RESISTANCE TO ANTIMALARIALS BY *PLASMODIUM FALCIPARUM* IN BURMA

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INTRODUCTION

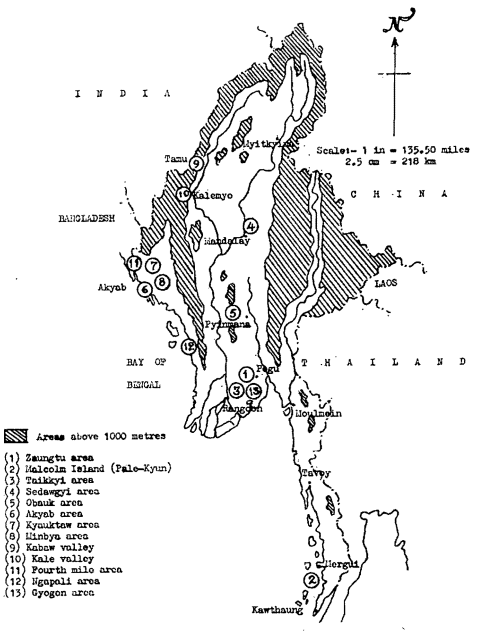
Chloroquine has been used as an antimalarial for some 30 years, but up to 1969 there were no records of studies on sensitivity of *Plasmodium falciparum* to a standard dose of chloroquine, though the existence of resistant infections in Burma had for some years been suspected on clinical grounds.

In 1969, studies undertaken at the Malaria Institute of Burma by the Chloroquine-Resistant Malaria Investigation Committee confirmed the presence of resistant *P. falciparum* infections. In these studies, the WHO extended field test for strain sensitivity to a standard regimen of chloroquine (WHO, 1967) was used on hospital patients in a non-endemic area under conditions precluding reinfection.

The present paper deals with further field trials which were carried out from 1971 to 1974 in 13 different endemic areas with persistent malaria transmission. It also includes the findings from a smaller trial conducted on five chloroquine-resistant falciparum infections at R I level found resistant falciparum malaria in Burma up to 1974, with indication of the vectors involved in these areas; on resistance to other antimalarials; and on the alternative antimalarials employed for these resistant cases.

MATERIALS AND METHODS

Between 1971 and 1974 chloroquine was administered under close supervision to 445 patients in 13 different endemic areas of Burma where persistent malaria transmission occurs, and where the total population involved is approximately 100,000 inhabitants (Map 1). Adult patients with falciparum



Map 1 — Distribution of chloroquine resistant falciparum infections in Burma up to 1974.

malaria (mixed infections were excluded) received 1.5 gm of chloroquine (base) in three divided doses over three days, while children received 25 mg/kg body weight of chloroquine (base) over the same period in doses divided as follows: 10 mg/kg on the first and second days and 5 mg/kg on the third day.

Thick and thin smears were taken from each patient just before the first dose of the drug was administered, and then daily for seven days. Afterwards, weekly blood

smears were taken over a period of three weeks. Smears were also taken from a patient on the day he had a recrudescence of fever if this occurred before the 14th day after administration of the first drug dose. The blood smears were stained with Giemsa, and for each thick smear at least 100 fields were examined for the presence of asexual stages of the parasite before it was considered to be negative. Parasite counts were performed against 400 leucocytes, with 8,000 leucocytes/c.mm being taken as the normal value for the study population.

Immediately after the patients had taken their last dose of chloroquine, the Dill and Glazko Eosin colour test was used to demonstrate any excretion of chloroquine in the urine. All patients with resistant infections gave a positive Dill and Glazko test. However, only falciparum cases recrudescing on or before the 14th day following the first drug dose were considered as resistant cases, since the trials were carried out under field conditions where reinfection could not be excluded in patients recrudescing after that time.

The five patients who had contracted falciparum malaria in different parts of Burma, and who were found to be resistant to a standard dose of chloroquine at R I level when they came to the Malaria Institute of Burma for treatment during the period 1971-1973, were given pyrimethamine at the following dosages; a young boy received 25 mg daily for three days, two adults received 50 mg daily for three days and the two other adults received 50 mg daily for five days. These five patients remained throughout the trial period in areas where the risk of reinfection could be precluded.

