

RESEARCH NOTE

FIRST REPORT OF *IN VITRO* SUSCEPTIBILITY OF *PLASMODIUM MALARIAE* THAI ISOLATES TO CHLOROQUINE

¹Peerapan Tan-ariya and ²Sataporn Pasuralertsakul

¹Department of Microbiology, Faculty of Science; ²Department of Parasitology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand

Emergence of *Plasmodium falciparum* resistance to antimalarials has forced researchers to develop techniques to assess malaria parasite responsiveness to the compounds used to eliminate the erythrocytic stages. Chloroquine, which was the drug of choice in the treatment of falciparum malaria was used in the development of the *in vitro* assessment of parasite sensitivity by Rieckmann in 1963, and later in the development of the *in vitro* microtest (Rieckmann, 1978). This technique is a very practical tool for monitoring parasite drug sensitivity in infected individuals as much as in communities in endemic areas.

In vitro assessment of *P. vivax* sensitivity to chloroquine was initiated much later by Brockelman *et al* (1989). During their studies on nutritional requirements of *P. vivax* aiming at results which may lead to the design of continuous cultivation of this parasite, they found that their technic was adequate to support *in vitro* growth and differentiation of *P. vivax* to complete the schizogony, and thus interference of growth by chloroquine can be interpreted as chloroquine responsiveness. Brockelman's initiation was proven useful since there have been several case reports of chloroquine-resistant *P. vivax* from some parts of the world, *ie* Papua New Guinea (Schuurkamp *et al*, 1989, 1992; Rieckmann *et al*, 1989; Whitby *et al*, 1989; Collignon, 1991), Irian Jaya (Baird *et al*, 1991; Murphy *et al*, 1992), after either therapeutic or prophylactic drug regimens.

As *P. malariae* is one of the four species of human malarial parasites in Thailand, and chloroquine is the drug of choice for the treatment, it would be beneficial to learn about the nature of its chloroquine responsiveness. We therefore carried out the *in vitro* microtest for chloroquine sensitivity using *P. malariae*.

Three blood samples infected with *P. malariae* were assayed in this study. Briefly, 1.0 ml of venous

blood was collected from patients into a 1.5 ml Eppendorf centrifuge tube containing 0.5 ml of Waymouth medium (GIBCO, New York) and 20 IU heparin. The sample was centrifuged at 500 g for 5-7 minutes at 4°C. After discarding the supernatant, an aliquot of 0.2 ml of packed cells was transferred to a test tube containing 3.8 ml of test medium (RPMI 1640: Waymouth at ratio 2:1) supplemented by 10% (v/v) normal AB serum to make a 5% cell suspension. A sample of 100 µl of the suspension was then pipetted into each well of chloroquine-predosed microtiter plate (WHO, Manila, Philippines), using four replicates of each drug concentration. The plate was shaken gently to redissolve the coated drug and incubated at 37.5 - 38.0°C in a candle jar. After 24 hours of incubation, a thick blood film was made from the contents of each well, stained with Giemsa stain and examined microscopically. The number of schizonts was counted against 200 asexual stages. For one case which showed mixed asexual stages at time zero, the total count of schizonts from each well was reduced by the number of schizonts counted at time zero before analysis. The inhibitory concentration (IC) of chloroquine was calculated by using probit analysis (Grab and Wernsdorfer, 1983). The results were expressed as IC₅₀ and IC₉₀, which were defined as the concentrations of chloroquine producing 50% and 90% inhibition of schizonts, respectively.

The initial parasitemia of three *P. malariae* isolates ranged from 0.02% to 0.05%, and most of parasites appeared as ring or band forms. Observation of the growth of *P. malariae* after 24 hours of incubation revealed rapid development of ring and band forms into immature and mature schizonts. The success of growth into multinucleate schizonts correlated inversely with the level of chloroquine as shown in Fig 1. The number of schizonts per 200 asexual stages in control wells without drug of three isolates *ie* PM-1, PM-2 and PM-3, were 108, 96 and 118, respectively. Fifty percent inhibition or greater

