SPECIAL REPORT*

DRUG RESISTANT MALARIA, WITH SPECIAL REFERENCE TO THAILAND

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Abstract. Drug resistance of malaria parasites is a major problem confronting efforts to treat and control malaria. Starting with chloroquine, the emergence of resistance to other drugs has led to multi-drug resistance patterns that pose increasing threats for the future. This report reviews work carried out over the past decades at the Hospital for Tropical Diseases, Bangkok, which monitors patients from many areas, including the Thai-Cambodian border, which harbors the world's most severe multi-drug resistant *Plasmodium falciparum*.

INTRODUCTION

Malaria still remains a major health problem of the world, particularly in the tropics. At present, it affects more than 200 million individuals and has a mortality of 1-2 million each year. The number of malaria cases in Southeast Asia has been increasing since the mid 1970s in spite of control programs. Failure to control the disease, death and the deteriorating malaria situation are due mainly to emergence of drug resistance of *Plasmodium falciparum*. Failure of vector control is also a contributing factor.

DEFINITION OF RESISTANCE OF HUMAN MALARIA TO DRUGS

Drug resistance in malaria is defined as the "ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject". Although this definition can be extended to all species of malaria parasite and all useful dosages of blood or tissue schizonticides, gametocytocides and sporonticides, in practice it is most commonly applied to the resistance of *P. falciparum* to the blood schizonticides, in particular the 4-aminoquinolines.

SPECTRUM OF RESPONSE OF MALARIA PARASITES TO DRUGS *IN VIVO*

The response of the parasites to antimalarials ranges from a low level of resistance with a loss of effect demonstrable only by occasional recrudescence (RI), to a high level of resistance at which the drug apparently has no suppressive effect on parasites (RIII) and results in severe malaria infections. In 1967, the WHO Scientific Group on Chemotherapy of Malaria proposed an arbitrary grading system based on the response to normally recommended doses of chloroquine, a slightly amended version of which is presented in Table 1. This grading is also used for other blood schizontocides and other species of human plasmodia but modification may be required for each drug on the basis of speed of action, half-life and the biological characteristics of the plasmodium species, *eg* the follow up period for chloroquine quinine or artemisinin derivatives should be 28 days but for mefloquine should be 42 days. The suggested grading system is based on the locally current threshold of microscopic patency of parasitemia. Parasites may persist in the blood in the absence

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of fever if the parasitemia patency threshold for fever is high. This is fequently found in patients with semi-immunity who have been living in highly endemic areas. With RI or even RII parasitological response, the patients are usually relieved from symptoms. However, clinical response becomes parallel to parasitological response when there is no immunity.

Although there is some disagreement about the application of the criteria to a drug with a very long half life such as mefloquine, and a very short one such as artemisinin derivatives, the grading system is still generally used for the purpose of comparison of drug efficacy.

IN VITRO SENSITIVITY

Attempts have been made to predict the *in vivo* response to a drug, on the basis of *in vitro* findings. It is likely that the *in vivo* response to a specific drug can be predicted with a certain amount of confidence on the basis of extensive comparative *in vitro-in vivo* studies caried out in nonimmune patients.

In the late 1960s, a simple *in vitro* test was developed to assess the susceptibility of *P.falciparum* to chloroquine and other antimalarial drugs by using 10-12 ml of venous blood (macro-test). This test measures the extent to which the maturation of ring forms to normal schizonts is inhibited after the incubation of parasitized blood at various drug concentrations for a period of 24-30 hours (Rieckmann *et al,* 1968). In this short-term culture system, a marked difference in the maturation of sensitive and resistant parasites is observed in the presence of drug plasma levels comparable to those observed after administration of the drug *in vivo.* Early studies showed that this technique was a quick and reliable method for estimating the presence, prevalence or degree of chlorquine resistance under field conditions. After considerable work, quite a number of *in vitro* tests have been developed. The "micro-test" (Rieckmann *et al,* 1978) has been evaluated quite extensively and is widely used to study the effects of antimalarial drugs and drug interactions. The correlation between *in vivo* and *in vitro* sensitivity tests has been established for some antimalarials *eg* quinine (Chongsuphajaisiddhi *et al,* 1981). However, the establishment of drug resistance should be confirmed in patients with adequate drug absorption and drug level in the blood (Karbwang *et al,* 1991 a,b). *In vitro* drug sensitivity tests are useful for epidemiological surveys, monitoring of drug response and prediction of resistance.

In the past decade several *in vitro* tests have been developed, namely modified "48-hour test" (Nguyen-Dinh and Payne, 1980), "semi-automated test", "visual micro-test" (Rieckmann, 1982) and "hypoxanthine incorporation test" (Desjardin *et al,* 1979; Webster *et al,* 1985).