In November 1971, 40 patients with falciparum malaria, most of whom were from Malcolm Island (Pale-kyun) and among whom 21 had R I level of chloroquine resistance, were given single doses of pyrimethamine with sulfadoxine. Adults received 50 mg pyrimethamine and 1 gm sulfadoxine, while children received smaller doses. Blood smears were taken one week after treatment and then once a fortnight for two more times.

A non-immune patient who had contracted falciparum malaria from Ngapali area in December 1973 and who was found to be resistant to a standard dose of chloroquine is of special interest as he was the first case found to be resistant also to a single dose of 75 mg pyrimethamine with 1.5 gm sulfadoxine.

The Burma (Thau.) strain, which was isolated in August 1971 from a semi-immune Burmese male (Clyde *et al.*, 1972), was induced in non-immune American volunteers at the Malaria Study Centre in Maryland and then tested against various antimalarials including chloroquine, pyrimethamine, proguanil, amodiaquine and quinine (Clyde *et al.*, 1973).

RESULTS

Out of 445 falciparum infections investigated between 1971 and 1974, 328 (73.7%) were sensitive while 117 (26.3%) were resistant to a standard dose of chloroquine. Of the resistant infections 113 were at R I level three at R II and one at R III (Table 1).

The five cases of falciparum malaria resistant to chloroquine at R I level were also found to be resistant to pyrimethamine: four at R I and one at R II level (Table 2). All five patients were cured by a single dose of pyrimethamine with sulfadoxine.

Of the 40 falciparum cases treated with a single dose of pyrimethamine with sulfadoxine, 39 became negative for asexual stages of the parasite by the seventh day after treatment. The one remaining patient showed the presence of asexual stages of the parasite up to the 21st day after treatment; but a blood smear taken from him again on the 35th day

Table 1

Chloroquine-resistant falciparum malaria in Burma up to 1974.

Serial No.	Areas where resistance of *P. falciparum* to chloroquine has been detected	Year when resistance was first detected	Number of falciparum cases tested by WHO standard procedure (month and year)	Number of cases found susceptible (S) or resistant to 3-day treatment (degree of resistance)*	Anopheline vectors responsible for transmission of malaria
1	Zaungtu area, Pegu Township, 64 km northwest of Pegu	1969	38 (February 1971)	31 (S) 5 (R I) 1 (R II) 1 (R III)	*A. balabacensis balabacensis* *A. minimus*
2	Malcolm Island (Pale-Kyun), Mergui Township, 137 km south of Mergui	1971	60 (November 1971)	36 (S) 24 (R I)	*A. balabacensis balabacensis* *A. sundaicus*
3	Taikkyi area, Taikkyi Township, 64 km north of Rangoon	1971	7 (August 1971)	2 (S)	*A. balabacensis balabacensis* *A. minimus*
4	Sedawgi area, Madaya township, 40 km north of Mandalay	1971	35 (January 1971)	15 (S) 20 (R I)	*A. balabacensis balabacensis* *A. minimus*
5	Obauk area, Taungdwingyi Township, 66 km west of Pyinmana	1972	23 (October 1972)	22 (S) 1 (R I)	*A. balabacensis balabacensis* *A. minimus*
6	Akyab area, Akyab Township	1972	54 (December 1972)	47 (S) 7 (R I)	*A. annularis*
7	Kyauktaw area, Kyauktaw Township, 129 km north of Akyab	1972	81 (December 1972)	59 (S) 22 (R I)	*A. annularis*
8	Minbya area, Minbya Township, 56 km east of Akyab	1972	47 (December 1972)	41 (S) 6 (R I)	*A. annularis*
9	Kabaw Valley, Tamu Township	1973	19 (October 1973)	14 (S) 4 (R I) I (R I)	*A. minimus* *A. balabacensis balabacensis*
10	Kale Valley, Kalemyo Township	1973	33 (October 1973)	24 (S) 8 (R II) 1 (R II)	*A. minimus* *A. balabacensis balabacensis*

Table 1 (Cont'd)

