Application of a simplified *in-vivo* test system for determining chloroquine resistance in *Plasmodium falciparum*

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A simplified in-vivo *test system was applied to detect chloroquine resistance in malaria patients in Shahjahanpur district (Uttar Pradesh) in India. In 27.6% of cases RIII resistance was observed. This* in-vivo *method is a simple and useful test for the early detection of chloroquine-resistant falciparum infections and for the management of these patients with alternative therapy.*

Introduction

Malaria remains a major threat to populations in several continents, despite the continued efforts of various national and international agencies to combat the infection. The problem is partly due to development of resistance in *Plasmodium falciparum* against chloroquine, a most effective and widely used antimalarial drug (*1*). Chloroquine resistance was first recorded in Thailand in 1957 (*2*), then spread to almost all parts of the world. To detect this resistance both *in-vitro* and *in-vivo* methods have been tried, but they pose certain difficulties.[a]

The various *in-vivo* methods at present in use are time-consuming and require daily follow-up during the first week of treatment; also patients have to wait for at least 7 days before starting the proper treatment (*2*). To overcome all these problems, Rieckmann proposed a simplified *in-vivo* method at an informal consultation in the WHO Regional Office in Manila, Philippines, in August 1988. According to this procedure, only two follow-up blood examinations are required and an infection can be declared resistant as early as the second day of drug administration. We have applied this test system to detect chloroquine resistance in *P. falciparum* at the Field Station in Shahjahanpur (Uttar Pradesh) and the results are reported here.

Materials and methods

Patients

Forty-seven fever cases, who were positive for *P. falciparum* (ring stages) by peripheral blood smear examination, and whose urine was negative for chloroquine by Dill & Glazko's method (*3*), were selected in our centre at Shahjahanpur for the detection of chloroquine sensitivity.

Simplified in-vivo *test system*

Chloroquine sensitivity/resistance was detected in these patients by Rieckmann's simplified *in-vivo* method,[b] which is summarized in Fig. 1. All patients were given 600 mg chloroquine phosphate orally; those below 14 years of age were given a dose of 10 mg/kg body weight. Blood smears had been collected before treatment and these were re-examined to determine asexual parasite densities per mm^3 of blood. The day of blood smear collection and drug administration was taken as day 0. On the next day, i.e., day 1, the same dose of chloroquine (as on day 0) was given. On day 2, the patients received 300 mg of chloroquine (or 5 mg/kg body weight) and their urine was tested again for chloroquine to confirm that they had taken the drug. Follow-up blood smears were taken on day 2 and the asexual parasite density was determined. If the level of parasitaemia detected on day 2 was more than 25% of that observed on day 0, the infection was considered to be resistant at the RIII level. These patients received alternative treatment with a single dose of sulfalene–pyrimethamine (Metakelfin) (*4*). If the patients were negative for malarial parasite on day 2 they were considered as S/RI.

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[a] **Grab, B. & Wernsdorfer, W.H.** *Evaluation of* in-vitro *tests for drug sensitivity in* Plasmodium falciparum: *probit analysis of log dose/response test from 3–8 points assay.* Unpublished document WHO/MAL/83.990, 1983.

[b] See accompanying article in this issue of the *Bulletin of the World Health Organization*, pages 759–760.