EMERGENCE OF DRUG RESISTANCE

The earliest description of drug resistance dates back to 1910 when resistance of *P.falciparum* to quinine was reported from Brazil (Nocht and Werner, 1910). Resistance to dihydrofolate reductase inhibitors such as pyrimethamine was noted soon after their introduction at the end of the Second World War. However, resistance to these drugs was of little consequence as other synthetic antimalarials were readily available and effective. Drug resistance became a major problem with the appearance of resistance in *P. falciparum* to the most potent and widely used synthetic antimalarial drug, chloroquine. The first evidence came almost simultaneously from Colombia, in South America and Thailand, in Southeast Asia in the late 1950s (Moore and Lanier 1961; Young and Moore 1961; Harinasuta *et al,* 1962).

THE SPREAD OF CHLOROQUINE RESISTANT *P.FALCIPARUM*

Chloroquine resistant *P.falciparum* in Thailand, first observed in an area near Cambodia in late 1950s, spread to the whole country within a decade.

It then spread in all directions, through Southeast Asia, South Asia, the Western Pacific and Africa while from Colombia it spread to the whole of Central and South America with the exception of Argentina, Paraguay and Peru which have practically no falciparum malaria.

Assuming that all the chloroquine-resistant parasites encountered in Asia, the Western Pacific

and Africa have originated from the primary focus in Thailand some 30 years ago, the spread has occurred through walk-over carriage of gametocytes from village to village, seeding local anophelines along the way or over long distances transfered by air, road, rail or ship. It appears that the periphery of the zone of resistance is constantly expanding, its parasite population composed of RI grade pressing out into the susceptible (S) grade parasite surroundings. These RI gametocytes hybridize in the mosquito with indigenous S gametocytes, both types having been picked up by the mosquito in a split feed or from a polyclonal carrier. If transmission conditions are favorable, an RI focus develops. Meanwhile, the central hardcore part of the zone of resistance is changing, losing its S strains through hybridization or selection and shifting in character progressively towards RIII. Intermediate zones between the periphery and the center are occupied mainly by RI and some RII strains, with a small residue of S on the verge of extinction. Such free hybridization occurs because several strains of parasite may coexist in an individaul host, all producing gametocytes picked up together by a feeding mosquito. Unless some dramatic intervention occurs, it is only a matter of time until these expanding hardcore areas meet and merge, consigning whole districts, countries or regions to the dominace of increasingly resistant grades of *P.falciparum,* posing a serious problem.

There is yet another factor. The level of indigenous *P. falciparum* sensitivity to chloroquine is gradually changing in areas remote from the resistant foci on a locally selective basis. Retrospective analysis of chloroquine treatment regimens in East Africa reveals that during the past 25 years the dosages needed to clear parasitemia have steadily risen. Between 1953 and 1980 the quantity of chloroquine necessary to clear 50% of trophozoites in children has increased from 1 to 3.5 mg (base)/Kg and, to clear 99.9%, from 2.5 to 15 mg (base)/Kg. These findings raise the question of whether or not this decrease in susceptibility indicates that the East African strains of *P.falciparum* have acquired RI resistance on their own. It may be asked if such apparent acclimatization to drug pressure has been accomplished by the mechanism of parasite adaptation or of mutation. It would appear that adaptation is involved for each stage of resistance, and mutation is necessary for the parasite to become resistant. The level of tolerance of these strains would level off until such time as (a) a mutation occurs locally to produce an indigenous resistant parasite, or (b) a resistant strain is introduced and hybridizes with local sensitive strains. East Africa is only an example, it is quite possible that similar changes have occurred in other parts of the world where chloroquine was used extensively through primary health care and community volunteer distribution agencies. The necessity to try to protect a new antimalarial such as artemether or artesunate from the same fate is incumbent upon all of us.

EFFICACY OF OTHER ANTIMALARIALS

Amodiaquine

Amodiaquine resitance was thought to be parallel to the distribution of chloroquine resistance; however, studies in Thailand (Noeypati manond *et al,* 1983; Looareesuwan *et al,* 1985) Colombia, Tanzania and Kenya (Watkins *et al,* 1984; WHO, 1984) revealed that the sensitivity to amodiaquine was superior to chlorquine, but a study carried out at the Bangkok Hospital for Tropical Diseases in 1986 showed no superiority (Bunnag, 1986, unpublished data). In some areas, such as the Philippines (Watt *et al,* 1985) and Pakistan (Khalig *et al,* 1987), amodiaquine and chloroquine have been shown to be equally ineffective. Furthermore, the potential toxicity of amodiaquine leading to agranulocytosis limits its use for the treatment of malaria (Hutton *et al,* 1986).

