

Investigations of in vitro and in vivo Chloroquine Sensitivity of Plasmodium falciparum in Yala Province

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OBJECTIVE: Chloroquine-resistant Plasmodium falciparum malaria is widespread in Thailand, approaching 100% prevalence in some areas. In the provinces of Yala, Satul and Narathiwat, Bourke, et al. (1966) reported finding a prevalence of chloroquine-resistant P. falciparum infections as high as 81%. This rate was based on the persistence of asexual parasitemias 3 days after the administration of 4 mg. chloroquine base per lb body weight. Although this dose is now considered subcurative and the follow-up period brief, Sandosham, et al. (1964), in reviewing results of earlier investigations, provided evidence that 4 mg per lb usually produced clearance of asexual chloroquine-sensitive parasites within 3 days.

Recently Andre, et al. (1972) reported that the prevalence of chloroquine-resistant P. falciparum in Kalantin province of Malaysia, adjacent to the study sites employed by Bourke and associates appeared to be much lower. Only 5 of 87 infected school children, (6%) had demonstrable parasitemias on the 7th day following conventional treatment with chloroquine (25 mg per kg). These results are identical to those obtained by McElvey, et al. (1971) 7 days after similar treatment of commonwealth troops stationed in Malaysia. Further in vivo studies by McElvey, et al., and limited in vitro investigations by Andre, et al., indicated the true prevalence was higher but less than has been reported in Thailand.

There is, therefore, some evidence that the prevalence rates of chloroquine-resistant falciparum malaria in Thailand and Malaysia are different. Furthermore, because of the proximity of the study sites used by Andre, et al., and Bourke, et al., it is conceivable that a transition zone between the higher and lower rates coincides with location of the Thailand-Malaysia border. This would not be too surprising since the border is more than just a political and religious boundary separating Buddhist Thailand and Moslem Malaysia. It is also the approximate position of a transition zone between 2 types of climate and is a widely recognized zoo-geographical boundary. On either side of the zone marked differences in flora and fauna exist. If a difference in rates of chloroquine resistance does exist, the border area would be a unique site to study the bionomics of chloroquine-resistant falciparum malaria.

The present investigations were initiated in an effort to obtain more recent data regarding the in vivo and in vitro chloroquine sensitivity of P. falciparum in southern Thailand and to compare the results with those obtained previously in both southern Thailand and Malaysia.

DESCRIPTION: Yala city was chosen as a study site because previous studies were conducted nearby and because of the support available from the provincial hospital and the district Malaria Eradication Center. Malaria infected subjects were referred for study from both sources. Other patients reported directly to the laboratory for diagnosis. Blood smears were made on all patients and checked for the presence of asexual parasites, their identification and the degree of parasitemia. Only subjects with P. falciparum infections were selected. Patients with parasitemias less than 1000 per cmm and those with mixed infections were excluded. Others excluded were patients under 6 years of age, those with a history of antimalarial treatment during the preceding 96 hours and those with renal or cerebral complications.

A total of 64 selected subjects were administered conventional doses of chloroquine (10 mg chloroquine base per lb. body weight) over 48 hours. Blood smears were examined daily for 7 days to determine parasitemias. If parasite levels and/or symptoms were not controlled by chloroquine, quinine was administered.

Further follow-up examinations on days 14 and 21 were performed on all subjects who were parasite free on the previous examination. All infections which recrudesced were treated with alternative antimalarial drugs.

At the time of subject selection, the mean level of trophozoite maturity was assessed. If the parasites were judged to be of adequate maturity, venous blood was drawn for an *in vitro* determination of the parasite sensitivity to chloroquine. The method used was that described by Rieckmann, *et al.* (1968) and employed by Colwell, *et al.* (1972). The test was considered successful if 5% or more of the trophozoites in the control vial matured to schizonts containing 3 or more nuclei. The parasites were arbitrarily considered to be chloroquine resistant if any schizonts were found in vials containing 0.6 millimicromoles or more of chloroquine base.

