

IN VITRO RESPONSE OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE AND MEFLOROQUINE IN SOUTHEAST MADAGASCAR

by

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ABSTRACT

46 isolates of *Plasmodium falciparum* collected in the Tolagnaro (Fort Dauphin) area of Southeast Madagascar were assessed with WHO *in vitro* micro-technique test kits to determine their susceptibility to chloroquine and mefloquine. The results of the tests indicated low grade resistance to chloroquine and satisfactory response to mefloquine.

INTRODUCTION

The resistance of *P. falciparum* to different anti-malarial drugs is present in many areas of the world where malaria is endemic. Chloroquine resistance has been reported in the Eastern, Northern, Central and Western areas of Madagascar (1-10). Resistance has not yet been documented in the Southern areas. In view of this, a study was conducted in Southeast Madagascar to determine the *in vitro* susceptibility of *P. falciparum* isolates to chloroquine and mefloquine.

MATERIALS AND METHODS

The study was conducted in the Tolagnaro and Manambaro districts, Southeast Madagascar among the school age population from 7 to 22 years old during the period April to June 1986.

The schools selected were Ambohimazava Lutheran School and the Lycée in Tolagnaro, Manantantely Lutheran School, 10 km west of Tolagnaro, and Manambaro Elementary (SFF) and Middle Schools (SAFF), 23 km west of Tolagnaro. Only students who had recent history of fever (within 14 days) and who denied taking antimalarials were selected for blood and urine screening. The students were weighed; thick blood films were made and stained with Romanowsky stain; urine was tested by the Dill-Glasko (11) and Lignin (12) tests for 4-amino-quinolines and sulphonamides respectively; spleen indices were determined using Hackett's method (13).

Cases having pure *P. falciparum* infections with more than 500 asexual stages per μ l were selected for the *in vitro* test. Blood cultures were set up on 46 patients who had parasite densities ranging from 560 to 104,000 per μ l. Concurrent with drawing blood for culture, thin and thick blood films were made for identification and

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quantization. Quantization of the malaria parasites was done by counting the number of parasites per 100 leucocytes and using a standard leucocyte count of 8000 per μ l.

All cases found positive were treated with three day standard treatment using 25 mg chloroquine base per kg body weight orally. At day 7, thick films were made to check for *in vivo* resistance. The 2 students who still had parasitemia after 7 days were treated with Fansidar.

In vitro tests were performed in duplicate according to instructions (14) using predosed micro-culture plates supplied by the World Health Organization (WHO). The concentration for chloroquine ranged from 1 to 32 picomoles per well and for mefloquine from 0,5 to 16 picomoles per well. *In vitro* tests were considered valid if at least 20 of 200 *P. falciparum* parasites counted in the control well had developed to the schizont stage with 3 or more nuclei. Schizonts developing *in vitro* in the presence of 5,7 picomoles of chloroquine indicated resistance and 4,0 picomoles of mefloquine normally completely inhibits schizont maturation (14).

All smears were referred for checking at the Parasitology Laboratory, Caribbean Epidemiology Centre (PAHO/WHO) in Trinidad and Tobago, West Indies.

RESULTS

211 cases were found positive during the initial thick film survey, with 210 *P. falciparum* and 1 *P. malariae*. Results of the initial malaria screen by thick blood film and spleen index are shown in Table I.

TABLE I

Results of initial malaria screen by thick blood and spleen indices

School	Students Screened	Thick Film No. Positive	Thick Film % Positive	SPLEEN INDICES							AEG	
				0	1+	2+	3+	4+	5+	Rate		
Manantantely	21	8	38%	-	-	-	-	-	-	-	-	-
Manambaro SAFF 1	61	31	51%	-	-	-	-	-	-	-	-	-
Manambaro SAFF 2	38	21	55%	20	8	3	6	1	0	47%	2	
Ambohimazava	119	37	31%	100	11	5	3	0	0	16%	1.6	
Lycée Tolagnaro	67	17	25%	55	9	3	0	0	0	18%	1.2	
Manambaro SFF	156	97	62%	46	59	30	18	3	0	71%	1.7	
TOTAL	462	211	46%	221	87	41	27	4	0	48%		

AEG : Average enlarged spleen

- : Spleen indices not made

19 of the 46 cultures were considered successful for chloroquine and 18 for mefloquine. 6 were lost because of incubation problems, 16 were contaminated, perhaps during field collection of the samples and 5 were discounted because of poor growth. The concentrations of the two drugs achieving 50 p. 100, 90 p. 100, 95 p. 100 and 99 p. 100 (EC50, EC90, EC95, EC99) schizont inhibition were determined by probit regression of the log dose response. Table II shows the results of the samples cultured.

