

## A Review of the Drug Sensitivity of Plasmodium falciparum in Thailand

By :

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**OBJECTIVE:** Twenty-five years ago, the introduction of chloroquine for suppression and cure of the human malarias appeared to offer a highly effective tool, in conjunction with vector control, for malaria eradication programs. However, the emergence of chloroquine resistant strains of Plasmodium falciparum had made the goal of eradication less tangible than that anticipated in earlier forecasts and has stimulated intensive studies on a search for alternative regimens for chloroquine. The purpose of this report is to review the historical aspects of chloroquine resistant falciparum malaria in Thailand with particular reference to its prevalence, geographic distribution and alternative treatment programs.

**DESCRIPTION:** The history of chloroquine resistant falciparum malaria in Thailand begins with the report of Professor Tranakchit Harinasuta and associates (1962) from the Faculty of Tropical Medicine in Bangkok in which they reported chloroquine treatment failures in nine patients infected with P. falciparum. At approximately the same time, an American serviceman on temporary duty in Thailand contracted falciparum malaria. During the next 7 months, he received multiple courses of chloroquine, each ranging from 1.5 to 2.1 gm base, without achieving a radical cure (Young et al., 1963). Infected blood from this patient was intravenously inoculated into non-immune volunteers and subsequent drug sensitivity studies demonstrated that this strain, called the JHK strain, was also resistant to mepacrine, proguanil, pyrimethamine and amodiaquine (Young et al., 1964). Quinine treatment was ultimately necessary to produce a radical cure in both the naturally and experimentally infected subjects.

During the next decade, studies of the prevalence and geographic distribution of P. falciparum strains resistant to chloroquine have been reported by investigators from the Faculty of Tropical Medicine and the SEATO Medical Research Laboratory, and the results are illustrated in Figure 1.

Harinasuta and colleagues (1965) administered a conventional course of chloroquine (i. e., 25 per kg) to infected patients who were hospitalized in Bangkok. Under the conditions of their study, prolonged follow-up examinations were possible and natural transmission was unlikely.

Only 2 (5%) of 42 patients experienced a radical cure. In the remaining 40 subjects, 28 exhibited an initial disappearance of parasitemia and symptoms followed by a recrudescence and 12 had only partial clearing of asexual parasites. In a subsequent report by Harinasuta et al. (1967), conventional chloroquine therapy was given to 65 hospitalized patients in Bangkok, who were acutely ill with P. falciparum infections. Not a single radical cure was achieved. Incomplete clearance of asexual parasites was observed in 40 patients and a recrudescence occurred in the remaining 25 patients who had experienced initial clearing. The subjects examined in the 2 reports by Harinasuta et al. resided in many areas of Thailand and these investigators suggested that strains of P. falciparum resistant to chloroquine were distributed throughout the entire nation.

While personnel of the Faculty of Tropical Medicine were concentrating their efforts on hospital based patients in Bangkok, investigators from the SEATO Medical Research Laboratory were conducting studies on the prevalence and geographical distribution of strains resistant to chloroquine in rural areas of Thailand. In 1966, Bourke and associates reported a reduced sensitivity of P. falciparum to chloroquine among residents of 3 southern provinces bordering Malaysia. They observed that 80% of 307 infected residents who were given a single dose of chloroquine (10 mg per kg) still exhibited asexual parasites 3 days later. Previous investigations in Southeast Asia which were reviewed by Sandosham et al. (1964) showed that a single dose of 10 mg per kg was generally sufficient to clear asexual parasitemias within 3 days.

These field studies were extended by Cadigan and colleagues (1968) to other provinces in the southern, central and northcentral areas of Thailand. Infected subjects were given 25 mg per kg of chloroquine base and followup blood smears were obtained one week after commencement of therapy. The proportions of unsatisfactory parasite responses ranged from 6% in the central province of Saraburi to 85% in the northcentral province of Loei.

During the 1970 summer peak of malaria transmission in Central Thailand, investigations of chloroquine resistant malaria were renewed at Phrabuddhabat Hospital, Saraburi Province, which was an area previously surveyed by Cadigan and associates (1968). The objective of these studies was to assess the reliability and reproducibility of a rapid *in vitro* technique described by Rieckmann *et al.* (1968) for detection of chloroquine resistant strains of *P. falciparum*. This technique consists simply of an *in vitro* cultivation of parasitized blood with glucose as the only added nutrient. The indicator response is the degree of trophozoite maturation to schizonts in vials containing zero or graded concentrations of chloroquine.

