

Parasitological response of *Plasmodium falciparum* infection to chloroquine treatment in malaria patients in Port Moresby

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SUMMARY

A 7-day in vivo test system was applied to assess the parasitological response to chloroquine treatment in patients with falciparum malaria in the Central Province and National Capital District of Papua New Guinea. 30 patients were investigated but only 23 took a full course of chloroquine and were completely followed up. Of the 23 patients, 13 (57%) were negative for malaria parasites on day 2, 4 (17%) had significantly reduced parasitaemia by day 2 and cleared parasites by day 7, and 1 (4%) showed a partial response (R2). In 5 (22%) of the patients resistance at the R3 level was observed. The indication from this study is that chloroquine should continue to be the first-line drug for the treatment of uncomplicated falciparum malaria. However, judicious use of chloroquine in uncomplicated falciparum malaria is required to halt the spread of chloroquine-resistant strains of *Plasmodium falciparum*.

Introduction

There is wide variation of response of *Plasmodium falciparum* to equal doses of chloroquine among different individuals. When a small dose of drug is administered in an area with chloroquine-susceptible parasites, the parasitaemia is cleared in some patients but not in others (1). In areas with chloroquine resistance, a standard treatment regimen of chloroquine (25 mg/kg for 3 days) causes parasites to disappear temporarily in some patients while there is only reduction in others or no response at all in a few (1,2).

These variations in the response to standard treatment with chloroquine are considered to be due to local variation in susceptibility of different strains of *P. falciparum* prevalent in the population. This is in accordance with the reports of in vitro observations where resistant strains multiply in the presence of higher drug concentrations than those affecting susceptible strains from the same local area (3). Other factors, such as chloroquine kinetics and host immunity, also contribute to the apparent

varying susceptibility of parasites to chloroquine. Individual variations of chloroquine concentrations in plasma after oral administration have an impact on the outcome of treatment and the classification of resistance in vivo (4). It would also be expected that the host immune response would be more effective when the parasitaemia levels are low.

The aim of the present study was to assess in vivo the parasitological response of *P. falciparum* malaria parasites to chloroquine treatment in the Central Province and National Capital District of Papua New Guinea (PNG). Data obtained from this study would provide useful information for future management of uncomplicated falciparum malaria in the region.

Subjects and Methods

Subject recruitment

The study was conducted at the Port Moresby General Hospital (PMGH) from April 1994 to March 1995. Patients presenting themselves at

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the Adult Outpatients Department were selected according to the following criteria:

- presence of asexual forms (single infection) of *Plasmodium falciparum*
- no evidence of complications such as liver dysfunction, cerebral signs, prostration, etc
- no history of ingestion of antimalarial drugs within 4 weeks of presentation
- no history of chloroquine allergy.

A full medical history, including drug (chloroquine) allergy, was obtained. Each patient had a full physical examination on initial presentation. On each follow-up visit axillary temperature was recorded, and thick and thin blood smears were taken for microscopy. Patients who had not been resident for at least 2 months in Central Province or National Capital District were excluded.

Assessment of parasitological response

Assessment of the parasitological response of *P. falciparum* infection to chloroquine was investigated by Rieckmann's simplified in vivo method (5). The in vivo parasitological response to chloroquine was graded according to the following criteria and definitions :

- **good response (S/R1):** parasitaemia level declines to less than 25% of the pretreatment level by day 2 and patient is free of asexual parasites on day 7
- **partial response (R1 early recrudescence or R2):** parasitaemia level declines to less than 25% of the pretreatment level by day 2 but asexual parasites are still observed on day 7; if parasites are absent on day 5 but return by day 7 this is considered as R1 early recrudescence but if parasites are also present on day 5 this is R2 resistance
- **poor response (R3):** parasitaemia level on day 2 is more than 25% of the pretreatment level.

Drug treatment and follow-up of patients

All patients were given a total of 1.5 g base of chloroquine phosphate orally over 3 days.

On the first day (day 0) 600 mg of chloroquine was given. On the next day (day 1) the same dose (600 mg) was administered and on day 2 the patients received 300 mg of chloroquine. On day 0 a blood smear was taken before drug administration. Follow-up blood smears were taken on days 2, 5 and 7. If the level of parasitaemia detected on day 2 was more than 25% of that observed on day 0 the response was considered poor (R3) and if parasitaemia which reduced in 2 days to less than 25% nevertheless persisted on day 7 the response was considered partial. In both instances alternative treatment with low-dose quinine (5 mg/kg every 8 hours for 3 days) and a single dose (3 tablets) of sulphadoxine-pyrimethamine combination (Fansidar) was instituted on day 2 and day 7, respectively.