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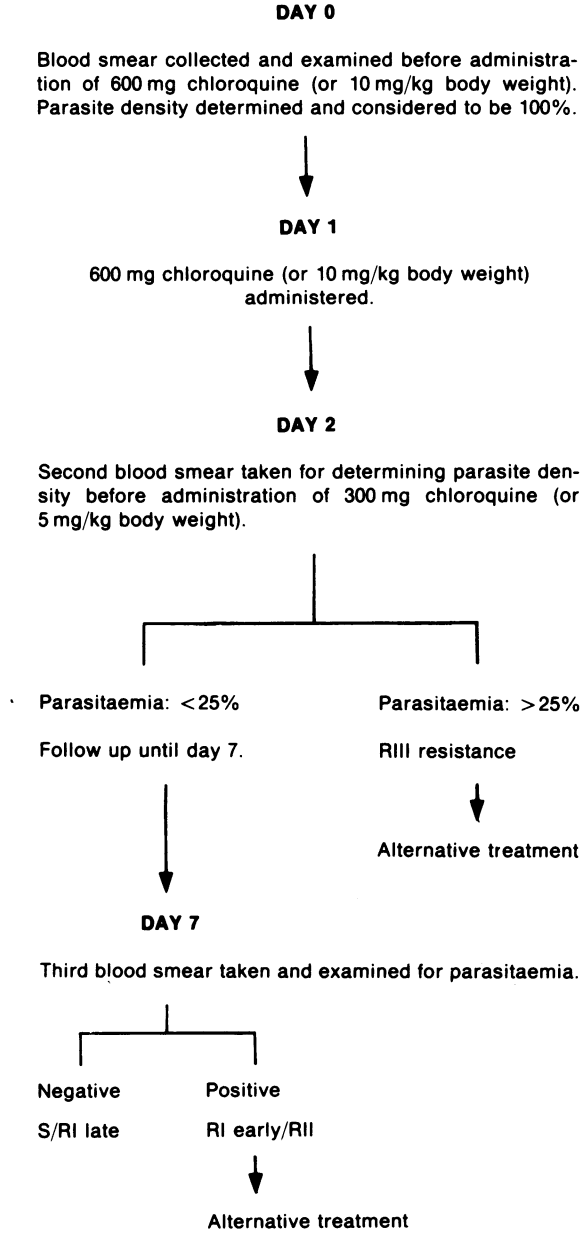
Fig. 1. **Simplified *in vivo* test system for detecting chloroquine resistance in *P. falciparum*.**



The patients whose parasite density on day 2 was less than 25% of that on day 0 had another blood smear taken on day 7; if the latter was negative for malarial parasites, the degree of resistance was S/RI late. If parasites were still present, RI early/RII resistance was presumed. These patients subsequently received alternative treatment with sulfalene–pyrimethamine.

Results

Out of 47 patients selected for drug sensitivity/resistance testing, 21 were negative for malarial parasite on day 2 post treatment, while the remaining 26 (55.3%) showed different degrees of resistance (Table 1). Of these, 53.2% were in the age group of 2–16 years. RIII resistance was detected in 27.6% of the total patients studied; only two of these were children aged 2 and 3 years. The individual responses observed in these 47 patients are presented in Table 2.

The urine of all 47 patients was negative for chloroquine before starting drug administration. All were positive for chloroquine after treatment and until day 7.

Of the 13 patients with an RIII level of resistance, to whom sulfalene–pyrimethamine was given, 5 were still having fever on day 7 of study. Blood smears made on this day were all positive. On inquiry it was found that 4 out of 5 patients had not taken the sulfalene–pyrimethamine given by us, as they had consulted some local practitioner. Thereafter they were asked to take the medicine. The fifth patient (S.N. 40), of RIII category, had taken the medicine on day 5 instead of day 3.

Discussion

Our study has clearly pointed out the usefulness of a modified *in-vivo* method to detect chloroquine-resistant strains. The results were in agreement with the results obtained in another group of patients using the *in-vitro* microtest (*5*). Of the 16 patients in this group, 6 (37.5%) were drug-sensitive and 10 (62.5%) were resistant; this difference is statistically not significant ($\chi^2 = 0.233$, $P > 0.05$). The *in-vivo* chemosensitivity assays are probably more relevant to a given field situation than *in-vitro* assays because they involve the host component of the drug effect, such as metabolism of the drug, host resistance, etc.

The present test system is more rapid than other *in-vivo* methods described in the literature (*2*). By this method a patient's infection can be identified as resistant (only RIII) as early as on the third day of drug administration. Hence by using this method one can manage the chloroquine-resistant patient's therapy in a more timely manner.

In conclusion, we believe that this *in-vivo* method may prove to be very useful for early detection of chloroquine-resistant *P. falciparum* infections, and it may provide information to improve the medical management of resistant falciparum cases.

