THE *IN VIVO* SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE IN THE RED RIVER BASIN, YUNNAN, CHINA

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Abstract. The sensitivity of *Plasmodium falciparum* to chloroquine *in vivo* was *examined* out in a small town in the Red River basin from 1995 to 1996. forty cases were recruited into the present study, 32 cases were completely followed-up. The results of the *in vivo* study revealed that only 3 (9%) cases were sensitive to chloroquine, 29 (90.6%) showed resistance to chloroquine, among whom 7 cases (22%) showed resistance at RI, 12 cases (38%) at RII, and 10 cases (31%) at RIII. It is suggested that *P.falciparum* endemic areas should stop using chloroquine and other 4-aminoquinolines in the Red River basin now. Qinhaosu and pyronaridine were recommended to use as the first line antimalarial drugs of *P.falciparum* infection in the basin.

INTRODUCTION

Malaria continues to be one of the most important public health problems in Yunnan, China today. Although considerable control has been achieved over the disease in the past three decades, a recent resurgence of falciparum malaria has been noted in some part of the province since the early 1980s. Fatal cases due to *Plasmodinm falciparum* have been reported (Zhou, 1994; Zhou *et al*, 1994). The highly endemic areas of *P.falciparum* have been extended from border areas between China-Myanmar, China-Lao to the middle areas of Yunnan Province, particularly in the Red River basin (Zhou, 1994).

Resistance of *P.falciparum* to chloroquine was first reported in Yunnan among semi-immune and non-immune (immigrant) patients at Mengdin county near the China-Myanmar border in 1974 (PLA Kunming Medical Research Institute, malaria research group, 1978). Subsequently, 16 sites in 14 counties near the China-Myanmar and China-Lao PDR borders were found to have *P.falciparum* resistance to chloroquine from 1979 to 1988 (Che *et al*, 1986; Yang *et al*, 1994). During this period of study, 530 falciparum malaria cases were studied *in vivo*, among 77.55% (411/530) cases showed resistance to chloroquine, the proportions of RI, RII and RIII were 38.30%, 25.28% and 13.96% respectively. Chloroquine resistance had been thought to be well established in most counties in border areas in Yunnan since the early 1980s. Consequently, the Yunnan health authority changed from chloroquine to sulfadoxine-pyrimethamine (SDX/PYR) and piperaquine as the first line of antimalarial drugs for *P.falciparum* infection in the China-Lao and China-Myanmar boder areas in1983 (Che *et al*, 1986).

P.falciparum was only sporadic in the Red River basin in the early 1980s. An *in vitro* study indicated the proportion of *P.falciparum* resistance to chloroquine was 52.2%, but all 37 cases in the *in vivo* study were sensitive to chloroquine in Xinpin County, upper part of the Red River in 1982(Che *et al*, 1986). Therefore, chloroquine has been continuing as the first line of antimalarial drug for falciparum malaria in the Red River basin for treating clinical patients, for mass prophylaxis at certain intervals for the general population at peak transmission season, and for presumptive treatment for suspected malaria cases. However, some practitioners have noticed increasing treatment failures with chloroquine, while others, for reasons unrelated to patient welfare, no longer prescribe chloroquine. An *in vitro* sensitivity of *P.falciparum* to available antimalarial drugs indicated that the proportion of *P.falciparum* resistance to chloroquine and piperaquine was 78.9% and 72.9%, respectively in this area in 1993 (Yang *et al*, 1994). No study assesses clinical response, an important consideration for developing a treatment policy. Therefore, we carried out clincal assessment the sensitivity of *P.falciparum* on chloroquine in the Red River basin.

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MATERIALS AND METHODS

Study site

The study was conducted in Manhao town, Jingpin county at the lower reaches of the Red River, approximately 100 km north of Vietnam, where Honghe Prefecture malaria surveillance station is located. Its environmental and malaria profiles are broadly similar to those of the rest of the Red River basin. Malaria transmission is perennial peaking from June to November. *P.falciparum* and *P.vivax* co-exist. The principal vector of malaria is *Anopheles minimus* and *An. sinensis* in the area.

