IN VITRO DRUG RESPONSE OF *PLASMODIUM FALCIPARUM* IN THE PHILIPPINES: INCREASED RESISTANCE TO AMODIAQUINE

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INTRODUCTION

A long term study has been carried out at San Lazaro Hospital, Manila, Philippines monitoring *in vitro* response of *Plasmodium falciparum* to antimalarial drugs. A previous publication from this study documented *in vitro* chloroquine resistance and also described results of assays against mefloquine, amodiaquine and quinine (Smrkovski *et al.*, 1985). The data for that publication was collected over the first 6 months of 1982. The study reported here is intended to update the *in vitro* response of Philippine *P. falciparum* strains to antimalarials.

MATERIALS AND METHODS

Specimens were collected from patients admitted to San Lazaro Hospital with *P. falciparum* malaria. The *in vitro* microculture technique was performed as previously described (Smrkovski *et al.*, 1985). Briefly, blood was collected in EDTA, washed twice by centrifugation in RPMI-1640 plus $NaHCO_3$. Packed cell were resuspended 1/10 (v/v) with RPMI 1640 + $NaHCO_3$ + 10% pooled human AB serum. Cultures were performed as described, in plates dosed by the technical staff of the World Health Organization. Urine was tested for sulpha compounds by use of the Lignin test (Bruce-Chwatt *et al.*, 1981) and for 4-aminoquinolines by the Dill-Glazko test (Lilijveld and Kartman, 1970).

Probit analysis was performed as described by Grab and Wernsdorfer (1983).

RESULTS

Specimens from 271 patients with *P. falciparum* infections were tested. Of these, isolates from 150 (55.4%) patients grew to greater than 10% schizonts. Urine tests for sulpha compounds were uniformly negative. The Dill-Glazko test for 4-aminoquinolines was positive in 48% of the cases.

Fig. 1 shows the results of the *in vitro* microculture drug response assays for approximately 80 specimens collected during 1984. Table 1 lists the effective dose 50 (ED_{50}) of the drugs tested during two consecutive years (1983-1984). The ED_{50} of chloroquine decreased by about 19% while that of quinine increased by a similar percentage. The ED_{50} of mefloquine remained unchanged. Over this same period there was a 38% increase in the amount of amodiaquine required to produce 50% inhibition of schizogony.

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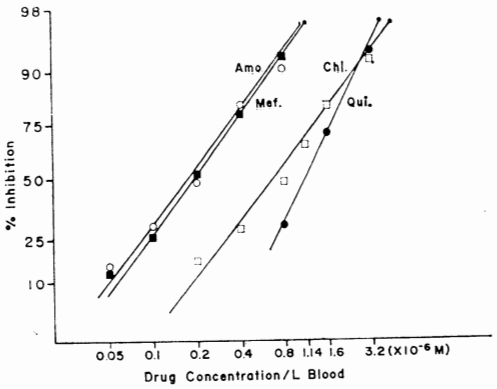

Fig. 1—Probit analysis of the response of 80 isolates of *Plasmodium falciparum* for the year 1984: chloroquine; amodiaquine; mefloquine; and quinine.

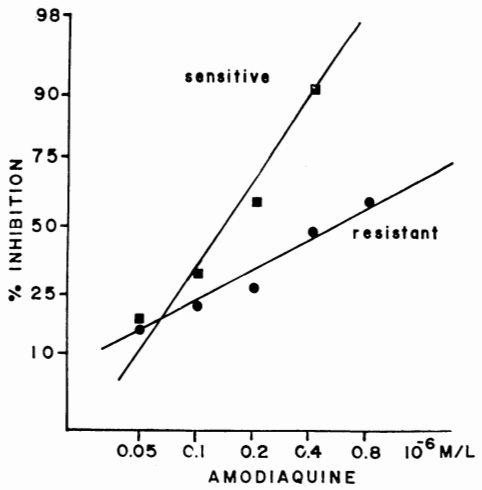

Fig. 2—Probit analysis of the response to amodiaquine of *in vitro* amodiaquine sensitive (63 samples) and amodiaquine resistant (18 samples) *Plasmodium falciparum* isolates.

Table 1

In vitro effective dose 50 ($\times 10^{-6}$ Molar/liter blood).

		1983 (N=66–69)	1984 (N=79–81)
Chloroquine	ED_{50}	0.84	0.68
	ED_{90}	2.8	2.6
Amodiaquine	ED_{50}	0.13	0.18
	ED_{90}	0.46	0.62
Mefloquine	ED_{50}	0.20	0.20
	ED_{90}	0.60	0.52
Quinine	ED_{50}	0.92	1.12
	ED_{90}	1.9	2.6

The percentage of specimens considered resistant using criteria established by WHO (schizonts in the presence of: 1.14×10^{-6} M chloroquine; 0.8×10^{-6}M amodiaquine; 12.8×10^{-6}M quinine; and 3.2×10^{-6}M mefloquine/liter of blood) is shown in Table 2. There was little change over the period tested in the percentage judged resistant to chloroquine, mefloquine, and quinine. Resistance to amodiaquine increased from 5.1% of cases in 1982 to 22.2% of all cases in 1984. Probit

Table 2

Percentage of specimens resistant *in vitro*.

	Chloroquine	Amodiaquine	Mefloquine	Quinine
1982	87.7%	5.1	6.8	3.4
	50/59*	3/59	4/59	2/59
1983	89.8	8.9	0	0
	62/69	6/67	0/66	0/69
1984	85.2	22.2**	1.2	1.2
	69/81	18/81	1/81	1/79

1982 data from reference 1
*No. resistant/total specimens
**Significant at 0.05 level.

