Health problems in the forested mountains of southern Viet Nam

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Citation for published version (APA):
Le, H. Q. (2004). Health problems in the forested mountains of southern Viet Nam.
Chapter 2

Artesunate with mefloquine at various intervals in non-severe falciparum malaria.

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American Journal of Tropical Medicine & Hygiene; 2004: in press
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ABSTRACT

To study the efficacy, tolerance, population pharmacokinetics and pharmacodynamics of artesunate followed by mefloquine at various intervals, 360 patients with falciparum malaria received 4 mg/kg artesunate and thereafter 15 mg/kg mefloquine simultaneously (A), after 8 h (B) or 24 h (C). Three dosages were completed with placebo. Follow up was 28 days.

All patients recovered rapidly except one case of failure within the first 24 h. Mefloquine pharmacokinetics was similar in the three regimens. Parasites reappeared in 26%, 26% and 33% of patients in groups A, B and C respectively. Early recrudescence was associated with high initial parasite density, slow parasite clearance and rapid mefloquine clearance and low plasma concentrations at day 28. Mefloquine plasma concentrations all reached therapeutic ranges, suggesting reduced parasite sensitivity.

In conclusion, there is no interaction between artemesunate and mefloquine with respect to tolerance, efficacy and pharmacokinetics. Single dose combination therapy with artemisinin drugs and 15 mg/kg mefloquine does not completely prevent parasite recurrence and may not prevent mefloquine resistance.
Chapter 2

INTRODUCTION

*Plasmodium falciparum* malaria was a major health problem in the remote areas of Viet Nam. A policy of early diagnosis and treatment of malaria, applying short drug combination regimens with artemisinin drugs and longer acting agents, was instrumental in bringing malaria under control in most areas of Viet Nam during the last decade. Several studies of artemisinin monotherapy and combination therapy were performed in Viet Nam, including combinations with quinine, sulfadoxine/pyrimethamine, doxycycline and mefloquine. (1-6) Single dose combination regimens with mefloquine appeared to be practical and became the first choice of treatment. (7) The Vietnamese policy on dosing mefloquine was conservative. While in nearby Thailand increasing resistance necessitated to increase the mefloquine dose to 25 mg/kg, a lower dose was applied in Viet Nam because it was assumed that the combination with artemisinin derivatives, would delay development of mefloquine resistance. Previous studies however, showed that a single dose of artemisinin plus 10 mg/kg mefloquine did not prevent recrudescence sufficiently (15%). (8) Without further evidence of resistance the recommended dose of mefloquine was increased to 15 mg/kg, a dose that initially worked well as monotherapy in Thailand. In combination with a single dose of 4 mg/kg artemunate it became standard therapy for falciparum malaria in large parts of Viet Nam. However, the efficacy of this regimen in Viet Nam was not confirmed at the time of its introduction and a possible interaction between artesunate and mefloquine was not yet confirmed. (9) (10)

In order to establish the efficacy of the single dose artesunate-mefloquine regimen for uncomplicated falciparum malaria in Viet Nam, and to study the tolerance, pharmacokinetics and pharmacodynamics of different timing of the mefloquine dose, a controlled randomized double blind study was performed in which a single dose of 4 mg/kg artesunate was followed by 15 mg/kg mefloquine, given at various intervals.

METHODS

Patient selection and treatment

From December 1997 to March 2001, subjects aged 6 years or older with a symptomatic *P. falciparum* (asexual blood stages with $1,000/\mu l < \text{parasitemia} < 200,000/\mu l$) infection were enrolled at five primary health care posts and one district hospital in Binh Thuan province, southern Viet Nam. Giemsa stained thick and thin blood smears were obtained for identification of asexual parasites by light microscopy prior to patient inclusion. Exclusion criteria included *P. vivax* infection, pregnancy, lactation, any sign of severe or complicated malaria, concurrent chronic disease, inability to take oral medication, known allergy to the study drugs and intake of antimalarial agents in the preceding period as follows: artemisinin or derivatives (e.g. artesunate) in the previous 24 hours; mefloquine, tetracycline or doxycycline in...
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the previous seven days or quinine in the previous 12 hours. Patients could be entered into the study for a second time if there were at least three months between the first and second date of entry.

