Artemisinin based combination therapy for malaria in Viet Nam
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CHAPTER 2

Artemisinin for treatment of uncomplicated falciparum malaria: is there a place for monotherapy?

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The efficacy of artemisinin monotherapy was studied in 227 patients with uncomplicated falciparum malaria. They all received artemisinin at $t = 0$ hr, $t = 8$ hr, and thereafter once daily; treatment was extended at random until they had taken either 5 days of artemisinin followed by 2 days of placebo ($A_5$), or 7 days ($A_7$) of artemisinin. The adult artemisinin dose was 500 mg; children aged $< 15$ years received 10 mg/kg per dose. The median (range) parasite clearance time was 39 (8–112) hr for $A_5$ and 43 (38–104) hr for $A_7$ ($P = 0.085$). The recrudescence rates were similar between the groups. The lowest parasite count achieved during treatment ($P_{\text{term}}$) was associated with the occurrence of recrudescence ($P = 0.046$, Cox regression model); it was lower for patients with a radical cure or late recrudescence than for early recrudescence ($P = 0.034$, t-test). Artemisinin monotherapy may offer rapid recovery and fast parasite clearance, but recrudescence is frequent. Extending the duration of monotherapy from 5 days to 7 days does not reduce recrudescence.
Introduction

Artemisinin and its derivatives have become standard treatment of falciparum malaria in Southeast Asia. Use of these drugs has increased in other parts of the world. One advantage of this class of antimalarials is that they can lead to rapid clinical recovery and clearance of parasites. Clinical failure caused by artemisinin-resistant Plasmodium falciparum strains has not yet been reported. Artemisinin is rapidly but incompletely absorbed, and its plasma elimination half-life is short (2.6 hr) \(^1\). This rapid elimination is probably one of the reasons why monotherapy is associated with high recrudescence rates.

Another reason for the high rate of recrudescence may be the time-dependent decline of plasma concentrations after repeated doses. This phenomenon has been observed in patients treated with artemisinin, artemether (with a concomitant increase of the plasma concentrations of its metabolite dihydroartemisinin), and dihydroartemisinin after intake of artesunate\(^3\). In comparison with primarily single-dose studies in healthy humans, the results indicate that the decline of plasma concentrations is drug related. In the case of dihydroartemisinin taken orally, data suggest that the decline of plasma concentrations down to values observed in healthy subjects is a disease-related effect (Le NH and others, unpublished data). A significant decline of plasma concentrations may counteract the benefits of prolonged therapy.

Because of the high rate of recrudescence associated with artemisinin monotherapy and the attendant risk of the development of drug resistance, current recommendations are to use artemisinin derivatives in combination with other antimalarial agents \(^7,8\). When monotherapy is deemed necessary, a minimum duration of therapy of 7 days has been recommended\(^8\). In reality, however, many other regimens are being applied \(^9\). An important reason for this is that the available artemisinin drugs in Southeast Asia are often packed in packages that contain the number of tablets required for 5 days of treatment, and sometimes one package may erroneously be considered a complete treatment course.

Some data suggest that the rate of recrudescence can be reduced by extending the duration of artemisinin monotherapy \(^10\). However, to our knowledge, a comparative, double-blind, randomized study comparing different durations of artemisinin monotherapy has not been performed \(^11\). Therefore, we compared 2 oral regimens of
Artemisinin monotherapy of different duration---5 days (A5) versus 7 days (A7)---in the treatment of uncomplicated falciparum malaria. The objectives were to assess the efficacy of both regimens in an area where there is a high prevalence of \textit{P. falciparum} resistant to chloroquine and sulfadoxine-pyrimethamine but not to mefloquine; and to study whether extending the duration of therapy would lead to better treatment results.

**Methods**

\textit{Patient selection and treatment.} The study was performed in a district hospital and several community health posts of Binh Thuan. Binh Thuan is a mountainous province in the south of Viet Nam, with high malaria endemicity. Transmission patterns are highly variable, mainly the seasonal forest fringe type of epidemiology, but with high transmission foci in the communities of ethnic minorities who inhabit the forested hill slopes. Significant acquired immunity is limited to these communities.