Serial No.	Areas where resistance of *P. falciparum* to chloroquine has been detected	Year when resistance was first detected	Number of falciparum cases tested by WHO standard procedure (month and year)	Number of cases found susceptible (S) or resistant to 3-day treatment (degree of resistance)*	Anopheline vectors responsible for transmission of malaria
11	Fourth Mile area, Maungdaw Township	1974	15 (February 1974)	13 (S) 2 (R I)	*A. minimus* *A. balabacensis balabacensis*
12	Ngapali area Sandoway township	1974	19 (April 1974)	12 (S) 7 (R I)	*A. sundaicus*
13	Gyogon area Hlegu Township	1974	14 (August 1974)	12 (S) 2 (R I)	*A. balabacensis balabacensis* *A. annularis*

* Total numbers tested (1971-74) : 445
Sensitive(S) : 328
Resistant (R) : 117 (RI : 113; RII : 3; RIII : 1).

Table 2

Response in five cases of chloroquine-resistant falciparum malaria to treatment with pyrimethamine.

Serial No.	Age of patient (in years)	Trophozoite count/c.mm before pyrimethamine administration	Dose of pyrimethamine administered	Day of reappearance of trophozoites after first drug dose	Parasite response
1	28 Burma (Thau.) strain	880	50 mg daily for 5 days	7	R I
2	31	40	50 mg daily for 5 days	9	R I
3	11	640	25 mg daily for 3 days	10	R I
4	26	320	50 mg daily for 3 days	12	R I
	27	22,000	50 mg daily for 3 days	Parasitaemia not cleared but reduced during one week	R II

no longer showed parasites, though the patient had not received any further treatment. This patient was among the 21 chloroquine-resistant infections at R I level included in the trial group of 40 patients; the other 20 resistant cases, however, were cleared of asexual stages of the parasite by the seventh day and remained so during the further observation period of four weeks (Tables 3 and 4).

A non-immune patient with chloroquine-resistant falciparum malaria was treated with a single dose of 75 mg pyrimethamine with 1.5 gm sulfadoxine. Recrudescence of fever and parasitaemia was noticed on the fifth day after treatment and the asexual parasite count which had been 48,000/c.mm before drug administration was now 284,000/c.mm. In this patient whose infection was resistant to standard doses of chloroquine and pyrimethamine with sulfadoxine, radical cure was obtained with 650 mg quinine given three times daily for 10 days.

The Burma (Thau.) strain when induced in non-immune American volunteers by Clyde *et al.*, (1973) exhibited resistance at RII/RIII levels to standard doses of chloroquine, pyrimethamine and proguanil and at R I level to a standard dose of amodiaquine. Clyde *et al.*, (1973) stated that it also showed R I/R II levels of resistance to 4.2 gm quinine (base) given in 5-7 days, but was sensitive to quinine when the dose was increased to 8.1 gm (base) given in 5-9 days. Even when the dose of proguanil was increased in one volunteer to 2.6 (base) given in 10 days, the infection remained resistant at R III level. When the dose of chloroquine was increased to 3 gm (base) over five days in three volunteers, resistance at RI level was seen in two of these volunteers while the response in the third was sensitive.

DISCUSSION

P. falciparum resistance to a standard dose

Table 3

Response in 40 cases of *P. falciparum* infection to treatment using pyrimethamine with sulfadoxine.

Number of patients	Blood-smear results for *P. falciparum* (asexual stages) after treatment		
	7th day	21st day	35th day
39	Negative	Negative	Negative
1	Positive	Positive	Negative

Table 4

Response in 21 cases of chloroquine-resistant falciparum infections to treatment using pyrimethamine with sulfadoxine.

Number of chloroquine resistant *P. falciparum* patients (R I level)	Blood-smear results for *P. falciparum* (asexual stages) after treatment		
	7th day	21st day	35th day
20	Negative	Negative	Negative
1	Positive	Positive	Negative

of chloroquine was present up to 14 days after administration of the first drug dose in 26.3% of the 445 cases studied under field conditions in 13 areas of Burma; 96.6% (113/117) of these were at R I level. If the results up to 28 days were included, the recrudescence rate at R I level would be higher but then reinfection could not be precluded in most of these cases.

