Artemisinin based combination therapy for malaria in Viet Nam
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CHAPTER 5
Artemisinin or chloroquine
for blood stage Plasmodium vivax malaria
in Viet Nam

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Chloroquine-resistant *Plasmodium vivax* has not yet occurred in Viet Nam. The efficacy of artemisinin for *P. vivax* was not established. We conducted a double-blind randomized study involving 240 inpatients with *P. vivax* malaria who received artemisinin (40 mg/kg over 3 days) plus placebo chloroquine (Art) or chloroquine (25 mg/kg over 3 days) plus placebo artemisinin (Chl). Patients were followed up with weekly blood smears for 28 days. In each group 113 cases were analysed. All patients recovered rapidly. The median (range) parasite clearance time of regimen Art was 24 h (8–72) and of Chl 24 h (8–64; P = 0.3). Parasites reappeared in two cases in each group on day 14, in eight cases in each group (7%) on day 16 and in 25 (23%) and 18 (16%) cases, respectively, at the end of 4-week follow-up (P = 0.3). The population parasite clearance curve followed a mono-exponential decline. The parasite reduction ratio per 48 h reproduction cycle was $2.3 \times 10^4$ for both regimens. We conclude that artemisinin and chloroquine are equally effective in the treatment of *P. vivax* infections in Viet Nam. Reappearance of parasites before day 16 (7%) suggests the emergence of chloroquine resistance. Three days of artemisinin monotherapy does not prevent recrudescence.
**Introduction**

In South-east Asia morbidity attributable to *Plasmodium vivax* prevails while *P. falciparum* gradually comes under control by applying artemisinin drugs with other control measures such as insecticide-treated bed nets \[1,2\]. This trend was also observed in Binh Thuan, a mountainous province in the south of Viet Nam (unpublished information, Binh Thuan Provincial Malaria Station). Chloroquine is the first-line drug for treatment of *P. vivax* in most areas of the world \[3\], although artemisinin and derivatives are also used in various regimens and they appear to be effective \[4\]. It was not yet known whether chloroquine resistant *P. vivax* is prevalent in Viet Nam. Artemisinin drugs are very active against *P. vivax* but the efficacy has not been firmly established. Therefore, this study aimed to establish and compare the efficacy of artemisinin and chloroquine in the treatment of blood stage infections of *P. vivax* in Viet Nam. Based on our own experience in pharmacodynamic modelling of the time course of *P. falciparum*, a 3-day regimen of artemisinin should be effective. Longer regimens were considered to conflict with good patient compliance.

**Methods**

*Patient selection and treatment*

From September 1997 to January 2000, subjects aged 15 years or older with a microscopically confirmed *P. vivax* (asexual stage) infection, presenting at eight primary health care posts in Binh Thuan province were enrolled. Exclusion criteria included *P. falciparum* infection, pregnancy, lactation, complications of malaria, underlying disease, inability to take oral medication, known allergy to the study drugs and intake of antimalarial agents in the previous week. After informed consent the treatment regimen was allocated by a computer-generated randomization code with 120 patients per treatment arm, to detect a difference between approximately 30% and 15% response rates ($\alpha = 0.05$ and $\beta = 0.20$). Patients were admitted to the health facility and treated with artemisinin 20 mg/kg on $t = 0$ (day 1) followed by 10 mg/kg o.d. on days 2 and 3 plus chloroquine placebo (Art) or with chloroquine 15 mg/kg on $t = 0$ followed by 5 mg/kg o.d. on days 2 and 3 plus artemisinin placebo (Chl). Artemisinin 250 mg capsules, chloroquine diphosphate 250 mg (150 mg base) tablets and placebos were obtained from Mekophar Company, Ho Chi Minh City, Viet Nam. Intake of all medication was supervised. Vomiting within 1 h after intake of the trial medication required re-treatment with a full dose.
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The contents of the medication was analysed in 12 remaining capsules of artemisinin and 10 tablets of chloroquine (Laboratories of ACE Pharmaceuticals BV, Zeewolde, the Netherlands). Primaquine base 0.25 mg/kg daily was administered for 5 days, starting after day 28.