Concurrent *in vivo* and *in vitro* responses to chloroquine were measured in 57 patients infected with *P. falciparum* (Colwell *et al.*, 1972 a). Treatment failures following conventional chloroquine administration were observed in 55 (96%) of the 57 subjects. All 57 individuals had parasites which exhibited chloroquine resistance *in vitro*. It is conceivable that in the two subjects who were cured, the additive effects of host immunity and treatment were sufficient to produce a radical cure even though the parasites were relatively resistant *in vitro*.

Investigations of the *in vitro* sensitivity of *P. falciparum* have also been conducted in Nong Khai (northeast), Trat (southeast) and Yala (south) provinces in Thailand. The subject examined in these 3 areas consisted primarily of acutely ill subjects seeking treatment at a local government health facility in the provincial capital city. However, 20 of the 43 subjects examined at Nong Khai were asymptomatic, infected residents of a remote rural village (Phone Phi Sai), who had not actively sought treatment from a government health facility. The proportions of resistant strains detected by the *in vitro* Rieckmann test at Nongkhai and Trat were approximately 90% (Hickman *et al.*, 1971). Utilizing the same technique in Yala Province (south), our current studies have yielded a similar high rate of chloroquine resistant strains.

Among the alternative treatments for chloroquine which have been investigated in Thailand are quinine, atabrine, sulfonamides, folic acid antagonists, tetracyclines and various combinations of these agents. Table 1 summarizes the radical cure rates obtained with these alternate regimens reported during the past decade.

Quinine still appears highly efficacious in controlling symptoms and promoting rapid clearance of asexual parasitemias of *P. falciparum* in acutely ill patients. However, few reports are available concerning the efficacy of quinine therapy alone in effecting radical cures of falciparum malaria. In addition, the optimal duration of quinine administration for effecting radical cures is still open to question. In Southeast Thailand, Colwell and associates (1972 b) examined the efficacy of a combination of quinine and chloroquine in acutely ill subjects. Thirty-six infected subjects were given a 3 day course of quinine at daily dose of 1.62 gm base, followed by a conventional (*i. e.*, 25 mg/kg) course of chloroquine. During the one month follow-up period, only 41% of these patients experienced a radical cure. Harinasuta *et al.* (1965) examined the efficacy of a 4 and 5 day course of quinine at the same daily dose. Although their patients exhibited initial clearing of asexual parasitemias, all experienced a recrudescence. More recently, the efficacy of a 7-10 day course of quinine was investigated in residents of Saraburi Province (Colwell *et al.* 1972 c). Thirty-four acutely ill patients, many of whom were severely ill with high densities of asexual parasites, were administered a 7-10 day course of quinine at daily dose of 1.62 gm base. All subjects experienced initial clearing of asexual parasitemias ranging from 1 to 6 days (mean clearance was 3.3 days). In 12 patients, blood smear examinations were obtained for one month after commencement of therapy and none exhibited a recrudescence of *P. falciparum*. These limited investigations suggest that quinine remains highly effective for treatment of falciparum malaria in Thailand. Only 1 isolated case of a poor response to quinine administration had been reported in Thailand (Pinswasdi and Charoenkwan, 1965).

Several investigations have been conducted to examine the effects of a single dose of a long acting sulfonamide, alone or in combination with pyrimethamine, against *P. falciparum* infections. Harinasuta *et al.* (1967) administered a single dose of sulphormethoxine (1000 mg) to 18 infected subjects who had recently experienced chloroquine treatment failures. Radical cures were reported in 11 of these individuals. When 50 mg of pyrimethamine was added to the sulfonamide, 17 of 19 infected individuals experienced radical cure. The latter combination was associated with a more rapid onset of action than the sulfonamide alone, as reflected by mean parasite clearance times of 2.9 and 5.7 days, respectively.

Tawaramorn and his naval medical colleagues (1970) also examined the effects of a single dose administration of sulphormethoxine and pyrimethamine at the same doses employed by Harinasuta *et al.* (1967). The majority of their infected subjects were marine and naval personnel who contracted *P. falciparum* in a single dose and radical cures were reported in all patients. The mean clearances of fever and asexual parasitemia in this study were considerably lower in comparison with the earlier report of Professor Harinasuta and associates (1967).

