underway. Results of a clinical trial of the combination of artesunate (300 mg given over 2.5 days) together with doxycycline (200 mg per day for 7 days) proved safe, well-tolerated and yielded a higher cure rate (Looareesuwan *et al,* 1994c).

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