**Patient follow-up**

A full blood count was performed before patient inclusion and on day 3. The parasitaemia was counted every 8 h until three negative smears had been obtained. Blood smears were repeated on days 8, 15, 22 and 29. The parasite density was expressed as the number of parasites per microlitre of blood, calculated from the ratio with the white blood cell count in the thick smear. Gametocytes were recorded but not enumerated and the slides of t = 0 h or t = 8 h were taken as baseline. All blood smears were retained and reviewed by an experienced technician at the Department of Parasitology of Cho Ray Hospital in Ho Chi Minh City. His results were taken as gold standard. Fever and parasite clearance times were defined as the time from t = 0 to the first of three consecutive normal temperature readings (<37.0°C axillary) or negative blood smears, respectively. Clinical and parasitological outcome were assessed separately. Clinical cure was defined as a disappearance of symptoms and of parasites. Parasitological cure was assessed according to WHO criteria for assessment of chloroquine efficacy in therapeutic trials of *P. falciparum*: RI-parasite clearance by day 8 but recrudescence before day 15 (early RI) or from days 15 to 29 (late RI); RII: initial reduction of parasite count (>75%) without clearance at day 8; RIII: no or less response; radical cure: clinical cure without recrudescence [5].

**Population dynamics of the parasite count and statistics**

The time course of parasitaemia was fitted to non-linear mixed effect population models (maximum likelihood method) using the statistical package S-Plus (v. 4.5, Math Soft Inc., Seattle, WA, USA) as described previously [6]. The variables treatment regimen, age, sex and body weight were allowed to affect the model. The models were compared by the Bayesian Information Criterion (BIC) [7]. The best model was used to estimate (restricted maximum likelihood method) the elimination rate constant (k), the initial parasite count [P(0)] and the time to achieve a reduction of the initial parasitaemia by 50%, (PC50) or by 90%, (PC90). P(0), k and the duration of treatment converge in the parasitaemia at the end of drug exposure P(term). For artemisinin P(term) can be projected assuming that exposure lasted until 12 h after the last dose. The long half time of chloroquine precludes this projection. Patient data were analysed with the statistical package SPSS (v. 10.05, SPSS Inc. Chicago, IL, USA). Outcome was expressed as the proportion of patients who completed 28 days of follow-up.
Extreme case scenarios such as re-allocating patients who were lost to follow-up was used as an alternative of intention to treat analysis. Patients who, upon review of the blood slides, appeared to be erroneously included were excluded from the analysis.

Contingency tables and chi-square tests with continuity correction were applied to categorical variables. Numerical variables were tested for normality and Student's t-test or non-parametric tests were applied for comparison. Parasite clearance and recrudescence were analysed with survival analysis (Kaplan–Meier plots and the Cox proportional hazard model). Statistical significance was accepted when $P < 0.05$.

**Ethical approval**

Informed consent was obtained from all patients who participated in the study before randomization. The study protocol was approved by the scientific board and medical ethics committee of Cho Ray Hospital, Ho Chi Minh City.

**Results**

A total of 240 patients were recruited. Fourteen erroneously included patients with *P. falciparum* infection at baseline were excluded from the analysis. Of the remaining 226 patients, seven (five Art, two Chl) were lost to follow-up because they had left the study area. The patient characteristics are shown in Table 1. Except for a slight difference in sex ratio, there were no significant differences between the two groups. On day 14, parasites had recurred in two patients in each group, on day 16 this had increased to eight patients in each group (7%).

The remaining artemisinin capsules contained 249.0 mg (SD: ±0.7, range: 237.5–252.5), the chloroquine tablets 257.0 mg (±1.8, 237.5–262.5). Tolerance to the study medication was good. Some mild effects of the medication were recorded but these could also be attributed to malaria. Vomiting of trial medication did not occur.