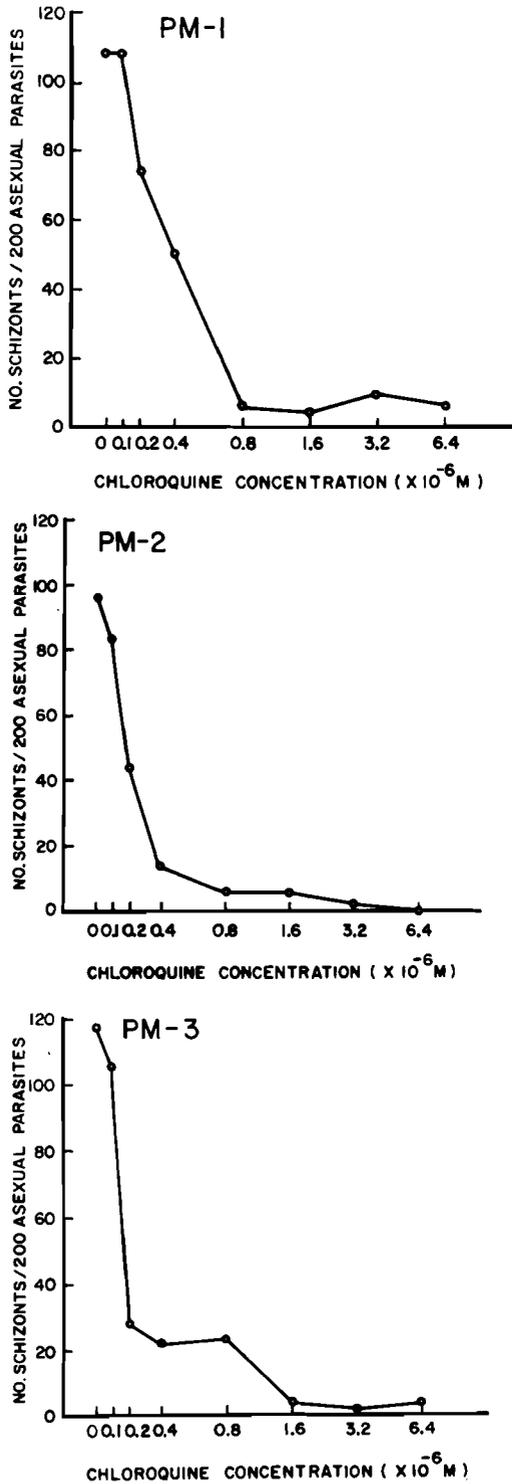


Fig 1—Chloroquine susceptibility of three *P. malariae* isolates as evaluated by schizont maturation inhibition.

was found at $0.2 - 0.4 \times 10^{-6}$ M. Ninety percent inhibition or greater was achieved at $0.8 - 1.6 \times 10^{-6}$ M. Fig 2 shows the median regression line of dose-response curves of each of the 3 isolates. The ID_{50s} and ID_{90s} are summarized in Table 1. The ID_{50s} of the three isolates ranged from 0.10×10^{-6} M to 0.20×10^{-6} M and the ID_{90s} from $0.37 - 0.77 \times 10^{-6}$ M chloroquine.

After blood collecting, all patients received a standard dose of chloroquine at a total dose of 1,500