Sulfadoxine/pyrimethamine

The combination of sulfadoxine (PABA inhibitor) with pyrimethamine (dihydrofolate reductase inhibitor) has been shown to potentiate antimalarial activity and produce radical cure of malaria infections. This combination was effective when it was first introduced in late 1960s as a single dose treatment for acute uncomplicated falciparum malaria (Harinasuta *et al,* 1967). Parenteral sulfadoxine/pyrimethamine was effective in acute uncomplicated and severe falciparum malaria in 1971 (Harinasuta *et al,* 1988). Over the years, resistance has developed in many parts of the world. Increasing failure rates were reported from Colombia, Brazil, Venezuela, Indonesia,

Malaysia, Papua New Guinea, Kenya (WHO, 1984), Myanmar and Thailand (Chongsuphajaisiddhi and Sabchareon, 1981; Pinichpongse *et al,* 1982; Reacher *et al,* 1981; Bunnag and Harinasuta, 1986). In some parts of Thailand, it is totally ineffective.

Quinine

The earliest documentation of quinine resistance was from Brazil (Nocht and Werner, 1910) where quinine 25.5 gm base given over 21 days was ineffective in curing the infection. Increased quinine resistance was reported from several parts of the world such as Tanzania (WHO, 1984), Vietnam (Hall, 1972) and Thailand (Bunnag and Harinasuta, 1986; Chonsuphajaisiddhi *et al,* 1983; Pinichpongse *et al,*1982); however, most of the resistance is at the RI level. During the past decade minimal inhibitory concentrations (MIC) of quinine for *P.falciparum* parasites have risen especially on the Thai-Cambodian and Thai-Myanmar borders. In this situation and in severe malaria, a loading dose of 20 mg of quinine dihydrochloride per kg body weight and maintenance doses of 10 mg per kg given every 8 hours are required (White *et al,* 1983). Persistent parasitemias after a loading dose of quinine treatment are not uncommon (Looareesuwan *et al,* 1990). It was observed that the MIC of quinine must be maintained for seven day to effect radical cure of *P.falciparum* infections (Chongsuphajaisiddhi *et al,* 1981). Since it takes two to three days to achieve steady state plasma levels of quinine when 10 mg/kg is given orally at 8 hours intervals, quinine must be given for at least seven days. In Thailand the cure rate of quinine dropped to 73% in 1986; tetracycline was then added and the cure rate rose to 100% (Bunnag *et al,* 1986). This is due to higher plasma quinine concentrations (Karbwang *et al,* 1990). A recent study revealed a cure rate of only 90% (Looareesuwan *et al,* 1992a). The cure rate has declined slowly. In children in whom tetracycline is contraindicated, increasing the dose of quinine (15 mg/kg) at a time when quinine clearance was accelerated (the second half of the treatment period) improved the cure rate (Chongsuphajaisiddhi *et al,* 1983).

Quinidine

Quinidine, the diastereoisomer of quinine, has a lower MIC for *P.falciparum* in Thailand (Bunnag *et al,* 1987a). It is as effective as or perhaps more effective than quinine for the treatment of falciparum malaria. Open studies of oral quinidine sulfate and parenteral gluconate in acute uncomplicated and severe falciparum patients in Thailand showed quinidine to be effective (White *et al,* 1981; Phillips *et al,* 1985; Druilhe *et al,* 1988; Bunnag *et al* 1987b; Sabchareon *et al,* 1988). A randomized clinical trial of quinine versus quinidine treatment for 5 days in acute uncomplicated falciparum malaria revealed a significant higher cure rate in the quinidine-treated group (25% vs 61%; $p < 0.05$, Suntharasamai *et al,* 1984). The combination of 3 cinchona alkaloids consisting of one-third each of quinine, quinidine, and cinchonidine in both adults and children suffering from acute uncomplicated falciparum malaria was also effective (Bunnag *et al,* 1989; Chongsuphajaisiddhi *et al,* 1987; Karbwang *et al,* 1992a). Quinidine slow release at a dosage of 19 twice daily for 7 days yielded a cure rate of 100% (Bunnag *et al* 1987b). However cardiovascular side effects were found more frequently with quinidine than quinine. Quinidine is not used for treatment of malaria in Southeast Asia where quinine is available.

Mefloquine

Mefloquine, a synthetic 4-quinolinemethanol, was developed by the US Army antimalarial drug development program. It has been registered in some countries in Europe since 1985. It was thought to be an ideal antimalarial. It has a long half-life of 25-30 days, therefore it is administered as a single oral dose. Its action is rapid and it is effective against all species of human malaria parasites, including chloroquine-resistant and quinine resisant *P.falciparum* (Harinasuta *et al* 1983, 1977, 1990). In an effort to delay development of resistance to mefloquine, the World Health Organization and F Hoffmann La Roche Ltd Basel, Switzerland advocated the combination of mefloquine and sulfadoxine-pyrimethamine (Fansimef®). The combination has been registered by this trade name in Thailand since 1984. The evidence that the combination could delay development of resistance came from studies using a mouse model (Peter and Robinson, 1984) and an *in vitro* study (Brockelman *et al* 1989) which have not been generally accepted. A clinical trial conducted in Thailand in 1983-84 showed high efficacy of Fansimef® (Harinasuta