The radical cure and recrudescence rates are shown in Table 2. The radical cure rates were 77% (83 of 108) and 84% (93 of 111). In the best case scenario the cure rate was 78% (88 of 113) in group Art and 84% (95 of 113) in group Chl; in the worst case scenario the ratios were 73% (83 of 113) and 82% (93 of 113), respectively. Although the cure rate in group Chl was slightly higher than in group Art, the differences did not reach statistical significance. All cases with recrudescence were treated successfully according to routine practice, usually with a second course of chloroquine plus primaquine 15 mg base o.d. for 14 days without follow-up after parasite clearance.
Artemisinin or chloroquine for vivax malaria

Table 1. Characteristics of patients with *Plasmodium vivax* infections treated with artemisinin or chloroquine.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ARTEMISININ (ART)</th>
<th>CHLOROQUINE (CHL)</th>
<th>p-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients included</td>
<td>113</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Number lost to follow up</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>26/87</td>
<td>13/100</td>
<td>0.03a</td>
</tr>
<tr>
<td>Median age (y) (Range)</td>
<td>27 (15-64)</td>
<td>27 (15-59)</td>
<td>0.4b</td>
</tr>
<tr>
<td>Median height (cm) (Range)</td>
<td>160 (111-175)</td>
<td>161 (135-175)</td>
<td>0.4b</td>
</tr>
<tr>
<td>Median weight (kg) (Range)</td>
<td>50 (31-62)</td>
<td>49 (39-69)</td>
<td>0.7b</td>
</tr>
<tr>
<td>Geom. mean P₀c (μl)</td>
<td>4057</td>
<td>3537</td>
<td>0.1c</td>
</tr>
<tr>
<td>95% CI</td>
<td>3248-5068</td>
<td>2872-4355</td>
<td>-660,1732f</td>
</tr>
</tbody>
</table>

a: Chi square test, b: Wilcoxon rank sum test, c: P₀: baseline parasite count; d: 95% CI: 95% confidence interval; e: Student’s t-test; f: 95% CI of the difference.

Table 2. Treatment response of *P. vivax* infections, treated with artemisinin or chloroquine.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Artemisin (Art)</th>
<th>Chloroquine (Chl)</th>
<th>p-Value; Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients included</td>
<td>113</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Radical cure (no.)</td>
<td>83 (77%)</td>
<td>93 (84%)</td>
<td>0.3; OR (95% CI): 0.64 (0.31, 1.33)</td>
</tr>
<tr>
<td>Recrudescence (no.)</td>
<td>25 (23%)</td>
<td>18 (16%)</td>
<td></td>
</tr>
<tr>
<td>Early (&lt; 14th day)</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Late (&gt; 14th day)</td>
<td>23</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Lost to follow up (no.)</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Median FCT (h), Range</td>
<td>16</td>
<td>16</td>
<td>0.2b</td>
</tr>
<tr>
<td>Median PCT (h), Range</td>
<td>24</td>
<td>24</td>
<td>0.3b</td>
</tr>
<tr>
<td>Mean K (h⁻¹), 95% CI</td>
<td>0.1747</td>
<td>0.1728</td>
<td>0.772</td>
</tr>
<tr>
<td>Geometric mean PC50 (h)</td>
<td>0.1661, 0.1832</td>
<td>1629. 1826</td>
<td>-0.015, 0.011d</td>
</tr>
<tr>
<td>Geometric mean PC90 (h)</td>
<td>14.6</td>
<td>14.7</td>
<td>0.749</td>
</tr>
<tr>
<td>Geometric mean PC90 (h)</td>
<td>13.6, 15.7</td>
<td>13.6, 15.9</td>
<td>-1.58, 1.55d</td>
</tr>
<tr>
<td>Geometric mean PC90 (h)</td>
<td>24.2</td>
<td>24.3</td>
<td>0.749</td>
</tr>
<tr>
<td>95% CI</td>
<td>22.7, 25.8</td>
<td>22.7, 26.1</td>
<td>-2.33, 2.17d</td>
</tr>
</tbody>
</table>