All patients presenting with signs or symptoms possibly related to malaria were evaluated for eligibility for entering the study. Inclusion criteria were uncomplicated falciparum malaria with parasitemia of 1,000--100,000/µL (0.02--2%) and aged > 6 years. Exclusion criteria included pregnancy, lactation, complicated malaria, inability to take orally administered medication, known allergy to artemisinin or derivatives and verbal confirmation of the intake of artemisinin derivatives in the previous 24 hr. and mefloquine, tetracycline, or doxycycline in previous 7 days or quinine in the previous 12 hr. Informed verbal consent was obtained before randomization from all participating patients. The medical ethics committees of the Academic Medical Center, Amsterdam, and Cho Ray Hospital, Ho Chi Minh City, approved the study protocol.

Closed envelopes containing computer-generated randomization codes were consecutively drawn after inclusion. One hundred twenty randomized numbers were originally allocated to each of the treatment regimens. Taking into account the patients lost to follow-up, the sample size of 240 patients, subdivided in 40 children and 200 adults, aimed at detecting a reduction in cure rate of at least 10% in the 2 regimens with statistical significance at \( \alpha = 0.05 \) and \( \beta = 0.2 \) (power 0.8). Adult patients (≥ 15 years) received artemisinin 500 mg at \( t = 0 \) hr and \( t = 8 \) hr and thereafter 500 mg once daily for either 6 consecutive days (A7, last dose give at \( t = \).
144 hr) or 4 days followed by 2 days of placebo (A5, last dose given at $t = 96$ hr). Children ($< 15$ years) received 10 mg/kg per dose. Artemisinin capsules of 250 mg and 100 mg and placebo capsules were obtained from Vidipha Company, Ho Chi Minh City, Viet Nam.

**Patient follow-up.** All patients were admitted to the district hospital or health posts. Vital signs were recorded every 8 hr, and physical examinations were performed every day. A full blood count was performed before patient inclusion and on the third day thereafter. Giemsa-stained thick and thin blood smears were obtained for identification and counting of asexual parasites by light microscopy before patient inclusion and every 8 hr after start of treatment until 3 negative smears had been obtained and the patient was discharged. After that, blood smears were taken 7, 14, 21, and 28 days after the start of treatment on an outpatient basis. Either 500 parasites were counted against the corresponding leucocytes or the parasites were counted against 1,000 leucocytes. The parasite count was calculated as the ratio with the white blood cell count and expressed per milliliter of blood. The presence of gametocytes was recorded but not enumerated. Gametocytes present in the slides of $t = 0$ hr or $t = 8$ hr 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.

Fever and parasite clearance times were defined as the time from initiation of treatment to the first of 3 consecutive normal temperature readings ($< 37.0^\circ$C axillary) or negative blood smears, respectively.

The parasite clearance rate ($K_e$), elimination half life ($t_{1/2e} = 0.693/K_e$), parasite count at the end of therapy ($P_{term}$), and replication rate after treatment were assessed as described previously.\(^{[12]}\) In brief, $P_{term}$ is a simulated value, in most cases far below the detection limit. A line connecting $P_{term}$ and the parasite count of patients with a recrudescence represents the replication rate after treatment.

Clinical and parasitological outcome were assessed separately. Clinical failure was defined as no clinical improvement necessitating additional treatment within the first 48 hr of treatment (early failure) or after 48 hr of therapy (late failure). Additional therapy of patients who failed to respond to treatment consisted of artemunate with mefloquine or quinine. Parasitological response was defined as follows. Radical cure means parasite clearance by Day 7 without recrudescence up to Day 28. R1 is initial disappearance of parasites with recrudescence before Day 14 (early R1) or from Day
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14 to Day 28 (late R1). R2 is an initial decrease of parasite count to < 25% of the initial value, followed by resurgence, without clearance by Day 7. R3 is no response or a small decrease of parasitemia to not less than 25% of the initial value, assessed at 48 hr after initiation of therapy.

A symptom was regarded to be a side effect possibly related to a study drug when it occurred after initiation of therapy or, if already present before the first dose, increased in intensity thereafter. Persistence of a symptom, although present before therapy, until 2 or more days after defervescence and parasite clearance was recorded as a possibly drug-related prolonged symptom.