The sample size was sufficient to detect a difference between approximately 30% and 15% response rates with statistical significance at \( \alpha = 0.05 \) and \( \beta = 0.2 \). Artesunate (PlasmoTrim® Lactabs) 50 mg, and mefloquine (Mephaquine®) tablets 250 mg and mefloquine placebo tablets were obtained from Mepha AG, Aesch, Switzerland.

All patients were admitted. After informed consent the treatment regimen was allocated by drawing an envelope, which included a computer-generated randomized dosage regimen, the mefloquine and placebo tablets and a timetable for drawing blood samples, starting just before the first drug was administered (t0). All patients received an initial dose of 4 mg/kg artesunate. Mefloquine was administered simultaneously, (regimen A), 8 h later (regimen B) or 24 h later (regimen C). By breaking tablets the dose nearest to 15 mg/kg bodyweight was chosen. Mefloquine placebo was given to complete three dosages for all patients. Medication was taken under supervision. Vomiting within 1 hour after intake of trial medication was reason for withdrawal and installation of another treatment. These patients would be evaluated for tolerance and for efficacy on intention to treat basis.

Written informed consent was obtained from all patients or their parents. The study protocol was approved by the research committee of Cho Ray Hospital, Ho Chi Minh City, and the medical ethics committee of the Academic Medical Center, Amsterdam.

Patients follow up

The parasite count was determined every eight hours until three negative smears had been obtained. Blood smears were repeated on 7, 14, 21 and 28 days after t0. Parasites were counted against 200 white blood cells in the thick smear, and, by recalculating it to the patient’s total leucocyte count, expressed as the number of parasites per \( \mu l \) of blood. A full blood count was determined on t0 and t = 48 h. All blood smears were retained and reviewed by an experienced technician at the department of parasitology of Cho Ray Hospital. His results were taken as gold standard in case of conflicting results.

Blood was collected four times for measuring mefloquine plasma concentrations. In order to ensure variable sampling points for a population pharmacokinetic analysis, the first 48 hours were divided in intervals of four hours from which the first two sampling points were chosen at random, starting at t=4 for regimen A, t=12 for regimen B and t=28 for regimen C. Additional samples were collected at \( t = 168 \) h (7 days, \( C_d7 \)) and day 28 (\( C_d28 \)). The selection procedure of sampling times was unknown to researchers and clinicians, in order to avoid that clever deduction would reveal the treatment regimen. Blood was drawn into heparinized tubes, centrifuged immediately and plasma was stored at minus 30°C.
untll analysis. Mefloquine, and its inactive carboxy metabolite, were measured using an high-performance liquid chromatography as described previously.(11) Mefloquine recovery of this assay was >95% with a detection level of 50 μg/L or lower. Patients who suffered from a recurrence of parasites received additional medication. They returned on day 28 for determination of the mefloquine plasma concentration Clinical and parasitological outcome were assessed separately.(12;13) “Cure” was defined as a disappearance of symptoms and of parasites; deterioration of the patient’s condition within the first 24 h after initiation of therapy was classified as “early clinical failure”, and later as treatment failure, irrespective of parasitological outcome. Early clinical failure was not attributed to failure of study drugs. Parasitological response was defined as follows: parasite recurrence: parasite clearance within 7 days but reappearance within the first 14 days after t0 (early recurrence, ER) or from day 14 until 28 (late recurrence, LR); R-II: initial reduction of parasite count (>75%) without clearance within the first 7 days; R-III: no or less response. Molecular techniques to distinguish re-infection from recrudescence were not available. Fever and parasite clearance times (FCT and PCT) were defined as the time from t0 to the first of three consecutive normal temperature readings (< 37.0°C axillary) or negative blood smears respectively.

Early clinical failure was treated with intravenous artesunate, parasite recurrence with proguanil plus atovaquone (Malarone®). To avoid interference with the pharmacokinetics, no mefloquine was prescribed to treat recrudescence.

Analysis of data

The time course of the parasite count was fitted to a mono-exponential elimination model. Log-linear mixed effects model were applied using maximum likelihood (ML) techniques in S-plus (v. 4.5, Math Soft inc., Seattle) as explained in previously.(14) The model estimates of the initial parasite count, P(0)est and the parasite elimination rate constant, k, were generated for every patient using restricted maximum likelihood method (REML) and from this the time to clear 50% (PC50, parasite elimination half life) and 90% (PC90) of parasites were calculated. The interaction with the variables regimen, age, sex, artesunate, mefloquine dose and pharmacokinetic parameters were entered as covariates. The mefloquine pharmacokinetics were analyzed in a similar way with non-linear mixed effects models to estimate the pharmacokinetic parameters for every individual.

