Changes in the resistance of *Plasmodium falciparum* to chloroquine in Hainan, China

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In 1979, in view of the widespread resistance of *Plasmodium falciparum* to chloroquine in the island of Hainan, China, its use as an antimalarial was suspended throughout the island. A longitudinal survey of the chloroquine-sensitivity of *P. falciparum* was carried out over the period 1981–91 to investigate whether its resistance had changed from the 1979 level.

In-vitro assays were carried out every 2–3 years, while in-vivo tests were performed annually over the period 1981–83 and also in 1991. Resistance to chloroquine declined progressively after its use had stopped. The in-vitro tests indicated that the rate of chloroquine-resistant *P. falciparum* was 97.9% in 1981, but dropped to 60.9% in 1991 (P < 0.001). The mean concentration of chloroquine for complete inhibition of schizont formation was 10.4 pmol/µl in 1981, but decreased to 3.0 pmol/µl in 1991 (P < 0.001). The proportion of samples taken from malaria cases that required high concentrations (>6.4 pmol/µl) of chloroquine for complete inhibition of schizont formation was 83.3% in 1981, but only 17.4% in 1991 (P < 0.001); at low concentrations (<1.6 pmol/µl), the corresponding proportions increased from 4.2% in 1981 to 60.8% in 1991 (P < 0.001). In the 4-week in-vivo test, the rate of chloroquine-resistant *P. falciparum* decreased from 84.2% in 1981 to 40% in 1991 (P < 0.001). RII + RII cases represented 59.4% of the total resistant cases in 1981, but decreased to 37.5% in 1991 (0.02 > P > 0.01).

The first case of chloroquine-resistant *Plasmodium falciparum* malaria was detected in Ya County, Hainan island, in 1974. Subsequently, a large-scale survey of the resistance of *P. falciparum* to chloroquine was conducted throughout the island over the period 1975–78. The results demonstrated that such resistance was spreading rapidly and that by 1978 it was fairly extensive. The situation was most serious in south-western Hainan, where there were more chloroquine-resistant malaria cases, and the degree of resistance was generally high; the proportion of RIII malaria cases reached 30% (1, 2). In view of this, health officials in Hainan stipulated that chloroquine use should be stopped throughout the island beginning in 1979 and that piperaquine should replace it for malaria therapy and prophylaxis. In order to monitor any changes in the resistance of *P. falciparum* to chloroquine following the cessation of its use we conducted a longitudinal survey over the period 1981–91 in Ledong county, Hainan, where a high degree of chloroquine resistance had existed.

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**Materials and methods**

The *in-vitro* microtest for assessing the response of *P. falciparum* to chloroquine was performed according to the standard WHO protocol (3, 4). The microtitration plates and culture medium used in the test were prepared by the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine; these preparations have the same effectiveness as those provided by WHO. The growth and chloroquine concentration required to completely inhibit schizont formation were in agreement with the results of WHO field trials, while the harvesting period used was 2–7 hours, shorter than the WHO period (6–8 hours). Growth of *P. falciparum* in wells where the level of chloroquine was >8 pmol/µl was taken to indicate resistance to chloroquine.

The WHO 28-day test was used for *in-vivo* observations (5). Chloroquine phosphate tablets were obtained from the Shanghai Fourteenth Pharmaceutical Factory. Falciparum malaria patients were hospitalized for 7 days; follow-up blood examinations were carried out every week for 3 consecutive weeks.

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DDT residual spraying was performed over the period 1981–83 to prevent reinfections, but was abandoned from 1986 onwards (9).

The majority of the 216 study subjects to be assessed were outpatients at the county hospital and were aged 5–61 years. Most were Li ethnic minority; a few were Han or temporary workers. None had received any antimalarials, sulfonamide or sulfone drugs, according to their histories. Examination of blood samples indicated that all the study cases were infected with the single species *Plasmodium falciparum*. The density of asexual parasitaemia was in the range 1000–80 000/mm³. “O” type red blood cells from a healthy person with no history of malaria were used to dilute the parasite density to 30 000–50 000/mm³ in instances of high parasitaemia (10, 11). Haskins’s method detected no traces of chloroquine and the lignin test detected no traces of sulfonamide in samples of urine from any study subject. All the tests were conducted in July–September, the peak malaria transmission season in Hainan.

## Results

A gradual decrease in the proportion of *Plasmodium falciparum* samples that were resistant to chloroquine (resistance rate) occurred over the study period. The results of the in-vitro microtest indicated that the resistance rate declined from 97.9% in 1981 to 60.9% in 1991 (P < 0.001) (Table 1).

The in-vivo test indicated that the resistance rate of *Plasmodium falciparum* to chloroquine decreased from 84.2% in 1981 to 40.0% in 1991 (Table 2).

Also, in the in-vitro test, *Plasmodium falciparum* samples from 83% of falciparum malaria cases exhibited complete inhibition of schizont formation at high concentrations of chloroquine (>6.4 pmol/µl) in 1981; the proportion fell to 17.4% in 1991. In contrast, samples from 4.2% of falciparum malaria cases exhibited complete inhibition of schizont formation at low chloroquine concentrations (<1.6 pmol/µl) in 1981; the proportion increased to 60.8% in 1991. Furthermore, the in-vitro microtest indicated that the mean concentration of chloroquine required to inhibit schizont formation fell from 10.4 pmol/µl in 1981 to 3.0 pmol/µl in 1991, a reduction of 71.2% (P < 0.001) (Table 3).

The results of in-vivo tests indicated that clearance of *Plasmodium falciparum* asexual forms in the blood of falciparum malaria cases took a mean of 72 hours in 1981, but had decreased to 59 hours in 1991. Concurrently, the proportion of patients exhibiting RII and RIII resistance fell from 59.4% in 1981 to 37.5% in 1991 (0.02 > P > 0.01) (Table 4).