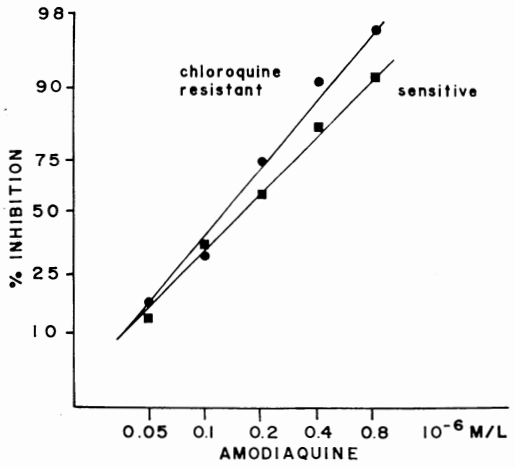

Fig. 3—Probit analysis of the response to amodiaquine of *in vitro* chloroquine sensitive (19 samples) and chloroquine resistant (150 samples) isolates.

analysis of the drug response of the sensitive versus resistant isolates (Fig. 2) indicates an ED_{50} of 0.14×10^{-6} and 0.54×10^{-6}, respectively.

The possibility of cross resistance between amodiaquine and chloroquine was explored by comparing the response of both chloroquine sensitive and resistant strains to amodiaquine. Probit analysis (Fig. 3) shows identical responses of both groups of isolates to amodiaquine.

DISCUSSION

Resistance of *P. falciparum* to antimalarials was first observed in the Philippines to amodiaquine in 1968 (Shute and Sangalang, 1970) and to chloroquine in 1971 (Ramos *et al.*, 1971). Valera and Shute (1976) used the *in vitro* test in 1976 comparing chloroquine response to *in vitro* follow up. Smrkovski *et al.*, (1985) performed a more extensive study at San Lazaro Hospital in Manila looking at the *in vitro* response to chloroquine, quinine, amodiaquine, and mefloquine as well as the *in vivo* response to chloroquine.

We studied 270 patients with *P. falciparum* infection over a period of 2 years at San Lazaro Hospital. *In vitro* resistance to chloroquine was 85.2% for 1984 which compares closely to the 84.7% seen in 1982 (Smrkovski *et al.*, 1985) and 89.8% in 1983. Self administration of antimalarials is common in the Philippines, and a significant number of patients may have failed treatment. The most popular antimalarial in use is chloroquine which is widely available and *in vivo* resistance to chloroquine is widespread (Brandling-Bennet *et al.*, 1981). Of those patients submitting urine samples, 48% were positive for 4-aminoquinolines using the Dill-Glazko test.

No increase in percentage of isolates resistant to either quinine or mefloquine was seen. There was, however, an increase in ED_{50} for quinine. The ED_{99} of quinine in this study was 4.4×10^{-6} compared to 12.8×10^{-6} M/liter blood established by WHO as the level indicating resistance *in vitro*.

The *in vitro* response to chloroquine observed in this study is consistent with results reported by WHO in areas with RI and RII resistance ($ED_{50} = 0.84 \times 10^{-6}$ M/liter blood compared to 1.14 and 0.6×10^{-6} in areas of Indonesia and 1.0×10^{-6} in Orissa, India (Wernsdorfer, 1982).

There was a marked decrease in response to amodiaquine in 1984 as compared to the previous year. The ED_{90} increased by approximately 35% from 0.46 to 0.62 while the percentage of isolates resistant in the assay (schizogony at 0.8×10^{-6} M/liter blood) increased from 8.9% to 22.2%. The ED_{50} of the resistant strains was approximately four fold higher than that seen in the sensitive strains.

Amodiaquine resistance was demonstrated in the Philippines in 1968 (Shute and Sangalang, 1970), but has not been reported to any great extent until recently (Watt *et al.*, 1986).

Both chloroquine and amodiaquine have been widely used over the past 30 years in the treatment of falciparum malaria in the Philippines. Until recently, chloroquine was the more popular of these two aminoquinolines, being the drug of choice at San Lazaro Hospital, the infectious disease hospital in the Philippines, and of the Philippine Malaria Eradication Service. The increase in *in vitro* resistance to amodiaquine seen in this study is coincident with a general trend over the past years towards the use of amodiaquine in the Philippines. It has been the drug of choice for falciparum malaria for the Philippine Malaria Eradication Service since 1983 (Rivera, D., Malaria Eradication Service, pers. commun.). A more recent study (Watt *et al.*, 1986) has demonstrated a high incidence of both RII and RIII resistance in patients at San Lazaro Hospital. It has been suggested that there is a degree of cross resistance between chloroquine and amodiaquine (Smrkovski *et al.*, 1985). Our data gives no evidence for this. The responses of both chloroquine resistant and chloroquine sensitive strains to amodiaquine were identical *in vitro*. The possibility exists that the observed resistance to amodiaquine is due to increased drug pressure. These results, along with the recent amodiaquine treatment failures in falciparum patients in the Philippines, make it apparent that amodiaquine use should be more closely monitored, and that further studies will be required to determine its efficacy in the Philippines.

SUMMARY

A long term study was carried out at San Lazaro Hospital, Manila, Philippines, monitoring the *in vitro* response of *Plasmodium falciparum* to chloroquine, amodiaquine, mefloquine, and quinine. The *in vitro* effective dose giving 50% inhibition of schizogony was: 0.68×10^{-6} M/liter blood for chloroquine; 0.18×10^{-6} for amodiaquine; 0.2×10^{-6} for mefloquine; and 1.12×10^{-6} for quinine. The percent of isolates determined to be resistant *in vitro* was 85.2% for chloroquine, and 1.2% for both mefloquine and quinine. These figures were relatively unchanged over the course of 3 years studied. The *in vitro* resistance rate to amodiaquine increased from 5.1% in 1982 to 22.2% in 1984.

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