Table 1

Grading of resistance of asexual parasites *(P.falciparum)* to blood schizontocidal drugs *(in-vivo* test)

Response	Recommended symbol	Evidence
Sensitivity	S	Clearance of asexual parasitemia within 7 day of initation or treatment, without subsequent recrudescence
Resistance	RI	Clearance of asexual parasitemia as in sensitivity, followed by recrudescence
	RII	Marked reduction of asexual parasitemia, but no clearance
	RIII	No marked reduction of asexual parasitemia

et al, 1987). The combination has not delayed the development of resistance (Thimsarn *et al,* 1990). Furthermore, severe adverse effects have resulted, presumably from the sulfadoxine component when the combination was used for malaria prophylaxis (Miller *et al,* 1986), favoring the use of mefloquine as a single component. Treatment failures have been reported in many parts of the world. A higher dose of mefloqine (25 mg/kg) given in 2 divided doses 6 hours apart, achieved a cure rate of 69% in 1988-89 which equals to 3 tablets of Fansimef® on the Thai-Cambodian border (Bunnag and Harinasuta, unpublished data). The reduction of cure rates of mefloquine and Fansimef® is shown in Tables 2 and 3. In chil dren, 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 (Chongsuphajaisiddhi *et al,* 1987; Nosten *et al,* 1991).

Halofantrine

Halofantrine, a phenanthrine methanol, is a product of the US Army antimalarial drug development program and has been co-developed for marketing by Smith Kline Beecham Pharmaceuticals. It has been shown in initial studies to be effective against multidrug resistant *P.falciparum* both in Thailand (Boudreau *et al,* 1988; Chitchang and Wongteptein, 1989) and Africa (Watkins *et al,* 1988; Wirama *et al,* 1988). However, studies in Thailand in 1988 and 1991 showed that halofantrine (1,500 mg given in three devided doses at 6 hours apart) cured only 30%-70% even though this drug had not been widely used (Bunnag *et al, 1990;* Shanks *et al,* 1992; Timasarn, unpublished data). The low cure rates are due to inadequate absorption (Karbwang *et al,* 1991c). Halofantrine is absorbed well when taken with a meal (Shanks *et al,* 1992), but that is difficult to accomplish during the acute phase of malaria.

Qinghaosu (artemisinin) derivatives

Qinghaosu, a sesquiterpene lactone peroxide, extracted from leaves and flowers of the qinghao plant *(Artemesia annua* L., sweet wormwood, annual wormwood), has been used in Chinese traditional medicine for more than 2000 years (Klayman, 1985) to treat chills and fever, probably malaria. The active constituent, artemisinin, was isolated in 1972. Qinghaosu derivatives are very effective in killing young blood forms of the parasites; however, recrudescent rates are high (Juang *et al,* 1981; Li *et al,* 1984, Arnold *et al* 1990; Bunnag *et al,* 1991a,b,c; Looareesuwan *et al,* 1992b). Attempts have been made to reduce the high recrudescent rates by increasing the dose and/or lenghtening the period of drug administration or using drug combinations. Artemisinin and mefloquine have been shown to be synergistic *in vitro* and in animal malarias (Chawira *et al,* 1987a,b). Two dervatives, artemether and artesunate are now widely used. Other qinghaosu deri-

Table 2

Efficacy of antinalarial drugs for the treatment of *P. falciparum* malaria at the Bangkok Hospital for Tropical Diseases, Thailand during 1978-1989.

Year	Regimen	Response %			
		S(%)	RI(%)	RII(%)	RIII(%)
1978-1979	CQ1.5	15	47	22	16
	SP_2	35	53	8	4
	Q7	94	6	0	0
1979-1980	SP_2	18	23	50	9
	SP_3	22	63	7	7
	Q7	86	14	0	0
1980-1981	SP_2	6	45	34	14
	Mt_2	8	54	28	10
	$Q7SP_3$	76	24	0	0
	M_4	100	0	0	0
	M_3	93	7	0	0
	M_5	95	5	0	0
1981-1984	CQ1.5	0	39	46	15
	CQ1.5T7	75	10	10	5
	SP_3T7	75	25	0	0
	Q7T7	99.5	0.5	0	0
	Qd7	99.5	0.5	0	0
	QdSr7	100	0	0	0
	MSP_1	63	37	0	0
	MSP_2	93	7	0	0
	MSP_3	98	2	0	0
	M_3	80	20	0	0
1985-1986	QQdCn400 IV	85	15	0	0
	CCdCn600 IV	100	0	0	0
1985-1986	QQdCn400	67	33	0	0
	QQdCn600	100	0	0	0
	M_3	86	11	3	0
	MSP_3	81	16	3	0
1986-1987	Q7	73	27	0	0
	QQdCn500	83	17	0	0
1987-1988	Q7 600	90	10	0	0
	Q5T7	88	12	0	0
	Q7T7	100	0	0	0
1988-1989	Q7T7	93	7	0	0
	M_3	66(51)*	22	8	4
	M_5	76(69)*	20	2	2
	MSP_3	79(69)*	17	2	2
	Q7T7	93	7	0	0
	H 2 × 3 (E)	56(29)*	36	8	0
	$M_3 + {}_2$	84(79)*	12	4	0