## Discussion

The resistance of *Plasmodium falciparum* to chloroquine, which has had considerable impact on malaria control, is an issue that is eliciting global concern (12). The possibility that the sensitivity of *P. falciparum* to chloroquine could resume after its use has been stopped for a period of years is attractive. WHO has considered such a possibility, but no clear evidence to support this hypothesis has previously appeared (13). Nevertheless, in Vietnam the susceptibility of *P. falciparum* to chloroquine increased after the drug’s use was stopped for 10 years (14). Also, in Thailand, the sensitivity of *P. falciparum* to chloroquine increased markedly over the period 1978–86 after alternative antimalarials were used (15). Our results also document a gradual increase in the sensitivity of *P. falciparum* to chloroquine in Hainan as a consequence of the decision made in 1979 to stop using the drug following the discovery of the first case of chloroquine-resistant falciparum malaria in 1974.

Since 1979, piperaquine has been used in place of chloroquine. Almost all doctors and health workers in Hainan have treated malaria patients with piperaquine, and more recently, with piperaquine or

### Table 2: Changes in the resistance rate of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the in-vivo test

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases assessed</th>
<th>No. of sensitive cases</th>
<th>No. of resistant cases</th>
<th>Resistance rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>38</td>
<td>6</td>
<td>32</td>
<td>84.2</td>
</tr>
<tr>
<td>1982–83</td>
<td>32</td>
<td>5</td>
<td>27</td>
<td>84.4</td>
</tr>
<tr>
<td>1986–89</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>40.0</td>
</tr>
</tbody>
</table>


### Table 1: Changes in the resistance rate of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the in-vitro test

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases assessed</th>
<th>No. of sensitive cases</th>
<th>No. of resistant cases</th>
<th>Resistance rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>48</td>
<td>1</td>
<td>47</td>
<td>97.9</td>
</tr>
<tr>
<td>1982–83</td>
<td>45</td>
<td>4</td>
<td>41</td>
<td>91.1</td>
</tr>
<tr>
<td>1986–89</td>
<td>77</td>
<td>15</td>
<td>62</td>
<td>80.5</td>
</tr>
<tr>
<td>1991</td>
<td>46</td>
<td>18</td>
<td>28</td>
<td>60.9</td>
</tr>
</tbody>
</table>
Changing resistance of *Plasmodium falciparum* to chloroquine in Hainan, China

Table 3: Changes in the degree of resistance of *Plasmodium falciparum* to chloroquine in Hainan, 1981–91, according to the *in-vitro* microtest

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases assessed</th>
<th>IC_{50} (pmol/μl)*</th>
<th>Mean concentration for complete inhibition of schizont formation (pmol/μl)</th>
<th>% of cases showing complete inhibition of schizont formation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>48</td>
<td>1.3 (1.0–1.5)*</td>
<td>10.4</td>
<td>&lt;1.6 pmol/μl</td>
</tr>
<tr>
<td>1982</td>
<td>45</td>
<td>0.8 (0.4–1.5)</td>
<td>6.1</td>
<td>&gt;6.4 pmol/μl</td>
</tr>
<tr>
<td>1986–89</td>
<td>77</td>
<td>0.5 (0.3–0.9)</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>46</td>
<td>0.3 (0.2–0.4)</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

* IC = inhibitory concentration.

During the period 1981–83, the concentration of chloroquine for complete inhibition of schizont formation decreased from 10.4 pmol/μl in 1981 to 3.0 pmol/μl in 1991, indicating a slight decrease in resistance intensity. However, even as resistance decreased to a slight extent, it still remained high, at 1.6% of cases showing complete inhibition of schizont formation.

For the surveillance of chloroquine resistance and any changes that may exhibit, the *in-vitro* microtest is more sensitive than the *in-vivo* test. A decrease in the concentration of chloroquine required for complete inhibition of schizont formation, even to a slight extent, reflects the change of resistance intensity, which preceded the decrease in resistance rate.

Although in Hainan about half the symptomatic cases of falciparum malaria could currently be cured by chloroquine, it should still not be reinstated as an antimalarial to avoid progressive development of drug resistance. Further studies are planned to determine the extent to which the sensitivity of *P. falciparum* to chloroquine can be restored.

Acknowledgement

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Résumé

*Modifications de la résistance de Plasmodium falciparum à la chloroquine à Hainan, Chine*

la chloroquine est tombée de 97,9% en 1981 à 60,9% en 1991 (p<0,001). La concentration moyenne de chloroquine nécessaire pour l'inhibition complète de la formation de schizontes était de 10,4 pmol/µl en 1981, et n'était plus que de 3,0 pmol/µl en 1991 (p<0,001). La proportion de prélèvements pris chez des malades et pour lesquels une forte concentration de chloroquine (>6,4 pmol/µl) était nécessaire pour obtenir l'inhibition complète de la formation de schizontes était de 83,3% en 1981, et de 17,4% en 1991 (p<0,001); pour les faibles concentrations (<1,6 pmol/µl) les proportions correspondantes étaient de 4,2% en 1981 et de 60,8% en 1991 (p<0,001). Dans le test in-vivo de 4 semaines, la proportion de P. falciparum chloroquinorésistant passait de 64,2% en 1991 à 40% en 1981 (p<0,001). Les cas RII + RIII représentaient 59,4% de l'ensemble des cas résistants en 1981, contre 37,5% en 1991 (0,02>p<0,01). Une résurgence de la population de P. falciparum sensible à la chloroquine a donc eu lieu à Hainan après l'arrêt prolongé de l'utilisation de ce médicament.

References