Blood film examination

Blood films for malaria parasites were stained with buffered Giemsa (20%). During the initial screening procedure, the number of parasites was calculated against 1000 red blood cells and parasitaemia was expressed as a percentage. Parasitaemia expressed as a percentage has certain advantages, including convenience and rapidity of screening. However, at the end of the study period all blood films were reexamined to determine asexual parasite densities per μ l of blood.

Results

Out of 30 patients recruited only 23 (77%) completed the study. 3 patients withdrew on day 1 due to chloroquine-induced pruritus and 4 defaulted. Of the 23 patients who completed the study, 13 (57%) were negative for malaria parasites on day 2 after the start of treatment and free of asexual parasites on day 7, in 4 (17%) parasitaemia declined to less than 25% of the pretreatment level by day 2 and was absent on day 7, 1 (4%) showed a partial response, and the remaining 5 (22%) showed R3 resistance (Table 1). The individual responses of these 23 patients are listed in Table 2. Patients with poor (R3) and partial responses to chloroquine responded well to the alternative treatment with low-dose quinine and Fansidar, and had negative blood films for malaria parasites on day 7 and day 14, respectively.

TABLE 1

IN VIVO RESPONSES OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE TREATMENT IN 23 PATIENTS WITH COMPLETE FOLLOW-UP

Response	No of cases	Percentage
S/R1: good response	17	73.9
S/R1 rapid*	13	56.5
S/R1 standard	4	17.4
R2: partial response	1	4.3
R3: poor response	5	21.7

* Patients who had a negative film for malaria parasites on day 2 after treatment

Notes:

S is susceptible and R resistant, according to definitions applied in the standard 28-day in vivo test.

In both S and R1, parasitaemia declines to less than 25% of the pretreatment level by day 2 and is negative on day 7. In R1, recrudescence of parasitaemia occurs by day 28. The 7-day in vivo test cannot distinguish between S and R1.

R2 is where parasitaemia declines to less than 25% of the pretreatment level by day 2 but persists until day 7.

R3 is where the parasitaemia level on day 2 is more than 25% of the pretreatment level; this indicates the most severe degree of resistance.

Discussion

This study shows that chloroquine treatment is still effective in the chemotherapy of uncomplicated falciparum malaria despite the clinical impression of a high prevalence of chloroquine-resistant malaria in the region. This is evident by a good response to chloroquine in 74% of patients, with 57% being aparasitaemic on day 2 after the start of treatment. Only one patient (4%) showed a partial response and required alternative drug intervention on day 7.

However, 22% responded poorly (R3) to the chloroquine treatment given. This group required an alternative drug intervention with low-dose quinine and Fansidar on day 2 for complete clearance of parasitaemia. It is not clear whether this subpopulation of patients represented true drug treatment failure since other factors, such as the pharmacokinetics of chloroquine, not measured in this study, also contribute to the final outcome of parasitaemia clearance. Most of these poor responders had

an initial parasitaemia greater than 30,000 parasites/ μ l, which may have affected the rate of decline in parasitaemia. However, correlation between the level of initial parasitaemia and subsequent response to drug treatment is often poor (6,7). Factors such as host immunity, virulence of the infecting strains and the proportion of naturally resistant parasites are more important determinants for severity and response to drug treatment than the density of parasitaemia per se. Moreover, there is no evidence to support a relationship between the infecting dose and the severity of the resulting malaria infection (8). Though the factors contributing to the variation in drug response and the causes of the wide spectrum of disease severity in malaria may not have yet all been elucidated, the resistance and virulence of parasite strains of *P. falciparum* are certainly important. In a mixed population of parasite strains (susceptible and resistant) the susceptible strains would be killed in the early part of the treatment, making way for more resistant and more virulent strains to multiply in the presence of chloroquine, thus conferring

TABLE 2

PARASITOLOGICAL RESPONSE OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE TREATMENT IN THE 7-DAY IN VIVO TEST IN 23 PATIENTS