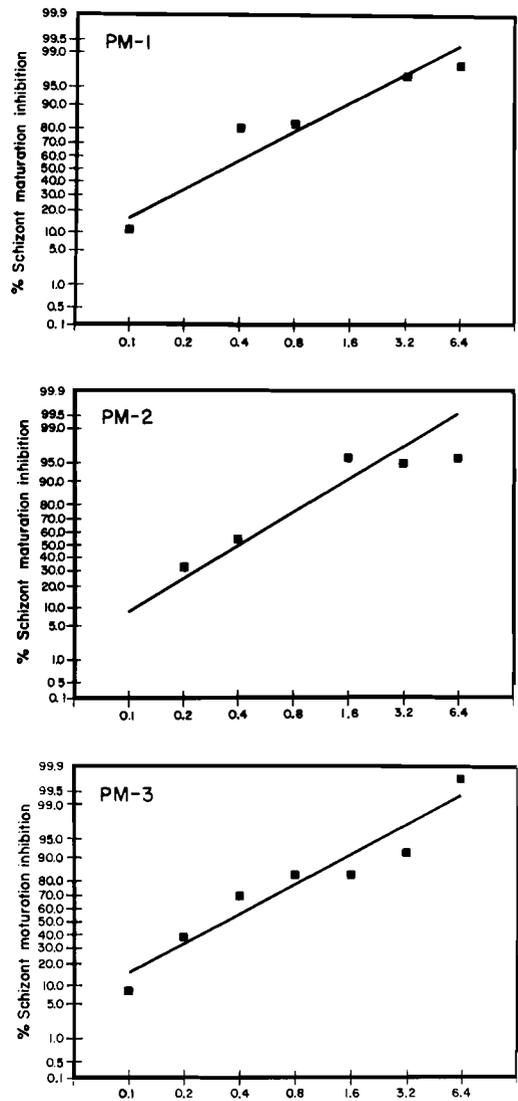


Fig 2—Log concentration-response curves for the schizont maturation inhibitory effect of chloroquine in three *P. malariae* isolates.

Table 1

The inhibitory concentrations of chloroquine to produce 50% schizont maturation inhibitions (IC₅₀) and 90% schizont maturation inhibition (IC₉₀) of three isolates of *Plasmodium malariae*.

| Isolates | Inhibitory concentrations | |
|----------|---|---------------------------|
| | IC ₅₀ (× 10 ⁻⁶ M) | IC ₉₀ (× 10 M) |
| PM-1 | 0.13 | 0.46 |
| PM-2 | 0.20 | 0.77 |
| PM-3 | 0.10 | 0.37 |

mg (25 mg base/kg body weight) over 48 hours (chloroquine phosphate, Government Pharmaceutical Organization; 1 tablet contains 150 mg base). The drug was administered as 600 mg base (10 mg base/kg body weight) at 0 hour and followed by a 300 mg base (5 mg base/kg) at 6 hours, 24 hours and 48 hours. Observation of daily thick blood films for 14 days indicated that parasite clearance times in the three *P. malariae* infected patients were within 24 - 48 hours (Fig 3). Recrudescence of *P. malariae* was not detected in any patients.

Since this report demonstrates for the first time the application of the *in vitro* microtest technic for testing the susceptibility of *P. malariae* to an antimalarial, we cannot compare our results with those of other studies. However, it was found that all three patients responded well to the standard treatment of chloroquine (Fig 3) and no recrudescence was observed. It can thus be concluded that these three *P. malariae* isolates are chloroquine sensitive strains. The ID_{50s} and ID_{90s} observed from this study may be used as baseline data of a range of inhibitory doses (ID₅₀ and ID₉₀) of chloroquine-sensitive *P. malariae*.

Though the *in vitro* test cannot be related directly to the *in vivo* results, it is a useful tool for epidemiological survey and for monitoring changes of antimalarial efficacy after a period of clinical use. However, in order to obtain more solid data of *in vitro* sensitivity of *P. malariae* Thai isolates to chloroquine, more cases of *P. malariae* infected patients need to be tested.