Further analysis of the patients’ data was carried out using SPSS (v. 11.0, SPSS Inc. Chicago, Ill). Intention to treat analysis included survival analysis and calculation of extreme case scenarios in which patients who were lost to follow were reallocated to either complete cure or to parasite recurrence.(15) Contingency tables and Chi square tests with continuity correction were applied to categorical variables. Numerical variables were tested for Normality before it was decided whether to use analysis of variance (ANOVA), or the Kruskal-Wallis test, to compare groups. Parasite clearance and recrudescence were analyzed with survival analysis (Kaplan
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Meier plots and the Cox proportional hazard model. Correlation was expressed by Pearson's correlation coefficient, r. Statistical significance was accepted if p<0.05.

A blinded interim analysis of outcome, was performed midway the study by a statistician not involved in the study. Differences between the three treatment groups were not found at that time.

RESULTS

Three hundred sixty patients were recruited. Tolerance to the study medication was good. The main complaints on 

t0 were dizziness (n=202), muscle pain (n=163), anorexia (n=160), arthralgia (n=120), nausea (n=82), tremor (n=66), dry mouth (n=51) and vomiting (n=24), all equally distributed over the three treatment groups. During the first 24 h nine additional patients vomited, four in group A, and five in group C, the latter before receiving mefloquine. Vomiting of trial medication did not occur. All symptoms subsided within three days and no probable or certainly drug related adverse effects were recorded.

![Figure 1: Patient flow sheet. Numbers in cadres are eligible for analysis of outcome. Numbers in circles only for intention to treat or survival analysis.](image-url)
The patient flow sheet (figure 1) shows which patients were used for analysis of proportions and which patients were only eligible for the extreme case scenarios and survival analysis. Twenty-three patients were excluded from the analysis because upon review of the blood slides the parasite count at baseline was below 1000/μl. One patient in group C deteriorated within 8 hours after start of therapy and was classified as an early clinical failure. Parasites were still detectable on day 7 in three patients. They had recovered completely and were classified as clinical cure with parasitological R2 response. Three patients left before any endpoint was reached and two patients left after parasite clearance but did not return on day 7 so that an R2 response could not be excluded. There was no significant difference among the three groups with respect to initial treatment outcome. Of the remaining 328 patients who were initially cured and completed parasite clearance before day 7, 11 cases were lost during follow up at some stage. Their results were entered into extreme case scenarios. The radical cure rates were in a best case scenario 75% in group A, 75% in group B and 69% in group C (Chi-Square test, p = 0.49). In a worst case scenario the ratios were 72%; 73% and 63% (Chi-Square test, p = 0.22) respectively. The rate of recrudescence was not associated with the patients' date of enrollment. The proportional cumulative parasite clearance and recrudescence curves are shown in figures 2. The mean (95% c.I.) PCT and FCT were 40.6 (38.5 – 42.8) h and 23.7 (22.4 – 25.0) h respectively.

Figure 2. Kaplan-Meier curve showing the survival function of *P. falciparum* parasitemia (panel a) and recrudescence (panel b), treated with regimens A (solid line), B (gray line) or C (broken line).
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Figure 3. Parasite population dynamics, treated by A4M3 combination regimens and stratified by outcome. *Observed values (blue line)*; *RI early recrudescence (gray line)*; *RI late recrudescence (green line)*; *Radical cure (red line)*.