Table 1: **Chloroquine sensitivity of *Plasmodium falciparum* in 47 patients, by simplified *in-vivo* test system**

No.	Age (years) and sex	Parasitaemia/mm³ on: Day 0	Day 2	Day 7	Degree of resistance
1.	24 M	3500	Nil	Nil	S/RI
2.	35 M	6500	Nil	Nil	S/RI
3.	6 F	50500	Nil	Nil	S/RI
4.	14 F	170000	Nil	Nil	S/RI
5.	40 F	850	Nil	Nil	S/RI
6.	50 M	4100	Nil	Nil	S/RI
7.	5 F	14600	Nil	Nil	S/RI
8.	25 F	60000	Nil	Nil	S/RI
9.	28 F	11100	Nil	Nil	S/RI
10.	14 F	6500	Nil	Nil	S/RI
11.	40 F	1000	Nil	Nil	S/RI
12.	42 F	7000	Nil	Nil	S/RI
13.	10 F	7400	Nil	Nil	S/RI
14.	3 F	5750	Nil	Nil	S/RI
15.	6 F	20500	Nil	Nil	S/RI
16.	6 F	12400	Nil	Nil	S/RI
17.	48 F	700	Nil	Nil	S/RI
18.	48 F	9500	Nil	Nil	S/RI
19.	45 M	8000	Nil	Nil	S/RI
20.	6 M	13500	Nil	Nil	S/RI
21.	4 M	27900	Nil	Nil	S/RI
22.	38 M	6500	350 (5.4)[a]	Nil	S/RI late
23.	18 M	1650	150 (9.1)	Nil	S/RI late
24.	4 M	45000	2500 (5.6)	Nil	S/RI late
25.	7 M	9500	1000 (10.5)	Nil	S/RI late
26.	42 M	9500	1400 (14.7)	Nil	S/RI late
27.	10 F	2850	250 (8.8)	Nil	S/RI late
28.	3 F	45850	2450 (5.3)	Nil	S/RI late
29.	10 M	5900	1350 (22.9)	Nil	S/RI late
30.	15 M	10500	300 (2.9)	Nil	S/RI late
31.	40 F	3950	100 (2.5)	Nil	Probably S/RI late
32.	18 M	9750	2000 (20.5)	1100 (11.3)	RI early/RII
33.	22 M	2000	250 (12.5)	400 (20.0)	RI early/RII
34.	10 M	7500	450 (6.0)	1500 (20.0)	RI early/RII
35.	8 F	5000	4650 (93.0)	Nil	R III
36.	7 F	13450	5700 (42.4)	Nil	R III
37.	38 F	750	300 (40.0)	Nil	R III
38.	4 M	3250	1050 (32.3)	Nil	R III
39.	3 F	4600	2450 (53.3)	Nil	R III
40.	26 F	20500	6250 (30.5)	1750 (8.5)	R III
41.	11 M	1100	2100 (190.9)	2000 (181.8)	R III
42.	14 M	1100	2000 (181.8)	2200 (200.0)	R III
43.	22 M	5650	1700 (30.1)	Nil	R III
44.	2 M	8100	7300 (90.1)	1750 (21.6)	R III
45.	60 M	300	150 (50.0)	Nil	R III
46.	15 F	25900	9200 (35.5)	Nil	R III
47.	16 M	5000	3000 (60.0)	2000 (40.0)	R III

[a] Figures in parentheses indicate the percentage parasitaemia, 100% being the level on day 0.

Table 2: **Different degrees of resistance to chloroquine treatment shown by *P. falciparum* parasites in 47 patients**

Response	No. of cases	Percentage
S/RI[a]	21	44.7
S/RI late	10	21.3
RI early/RII	3	6.4
RIII	13	27.6

[a] These were patients who were negative for malarial parasites on day 2 after treatment.

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Résumé

Utilisation d'un système de test simplifié *in vivo* de détermination de la résistance de *Plasmodium falciparum* à la chloroquine

Un système de test *in vivo* (modifié par Rieckman) a été utilisé pour détecter la résistance à la chloroquine chez des malades atteints de paludisme dans le district de Shahjahanpur (Uttar Pradesh) en Inde. Dans 27,6% des cas, une résistance RIII a été observée. L'efficacité de ce système, comparée avec les résultats obtenus dans un autre groupe de malades en utilisant le micro-test *in vitro*, n'a pas montré de différence significative ($P > 0,05$). Cette méthode *in vivo* est un test simple et utile pour la détection précoce des infections à falciparum chloroquinorésistantes et pour le traitement des malades atteints par des thérapeutiques de remplacement.

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