THE *IN VIVO* AND *IN VITRO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO QUININE

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INTRODUCTION

In Burma due to the widespread occurrence of chloroquine resistant falciparum malaria (Aung Than Batu *et al.*, 1973; Franco Tin *et al.*, 1973) and the emergence of resistance to sulphadoxine/pyrimethamine combination (Franco Tin, 1981; Kyaw Win *et al.*, 1983, Maung Maung Wint *et al.*, 1983), quinine is now being increasingly used for multidrug resistant malaria especially in severely ill patients. However, reduced efficacy to quinine has been observed in hospitalized patients (Mya Oo *et al.*, 1984) and demonstrated in apparently healthy patients with *Plasmodium falciparum* infection in whom blood level of quinine were also measured (Myint Lwin and Myint Oo, 1984).

The present study was thus carried out to assess the efficacy of oral quinine therapy in *P. falciparum* infected patients in relation to *in vitro* sensitivity test.

MATERIALS AND METHODS

Patients presenting themselves for treatment of malaria to an out-patient clinic in Rangoon and to the Tharrawaddy township hospital, 80 miles north of Rangoon with the exception of severely ill patients and pregnant women were initially screened. Those with asexual *P. falciparum* only, having parasite count between (5,000-150,000/ml/c.mm of blood) with no history of taking antimalaria drugs during the past 14 days were accepted, and tested for the presence of antimalaria drugs, quinine, chloroquine, amodiaquine and sulphonamides in urine. All those positive were excluded. 20 patients were so selected and included in the study, 19 males and one female between the ages of 18 to 59 years.

The method of Dill and Glasko was used to detect 4 amino-quinolines, Mayer-Tanret reagent for quinine and Lignin test for sulphonamides in the urine as described (WHO, 1981).

Quinine was given orally as quinine sulphate (obtained from WHO), 9-10 mg/kg 8 hourly for 10 days.

The *in vivo* test for the response of *P. falciparum* to quinine was performed for 28 days. Blood slides were taken on days 0, 2, 4 and 7 in all cases and on days 9, 12, 14, 21 and 28.

The *in vitro* micro test was done according to the method described by Rieckmann *et al.*, (1978), using precharged plates supplied by WHO. Wells A-H of the plate were charged with O (control), 4, 8, 16, 32, 64, 128 and 256 p. mol of quinine.

One ml of venous blood was obtained with ACD and 100ul was then transferred to each well of the test line in the plate. The plate was gently agitated to ensure that the quinine deposit was dissolved in the blood medium mixture and then incubated along with the vial at 38.5°C in a candle jar.

Maturation was verified by checking the contents of the vial after 22 hours. When incubation was estimated to be adequate (24-26 hours), the plate was removed and standard blood films were prepared, dried

for 48 hours and stained with 2% Giemsa for 30 minutes. Schizonts were counted against 200 asexual parasites and the maturation of schizonts were expressed either as a direct count or as a percentage of the mean count in the controls.

Plasma quinine level was monitored by benzene extraction method (Cramer and Isaksson, 1965), 1 to 3 hours after quinine administration on days 1, 3, 5 and 7.

RESULTS

The results of the *in vivo* test is shown in Table 1. In 12 subjects (60%) parasites are cleared within 7 days without recrudescence and in 5 subjects (25%) between 7-12 days. These 2 groups are deemed to be sensitive to quinine and denoted as S_I and S_{II} respective ly. In the 3rd group comprising 3 subjects (15%) parasites are cleared within 7 days, but reappeared within 28 days corresponding

Table 1

The response of *Plasmodium falciparum* to oral quinine 9 to 10 mg base per kg tds for 10 days.

Patient Age (yr)	Wt. (kg)	Total quinine dose (gm) for 10 days	*In vivo* test: Number of asexual parasites per ul blood on days 0	2	4	7	9	12	14	21	28	Grade of resistant
25	48	12	12625	150	75							S_1
26	54	13.8	12225	525						50	50	R_1
30*	32	6.9	8025	150	75							S_1
34	40	10.4	12825	225	25							S_1
18	52	15.5	23925	350	175			50	75			R_1
31	48	13.8	14025	100	25							S_1
36	44	13.8	32325	50	50							S_1
49	49	13.8	16175	100					50	50	200	R_1
18	46	13.8	20325	175	100							S_1
18	53	15.5	10150									S_1
26	48	13.8	18550									S_1
37	59	17.3	2900									S_1
22	52	15.5	700	200	150	100						S_2
59	49	13.8	141675	250								S_1
37	68	15.5	6200	275	75							S_1
39	48	13.8	47388	1100	675							S_1
28	50	15	6000	1200	5000	500	275					S_2
37	49	15	6000	3850	475	1250	975					S_2
23	43	13.8	6000	1700	–	1275						S_2
15	45	13.8	6000	–	–	325	150					S_2