Statistical analysis. The individual data were analyzed with the aid of the statistical package SPSS version 9 (SPSS Inc., Chicago, IL). All statistics concerning parasitemia were performed with log transformation. Clinical outcome was analyzed with contingency tables and the chi-square test with continuity correction for categorical parameters and with Student's t-test or nonparametric tests for numerical parameters. Parasite clearance and recrudescence were analyzed with survival analysis (log rank test and Cox's proportional hazard model). P < 0.05 was associated with statistical significance.

Results

The inclusion of patients proceeded more slowly than anticipated because of the rapid decline of malaria in the region, which started in 1994. When 229 patients (200 adults and 29 children aged < 15 years) were included, study enrollment stopped.

Baseline patient characteristics are shown in Table 1. We excluded 2 children who, upon review of the slides, were found to have vivax malaria, leaving 227 patients for analysis. Some patients with hyperparasitemia (> 100,000/µL) were enrolled. They were included in the analysis because there were no complications of malaria. There were no significant differences between the 2 groups.
Table 1
Characteristics of patients treated with artemisinin monotherapy of different duration for uncomplicated falciparum malaria*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>5 days</th>
<th>7 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>115</td>
<td>112</td>
<td>227</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>98/17</td>
<td>95/17</td>
<td>193/34</td>
</tr>
<tr>
<td>Median age, yr (range)</td>
<td>25 (4--58)</td>
<td>25 (7--50)</td>
<td>25 (4--58)</td>
</tr>
<tr>
<td>Median weight, kg (range)</td>
<td>49 (15--62)</td>
<td>50 (17--65)</td>
<td>49 (15--65)</td>
</tr>
<tr>
<td>Artemisinin dose, mg/kg (95% CI)</td>
<td>10.2 (10.0--10.3)</td>
<td>10.3 (10.0--10.5)</td>
<td>10.2 (10.1--10.4)</td>
</tr>
<tr>
<td>GM initial parasitemia</td>
<td>12,589</td>
<td>12,861</td>
<td>12,725</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(10,251--15,460)</td>
<td>(10,092--16,391)</td>
<td>(10,869--4,895)</td>
</tr>
</tbody>
</table>

* CI = confidence interval; GM = geometric mean asexual stage per microliter.

All patients tolerated the medication well. No significant adverse effects of the trial medication were noted. Three patients experienced prolonged headaches, and 2 patients experienced diarrhea after taking medication.

Some outcome parameters are shown in Table 2. There was a difference in $K_A$ and the derived $t_{1/2A}$ between the 2 regimens, but when adults were analyzed separately from children, this difference disappeared. On average, the elimination rate in regimen A7 was somewhat slower.
Table 2
Outcome parameters of artemisinin monotherapy of different duration in patients with uncomplicated falciparum malaria*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Artemisinin monotherapy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>5 days</td>
<td>7 days</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>115</td>
<td>112</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radical cure</td>
<td>86</td>
<td>87</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Recrudescence</td>
<td>28 (24%)</td>
<td>24 (21%)</td>
<td>52 (23%)</td>
<td>0.6†</td>
</tr>
<tr>
<td>Early</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Late</td>
<td>19</td>
<td>18</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Median PCT, hr (range)</td>
<td>39 (8-112)</td>
<td>43 (38-104)</td>
<td>41 (8-112)</td>
<td>0.085</td>
</tr>
<tr>
<td>Median t₁/₂ₑₜ, hr (range)</td>
<td>5.1 (1.5-18.3)</td>
<td>6.2 (1.9-21.7)</td>
<td>5.5 (1.5-21.7)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

* PCT = parasite clearance time; R2 = initial decrease of parasite count to < 25% of the initial value, followed by resurgence, without clearance by Day 7; t₁/₂ₑₜ = parasite elimination half-life.

† Chi-square test for radical cure versus recrudescence.

One patient each in both groups had an R2 response. These 2 patients required additional treatment, although they showed signs of clinical recovery. All other patients had an uneventful recovery. There were no clinical failures and no R3 responses. Recrudescence occurred in 23% of the total study population. No significant difference in recrudescence rates was observed between the 2 treatment groups. Recrudescent cases were successfully treated with artesunate-mefloquine or artesunate-quinine combinations.