The best population model of the time course of the parasite count, is described by the mono-exponential function:

\[ P(t) = P(0)_{\text{est}} \cdot e^{-k_{p}t} \quad (1) \]

in which \( P(t) \) is the parasitemia as a function of time and \( P(0)_{\text{est}} \) the fitted value of the initial parasite count (the intercept with the ordinate). The linear mixed effect model was improved when a lag time of three hours was introduced, which also explains the difference between the observed initial parasite count, \( P(0)_{\text{obs}} \), and \( P(0)_{\text{est}} \) (figure 3). There was no significant effect of age, body weight, sex, and treatment regimen on the goodness of fit of the model. However, when patients with complete cure, early and late recrudescence were compared, the parasite clearance dynamics were significantly different. The parameter estimates are shown in Table 2. Patients with an early recrudescence had a significantly slower parasite clearance and higher \( P(0)_{\text{est}} \) than patients with radical cure or late recrudescence and this was confirmed in a Cox survival model of parasite recurrence (\( p < 0.001 \) for \( P(0) \) and \( P < 0.001 \)). In terms of relative risks, this means that for every hour prolongation of the PC50, the risk of parasite recurrence increases 1.36 times (95% c.l. 1.06 – 1.21), and for every ten-fold increase of \( P(0) \) the risk of recurrence increases 1.7 fold (95% c.l. 1.3 – 2.1).

After exclusion of plasma samples with illegible identification codes or leakage, plasma samples of 250 patients were available for measurement of mefloquine concentrations of which 179 had a complete series of four measurements, available for population pharmacokinetics.

A two compartment elimination model with first order absorption yielded the best fit, in formula:

\[ P(0) = P(0)_{\text{est}} \cdot e^{-k_{p}t} \]
\[ C(t) = C_{01}e^{-k_1t} + C_{02}e^{-k_2t} - (C_{01} + C_{02})e^{-k_at} \quad (2) \]

in which \( C(t) \) is the plasma concentration (\( \mu g/L \)), \( t \) is the time after intake of the mefloquine dose (h), \( k_1 \) and \( k_2 \) are the distribution and elimination rate constants (h\(^{-1}\)) with their respective intercepts \( C_{01} \) and \( C_{02} \) (\( \mu g/L \)), \( k_a \) is the absorption rate constant (h\(^{-1}\)). With four data points per patient a maximum of four kinetic parameters can be estimated. Therefore the terminal elimination \( k_2 \) and its intercept \( C_{02} \) were fitted first and their mean value was entered as constants in the population model. For \( k_2 \) the value was 0.00217 h\(^{-1}\), corresponding to an elimination half life of 319 h (13 days). \( C_{02} \) was 1506 \( \mu g/L \). The maximum plasma concentration, \( C_{\text{max}} \), was derived numerically. The area under the concentration time curve, from \( t=0 \) until infinity (AUC\( _{0-\infty} \)) was calculated according as:

\[ \text{AUC}_{0-\infty} = \frac{C_{01}}{k_1} + \frac{C_{02}}{k_2} - \frac{(C_{01} + C_{02})}{k_a} \quad (3) \]

The pharmacokinetic data are specified per treatment regimen in table 1. Figure 4 shows that the fitted individual concentration time curves are close to the observed values but tend to underestimate \( C_{\text{max}} \) in the lower ranges and \( C_{28} \). The plasma concentrations on day 7, observed as well as estimated by the pharmacokinetic model, were lowest in group A and highest in group C, due to the difference in mefloquine dosing time, but this did not reach statistical significance (data not shown). The observed mefloquine plasma concentrations on day 28 showed a similar trend with the highest values in group C and the lowest in group A (table 1).

**Table 1.** Characteristics of patients with *Plasmodium falciparum* infections treated by artesunate with mefloquine given at various intervals.

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>Treatment regimen</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>No. of patients for evaluation</td>
<td>114</td>
<td>110</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>14 / 100</td>
<td>13 / 97</td>
</tr>
<tr>
<td>Age</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>(years, mean [range])</td>
<td>[12 - 58]</td>
<td>[9 - 51]</td>
</tr>
<tr>
<td>Body weight (kg, median [range])</td>
<td>52.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Body temperature (°C, mean [range])</td>
<td>39.5</td>
<td>39.5</td>
</tr>
<tr>
<td>P(0)(_{obs}) (10(^3)/μL, GM [95% CI])</td>
<td>10.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Artesunate dose (mg/kg, mean, [range])</td>
<td>3.94</td>
<td>3.90</td>
</tr>
<tr>
<td>Mefloquine dose (mg/kg, mean, [range])</td>
<td>14.78</td>
<td>14.65</td>
</tr>
</tbody>
</table>
**Artesunate with mefloquine for falciparum malaria**