Criteria for selecting patients

The study was carried out from August to October 1995, and from July to October 1996. All patients with febrile episodes attending the malaria surveillance station who fulfilled the following criteria during the study periods would be admitted to the study after fully informed consent obtained: (1) they had diagnosis of mono-infection with *P.falciparum* based on clinical signs and symptoms, and confirmed by microscopic examination of Giemsa-stained thick and thin blood films; (2) they exhibited asexual parasitemia levels from ≥ 1,000; (3) they did not receive antimalarial therapy within the preceding seven days, and urine samples from patients were negative for 4-aminoquinolines in the Haskin urine test (Bruce-Chwatt, 1981). Patients would be excluded if they had severe malaria, as defined by World Health Organization (WHO, 1990), and chronic medication or one suggestion of possible allergy to drugs tested in present study, if they had a history of antimalarial drug treatments within the preceding week, if they had chronic diarrhea, or if they were pregnant.

Blood slide examination

Thick and thin blood smears were collected from fingerprick blood samples. The films were examined every day after taking the drugs from day 0 to day 7, on the day 14, 21 and 28. Each time two slides were made, and one for immediately examining and another one for checking. Thick films were stained with Giemsa stain and examined under microscope at 1,000 x magnification. Parasites were counted against 300 leukocytes. Parasite density was estimated using assumed leukocyte count of 8,000 parasites/μl. The thick smear was used to quantify the density of parasitemia and the thin one used for identify parasite species.

Urine test for antimalarials

Haskin urine test for 4-aminoquinolines was carried out for each patient. Urine samples of the patients were checked on day 0 to ensure that they had not taken the anti-malarials prior to the treatment. Urine samples were checked again for second time at 48 hours for each patient recruited into the study after the patients had been taking chloroquine for assurance of absorption of the drug.

Drug regimens

Patients received 25mg of chloroquine base/kg given in three doses: 10 mg/kg at admission, follows by 10 mg/kg 24 hours later, and 5 mg/kg 48 hours later. The young children received the exact dosages of crushed tablets mixed with water, sugar given by mouth using syringe. Chloroquine was provided by Shanghai Pharmaceutical Company (coded 11012). Patients taking the drugs were under field staff supervision to make sure that they would have swallowed the drugs and had not vomited. If vomiting occurred during the first 30 minutes, the full dose was repeated. Patients vomiting between 30 minutes and 1 hour were given half the initial dose.

Treatment of chloroquine resistant strain

Once a patient treatment with chloroquine showed evidence of failure (resistant to chloroquine), he or she would be treated with artesunate at total dosage of 500mg for 5 days and primaquine 67.5 mg for 3 days, and children would decrease the dosages properly.

Definition of outcome

The sensitivities of chloroquine were determined by World Health Organization criteria (Bruce-Chwatt, 1981): (a) If no asexual parasites are found by day 6 and parasitemia does not reappear by day 28, the parasites are sensitive (S); (b) If asexual parasites disappear as in (a) but return within 28

days, the parasite resistance is at the RI level; (c) If the asexual parasitemia does not clear but is reduced to 25% or less of the orginal pre-test level during the first 48 hours of treatment, the parasites are resistant at the RII level; (d) If asexual parasitemia is reduced by less than 75% during the first 48 hours of it continues to rise, the parasites are resistant at the RIII level.

RESULTS

Forty-three *P. falciparum* cases fulfilled the inclusion criteria and were enrolled into present study. Ten cases were lost follow-up and one case took other antimalarial during follow-up. Therefore, the eleven cases were excluded from the analysis. 32 cases remained for the present analysis, of those 22 cases were male and 10 female. 15 of 32 cases were local inhabitants and 17 of them were temporary immigrants from other counties, most of who were with little immunity to malaria. Four cases were under 10 years old, the youngest patient was 6 years old and the oldest 60. The *in vivo* test indicated that 3 cases were sensitive to chloroquine, 7 cases at resistance level R1, 12 cases at R2 and 10 cases at R3. The results suggest that the proportion of *P. falciparum* resistance to chloroquine was 90.6%. The detailed description and sensitivity are shown in Table 1.

The relationship between the level of resistance to chloroquine and population sources indicated that immigrant groups tended to have a higher level of resistance (Table 2). This is probably because the local residents have higher immunity to malaria

Table 1

Characteristics of 32 patients and the resistance level to chloroquine.