* Female patient, others all male.

to R_I level of resistance of WHO extended 28 day test of chloroquine. Subjects exhibiting these 3 patterns of parasitaemia have been grouped and shown in Fig. 1. The mean plasma quinine level on days 1, 3, 5, 7 was between 7-11 mg/litre in all the subjects.

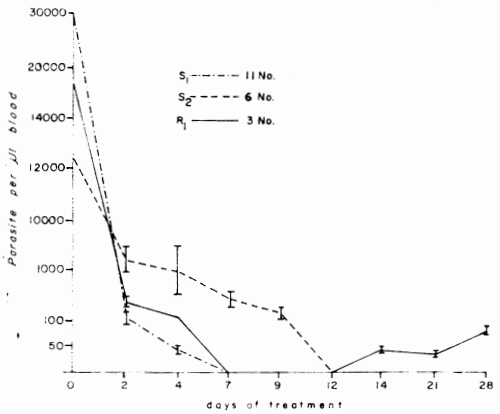

Fig. 1—The *in vivo* response of *P. falciparum* to oral quinine (9-10 mg/kg T.D.S. for 10 days).

The *in vitro* effect of quinine on maturation of schizont is shown in Fig. 2. Complete inhibition of schizont maturation is seen within the range of 32-128 p.mol/well. The majority are inhibited at 64 p.mol/well and 3 isolates at 128 p.mol/well. The mean MIC of quinine is calculated as 4.3 mg/litre.

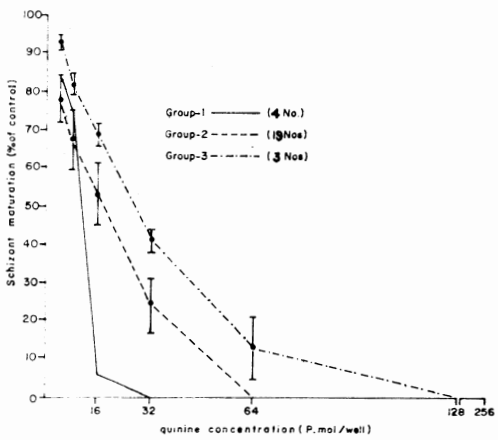

Fig. 2—Effect of quinine on maturation of parasite *in vitro*.

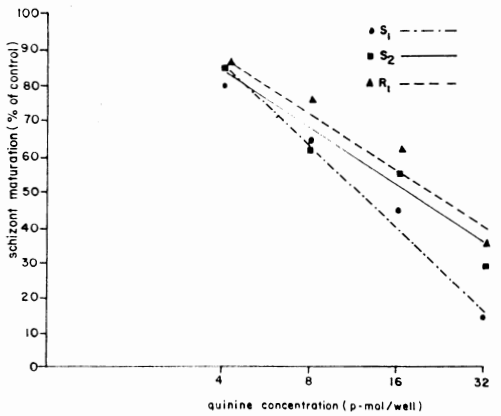

Fig. 3—The percentage of *P. falciparum in vitro* maturation as a function of quinine concentration, in cases classified as S1, S2 & R1 by the *in vivo* test (The actual observations and the log normal model fitted by the maximum-likelihood method).

The relationship between *in vivo* response of quinine and *in vitro* effect on schizont maturation in shown in Fig. 3. The *in vitro* schizont maturation inhibition curves are clearly different between isolates obtained from subjects denoted as sensitive (S_I & S_2) and R_I according to the *in vivo* test.

Thorough history was taken from the 20 patients from whom the isolates were obtained. Except for 4 patients from Tharrawaddy, the history revealed that most of the patients were resident in Rangoon and had acquired malaria infection during the course of travel to different places in Burma while some were resident elsewhere who had come to Rangoon for various reasons and presented themselves to the outpatient clinics. The places in Burma where the patients have most probably acquired infection are shown in Fig. 4.