a: Chi square test b: Wilcoxon rank sum test c: 95% CI: 95% confidence interval; d: 95% CI of the difference
Both regimens offered rapid parasite clearance and defervescence. There was no RII or RIII response. The proportional cumulative parasite clearance and recurrence rates are shown in Figure 1. There was no significant difference between the two regimens ($P = 0.1729$; odds ratio 0.65; 95% CI 0.35–1.21). Initial parasitaemia was a weak but significant predictor of parasite recurrence ($P = 0.010$; odds ratio 1.0001; 95% CI 1.0000–1.0001). Age, sex and body weight did not affect the hazard function.
Figure 2. Time course of the P. vivax geometric parasitemia for patients treated with artemisinin (solid line, crosses) and chloroquine (broken line, round dots) and population estimate for all patients (unbroken line, no markers). Negative blood smears were entered as the value 2.5 parasites/μl. The error bars indicate the 95% C.I. of the geometric mean.

Figure 2 shows the geometric mean of the observed parasite counts and the population elimination model. A mono-exponential elimination model yielded the best fit, described by the mathematical function: \( P(t) = P(0) e^{-kt} \) in which \( P(t) \) is the parasitaemia as a function of time. There was no significant increase in the parasitaemia or any lag time before the parasitaemia starts to decline. Entering the variables age, body weight, sex and treatment regimen did not improve the fit. The mean of the estimates is shown in Table 2. The overall mean (95% CI) value of \( k \) was 0.175 (0.167–0.180) per hour, corresponding to a parasite reduction ratio of \( 2.3 \times 10^4 \) per 48 h development cycle. The estimated model parameters [including P(term)] were not different for the outcome groups radical cure vs. recrudescence.

Gametocytes were detected at baseline in 91% of patients in group Art and 94% of patients in group Chl. In group Art 0%, 5.3%, 7.4% and 4.2% of patients were still positive on days 8, 15, 22 and 28, respectively. In group Chl this was the case for 0%, 4.2%, 6.3% and 3.2%, respectively. Most of the patients who remained positive for gametocytes also had a
recurrence of asexual parasites. There was no significant difference between the two groups regarding gametocytes. Among the remainders who had no gametocytes at baseline, none of these had gametocytes during follow-up, including one patient in group Art with recurrence of asexual parasites on day 8.

**Discussion**

This is the first double-blind randomized trial which shows that RI chloroquine resistance of *P. vivax* is prevalent in the south of Viet Nam. The definition of drug resistance can be based on clinical criteria or on blood or plasma concentrations. The study medication contained the correct doses and the bioavailability of chloroquine is so predictable that it was regarded sufficient to supervise intake of medication. The absence of blood concentrations was not considered to affect the conclusions of this study.