A comparative evaluation of a single dose administration of sulphormethoxine—pyrimethamine and sulfalene-trimethoprim was recently completed at Trat Provincial Hospital in Southeast Thailand (Chin *et al.*, 1971). Adult males were administered a single dose of sulphormethoxine (1000 mg)—pyrimethamine (50 mg) or sulfalene (1000 mg)—trimethoprim (500 mg). Radical cures were achieved in 91% of 34 subjects treated with the sulphormethoxine—pyrimethamine regimen and in 84% of 31 patients administered the sulfalene-trimethoprim regimen. These rates were not significantly different. The mean clearances of fever and asexual parasites in both treatment groups were similar and were comparable to those reported by Harinasuta *et al.* (1967).

In central Thailand, Benjapong *et al.* (1970) evaluated the antimalarial activity of a combination tablet of sulphamethoxazole (400 mg) and trimethoprim (80 mg) in doses recommended for treatment of bacterial infections. Administration of 2 tablets twice daily for one week resulted in a presumptive radical cure in 17 of 18 patients of whom two had experienced recent chloroquine treatment failures. Gametocidal effects were not observed and no significant toxic complications were reported. Although a single dose administration of a sulfonamide and folic acid inhibitor has its obvious advantages over multiple doses, further studies are warranted to assess therapeutic effectiveness and untoward side effects of these alternative regimens.

In addition to investigations of conventional drugs, the therapeutic efficacy of the tetracycline family of antibiotics had been examined in subjects residing at Trat Province in Southeast Thailand (Colwell *et al.*, 1971 b). Sixteen asymptomatic subjects of whom 8 had experienced treatment failures with chloroquine were given a 10 day course of oral tetracycline (250 mg every 6 hours) and blood smear examinations were accomplished for one month after commencement of therapy. Presumptive radical cures were demonstrated in 12 of 16. In the other 4 patients, tetracycline was discontinued within 72 hours because of the development of fever, headache and chills. The mean parasite clearance time of 4.7 days in the successfully treated patients is significantly longer than the clearance times associated with quinine and chloroquine treatment of susceptible strains (Handfield—Jones, 1949; Jefferey *et al.*, 1956).

The antimalarial activity of tetracycline was also evaluated in acutely ill subjects with *P. falciparum* infections in a subsequent phase of the preceding study (Colwell *et al.*, 1971 b). Because of the slow rate of tetracycline action, all subjects were given a 3 day course of conventional quinine therapy to control initial symptoms, followed by either a 10 day course of tetracycline or a conventional course of chloroquine. Treatment failures were observed in 15 (58%) of 36 patients treated with quinine—chloroquine, but in only one (3%) of 31 patients administered quinine—tetracycline. The difference in the failure rates was very highly significant. The single subject who experienced a treatment failure with quinine—tetracycline exhibited initial clearance of asexual parasites followed by a reappearance of trophozoites on the 28th day of observation, possibly reflecting reinfection rather than resistance. Although the prolonged

course of tetracycline and its relatively delayed rate of action limit its antimalarial efficacy, its lack of toxicity and high degree of effectiveness, particularly against resistant P. falciparum strains, warrant further study.

Minocycline, a new synthetic tetracycline analogue, was subsequently evaluated for its antimalarial efficacy at Prapokklao Hospital, Chantaburi Province in Southeast Thailand (Colwell *et al.*, 1971 d). The experiences of the staff physicians at this location indicate that this area is also highly endemic for chloroquine resistant falciparum malaria. Acutely ill subjects with P. falciparum infections were given a 3 day course of quinine to control initial symptoms, followed by the administration of 100 mg minocycline twice daily for one week. Presumptive radical cures were observed in all but one of 28 patients. The single subject with a treatment failure exhibited a reappearance of trophozoites on the 24th day of observation, and, hence, could have been reinfected. Additional studies are needed to evaluate the toxic side effects of this new tetracycline analogue in man. Rieckmann and associates (1971) have recently reported that tetracycline courses of 5 or 7 days were highly effective in terminating P. falciparum infections in partially immune volunteers who had low density asexual parasitemias. Similar to our studies, these investigators observed delayed clearances of fever and asexual parasitemias with tetracycline treatment alone. In an effort to reduce cost and duration of quinine-tetracyclines combinations, yet maintain effectiveness, we have renewed our *in vivo* drug sensitivity studies at Phrabuddhabat and Trat. Acutely ill adults infected with P. falciparum were given quinine (1.62 gm) for only one day and, at the same time, tetracycline therapy was initiated at a dose of 250 mg, 4 times a day, for only 7 days. A presumed radical cure was demonstrated in 25 of 29 patients who were followed for at least one month.