PROGRESS. The results of post-treatment examinations to determine *in vivo* chloroquine sensitivity of *P. falciparum* in Yala province are summarized in Table 1. A total of 42 patients were examined daily for 7 days. Of these, 3 failed to respond to treatment either clinically or parasitologically and 14, although they did respond, were parasitized on day 7. Thus, 40.5% of the infections demonstrated RII-RIII type chloroquine resistance. Of the 25 subjects who were parasite free on day 7, 21 were examined on day 14 and if negative again on day 21. Asexual parasitemias of *P. falciparum*, suggestive of RI type chloroquine resistance recurred in all but one. The cumulative prevalence of chloroquine-resistant *P. falciparum* was 97%. Since patients were rarely hospitalized more than 5 days, it is possible that some of the later recrudescences were, in fact, reinfections.

The prevalence of chloroquine-resistant *P. falciparum* in Yala as determined by the *in vitro* technique was similar to that obtained *in vivo*. The *in vitro* test was successfully conducted with the blood of 42 patients, 25 of whom subsequently participated in the *in vivo* portion of the study. The corresponding *in vivo* and *in vitro* results are presented in Tables 2 and 3. With regard to chloroquine resistance, the results are in complete agreement, both being 100%. As in previous studies (Colwell, *et al.*, 1972), however, the degree of resistance as determined clinically (RI or RII) could not be correlated with the growth patterns that occurred in vials containing different concentrations of chloroquine. Individual results of *in vitro* tests where *in vivo* data was not obtainable are presented in Table 4. The prevalence, overall, of *in vitro* chloroquine resistant *P. falciparum* was 97%, the same as was determined using the *in vivo* technique.

The *in vivo* results compare favorably with those obtained previously in southern Thailand (Bourke, *et al.*, 1966) and are markedly higher than those obtained more recently in Malaysia (McElvey, *et al.*, 1971; Andre, *et al.*, 1972). In the latter studies, only 6% of the subjects studied had infections that could be classified as chloroquine resistant 7 days after treatment was initiated. Further studies by McElvey, *et al.*, showed that 47% of the patients that were parasite free on day 7 recrudesced before day 32 resulting in an overall prevalence of chloroquine resistance of 50%. The possibility of reinfection was excluded in the latter study.

Differences in the number of patent infections found on day 7 and in the overall prevalence of resistance suggest that there is a difference in the prevalence and degree of chloroquine resistance in the extreme south of Thailand and in Malaysia. It is conceivable that the differences may be somewhat exaggerated by reinfections which, if they occurred, were unavoidable in the current investigation.

It is also true that the subjects studied in Yala province represented a different population than did those studied in Malaysia. The fact that subjects selected at Yala were seeking treatment implies that they may have been more ill than those studied in Malaysia and some may have already been treated unsuccessfully with chloroquine. Both variables may have influenced the results in such a way as to suggest a higher prevalence of chloroquine resistance. On the other hand, the level of acquired immunity in the Yala patients who were predominantly young adults (mean age: 25.1 years) was probably greater than that in either the school children examined by Andre, *et al.* (1972) or the commonwealth troops studied by McElvey,

et al (1971). Higher levels of immunity may improve the efficacy of chloroquine and, thus, result in a reduced prevalence of chloroquine-resistant falciparum malaria. Obviously further study will be required to more fully evaluate the situation, but these preliminary results strongly suggest that a gradient in the prevalence of chloroquine-resistant P. falciparum does exist in the Thailand-Malaysia border area.