( )* = Thai-Cambodian border (Eastern part of Thailand)

Am1.5T7 = Amodiaquine 1.5 g in 48 hours together with tetracycline 250 mg q.i.d. for 7 days
CQ1.5 = Chloroquine 1.5 g base in 48 hours
CQ1.5T7 = Chloroquine 1.5 g base in 48 hours together with tetracycline 250 mg q.i.d. for 7 days
SP_2 = SP (Fansidar®) 2 tabs, single dose (1 tab = Sulfadoxine 500 mg + Pyrimethamine 25 mg
SP_3 = SP 3 tabs, single dose
SP_3T7 = SP 3 tabs together with tetracycline 250 mg q.i.d. for 7 days
Mt_2 = Metakelfin 2 tabs (1 tab = sulfalene 500 mg + pyrimethamine 25 mg) single dose
Q7 = Quinine 500 mg base (in children = 10 mg base. kg^{-1} t.i.d. for 7 days)
Q7 600 = Quinine 600 mg base t.i.d. for 7 days
$Q7SP_3$ = Quinine 500 mg base (in children = 10 mg kg^{-1}) t.i.d. for 7 days together with SP (Fansidar®) 3 tabs
Q5T7 = Quinine 500 mg base t.i.d. for 5 days together with tetracycline 250 mg q.i.d. for 7 days
Q7T7 = Quinine 500 mg base t.i.d. for 7 days together with tetracycline 250 mg q.i.d. for 7 days
Qd7 = Quinine sulphate 500 mg t.i.d. for 7 days
QQdCn7 = Combinationofquinine + quinidine + cinchonine (Falcimax™) (1/3+1/3 + 1/3) 500 mg t.i.d. for 7 days
QQdCn400 = Combination of quinine + quinidine + cinchonine (1/3 + 1/3 + 1/3) 400 mg t.i.d. for 7 days
QQdCn600 = Combination of quinine + quinidine + cinchonine (1/3 + 1/3 + 1/3) 600 mg t.i.d. for 7 days
M = Mefloquine 1 tab = 250 mg base (Lariam®)
M_2 = Mefloquine 2 tabs = 500 mg base (Lariam®)
M_4 = Mefloquine 4 tabs = 1,000 mg base (Lariam®)
MSP = MSP 3 tabs = M 750 mg + S 1,500 mg + P 75 mg
$M_3 + {}_2$ = Mefloquine 750 mg (3 tabs) followed by 500 mg (2 tabs) 6 hours later
H 2 × 3 = Halofantrine 2 tabs (1 tab = 250 mg) every 6 hours for 3 doses
D-B-R = Double Blind Randomised
IV = Intravenous

Modified from Bunnag and Harinasuta, 1986.

vatives include artemisinin available as capsules of powder for oral use or as suppositories, arteether, formulated in sesame oil under development as part of a TDR-financed collaborative research program with the Walter Reed Army Institute of Research, and artelinic acid, a water soluble compound under development by the Walter Reed and F Hoffmann-La Roche Ltd Basel). Currently arteether is undergoing a phase I clinical trial in the Netherlands and a phase II clinical trial is being planned. The arteether development is intended for use in treatment of severe malaria.

Table 3

Efficacy of antimalarials in children

Year	Regimen	No.of patients	% Response			
			S	RI	RII	RIII
1971-1977	CQ1.5	25	4	44	28	24
	SP_2	30	77	17	7	0
	$SP_2CQ1.5$	23	66	26	9	0
	SP_Q3	31	68	29	3	0
	SP_2Q5	32	91	9	0	0
1980	SP_2	27	7	15	63	15
	SP_3	18	11	89	39	11
	SP_2Q5	23	57	44	0	0
1981	Q7	28	75	21	4	0
	$Q7SP_3$	26	62	38	0	0
	Q4 + Q3	23	87	13	0	0
1982-1984	M 17-33 mg/kg	82	98	2	0	0
1986	MSP 7.1-12.5 mg of M/kg	33	91	9	0	0
	MSP 15.2-17.9 mg of M/kg	38	97	3	0	0
	MSP 18.1-23.0 mg of M/kg	19	95	5	0	0
	MSP 23.3-33.3 mg of M/kg	27	100	0	0	0
1986-1988	Q4-Q4	25	92	8	0	0
	Q7E7	28	93	7	0	0
1987	Q7	30	57	37	7	0
	QQdCn7*	30	70	30	0	0
	QQdCn7**	30	97	3	0	0
1989	Q7	31	74	23	1	0
	Qd7	30	87	13	0	0
	QQd7	30	87	13	0	0
	Q4 + Q4	19	90	10	0	0
	Q7E7	17	94	6	0	0