**PROGRESS:** Chloroquine resistant falciparum malaria appears to be distributed throughout all of Thailand and, in areas of intensive study, the majority of these strains are resistant to conventional doses of chloroquine. At localities in which low rates of resistance have been reported, these estimates may be conservative because subcurative doses (*i. e.*, 10 mg base/kg) of chloroquine were administered and/or prolonged follow-up examinations were not accomplished. According to recommendations of the World Health Organization (1968), chloroquine sensitivity should not be presumed until blood smear examinations remain negative for asexual parasites of P. falciparum for at least 28 days after commencement of chloroquine treatment at a dose of 25 mg per kg body weight.

Other considerations, in addition to inadequate follow-up examinations, may obscure interpretation of *in vivo* drug sensitivity studies. These include lack of drug intake supervision, exposure to reinfection and partial immunity of the sample populations. These restrictions of *in vivo* studies can be eliminated by the utilization of a simplified *in vitro* technique for detection of drug resistance. Within certain limitations, the simplified *in vitro* technique described by Rieckmann and colleagues (1968) for detection of chloroquine resistant falciparum malaria proved in our investigations to be a rapid and reliable test that demonstrated a qualitative index of chloroquine resistance in Central Thailand (Colwell *et al.*, 1971 a).

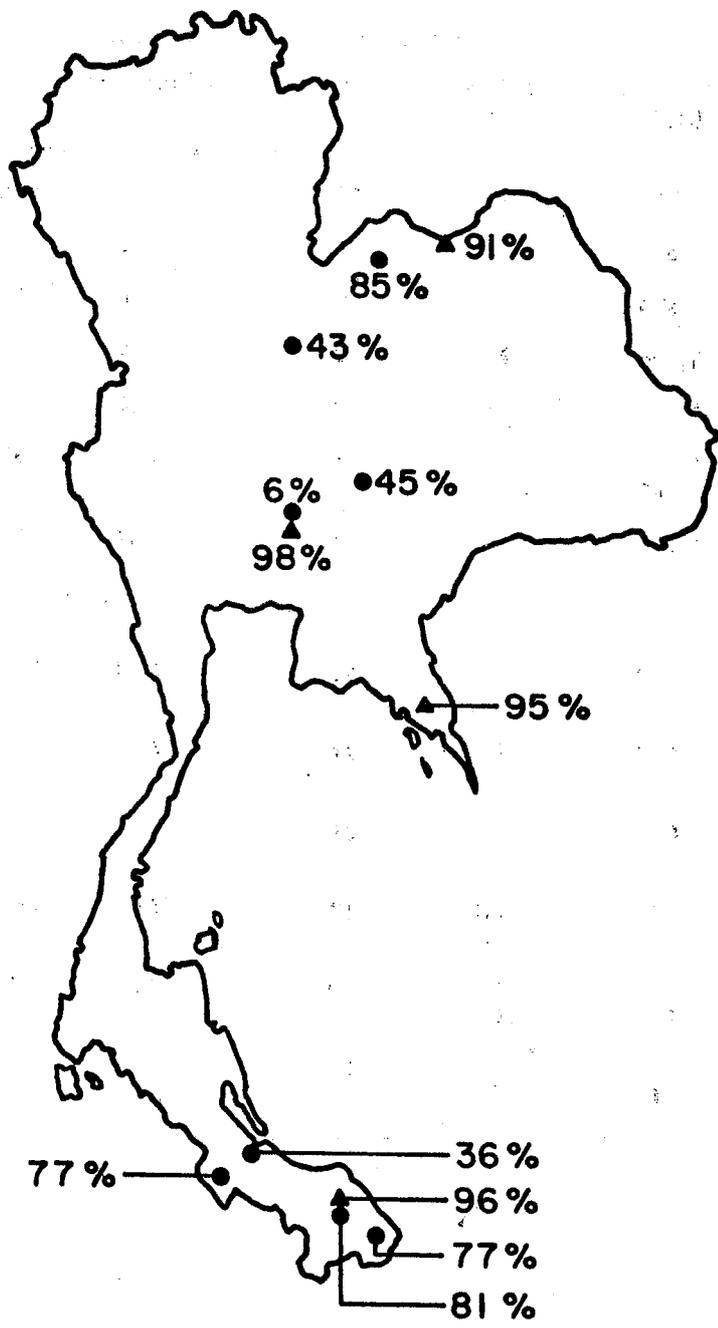
Among the alternative treatments for infected residents in endemic areas for chloroquine resistant falciparum malaria, the more promising regimens, with regard to cost, toxicity and efficacy are the single dose administration of sulphamethoxine-pyrimethamine and the multiple dose administration of quinine-tetracycline. The single dose schedule is particularly attractive for mass control campaigns wherein repeated supervision of drug intake is obviated. Although preliminary investigations of the efficacy of the sulfonamide-folate inhibitor combination in Thailand have demonstrated a high degree of efficacy, several disadvantages of this combination could preclude its mass use. Among the disadvantages are the lack of sporontocidal activity, the potential emergence of P. falciparum strains resistant to this regimen, the potential emergence of Neisseria meningitidis resistant to sulfonamides and toxic side effects. The later possibilities associated with this regimen are bone marrow suppression, gastrointestinal irritability and the Stevens-Johnson syndrome.