**Table 1. (cont.)**

<table>
<thead>
<tr>
<th>Mefloquine pharmacokinetics</th>
<th>Treatment regimen</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>No. of patients for evaluation</td>
<td>72</td>
<td>56</td>
</tr>
<tr>
<td>$T^{\frac{1}{2}}_{\text{abs}}$ ($h$, med., [range])</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>$C_{\text{max}}$ ($\mu g/L$, GM, [range])</td>
<td>2590</td>
<td>2768</td>
</tr>
<tr>
<td>$AUC_{0-x}$ ($mg/L-h$, median, range)</td>
<td>838</td>
<td>869</td>
</tr>
<tr>
<td>$T^{\frac{1}{2}}_{\text{el}}$ ($h$, med., [range])</td>
<td>322</td>
<td>326</td>
</tr>
<tr>
<td>$C_{28}$ ($\mu g/L$, GM [range])</td>
<td>316</td>
<td>352</td>
</tr>
</tbody>
</table>

$P(0)_{\text{obs}}$: observed initial parasite count; $AUC_{0-x}$: area under the mefloquine plasma concentration time curve; $C_{28}$: observed mefloquine plasma concentration on day 28; med.: median; GM: geometric mean; 95% CI: 95% confidence interval; \(a\): Chi-square test; \(b\): Kruskal–Wallis test; \(c\): Analysis of Variance.

**Table 2. Dynamics of *P. falciparum* clearance, after treatment with artemunate and mefloquine, by outcome.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outcome</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radical cure</td>
<td>Late recrudescence</td>
</tr>
<tr>
<td>$P(0)_{\text{obs}}$ ($10^9/\mu L$, GM [95% CI])</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>($10^9/\mu L$, GM [95% CI])</td>
<td>[8.4-11.4]</td>
<td>[7.3-13.2]</td>
</tr>
<tr>
<td>$P(0)_{\text{fig}}$ ($10^9/\mu L$, GM [95% CI])</td>
<td>13.9</td>
<td>15.9</td>
</tr>
<tr>
<td>($10^9/\mu L$, GM [95% CI])</td>
<td>[12.1-15.9]</td>
<td>[11.9-21.1]</td>
</tr>
<tr>
<td>PC50 ($h$, mean [95% CI])</td>
<td>5.6</td>
<td>5.7</td>
</tr>
<tr>
<td>($h$, mean [95% CI])</td>
<td>[5.4-5.9]</td>
<td>[5.2-6.3]</td>
</tr>
<tr>
<td>PC90 ($h$, mean [95% CI])</td>
<td>18.7</td>
<td>19.0</td>
</tr>
<tr>
<td>($h$, mean [95% CI])</td>
<td>[17.8-19.5]</td>
<td>[17.2-20.9]</td>
</tr>
<tr>
<td>PCT ($h$, mean [95% CI])</td>
<td>38.2</td>
<td>43.5</td>
</tr>
<tr>
<td>($h$, mean [95% CI])</td>
<td>[35.6-40.8]</td>
<td>[39.0-48.0]</td>
</tr>
</tbody>
</table>

32
Varialbe & Radical cure & Late recrudescence & Early recrudescence & p-value \\
\hline
Mefloquine pharmacokinetics & & & & \\
\hline
T½\text{abs} \hspace{1cm} (h, med. [range]) & 4.8 & 4.8 & 4.8 & 0.9 \text{b} \\
\hline
C\text{max} \hspace{1cm} (\mu g/L, GM [range]) & 2631 & 2714 & 2259 & 0.633 \text{a} \\
\hline
AUC\text{0-\infty} \hspace{1cm} (mg/L-h, med.[range]) & 814 & 804 & 790 & 0.554 \text{b} \\
\hline
T½\text{el} \hspace{1cm} (h, med., [range]) & 318 & 408 & 220 & 0.001 \text{b} \\
\hline
C\text{d28} \hspace{1cm} (\mu g/L,GM, [range]) & 353 & 427 & 206 & 0.001 \text{a} \\
\hline

\text{P(0)}_{\text{obs}}: \text{observed initial parasite count}; \text{P(0)}_{\text{est}}: \text{initial parasite count estimated by a mixed effects population model of the parasite clearance}; \text{PC50 and PC90}: \text{time to reduction of initial parasite count by 50\% and 90\% respectively}; \text{PCT}: \text{parasite clearance time}; \text{med.}: \text{median}; \text{AUC\text{0-\infty}}: \text{area under the mefloquine plasma concentration time curve}; \text{C\text{d28}}: \text{observed mefloquine plasma concentration on day 28}; \text{GM}: \text{geometric mean}; \text{95\% CI}: \text{95\% confidence interval}; \text{a}: \text{Analysis of Variance}; \text{b}: \text{Kruskal-Wallis test}.