TABLE II

Analysis of *in vitro* tests with chloroquine and mefloquine in
P. falciparum, Madagascar 1986

	Chloroquine	Mefloquine
Number of isolates (successful tests)	19	18
Number of isolates with complete inhibition of schizont maturation at :		
0.5 picomol/well	—	0
1 picomol/well	0	1
2 picomol/well	2	9
4 picomol/well	9	17
5.7 picomol/well	15	17
8 picomol/well	17	17
16 picomol/well	19	17
32 picomol/well	19	—
Regression showing effective inhibitory concentrations for :		
EC 50	1.4 pmol	0.6 pmol
EC 90	3.8 pmol	1.6 pmol
EC 95	4.8 pmol	2.2 pmol
EC 99	7.9 pmol	3.7 pmol

DISCUSSION

15 of the 19 successful chloroquine isolates showed complete inhibition while 4 showed schizont maturation at 5.7 picomol chloroquine/well, indicative of resistance. However, all 19 isolates were fully inhibited at 16 picomol, denoting a low degree of resistance. This is also indicated by the EC90 and EC99 values of 3.8 and 7.9 picomoles respectively.

All but one mefloquine isolate were completely inhibited at 4 picomol mefloquine/well, indicative of high sensitivity. One isolate showed 3 schizonts at 5.7 picomoles and 1 schizont at 8 and 16 picomoles mefloquine. Therefore, further *in vitro* mefloquine sensitivity studies are needed to obtain information on possible shifts in the existing results.

Incidental findings during the blood screen and spleen indexing reveal a higher prevalence of parasitemia and higher spleen rate in the Manambaro schools than in the Tolagnaro schools.

In conclusion, the results of the investigation to determine the *in vitro* response to *Plasmodium falciparum* to chloroquine and mefloquine indicated low grade resistance to chloroquine and satisfactory response to mefloquine.

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RESUME

De nombreux rapports ont été publiés sur la résistance du *Plasmodium falciparum* dans les différentes régions de Madagascar, mais aucun concernant le Sud.

46 isoléments de *P. falciparum* recueillis à Tolagnaro (Fort Dauphin) région de l'extrême Sud de Madagascar, sont étudiés *in vitro* par la méthode de micro-culture de l'O.M.S. pour déterminer leur sensibilité à la chloroquine et à la méfloquine.

L'étude a été concentrée dans les écoles de Tolagnaro et de Manambaro. Seuls les écoliers qui ont des antécédents récents de fièvre et qui ont nié la prise d'un antipaludéen sont sélectionnés pour le dépistage. On a pesé les élèves; on a effectué des gouttes épaisses; on a testé les urines respectivement aux 4-aminoquinoléines et aux sulphonamides; on a déterminé les indices spléniques. 46 cas ayant uniquement du *P. falciparum* allant de 540 à 104.000 parasites par μ l ont été mis en culture. Tous les cas positifs ont été suivis au traitement standard de la chloroquine et les gouttes épaisses sont refaites au 7^e jour. Les deux cas montrant encore une parasitémie ont été traités au Fansidar.

Voir Tableau I pour une répartition des résultats des écoles. Parmi les 211 cas positifs découverts lors du dépistage initial, il y avait 210 *P. falciparum* et 1 *P. malariae*.

Parmi les 46 mis en culture, 19 ont été réussies pour la chloroquine et 18 pour la méfloquine; les résultats sont présentés au Tableau II. Les échantillons manifestaient une croissance à 5,7 picomol chloroquine/godet, indicatif d'une résistance. Tous les 19 isoléments étaient entièrement inhibés à 16 picomole chloroquine par godet. Ce qui dénote un degré de résistance bas. Ceci aussi est indiqué par les valeurs EC90 et EC99 des 3,8 et 7,9.

Tous les isoléments sauf un, étaient complètement inhibés à 4 picomol méfloquine/godet, indicatif d'une haute sensibilité. Un isolement montrait une maturation à 16 picomoles, mais étant donné qu'il y avait seulement 1 schizonte à 8 et 16 picomoles, il est indiqué d'étendre l'étude *in vitro* de la méfloquine pour

obtenir plus d'informations à propos de la sensibilité naturelle de la méfloquine et d'une éventuelle forme d'hétérogénéité.

En conclusion, les résultats indiquaient un bas degré de résistance à la chloroquine et une réponse satisfaisante à la méfloquine.

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