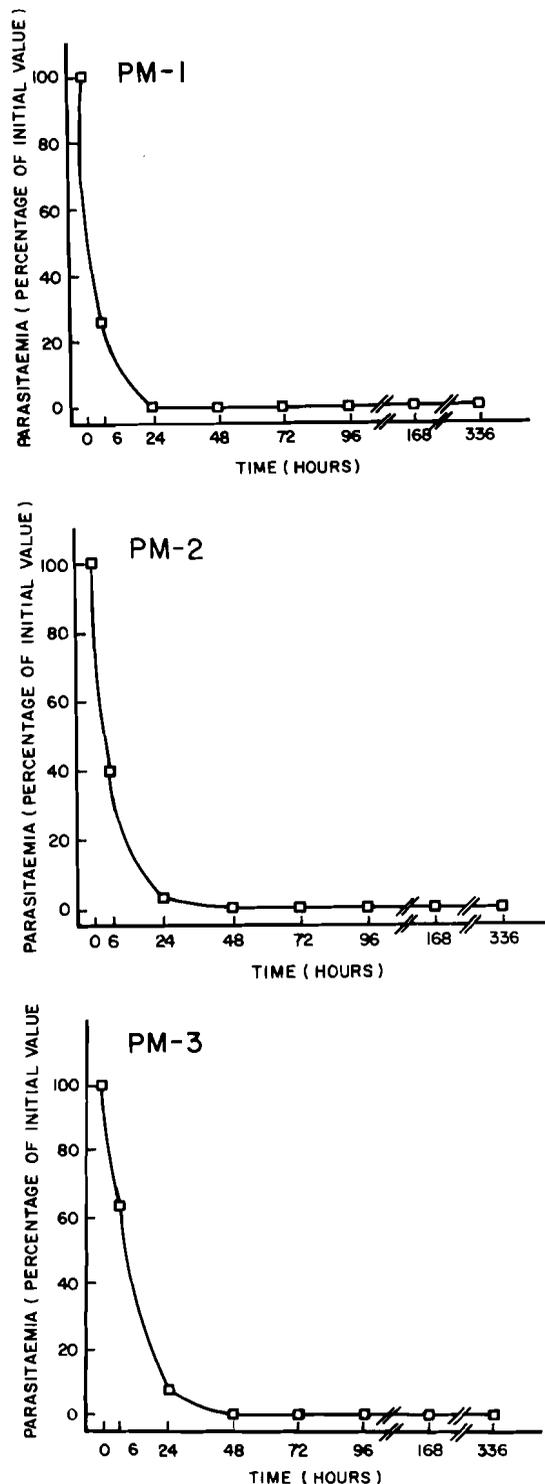


Fig 3-Parasite clearance time (hour) in three *P. malariae* patients.

ACKNOWLEDGEMENTS

This work was partially supported by UNDP/WORLD BANK/WHO Special Programme for Research Training in Tropical Diseases. We are grateful to Dr CR Brockelman for critical reading and reviewing the English version of the manuscript.

REFERENCES

- Baird JK, Basri H, Purnomo, *et al.* Resistance to chloroquine by *Plasmodium vivax* in Irian Jaya, Indonesia. *Am J Trop Med Hyg* 1991; 44 : 547-52.
- Brockelman CR, Tan-ariya P, Bunnag D. Development of an *in vitro* microtest for the assessment of *Plasmodium vivax* sensitivity of chloroquine. *Southeast Asian J Trop Med Public Health* 1989; 20 : 41-7.
- Collignon P. Chloroquine resistance in *Plasmodium vivax*. *J Infect Dis* 1991; 164 : 222-3.
- Grab B, Wernsdorfer WH. Evaluation of *in vitro* tests for drug sensitivity in *Plasmodium falciparum*: probit analysis of logdose/response test from 3-8 points assay. *WHO/MAL/83.999*, 1983; p 1-18.
- Murphy GS, Basri H, Purnomo, *et al.* Vivax malaria resistant to treatment and prophylaxis with chloroquine. *Lancet* 1992; 341 : 96-100.
- Rieckmann KH, Campbell GH, Sax LJ, Mrema JE. Drug sensitivity of *Plasmodium falciparum*. An *in vitro* microtechnique. *Lancet* 1978; 1 : 22-3.
- Rieckmann KH, Davis DR, Hutton DC. *Plasmodium vivax* resistance to chloroquine? *Lancet* 1989; 2 : 1183-4.
- Schuurkamp GJ, Spicer PE, Kereu RK, Bulungol PK. A mixed infection of vivax and falciparum malaria apparently resistant to 4-aminoquinoline: a case report. *Trans R Soc Trop Med Hyg* 1989; 83 : 607-8.
- Schuurkamp GJ, Spicer PE, Kereu RK, Bulungol PK, Rieckmann KH. Chloroquine-resistant *Plasmodium vivax* in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1992; 86 : 121-2.
- Whitby M, Wood G, Veenendaal JR, Rieckmann K. Chloroquine-resistant *Plasmodium vivax*. *Lancet* 1989; 2 : 1395.