There was no significant difference between Groups A5 and A7 in the proportional cumulative parasite clearance and recrudescence (Figure 1). There was a trend toward an effect of treatment regimen on parasite clearance ($P = 0.069$, relative risk [RR], 1.281, 95% confidence interval [CI], 0.981--1.6735) where A5 was associated with a slightly more rapid rate. This was independent of weight, age, and initial parasite
count, which itself had a significant effect on parasite clearance \((P < 0.001, \text{RR}, 0.8, 95\% \text{ CI}, 0.7--0.9)\). Recrudescence rates were similar in the 2 treatment regimens \((P = 0.6, \text{RR}, 1.17, 95\% \text{ CI}, 0.7--2.0)\). There was a slight effect of the initial parasite count on outcome \((P = 0.047, \text{RR}, 1.3, 95\% \text{ CI}, 1.0--1.6)\) in such a way that independent of treatment regimen, a higher initial parasite count was slightly associated with a higher risk of recrudescence.

![Graph showing parasite clearance and recrudescence](image)

**Figure 1.** Kaplan-Meier curves of the cumulative parasite clearance (left) and recrudescence (right) in patients with uncomplicated falciparum malaria treated with artemisinin monotherapy for 5 (A5, continuous line) or 7 (A7, broken line) consecutive days.

The simulation of the time course of the parasitemia is shown in Figure 2. Overall, \(P_{\text{term}}\) was significantly lower for patients with radical cure or late recrudescence than with early recrudescence \((P = 0.034, \text{t-test})\). When \(P_{\text{term}}\) was calculated for 2 different lengths of effective treatment, this effect was similar. In a Cox regression model in which recrudescence is not subdivided into early and late, the value of \(P_{\text{term}}\) had a small but statistically significant effect on the hazard function of recrudescence \((P =\)
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0.046, RR, 1.105, 95% CI, 1.002-1.22). The geometric mean (95% CI) values of $P_{\text{term}}$ were $3.3.10^{-3}/\mu L$ (8.6.10^{-4} to 1.2.10^{-2}) for patients with a radical cure, $2.9.10^{-3}/\mu L$ (2.5.10^{-4} to 3.5.10^{-2}) for late recrudescence, and $3.4.10^{-1}/\mu L$ (2.2.10^{-2} to 5.2) for early recrudescence. The discriminating power for radical cure from recrudescence was low.

At baseline 30 (13%) patients had gametocytes. Of these, 24 (80%), 15 (50%), 11 (36.7%), and 10 (34%) were still gametocytemic at Weeks 1, 2, 3, and 4, respectively. Among the remainder who had no gametocytes at baseline, 9 of 193 (5%), 5 of 192 (3%), 1 of 176 (0.5%), and 1 of 150 (0.6%) were gametocytemic on Days 7, 14, 21, and 28, respectively.

![Figure 2](image)

**Figure 2.** Geometric mean simulated parasite count during treatment with artemisinin for 5 days (A5) or 7 days (A7). Patients with radical cure and late and early recrudescence are shown. The limit of detection is set at 5 parasites/µL. Arrows indicate dosing times of artemisinin; shaded areas indicate the duration of effective drug therapy of the 2 regimens at the end of which $P_{\text{term}}$ was calculated.
Discussion

In treating uncomplicated falciparum malaria, artemisinin monotherapy led to rapid clinical recovery and parasite clearance. It was safe and well tolerated. However, the rate of parasite recrudescence was unacceptably high.

During the study period, diagnosis and treatment of malaria were offered free of charge by official health care providers and so prereferral drug use was very limited. A prohibition of the treatment of malaria in the private sector was important in this matter. There was no significant difference between the 2 treatment regimens in parasite elimination rates, although the parasite clearance time was observed to be slightly more rapid in regimen A5. This was likely a matter of chance because throughout the time of parasite clearance, the 2 regimens were similar, and so should have been parasite elimination. Parasite clearance time is a rather crude measure of efficacy and so is probably also the estimate of the parasite clearance rate.