Artesunate

Artesunate is the sodium succinyl salt of artemisinin formulated either as tablets (50 mg tablet) or as a dry powder of artesunic acid for injection (supplied in a 60-mg vial with an ampoule of 5% sodium bicarbonate 1 ml). The powder is dissolved in the sodium bicarbonate and then diluted in 1 ml of normal saline and must be used within 3 minutes, by intravenous or intramuscular injection. Artesunate is manufactured by Guilin No. 2 Pharmaceutical Factory, Guangxi, China. It has rapid antimalarial activity with the clearance of over 90% of parasitemias in 24 hours after initiation of treatment (Li *et al,* 1982; Bunnag *et al,* 1991a,b,c; Looareesuwan *et al,*1992b,c). However, the recrudescent rates were high, up to 100% depending upon the dose and duration of treatment. A recent study in Thailand showed that oral artesunate at a total dose of 600 mg given over 5 days had a cure rate of 88-90% (Bunnag *et al* 1991a,b,c; Looareesuwan *et al,* 1992b) but the efficacy increased in both acute uncomplicated and recrudescent falciparum malaria infections when mefloquine was added (Looareesuwan *et al,* 1992b,c). Both oral and parenteral preparations of artesunate are licensed in Thailand. To reduce the high recrudescent rate, combination of

artesunate with other antimalarials is being investigated.

Artemether

Artemether (60 mg ampoule) is the methyl ether derivative of artemisinin formulated in peanut oil for intramuscular injection and licensed in Thailand. Artmether is manufactured by Kunming Pharmaceutical Factory, Kunming, China. Oral artemether tablet and capsule of 50 mg) is undergoing clinical trial in Thailand. As with artesunate, the parasite clearance is rapid, over 90% clearance of parasitemias in 24 hours after initiation of treatment (Bunnag et al, 1991a; 1992a,b; White *et al,* 1992; Karbwang *et al,* 1992a,b,c). Unfortunately, the recrudescent rate was also high. Artemether in combination with other antimalarials is under study in Myanmar (Naing *et al,* 1988; Shwe *et al,* 1988, 1989) and Thailand.

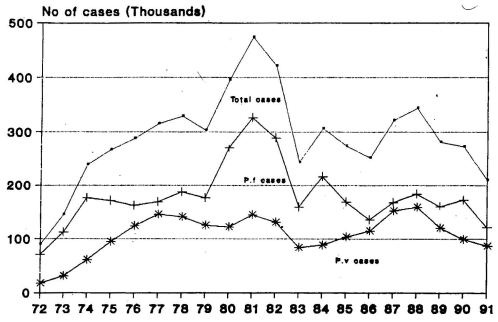

Fig 1—Malaria cases and parasite species Thailand.

(Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health, Bangkok, Thailand.)

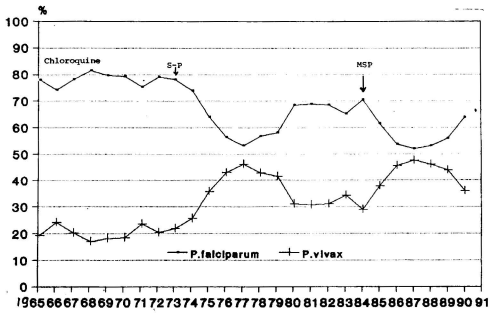

Fig 2—Proportion of *P.falciparum* and *P.vivax* in Thailand.

Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health, Bangkok, Thailand.

CURRENT STATUS OF DRUG RESISTANCE IN THAILAND

Fig 1 shows the number of malaria cases in 1972 to 1991; The malaria cases increased since the early 1970s when *P.falciparum* resistance to chloroquine became severe. Fig 2 shows the percentage of *P.falciparum* and *P.vivax.* Whenever *P.falciparum* was sensitive to the blood schizontocide used, the number or percentage of falciparum malaria cases decreased and whenever *P.falciparum* became resistant to the blood schizontocide used the number or percentage of falciparum malaria cases increased.