These findings are similar to those made in South America, especially in Brazil, Venezuela and Colombia, and in other countries of Southeast Asia, such as Thailand and Viet-Nam, where recrudescence of infection after a normal initial response to a standard chloroquine course (WHO, 1967) seems to be the common manifestation of resistance. This does not necessarily imply that the drug has ceased to be useful for the treatment of semi-immunes in malaria endemic areas of Burma where only 3.4% (4/117) of falciparum infections are resistant at R II/R III levels. In the future, however, the problem of parasite resistance may become more widespread, and consequently the campaign against malaria more difficult, in countries of South America and Southeast Asia where malaria continues to be an important public health disease, not only because of the financial, operational and logistic difficulties encountered but also because the application of residual insecticides alone has failed to interrupt effectively transmission in most of these areas of stable malaria.

In 1973, Burma's population was 28.886 million, and of these about 10 million are living in the endemic areas of the country. The 13 endemic areas included in the present study have a total population of approximately 100,000 but some patients with chloroquine-resistant infections contracted from other areas of Burma where malaria is still endemic, also came to the Malaria Institute of Burma for treatment. It is therefore assumed that a similar percentage (i.e. 26.3%) of falciparum infections from these other endemic parts of Burma may be resistant to a standard dose of chloroquine.

It is also presumed that chloroquine-resistant infections may exist in certain neighbouring parts of Bangladesh and India. In Fourth Mile area of Maungdaw township, which is close to the Bangladesh border, two cases of chloroquine-resistant infections at R I level were detected, and in the Kabaw valley in Tamu township, which is close to the Indian border, five resistant cases at RI/RII levels were found. Both these endemic areas of Burma are not more than 8 km away from the respective bordering parts of Bangladesh and India where similar topographical and possibly epidemiological conditions exist. Moreover, the presence of a few chloroquine-resistant falciparum infections have been reported from certain parts of India, and more recently from Bangladesh (Rosenberg and Maheswary, 1976).

Pyrimethamine-resistant infections may be more widespread in Burma than hitherto suspected. This impression, however, is based only on the very small sample of five patients with chloroquine-resistant falciparum infections at R I level which were found to be resistant also to pyrimethamine at RI/RII levels.

Proguanil-resistant falciparum was not investigated but in a non-immune volunteer the Burma (Thau.) strain was found to be resistant at R III level even when the usual dose of 1.3 gm proguanil (base) given in five days was increased to 2.6 gm (base) given in 10 days (Clyde *et al.*, 1973).

On the basis of the observations made in these studies, any of the following regimens are proposed for the management of individuals with falciparum malaria resistant to standard doses of chloroquine, pyrimethamine and proguanil.

In most of these individuals, parasites may be eliminated by increasing, for adults, the chloroquine dose and duration of treatment to 2.4-3 gm (base) to be given in 5-6 days. Discretion should be exercised, however, in giving radical treatment to semi-immunes from endemic areas where no antimalarial measures are undertaken as such treatment could abolish their semi-immune status, and consequently on their return to these areas, where they will surely get re-infected, they may suffer more severely from the disease.

Pyrimethamine, though not effective against chloroquine-resistant infections when used alone, was very effective against most of these resistant cases when combined with sulfonamides, due to a synergistic action. The five patients with chloroquine and pyrimethamine resistant falciparum malaria were eventually cured by a single dose of pyrimethamine with sulfadoxine, the dose for adults being 50 mg of pyrimethamine and 1 gm of sulfadoxine. Out of the group of 40 patients (Tables 3 and 4), this combination showed very slow action in only one. In another patient, a non-immune who had contracted malaria in Ngapali area, resistance was noticed at RI level, to 75 mg pyrimethamine with 1.5 gm sulfadoxine.

The combination of 100 mg dapsone and 12.5 mg pyrimethamine given weekly for four weeks also gave very good results in some adults with chloroquine-resistant infections.

