RESEARCH NOTE

IN VITRO SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES IN THAILAND TO QUININE AND CHLOROQUINE, 1984-1990

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Until now, quinine has been used worldwide as the drug of choice for the emergency treatment of severe and complicated malaria. In fact, the long-standing dependability of quinine has created a context of clinical confidence and reduced the perceived threat of *P. falciparum* recrudescence where quinine and quinidine are available. However, in a situation of substantial loss of efficacy, it is conceivable that the life-saving properties of quinine may be challenged. We have therefore analysed data on the *in vitro* sensitivity to quinine of *Plasmodium falciparum* (pf) isolates collected from various parts of Thailand from 1984 to 1990. The investigation included also the *in vitro* response to chloroquine. Details of materials and methods of the study can be found in an earlier report on the *in vitro* sensitivity of pf to mefloquine and halofantrine (Wongsrichanalai *et al,* 1992). Basically, we used two *in vitro* methods to determine the susceptibility of pf isolates, one using morphological reading (Rieckmann *et al,* 1978; Childs and Pang, 1988) and the other measuring radioisotope incorporation (Desjardins *et al,* 1979; Webster *et al,* 1985). For both methods, inhibition of growth or schizont maturation is expressed as the 50-percent inhibitory concentration (IC50). The diphosphate salts of quinine and chloroquine were used in all tests.

Quinine

According to our morphology-technique data, quinine IC50s varied moderately from year to year in 1984-1989 for isolates from Borai, a south-eastern district of Trad Province on the Thai-Cambodia border, and slightly for elsewhere in Thailand (Table 1). Log-probit analysis (Grab and Wernsdorfer, 1983; Litchfield and Wilcoxon, 1949) showed all regression lines to be parallel within experimental error (Litchfield and Wilcoxon, 1949). There was no statistical significance in the potency ratio estimate of any paired lines, either for Borai or elsewhere between 1984 and 1989 (data not shown). However, in 1990 the isolates from Borai showed a much larger IC50 value (354.2 ng/ml) than in 1989 (175.0 ng/ml) as measured by the radioisotope technique (Table 1). The IC50 for elsewhere in 1989 (222.0 ng/ml) corresponded roughly to that found at Borai in the same year. A large increase of the quinine IC50 in 1990 was also observed in a separate study at Borai by the Thai Malaria Division (unpublished observation).

The high IC50s appeared characteristic of Thai pf isolates. For Borai, the earlier and minor fluctuation of IC50s probably reflected various combinations of pf strains with different degrees of quinine sensitivity in the isolates sampled. However a high proportion of resistant pf isolates (resistant phenotypes) became apparent. The flatter slope with raised IC95 in 1989 (morphology data) and the large increase in the IC50 from 1989 to 1990 (radioisotope data) suggested a serious trend. This may be explained by drug pressure because of the increasing incidence of severe and complicated malaria among gem miners admitted to district hospitals, requiring quinine-tetracycline therapy. The malaria mortality rate in Trad Prov-

Table 1

Quinine IC50 and IC95 of *P. falciparum* isolates collected from Borai and elsewhere (1984-1990) determined by morphology and radioisotope techniques. (1 ng/ml ≃1.938 nM/l).

Year	Borai				Elsewhere			
	N	Slope	IC50	IC95	N	Slope	IC50	IC95
Morphology technique								
1984	15	1.97	134.2	921.6				
1985	23	2.24	160.4	871.2	127	2.27	188.6	1002.8
1986	13	2.66	151.5	629.3	25	2.66	198.4	822.2
1987	-	-	-	-	29	2.79	195.0	757.4
1988	8	2.60	188.2	808.9	-	-	-	-
1989	12	1.96	156.6	1080.2	-	-	-	-
Radioisotope technique								
1989	12		175.0 (95% CI = 168.2-181.8)		23		222.0 (95% CI = 215.8-228.2)	
1990	14		354.2 (95% CI = 337.9-370.5)		-		-	

ince doubled between 1989 and 1990 (Malaria Division, 1990). Drug pressure was believed to be responsible for the reduced quinine sensitivity in different parts of Thailand from 1982 to 1984 following extensive use of the drug for routine treatment of falciparum malaria (Suebsaeng *et al,* 1986).

Desjardins reported an IC50 for quinine in the multi-resistant Vietnam Smith strain of 133 ng/ml (Desjardins *et al,* 1979). The quinine IC50s of 175.0 ng/ml in 1989 and 354.2 ng/ml in 1990 represented a serious trend and suggested that it was important to closely monitor the quinine sensitivity pattern in Thailand, particularly in the Borai area.