In Thailand, monitoring of efficacy of antimalarial drugs has been carried out in the hospitals and in the field. The results are shown in Tables 2 and 3. It is obvious that *P.falciparum* in Thailand developed resistance to all kinds of blood schizontocides; the longer the half life of the drug the sooner the parasite develops resistance to the drug. Thus the $t^{1/2}$ of chloroquine, mefloquine, sulfadoxine and pyrimethamine are about a month, 3 weeks, 200 hours and 80 hours, respectively; the shorter the $t^{1/2}$ of the drug the longer time the parasite takes to develop resistance, *eg* quinine whose $t^{1/2}$ is 11-16 hours. It is hoped that it would take a long time for *P.falciparum* to develop resistance to artemisinin and its derivatives as their half lives are very short.

RESISTANCE OF *PLASMODIUM VIVAX* TO CHLOROQUINE

Recently, there have been reports from Indonesia and Papua New Guinea of chloroquine resistant strains of *P.vivax* (Schwartz *et al,* 1991; Gerrit *et al,* 1992). However, confirmation in well-designed studies is needed, as relapse in vivax malaria can be confused with recrudescence. Regardless, chloroquine is still effective for the treatment of *P.malariae* and *P.ovale.* In Thailand *P.vivax* is sensitive to chloroquine; a single dose of 300 or 450 mg base of chloroquine can clear parasitemia

within 4-5 days (Harinasuta, unpublished data).

MECHANISM OF DRUG RESISTANCE

A number of different mechanisms theoretically can account for changes in drug sensitivity: physiological adaptation, selection of mixed sensitive and resistant parasite populations, spontaneous mutation and subsequent selection of resistant in gene expression; introduction of resistance transfer factors or other plasmids. Spontaneous gene mutation is considered to be the most likely mechanism for *Plasmodium* species, though other factors also play a role. Subcurative blood levels of drug tend particularly to encourage the survival of tolerant strains of parasites whose mutation to drug resistance may thereby be facilitated. Drug pressure may occur in both the human and the mosquito hosts, especially with slowly-excreted drugs. Chloroquine is retained in mosquito tissues after a blood meal taken from a chloroquine-treated person, and this exerts drug pressure on the sporogonic phase, contributing to development of resistance.

Mutations may be one-step, where total sensitivity is replaced by RIII resistance in a single step, or multi-step where each successively higher drug resitance level requires a different mutation for resistance to develop up to that level. Linked paris or groups of mutations may occur together, accelerating the process of multi-step mutations.

The mutation rate for *P.vivax* appears to be lower than that of *P. falciparum* and *P.vivax* densities are also much less, thus fewer mutant organisms will be avaible for selection under drug pressure during a given infection.

Resistance to dihydrofolate reductase (DHFR) inhibitors

The mechanism of resistance to antifolates, pyrimethamine, proguanil and cycloguanil in *P.falciparum* is due to mutations within the dihydrofolate reductase (DHFR) gene. The mutations cause the enzyme to be less susceptible to inhibition by the drug. The activity of pyrimethamine in *P.falciparum* is decreased because of lesser affinity of the parasite DHFR to pyrimethamine.

Resistance to PABA competitors

The sulphones and sulphonamides act at an earlier stage of folate synthesis than do the DHFR inhibitors, being conpetitive antagonists of para-aminobenzoic acid (PABA). Their molecules mimic PABA in structure and compete with it for the enzyme, dihydropteroate synthetase, that is responsible for coupling pteridine to PABA and forming dihydropteroate (the direct precursor of dihydrofolate). Resistance to this process has developed as a one-step mutation in experimental rodent malaria. With respect to *P.falciparum,* there are numerous reports of resistance, particularly to sulfadoxine. It must not be overlooked, however, that some alleged cases of resistance represent drug failure due to abnormal metabolism of sulfonamides. Parasite transfer studies in volunteers showed that failure to clear parasitemia was not attributable to the parasite, but to human metabolic activity in rapidly acetylating the drug, resulting in rapid urinary excretion or in excessive binding to plasma protein from which the drug is subsequently released in small quantities over a prolonged period of time. In pathological conditions where plasma protein levels are raised, it is evident that more will be avilable to bind the drug.

The mechanism of resistance has been suggested to involve the parasite's ability to use an alternative pathway of obtaining folate precursor, bipassing PABA-utilisation, and thus circumventing the drug which is a PABA-analog.

Resistance to chloroquine

Many studies have shown chloroquine resistant strains of *P.falciparum* to be able to accumulate chloroquine in lesser amounts than sensitive ones. Nevertheless, the rate of uptake of chloroquine into the parasites has been demonstrated to be similar between sensitive and resitant strains. However, the efflux of chloroquine in resistant strains was shown to be 40-80 times greater than in sensitive strains. This efflux of chlorquine can be inhibited by verapamil and upon this inhibition, there is a reversal of chlorquine resistance. The concentration of verapamil required to reverse chloroquine resistance is similar to that required to reverse the multidrug resistance (MDR) phenotype of mammalian tumor-cells. Tumor

cells of the MDR phenotype express a P-glycoprotein which acts as an energy dependent drug efflux pump. These cells appear to be cross-resistant because the P-glycoprotein pump transports pharmacologically unrelated compounds, the phenotype is thus termed multidrug resitance. It has been shown that the MDR gene is amplified in some but not all chloroquine resistant strains of *P.falciparum* and not in sensitive strains. The MDR gene in *P.falciparum* has also been found to encode a P-glycoprotein. Overproduction of P-glycoprotein was found in some chloroquine resistant parasites in which strains these is a greater efflux of chloroquine than in sensitive strains.