SUMMARY: In vivo and in vitro studies were conducted in Yala province to determine the chloroquine sensitivity of P. falciparum in southern Thailand. By the seventh day after conventional treatment with chloroquine was initiated, 40.5% of the infections studied were either still patent or had been treated with other drugs because of an unsatisfactory patient response. By day 21, all but one of the remaining infections studied had presumably recrudesced although the possibility of reinfection could not be entirely excluded. The overall prevalence of both in vivo and in vitro chloroquine resistance was 97.4%. This is comparable to the prevalence found earlier in Thailand (Bourke, et al., 1966) and is higher than reported recently in Malaysia (McElvey, et al., 1971; Andre, et al., 1972). The evidence suggests that there may be a prevalence gradient in the area of the Thailand-Malaysia border. If true, the area may be a unique site in which to study the bionomics of chloroquine-resistant falciparum malaria.

Table 1.
Clinical response of P. falciparum infections to conventional chloroquine therapy

Response	No. of subjects	Per cent
RIII	3	7.1
RII	14	33.3
RI	20	47.6
S-7*	4	9.5
S-21**	1	2.4

* No recrudescence through day 7. No further follow-up.

** No recrudescence through day 21. No further follow-up.

Table 2.
Effect of chloroquine upon in vitro maturation of P. falciparum in subjects with an RII-RIII clinical response to chloroquine

Subject Number	Parasite count (per cmm)	Chloroquine Concentration*						
		0	0.4	0.6	0.9	1.35	2.02	3.04
1	16,350	84**	73	65	53	34	5	1
2	1,560	76	70	66	61	47	11	5
3	14,070	68	52	47	0	1	0	0
4	3,460	27	12	24	30	10	15	2
5	296,600	21	17	15	8	4	3	0
6	55,200	18	12	12	5	4	3	2
7	39,170	9	5	4	4	4	3	2

* Millimicromoles per ml of added inoculum.

** % Schizonts with 3 or more nuclei.

Table 3.
 Effect of chloroquine upon in vitro maturation of P. falciparum in subjects with an
 RI clinical response to chloroquine.

Subject Number	Parasit Count (per cmm)	Chloroquine Concentration						
		0	0.4	0.6	0.9	1.35	2.02	3.04
8	6,650	89	80	34	57	38	4	1
9	1,175	81	79	76	74	40	57	1
10	2,250	56	25	28	1	0	0	0
11	7,080	50	20	14	1	0	0	0
12	1,990	50	42	39	24	10	3	1
13	9,470	48	51	43	21	10	13	0
14	2,660	40	28	35	20	6	2	0
15	24,450	35	23	29	31	6	6	1
16	13,820	22	0	15	2	0	1	0
17	13,640	14	13	10	5	1	0	0
18	27,070	12	9	8	7	4	3	5
19	13,210	9	9	7	6	1	1	0
20	40,760	8	8	5	5	3	1	0
21	1,780	8	4	5	8	2	1	0
22	44,200	7	7	6	5	1	0	0
23	5,930	7	3	7	6	3	2	1
24	21,640	5	4	5	3	3	0	0
25	9,070	5	5	5	3	1	3	1

Table 4.
Effect of chloroquine upon in vitro maturation of P. falciparum in subjects with an unknown clinical response to chloroquine.

Subject Number	Parasits Count (per cmm)	Chloroquine Concentration						
		0	0.4	0.6	0.9	1.35	2.02	3.04
26	3,220	75	73	85	36	10	6	0
27	3,410	74	60	64	13	1	0	0
28	2,400	60	50	48	52	0	6	0
29	27,550	44	11	21	7	2	2	0
30	17,490	44	17	19	10	2	0	1
31	6,970	43	44	40	18	7	5	0
32	12,380	30	13	23	20	7	8	1
33	7,840	28	19	16	10	1	1	0
34	7,490	26	24	20	18	12	7	2
35	25,140	25	10	25	10	2	0	1
36	5,640	19	11	9	9	6	7	0
37	36,630	16	17	12	13	6	2	0
38	29,060	16	4	4	1	0	0	0
39	34,010	8	0	0	0	0	0	0
40	14,640	7	5	7	7	4	2	1
41	56,230	6	3	4	1	1	1	1
42	5,540	6	4	6	0	0	0	0