Chloroquine

The patterns of chloroquine sensitivity were similar for Borai and elsewhere, so only the aggregate results are presented. There was a prominent trend of increasing slope values from 1984 to 1989 accompanied by a progressive decrease in both IC50 and IC95 (Table 2). Potency ratio estimates showed a significant difference (increase of sensitivity) between the lines for 1985 vs 1989 and 1986 vs 1989 (Table 3). However, observations in 1989 and 1990 (radioisotope technique), indicated that the trend towards increased sensitivity did not continue in 1990 (Table 2).

The wide distribution and high level of chloroquine resistance has been well documented in Thailand (Harinasuta *et al,* 1982). Chloroquine

Table 2

Chloroquine IC50 and IC95 of *P. falciparum* isolates from Thailand, 1984-1990, determined by morphology and radioisotope techniques. (1 ng/ml ≃ 1.940 nM/l).

Year	N	Slope	IC50	IC95
Morphology technique				
1984	30	1.80	179.7	1472.1
1985	138	2.15	159.4	927.1
1986	38	2.78	141.9	554.4
1987	29	2.42	114.2	544.8
1988	9	2.61	89.1	379.6
1989	12	2.65	78.7	329.1
Radioisotope technique				
1989	31		61.8 (95% CI = 58.7-64.8)	
1990	23		135.0 (95% CI = 128.1-141.8)	

sensitive isolates normally produce a steep regression with an IC50 well below 0.5×10^{-6} mol/liter blood (corresponding to 25.3 ng/ml of chloroquine diphosphate or 16 ng/ml of chloroquine base in blood-medium mixture) (Wernsdorfer and Payne, 1988). The progressive decline in the chloroquine IC50 found in this study may be explained by a reduction in chloroquine pressure countrywide. This confirms a prior observation on the decreasing trend of chloroquine resistance for the period 1982-1985 (Thaithong *et al,* 1988). In spite of the overall decline, it would be speculative to expect any therapeutically relevant reversal of resistance in nature. A clinical study conducted in Vietnam in 1982, after the drug was thought be not readily available in the country from 1975 seemed to suggest a natural reversal phenomenon (Jacquier *et al,* 1985). However, this was refuted by another report (Onori and Vu, 1986).

In Thailand, chloroquine was replaced by Fansidar for radical treatment of falciparum malaria in 1973. It remained in use for presumptive treatment until 1981 in some areas, and 1985 in the others. Today, chloroquine is used in Thailand for the treatment of *vivax* malaria, so the drug has never been completely withdrawn. As pf and *P. vivax* share the same ecology in Thailand, it is conceivable that some chloroquine pressure on pf has continued in spite of exhaustive case identification and species-specific medication.

These observations on quinine and chloroquine using *in vitro* techniques demonstrate the importance of such methods in evaluating patterns of drug response and confirm their value in the prospective definition of issues for clinical management in areas of multidrug resistance.

Table 3

Tests of parallelism of the regression lines and potency ratios of chloroquine for successive years, 1984-1989, for isolates collected from different areas in Thailand and tested by morphology technique.

Regression lines	SR*	f_{SR}	Parallelism	PR**	f_{PR}	Significant
1984 vs 1985	1.22	2.50	yes	1.13	2.46	no
vs 1986	1.56	2.41	yes	1.27	2.38	no
vs 1987	1.38	2.46	yes	1.57	2.45	no
vs 1988	1.48	2.52	yes	2.01	2.59	no
vs 1989	1.49	2.45	yes	2.27	2.45	no
1985 vs 1986	1.27	1.43	yes	1.12	1.55	no
vs 1987	1.13	1.50	yes	1.39	1.64	no
vs 1988	1.21	1.58	yes	1.78	1.80	no
vs 1989	1.22	1.49	yes	2.02	1.64	yes
1986 vs 1987	1.13	1.37	yes	1.24	1.53	no
vs 1988	1.06	1.45	yes	1.58	1.70	no
vs 1989	1.05	1.35	yes	1.80	1.53	yes
1987 vs 1988	1.07	1.52	yes	1.28	1.78	no
vs 1989	1.07	1.43	yes	1.45	1.62	no
1988 vs 1989	1.01	1.51	yes	1.14	1.78	no

* SR = slope ratio, f_{SR} = factor for slope ratio. Two regression lines are parallel within experimental error if SR is less than the corresponding f_{SR}. Parallel regressions may be compared for potency (Litchfield and Wilcoxon, 1949).

** PR = potency ratio, f_{PR} = factor for potency ratio. Drug efficacy of the groups compared are significantly different ($p < 0.05$) if a PR exceeds the corresponding f_{PR}.

The manifest decrease of pf sensitivity indicates the impending loss of quinine as a basic management tool in severe and complicated *falciparum* malaria in parts of Thailand. For the decreasing resistance of pf to chloroquine it remains to be seen whether this phenomenon is due to selective population replacement induced by other drugs or solely due to decreased chloroquine pressure.

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