Patient	Sex/age (years)	Parasitaemia/ μ l				Graded response
		Day 0	Day 2	Day 5	Day 7	
01	F/40	6500	Nil	Nil	Nil	S/R1
02	M/22	7000	Nil	Nil	Nil	S/R1
03	M/20	1650	Nil	Nil	Nil	S/R1
04	M/30	1100	Nil	Nil	Nil	S/R1
05	F/25	500	Nil	Nil	Nil	S/R1
06	M/16	400	Nil	Nil	Nil	S/R1
07	M/23	5000	Nil	Nil	Nil	S/R1
08	M/18	300	Nil	Nil	Nil	S/R1
09	M/20	4500	Nil	Nil	Nil	S/R1
10	F/15	21000	Nil	Nil	Nil	S/R1
11	M/A*	14000	Nil	Nil	Nil	S/R1
12	M/40	10150	Nil	Nil	Nil	S/R1
13	F/35	4000	Nil	Nil	Nil	S/R1
14	M/A*	8500	120 (1.4)**	Nil	Nil	S/R1
15	F/20	25800	2000 (7.8)	Nil	Nil	S/R1
16	M/55	10000	120 (1.2)	Nil	Nil	S/R1
17	M/45	5900	120 (2.0)	Nil	Nil	S/R1
18	F/18	45000	3250 (7.2)	500 (1.1)	200 (0.4) [¶]	R2
19	F/34	30000	12450 (41.5)+	500 (1.7)	Nil	R3
20	M/15	30450	10000 (32.8)+	Nil	Nil	R3
21	F/35	58000	19200 (33.1)+	3000 (5.2)	Nil	R3
22	M/35	29000	22500 (77.6)+	550 (1.9)	Nil	R3
23	F/22	25100	7300 (29.1)+	1250 (5.0)	Nil	R3

* Adult (age unknown)

** Figures in parentheses indicate the percentage parasitaemia, 100% being the parasitaemia level on day 0

[¶] This patient had a partial response (R2); the patient was treated with low-dose quinine and Fansidar starting on day 7

+ These patients were treated with low-dose quinine and Fansidar starting on day 2

on these parasite strains a biological advantage (9). This will promote the rapid spread of chloroquine-resistant strains of *P. falciparum* parasites.

The observed percentage of R3 resistance in this study is higher than that previously reported for Madang (6) and Central Province (including National Capital District) (10). The data of Schuurkamp (1993) indicated an overall resistance level of 63% (n=54) for the Central Province, with R3 resistance accounting for only 4%. For Madang, R3 resistance accounted for 7% with the overall resistance level being 71% (n=14). However, a more recent report (11) from the same area

showed a prevalence of R3 resistance similar to the one found in this study, indicating a 3- to 5-fold rise from previous levels in the prevalence of R3 chloroquine resistance in both regions. This raises some serious concern about the effectiveness of the standard chloroquine regimen against uncomplicated falciparum malaria. Hence the appropriateness of this standard regimen needs to be assessed fully in the light of increasing R3 resistance. In addition, continued monitoring of the response to chloroquine is necessary to appreciate the extent of R3 resistance in the country. This would also allow the trend of chloroquine resistance to be monitored over time. With this information, alternative chemotherapeutic

strategies can be devised to combat drug resistance in the future.

While chloroquine treatment appeared effective in most cases, intervention with alternative antimalarial drugs was required in others. In this study, the alternative drug treatment consisted of low-dose quinine and a single dose of Fansidar. Not only is this quinine regimen effective against low-level chloroquine-resistant falciparum malaria (10), but it also results in a low incidence of cinchonism, the most troublesome adverse effect of the standard quinine regimen, and thus has an advantage needed for patient compliance. However, we have yet to assess the potential of low-dose quinine as an alternative to the standard-dose quinine regimen. Meanwhile, the standard-dose quinine regimen in combination with Fansidar remains the recommended choice for chloroquine-resistant falciparum malaria.

In conclusion, the indications from this study are that chloroquine treatment is still effective in most cases of uncomplicated falciparum malaria but in some an intervention with alternative antimalarial drugs may be required for complete clearance of parasitaemia. Early detection of resistance, and management of potential chloroquine-resistant strains of *P. falciparum* in a more timely manner with low-dose quinine, may be the choices for the future.

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