For treatment of hospitalized patients, the combination of quinine and tetracycline is clearly superior, with regard to cost and toxicity, to the prolonged administration of quinine with or without pyrimethamine and sulpham drugs. Our studies clearly demonstrate that by controlling the initial symptoms with quinine, tetracycline is highly effective in producing a radical cure in acutely ill patients infected with chloroquine resistant falciparum malaria. No toxic side effects have been observed and recent studies have shown that the original 13 day course of quinine-tetracycline therapy can be reduced to 7 days without compromising the efficacy. A potential disadvantage of major importance in prospective investigations is the emergence of P. falciparum and pathogenic bacteria resistant to tetracycline.

**SUMMARY:** The historical aspects of chloroquine resistant falciparum malaria in Thailand with particular reference to its prevalence, geographic distribution and alternative treatments have been reviewed.

Chloroquine resistant strains of P. falciparum are distributed throughout all of Thailand and, in areas of intensive study, the majority of strains are resistant to conventional doses of chloroquine.

The most promising alternative treatments are a single dose administration of sulphamethoxazole—pyrimethamine and a multiple dose administration of quinine—tetracycline. The former combination is particularly attractive for mass control programs and the latter combination is suitable for hospitalized subjects with moderate to severe degrees of illness.



**DISTRIBUTION AND FREQUENCY OF CHLOROQUINE RESISTANT FALCIPARUM MALARIA IN THAILAND**

Table 1.  
Radical cure rates associated with alternative regimens employed for treatment  
of falciparum malaria in Thailand

Drug (s)	Daily dose (mg)	Duration (Days)	No. treated	Radical cure (%)	References
Mepacrine	200	7	12	66.7	Harinasuta et al. (1965)
Quinine	1,620	4	3	0	"
Quinine	1,620	5	3	0	"
Quinine	1,620	7	8	100.0	"
Quinine	1,620	7 - 10	12	100.0	Colwell et al. (1972)
Quinine	1,620	3	36	41.6	Colwell et al. (1972)
Chloroquine	1,500	2			
Fanasil	1,000	1	18	66.7	Harinasuta et al. (1967)
Fanasil	1,000	1	19	89.4	"
Pyrimethamine	50	1			
Fanasil	1,000	1	62	100.0	Tawaraman et al. (1970)
Pyrimethamine	50				
Fanasil	1,000	1	34	91.2	Chin et al. (1971)
Pyrimethamine	50	1			
Sulfalene	1,000	1	31	83.9	"
Trimethoprim	500	1			
Sulphamethoxazole	1,600	7.5	19	89.4	Benjapongs et al. (1970)
Trimethoprim	320	7.5			
Quinine	1,620	3	30	96.6	Colwell et al. (1972)
Tetracycline	1,000	10			
Quinine	1,620	3	28	96.4	Colwell et al. (1972)
Minocycline	200	7			

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