Table 2 shows that the mefloquine concentration was significantly different among the sub-groups with cure, early and late parasite recurrence, especially C\text{d28}, C\text{max} and AUC\text{0-\infty} were lower in patients with an early recrudescence but this did not reach statistical significance (ANOVA and Cox regression model of parasite survival). This also applies to the observed and estimated mefloquine plasma concentration on day 7 (data not given). The terminal elimination half-life was shorter and C\text{d28} was lower in patients with early parasite recurrence.

The minimum in vivo parasitocidal plasma concentration of a fully sensitive parasite strain, 500\mu g/L, is also shown in figure 4. It shows that for all patients the plasma concentration was above this value for a certain period. On day 28, the mefloquine plasma was still higher then 500 \mu g/L in 28\% of the patients with complete cure, 45\% in late recrudescence and 10\% in early recrudescence (p =0.037). Based on the population model, the estimated mefloquine plasma concentrations were above 500 \mu g/L during a mean interval of 507 (504-560) h. This was similar for the three regimens and treatment outcomes. However, this interval is a conservative.
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estimate since the model underestimates the observed terminal plasma concentrations and, consequently, also the interval of plasma concentrations above 500 μg/L.

![Graph showing mefloquine plasma concentrations over time]

**Figure 4.** The observed plasma mefloquine concentrations and the time course fitted by a population pharmacokinetic model. *Three patients are shown, with the highest (circles), median (triangles) and lowest (squares) mefloquine plasma concentrations, coincidentally, all treated by regimen C. The mean inhibitory concentration (500 μg/L) of a fully sensitive strain is also indicated. *MPC: Minimum in vivo parasidal concentration of sensitive strain 500mg/L.

**DISCUSSION**

This study shows that a single dose of artemisinin with mefloquine 15 mg/kg does not prevent recurrence of *P. falciparum* adequately in Viet Nam. Parasite recurrence was associated with a high initial parasitemia, slower parasite clearance and lower mefloquine plasma concentrations.

Early recurrence, during the first two weeks, is usually a true recrudescence, late parasite recurrences may include re-infections. Due to the long plasma residence time of mefloquine and the low incidence of malaria in our study area at present, over-estimation of the true recrudescence rate in this study is probably little. On the contrary, other studies indicated that the follow up after mefloquine treatment should be extended until 63 days.(16) Since the follow up in this study was only 28 days, the real parasite recurrence rate may be somewhat higher.

The pharmacodynamics of this antimalarial combination can be divided into two components, initial parasite elimination and complete eradication. The fast initial parasite killing and clinical recovery are effects of artemisinin and they were similar to
values found in other studies with artemisinin drugs. No synergy with mefloquine was observed. The explanation of the unacceptably high rate of incomplete parasite eradication is more complex. Recrudescence, a frequent complication of monotherapy with artemisinin drugs, is associated with high initial parasite counts and with slower parasite clearance rates, but not with resistance to artemisinin. Exposure to effective artemisinin blood concentrations is often not long enough due to their short plasma residence time and time dependent decline of blood concentrations. This is the pharmacodynamic basis for combining artemisinin drugs with longer acting agents, to ensure long suppressive drug concentrations.

Complete eradication of all parasites, therefore depended on exposure to mefloquine, but apparently this was insufficient. This may be caused by two factors, sub-therapeutic plasma concentrations and resistant parasites. The plasma concentrations depend on dose, bioavailability and disposition. The dose was strongly correlated with $C_{\text{max}}$ and AUC$_{0-\infty}$ but these were not associated with parasite recurrence. Variable disposition caused variation in plasma concentrations on day 28. Low plasma concentrations on day 28 were associated with parasite recurrence indicating that duration of exposure to parasitocidal plasma concentrations is important complementary to the height of peak concentrations.