The radical cure rate of 84% in this study is not sufficient although RII or RIII responses were not observed. In endemic areas recurrence of parasites may be a recrudescence or a re-infection or, in the case of *P. vivax*, a relapse. Genotyping could have assisted in distinguishing the two but this was not available. However, some assumptions can be made regarding early reappearance of parasites. Given an incubation time of 12–17 days and the low transmission rates of malaria in Binh Thuan, it is unlikely that first attacks of second infections were frequent. Therefore the early recurrence of parasites in this study is interpreted as a true recrudescence and for chloroquine this means RI resistance. Relapse of *P. vivax* malaria is also possible but day 16 is regarded as the minimum time after which relapses can occur. Under this assumption six more cases in each group would be classified as early reappearance and the rate of recrudescence in this study would be 7%, still higher than in a study in Thailand where no early recurrence was found during 4 weeks after treatment with chloroquine. When parasites reappear after more than 2 weeks, re-infection, relapse and true recrudescence cannot be distinguished. Nevertheless, because of the long half time of chloroquine, all recurrences within the first 4 weeks may point at some level of resistance. After a standard treatment course the total blood concentration of chloroquine plus its metabolite desethylchloroquine of chloroquine exceeds the minimum effective concentration of a sensitive strain for at least 7 days and often much longer. However, late parasite recurrence was also probably not a relapse in most cases because in nearby Thailand almost all relapses occurred more than 4 weeks after chloroquine administration.
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Chloroquine-resistant *P. vivax* was first seen in the Asia Pacific region \[13-16\]. Later reports appeared from other regions in Asia, Ethiopia and also from Guyana \[17-20\]. In Viet Nam *P. vivax* was still considered sensitive to chloroquine but there is no routine surveillance of treatment response of *P. vivax*. A recent study in Viet Nam did not reveal any chloroquine resistance, but because of the small sample size (29 patients) this does not contradict our findings \[21\]. In addition, chloroquine resistance may be masked because it is often combined with primaquine which also has some effect on asexual blood stages of *P. vivax* \[22,23\]. It is tempting to speculate that the emergence of resistant parasites is rather recent, because early recrudescence was infrequent and RII and RIII response were not (yet) observed.

In Thailand the response of *P. vivax* to chloroquine is good, although in vitro studies indicate declining sensitivity \[9,12-24\]. In contrast, chloroquine resistance, including RII and RIII response, was reported from Myanmar \[18\]. One explanation for these differences may be that in Thailand “drug pressure” is less than that in surrounding countries where chloroquine is still used for *P. falciparum*. Whether this also explains the variable response rate in Indonesia and the Philippines remains unclear \[25-29\].

For artemisinin drugs the situation is different. After treatment with artesunate, parasite clearance of *P. vivax* and *P. falciparum* was comparable \[30\]. Artesunate monotherapy for 5 days induced rapid recovery and parasite clearance, but in 63% of the patients the parasite reappeared before day 28 \[31\]. Three days of artesunate combined with primaquine for 14 days showed no recurrence of parasites. There is no information on the intrinsic activity of artemisinin on *P. vivax*, in terms of minimum effective concentrations, but the parasite reduction rate of *P. vivax* in this study is greater than that of *P. falciparum* \[6\]. This and the lower P(0) explain why in this study the recrudescence rate was similar to that of *P. falciparum* after 5 days of artemisinin monotherapy \[32\]. As stated for chloroquine, it is likely that the recurrences after artemisinin are true recrudescences.

The responses to artemisinin and chloroquine in this study were comparable although the cure rate was slightly lower for artemisinin. Interestingly, the parasite clearance rates were also similar. This is in contrast to *P. falciparum*. In Gambian children with severe malaria the effect of chloroquine was somewhat slower than of the artemisinin derivative artemether \[33\].

As a 3-day course of artemisinin is not superior to a standard course of chloroquine it is prudent to study how the efficacy of chloroquine can be improved. The combination of chloroquine and primaquine was shown to be very effective in areas with a high degree of chloroquine-resistant *P. vivax* \[23,34\]. Recently the Vietnamese policy advised to start a 5-day course of primaquine together with chloroquine on t = 0. The risk of G6PD deficiencies is
considered to preclude higher dosages or longer treatment courses of primaquine without further testing. Combinations of chloroquine with artemisinin or artemisinin with concomitant primaquine seem attractive options for further study.

In conclusion, artemisinin and chloroquine both offer rapid parasite clearance and recovery of *P. vivax* infections in Vietnam, but neither can prevent recrudescence; chloroquine probably because of resistance and artemisinin because of its intrinsic characteristics. Chloroquine resistance should be monitored and combinations of drugs should be studied further.

**Acknowledgements**

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