Characteristics	Value
No. of male	22
No. of immigrants	17
Mean (SD) age (year)	29.3 (15.5)
Mean (SD) weight (kg)	48.5 (14.7)
Mean (SD) temperature (°C) at enrollment	38.6 (1.4)
Parasitemia (parasites / μl)* at enrollment	4,303 (2,238 - 8,395)
Resistance (% of subjects)	
S	3 (9%)
RI	7 (22%)
RII	12 (38%)
RIII	10 (31%)

*Values given are geometric mean parasite density and (interquartile range)

Table 2

The relationship between chloroquine resistance and population sources.

Population	No. of population	S	RI	RII	RIII
Local residents	15	1	3	8	3
Immigrants groups	17	2	4	4	7

due to long term exposure to malaria infection. The higher level of resistance among immigrant groups was probably pertinent to the greater number of fatal cases among immigrant groups in the basin.

DISCUSSION

The present *in vivo* study suggested that the proportion of antimalarial resistance to chloroquine was 90.6% and the majority of resistance cases were at the resistance levels of RII and RIII. The results of the *in vivo* study were consitent with those *in vitro* studies in the same place in 1993 (Yang *et al,* 1994). The study was also evidence that the immigrant group with little immunity to malaria tend to have a worse response to the chloroquine, which might explain the finding that more fatal cases occurred among the immigration population in the Red River basin in recent years. However, it is hard to estimate exactly the proportion of *P. falciparum* resistance to chloroquine in the Red River basin due to the high proportion of the recruited cases in the present study who were immigrants. Patients who went to the Prefecture Malaria Surveillance Station might be highly selected, more sensitive cases might be treated by local village doctors. Ten lost cases seemed to have better responses to chloroquine when we looked at the first four day data among the lost cases in the present study. Nevertheless, the present study provides clear evidence that *P. falciparum* is highly resistant to chloroquine in the Red River basin.

All 29 chloroquine resistant cases were treated with artesunate and primaquine. The 28 of 29 cases were clinically cured. Only one case was clinically recrudescent with microscopically confirmed parasite slides.

In conclusion, *P. falciparum* is highly resistant to chloroquine in the Red River basin now. Chloroquine should not be used as first line antimalarial drugs of *P. falciparum* infection in the basin. We suggest that *P. falciparum* endemic areas should stop using chloroquine and other 4-aminoquinolines in the Red River basin. Qinhaosu and pyronaridine (Fu and Xiao, 1991) are recommended to use first line antimalarials of *P. falciparum* infection in the basin.

REFERENCES

Bruce-Chwatt LJ. Chemotherapy of Malaria, 2nd ed. World Health Organization, 1981.

Che LG, Chen WC, Yang HL, Huang KG. The distribution of chloroquine resistant *P. falciparum* strains in Yunnan Province. *Chin J Epidemiol* 1986; 7: 88-91.

Fu S, Xiao SH. Pyronaridine: A new antimalarial drug. *Parasitol Today* 1991; 7: 310 - 13.

PLA Kuanming Medical Research Institute, malaria research group. Clinical observation of the sensitivity of *Plasmodium falciparum* to chloroquine in Mendin, Yunnan Province. *Chin Intern Med* J 1978; 18: 247-9.

WHO. Severe and complicated malaria. *Trans R Soc Trop Med Hyg.* 1990; 84 (suppl): 1-22.

Yang HL, Yang PF, Yang YM. *In vitro* sensitivity of *Plasmodium falciparum* to melfoquine, quinine, amodiaquine, chloroquine, pyronaridine and sulfadoxine/pyrimethamine in south Yunnan. *Chin J Parasitol Parasit Dis* 1994; 12: 140-2.

Zhou DF. Prevalent outline of falciparum malaria and the resistant of *P. falciparum to* chloroquine in Yunnan Province. *Chin J Parasit Dis Contr* 1994; 12: 140-2.

Zhou DF. Prevalent outline of falciparum malaria and the resistant of *P. falciparum* to chloroquine in Yunnan Province. *Chin J Parasiti Dis Contr* 1994; 7: 84-7.

Zhou DF, Che LG, Su FC. The malaria situation on the frontiers of Yunnan Province, China. *Southeast Asian J Trop Med Public Health.* 1994; 25: 19-24.