Cross-resistance

The mode of action of chloroquine, quinine and mefloquine seems to be similar and cross-resistance between these drugs may exist. However, acquisition of chlorquine resistance by a parasite does not imply resistance to quinine or mefloquine. Nevertheless, it may predispose the parasite to resistance to these drugs or resistance to these drugs may be genetically linked to chloroquine resistance.

MEASURES TO COMBAT DRUG RESISTANCE

Limiting use of drug

The major obstacle to limiting the use of antimalarial drugs is their ready availability on the open market. It is probably a counsel of perfection to suggest that antimalarial drugs should only be imported and supplied through national health authorities. At least, governments should be able to control the distribution and/or sale of antimalarials in affected countries. Unfortunately drug control is rarely exercised in the developing countries, even if laws exist for this purpose. While it is certainly too late to do much to manage the use of existing antimalarial drugs, every effort should be made to restrict the importation and distribution of new compounds that become available. If this is not done, it is quite certain that new compounds will meet the same fate as the older ones, and in a short they will be rendered obsolescent by the emergence of resitant parasites. The current fate of Fansidar® in Thailand is a case in point.

Prevention of spreading of resistance

In relation to prevention of spreading of drug resistance, the complementary use of primaquine together with blood schizontocides is probably the most valuable measure that can be taken since this compound is equally effective upon the gametocytes, of drug-resistant and drug sensitive *P.falciparum* (Harinasuta, unpublished data). A dose of 30 mg base in adults weekly for 3-4 weeks is sufficient for this purpose.

Drug monitoring

In order to be forewarned of any possible change in the drug response pattern in a given area, to interpret its significance and to plan and implement appropriate operational responses, it is necessary to establish baseline data and an effective monitoring system. Such a system should monitor the sensitivity status related to all operational and advanced candidate blood schizontocidal drugs and be based on the use of *in vitro* and *in vivo* methods, and pharmacokinetic studies, if possible. The development of standardized methods of *in vivo* and *in vitro* tests for drugs other than 4-aminoquinolines, the provision of standardized material, and the establishment of threshold levels of *in vitro* response as related to *in vivo* response require urgent attention. In Thailand, such tests are done at national, institutional and community levels. Nationally, the Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health has monitored parasite sensitivity using mainly *in vitro* methods for the last decade in the field in scattered around the Thai-Combodian border, the Thai-Myanmar border and other endemic areas all over the country. At the institutional level, at the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University both *in vivo* and *in vitro* sensitivity monitoring have been carried out on in-patient volunteers, and, in collaboration with the Malaria Division, at malaria clinics on outpatient volunteers. Since 1980 the Bangkok Hospital for Tropical Diseases has investigated mefloquine, MSP and halofantrine sensitivity. The data are presented in Table 2. At present the efficacy of

artemisinin derivatives is under study. A clinical pharmacology laboratory has been established in order to measure the drug levels and carry out pharmacokinetic studies

Management of established drug resistance

Chloroquine resistant *P.falciparum* appears to have several biological advantages over chloroquine sensitive parasites and, once established, resistant parasites manifest a rapid geographical spread into receptive areas. Resistance is stable in the absence of drug selection pressure in experimental models and possibly also in *P.falciparum* in nature. It may thus not be sufficient simply to withdraw chloroquine and chemically related drugs and hope that the problem will resolve itself. Chloroquine resistance now often goes hand in hand with resistance to sulfadoxine-pyrimethamine. Multiple drug resistance thus poses a serious threat to health and life in such areas.

The following measures to combat drug resistance are indicated:-

A. Restriction of the use of all antimalarial drugs and limitation of their distribution by bringing them under the control of national health authorities.

B. Defining the way in which antimalarials should be used, and by whom.

C. Encourage the use of tested effective drug regimens (90-100% cure rate) for treatment.

D. Reserve adequate supplies of quinine-tetracycline or artemisinin derivatives for treatment of resistant infection in appropriate cases.

E. Undertaking intensive anti-vector measures in the most serious areas.

F. Establishment of a policy for the management of immigrants in and out of the affected areas.

G. Utilization of primaquine 30 mg weekly for 3-4 weeks to prevent transmission from gametocyte carriers.

H. Stimulation of basic, clinical and field research on new antimalarial drugs or drug combinations under health authority and/or university auspices, especially in collaboration with WHO or other international bodies.

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