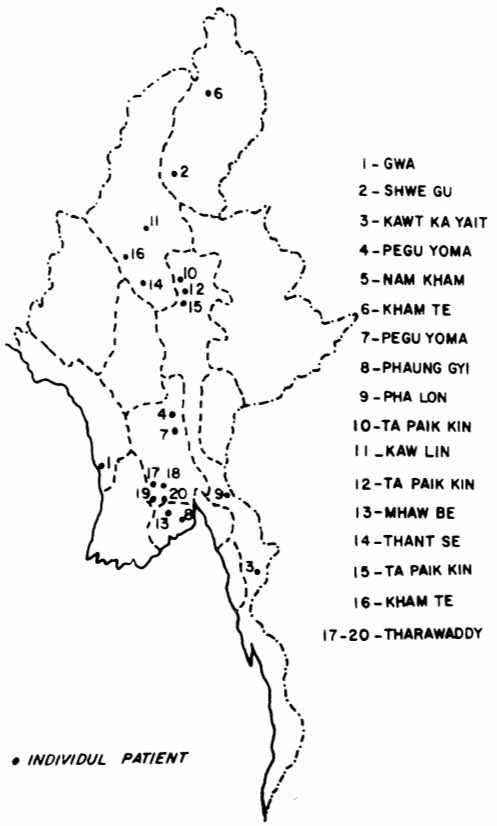

Fig. 4—Distribution pattern of the patients in *in vivo* and *in vitro* quinine study.

DISCUSSION

Persistence of parasitaemia atter standard dose of quinine have been increasingly seen by clinicians in Burma. Although blood level of quinine was not measured the probability was strong that they were true cases of quinine resistance. In a previous communication we reported that parasite persisted for seven days in two out of seven children given oral quinine 8 to 9 mg/kg 8 hourly for 7 days inspite of adequate blood level of quinine on days 1, 4 and 7 (Myint Lwin and Myint Oo, 1984) and confirm the previous findings that true instances of quinine resistance are being seen in Burma.

In the present study, oral quinine at 9-10 mg per kg 8 hourly for 10 days in adults (total dose of 14 g quinine base) gave complete parasite clearance up to 28 days in 17 out of 20 (85%) patients. This may be compared with complete parasite clearance in all non-immune subjects infected with the Burma (Tha U) chloroquine resistant strain of *Plasmodium falciparum* originating from the Tharrawaddy township who were treated with total dose of 8.1 g quinine base in 8 days. These results may also be compared with 96% cure obtained when oral quinine 10 mg/kg 8 hourly for 14 days in Thailand (Chongsuphajaisiddhi *et al.*, 1981).

The slope of schizont maturation inhibition curve appeared to differ between sensitive (S_1 & S_2) and R_I isolates. Sensitive (S_1 S_2) and resistant (R_I) isolates also seemed to require progressively higher concentration of quinine for complete inhibition with the exception of one isolate which though sensitive *in vivo* required 128 p.mol for schizont inhibition. The discrepancy may be due to the involvement of host factor *in vivo*. The sensitivity of the *in vitro* quinine test system could be increased if intermediate drug concentration between 32, 64 and 128 are also included.

The criteria used for determining sensitive or resistance in WHO extended 28 days field test which was originally developed for chloroquine sensitivity should not be applied to quinine. Whereas in the 28 day test for chloroquine sensitivity if the parasites do not disappear by day 7 they are deemed to be resistant. This conclusion should not be made in the case of quinine because of its slow action. As shown in this study, some parasite (denoted as S_2) disappeared only after 7 days and were completely cleared when followed up for 28 days. This may have not been noticed if the parasite is mistakenly concluded to be resistant (R_{II}) and another treatment is given without further observation of parasite

in the blood. The significance of the delayed clearance of the parasite designated as S_2 may need to be further investigated.

SUMMARY

The *in vivo* and *in vitro* sensitivity of *P. falciparum* to quinine were studied simultaneously on 20 isolates of *P. falciparum* from infected patients in Rangoon and in Tharrawaddy Township. The *in vivo* study showed 85% sensitive and 5% resistance at R_I level. The peak plasma quinine level in all the cases were above mean MIC on days 1, 3, 5 and 7.

Schizont maturation was inhibited at 128 p.mol/well in 15% of the cases but the rest were at or below 64 p.mol/well *in vitro* test. However, no relationship was detected between the *in vivo* and *in vitro* sensitivity of quinine.

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