These pyrimethamine-sulfonamide combinations are apparently effective, simple to administer and usually well tolerated, especially the pyrimethamine-sulfadoxine combination. However, these combinations are strictly reserved for chloroquine-resistant cases, not only because their possible long-term side effects are not yet known, but also because it is feared that their indiscriminate use for prolonged periods may hasten the emergence of resistant strains as a number of pathogenic micro-organisms have already developed resistance to sulfonamides. Few more cases of chloroquine-resistant falciparum malaria, resistant also to pyrimethamine-sulfadoxine and pyrimethamine-dapsone combinations have also come to our notice recently.

Quinine, at a dosage of 650 mg given three times daily for 10 days, brought a complete cure to a non-immune adult whose falciparum infection was resistant to standard doses of both chloroquine and pyrimethamine with sulfadoxine. Cure was also obtained in other similar cases treated with this drug. Though quinine was not well tolerated by most patients, the authors have never so far observed failure when the drug was administered at the above dosage.

In areas where chloroquine-resistant infections were detected, related entomological studies showed that *Anopheles balabacensis balabacensis* was not the only vecter involved (Table 1). Other vectors such as *A. minimus*, *A. sundaicus* and *A. annularis* either singly or together with *A.b. balabacensis*, were also responsible for the persistance of malaria transmission despite the application of residual insecticides in some of these areas.

Hence in our studies, resistant falciparum malaria infections are not confined to only *A.b. balabacensis* areas; it could be found in any area of persistant malaria transmission irrespective of the vector species. Verdrager *et al.*, (1976) has also stated that chloroquine-resistant falciparum malaria has been detected in almost all *A.b. balabacensis* areas, due to the high intensity of malaria transmission.

Most of the indigenous population where these trials were carried out did not come into contact with those who might have contracted the resistant strains from a neighbouring country, such as Thailand. Hence, though there is no conclusive proof, it is believed that

chloroquine-resistant falciparum infections are indigenous to these endemic areas, rather than being introduced from neighbouring countries as has been suggested (Clyde *et al.*, 1973; WHO, 1973); but this is a question open to debate.

We believe that the emergence of drug-resistant falciparum malaria is due to the high persistance of malaria transmission and due to drug pressure. In the various countries, the endemic areas where the problem of parasite resistance has been detected, all present some common factors: malaria has been endemic for years; population movements are common; chloroquine has been readily available since several years, being taken irregularly and, for the most part, inadequately; socio-economic conditions are similar; and malaria transmission has persisted in most of these areas despite the anti-malarial measures applied, such as residual insecticide spraying and/or drug administration.

SUMMARY

The presence in Burma of *P. falciparum* resistance to a standard dose of chloroquine was confirmed in 1969. Further trials conducted from 1971-74 showed chloroquine-resistant falciparum infections in 26.3% of the 445 patients tested.

Pyrimethamine resistance was found in a group of five chloroquine-resistant patients. The falciparum infections in these five patients and in another group of 40 patients, including 21 chloroquine-resistant cases were all cleared by a single dose of pyrimethamine with sulfadoxine.

A none-immune patient resistant to both chloroquine and pyrimethamine with sulfadoxine, was cured by 650 mg of quinine given three times daily for 10 days.

The Burma (Thau.) strain induced in non-immune American volunteers had been shown to be resistant to standard doses of chloroquine, amodiaquine, pyrimethamine, proguanil and also to quinine at a dosage of 4.2 gm (base) given in 5-7 days, but was reported as sensitive to an increased dosage (8.1 gm base in 5-9 days) of quinine.

Any of the following regimens are proposed for the treatment of adults with falciparum malaria resistant to standard doses of chloroquine, pyrimethamine and proguanil: (i) Chloroquine at an increased dosage of 2.4-3.0 gm(base) in 5-6 days; (ii) 50 mg pyrimethamine with 1 gm sulfadoxine, given in a single dose; (iii) 12.5 mg pyrimethamine with 100 mg dapsone, once a week for four weeks, (iv) 650 mg quinine given three times daily for 10 days.

The vectors involved in the 13 different endemic areas of Burma where chloroquine-resistant infections have been detected were identified, and the question of whether such infections are indigenous to these endemic areas or introduced from neighbouring countries was raised.

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