Synergy between artesunate and mefloquine was not detected, mainly because by the time that mefloquine starts to exert its activity, artesunate is no longer present in the blood. Mefloquine pharmacokinetics were not different between the three treatment groups, except for a difference in the observed $C_{d28}$. This difference is explained by the different timing of mefloquine administration, although the magnitude of the difference is not completely explained by the 8 and 24 h differences in dosing time and the estimated $t_{1/2e}$. Pharmacokinetic interactions between artesunate and mefloquine, as suggested by previous authors, were not found and hence do not explain the subtherapeutic mefloquine plasma concentrations in patients with parasite recurrence. Similarly, the improvement of bioavailability of mefloquine or artesunate during recovery from malaria, reported by Thai authors, was not confirmed in this study. Other potential sources of variation in drug disposition, not studied here, are stereoselective pharmacokinetics and pharmaceutical bioavailability. The pharmaceutical bioavailability of the mefloquine formulation, used in this study, is possibly lower than of other formulations but this does not explain inter-individual variation as a cause of parasite recurrence.

Incomplete eradication of parasites may also have been caused by resistance of parasites. The official definition of resistance is based on the administration and absorption of a therapeutic, non-toxic, dose of an anti-malarial drug, and not on plasma concentrations. The mefloquine dose used in this study (15 mg/kg) was lower than the therapeutic dose which is recommended for South East Asia (25 mg/kg) and that would preclude any statement on parasite resistance. However, the population pharmacokinetic model yielded a rather accurate description of the individual concentration time curves even at the extremes of the population and based on this we can draw some tentative conclusions. The observed mefloquine plasma
concentrations and the concentrations fitted by the model, even though this tends to underestimate the observed plasma concentrations on day 28, came all above the in vivo suppressive (chemoprophylactic) plasma concentration for a fully sensitive strain (500 μg/L to 625 μg/L) for a certain period of time even in the cases with early recrudescence. (31;32) This points at a reduced sensitivity of *P. falciparum* to mefloquine in southern Viet Nam.

Mefloquine resistance has not been documented in Viet Nam before. It may have been overlooked but the general view is that resistance is absent because mefloquine was used mainly in combination with artemisinin drugs. The Vietnamese drug policy has been restrictive towards use of antimalarial agents in the private health sector. Mefloquine was introduced in the public health sector in 1992 and always used in combination with artemisinin drugs. This is different from Thailand, where mefloquine resistance developed within several years of applying mefloquine monotherapy in highly endemic areas. (33-36)

Reduced mefloquine sensitivity is a reason for concern. Firstly because it would show that a policy to use mefloquine only in combination with artemisinin drugs may not prevent development of mefloquine resistance. (23) Secondly because a 15 mg/kg dose of mefloquine is not enough in Viet Nam, despite the induction of rapid parasite clearance by artesunate. This has implications for the use of mefloquine and artesunate in other regions. (37)

This study raises questions if a dose of 15 mg/kg mefloquine should be applied at all, even in areas without documented mefloquine resistance. Notably, in a non-scientific setting, subtherapeutic plasma concentrations will be common because the dose will not always be adapted exactly to body weight and pharmaceutical bioavailability is variable. Even in combination with artemisinin drugs such a low dose of mefloquine could lead to recrudescence and selection of resistant parasite strains. A related question is if only raising the mefloquine dose is sufficient to prevent recrudescence. The experience in Thailand showed that a regimen with 25 mg/kg mefloquine, divided over two days, plus three days of artesunate, is superior to single dose regimens, with respect to tolerance and efficacy. (38;39)

**CONCLUSION**

In conclusion, there is no effect of different dosing times of a 15 mg/kg dose of mefloquine within the first 24 hours after an initial single dose of artesunate. The pharmacodynamics of this low dose artesunate-mefloquine combination reflect the characteristics of artesunate mono-therapy, i.e. parasite recurrence being associated with high initial parasite counts and slower initial parasite clearance, and confirmed the association between parasite recurrence and lower mefloquine plasma concentrations. However, the data also suggest reduced mefloquine sensitivity of *P. falciparum* in Viet Nam. Therefore, the mefloquine dose should be raised to 25 mg/kg, in combination with artemisinin drugs, preferably given for three days.
REFERENCE LIST


Artesunate with mefloquine for falciparum malaria


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