No R3 resistance was observed in our study, and the 2 patients with an R2 response did not develop any complications of malaria. The recrudescence rates in regimens A5 and A7 were high, but it should be noted that late recrudescence cannot unambiguously be differentiated from reinfection in such an endemic area. This is illustrated in Figure 2, where the simulated parasite kinetics in radical cure and late recrudescence behave similarly.

Other studies into the effects of extending the duration of artemisinin monotherapy are limited. In a study from Viet Nam that compiled different noncomparative studies, the recrudescence rate was highest in patients treated for falciparum malaria with artemisinin for 5 or fewer days (50%), but it ranged 10--23% for patients receiving the drug for 5--10 days [10]. A compilation of studies from China and Thailand suggests that extending the duration of treatment is more effective [14]. In contrast, in this study, we show that extending the duration of artemisinin treatment from 5 to 7 days is not useful. It is not easy to explain the absence or presence of a difference between the 2 regimens because at their principal difference, 5 or 7 days of treatment, the parasitemia is already below the detection threshold. The significant effect of the initial parasite count on the parasite clearance is in this aspect just another way of expressing the kinetic relation between these parameters. Put simply, it takes longer to eliminate a greater biomass of parasites.
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To obtain more insight, we introduced the simulation of the time course of the parasitemia and the concept of $P_{\text{term}}$. As shown in Figure 2, there is the suggestion of an absolute minimum of the parasitemia. Below this threshold, early replication of asexual parasites would not occur anymore. However, late recrudescence does occur, and further studies will reveal whether this is real recrudescence or reinfection. A sufficiently low value of $P_{\text{term}}$ can be achieved in 2 ways. The first is to ensure effective blood concentrations for a long enough time. The second is to use drugs with fast parasite clearance such as artemisinin. In a previous study, we showed that extending the duration of quinine monotherapy was equally effective in lowering $P_{\text{term}}$ and reducing recrudescence, as was adding a single starting dose of artemisinin before administration of quinine $^{[12]}$. The critical value of $P_{\text{term}}$ to prevent early recrudescence was $\sim 1$ parasite/μL, comparable to the value found in this study.

In this study, extending artemisinin monotherapy was not beneficial. This may partly be explained by induction of enzymatic transformation of artemisinin and the consequent decline of blood concentrations after repeated doses $^{[3,4]}$. This could be confirmed by studying whether introducing drug-free intervals could overcome the problem of enzyme induction. There are a number of unanswered questions. Do antimalarials act on the few parasites remaining below the microscopic detection limit? If so, how do drugs act on those few remaining parasites? Do parasites hide from artemisinin? Are there dormant survivors? These are all questions concerning the eradication of trophozoites that need further study.

The effect of artemisinin on gametocytes in this study was limited. Gametocytes at baseline (before treatment) were cleared relatively slowly, and during follow-up, a minority of patients (9%) started to develop new gametocytes. Artemisinin has been shown to have some gametocytocidal effect for early-stage gametocytes both in vitro and in refeeding experiments $^{[15,16]}$. There is a suggestion that artemisinin and its derivatives may have an effect on the epidemiology of malaria by their effect on gametocytes $^{[17]}$. In this study, the effect could not be compared because both regimens contained artemisinin.

To date, resistance to artemisinin and derivatives has not been reported. However, extensive use of artemisinin drugs, particularly unsupervised and incomplete regimens, as was observed in Viet Nam $^{[9]}$, may contribute to the development of resistance in the future. Combination therapy may effectively combat this threat. Indeed, several studies have shown that combining artemisinin drugs with other
agents such as mefloquine or lumefantrine offers effective and practical treatment regimens\textsuperscript{[18,19]}. Disappointingly, these safe and effective alternatives are expensive. As long as this is the case, artemisinin monotherapy will inevitably be applied, and this requires careful follow-up of treatment efficacy.

In conclusion, artemisinin monotherapy is effective for the treatment of \textit{P. falciparum} malaria with a rapid clinical recovery, defervescence, and parasite clearance. But with both 5- and 7-day courses, the rate of recrudescence is high. A 7-day course is not superior to a 5-day course; this is probably an effect of declining plasma concentrations after repeated dosing. This should be interpreted as an additional argument for combination therapy.

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