Artemisinin based combination therapy for malaria in Viet Nam
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CHAPTER 3

CV8, a new combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine, compared with atovaquone-proguanil against falciparum malaria in Vietnam

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OBJECTIVES: To study a new combination, based on dihydroartemisinin and piperaquine (CV8) and atovaquone/proguanil (Malarone) for treatment of uncomplicated falciparum malaria in Viet Nam.

METHODS: Vietnamese adults with falciparum malaria were allocated randomly to treatment with dihydroartemisinin/piperaquine/trimethoprim/primaquine 256/2560/720/40 mg (CV8, n = 84) or Malarone 3000/1200 mg (n= 81), both over 3 days. Patients were followed-up for 28 days. All patients recovered rapidly. The mean (95% CI) parasite elimination half-life of CV8 was 6.8 h (6.2–7.4) and of Malarone 6.5 h (6.1–6.9) (P = 0.4). Complete parasite clearance time was 35 (31–39) and 34 h (31–38) (P = 0.9). The 28-day cure rate was 94% and 95%, respectively (odds ratio 0.84, 95% CI 0.18–3.81). No significant side-effects were found.

CONCLUSION: CV8 and Malarone are effective combinations against multi-drug resistant falciparum malaria. CV8 has the advantage of a low price.
Introduction

A combination of antimalarial drugs is advocated for the treatment of *Plasmodium falciparum* malaria, not only because of prevalent drug resistance but also to prevent further development of resistance\(^1,2\). The artemisinin drugs are ideal candidates for combination with other antimalarials (ACT) and resistance of clinical isolates of *P. falciparum* has not been documented so far. They offer rapid parasite clearance and clinical recovery but need to be combined with longer-acting drugs to prevent recrudescence\(^3\). Most importantly, artemisinin drugs can be produced at relatively low cost and offer an affordable alternative for quinine and sulfadoxine/pyrimethamine. Recently, dihydroartemisinin was combined with piperaquine, a bis-aminoquinoline that was first synthesized more than 30 years ago. Trimethoprim and primaquine were added in a fixed combination. The combination of these four agents was selected as the best of different combinations, systematically studied by Chinese researchers working in Viet Nam (Li Quao Guo, Anh Trinh Kim, unpublished observation). It is produced and marketed under the name CV8\(^\text{®}\) in Viet Nam. CV8 was studied in Viet Nam in some preliminary trials and was shown to be effective for treating *P. falciparum* infections\(^4,5\).

The Vietnamese Ministry of Health introduced CV8 in the National Malaria Control Program (NMCP) in 2000 (Decision 3952/QD-BYT of Minister of Health on 9 December 1999). Until that time artemisinin mono-therapy or single dose combinations of mefloquine with artemisinin or artesunate were used. The high recrudescence rates of these regimens, up to 25%, were the reason to change the policy to CV8 as a first line drug. Since then it has been used on a large scale as the first line of treatment of falciparum malaria in many regions in Viet Nam. Formal studies with CV8 were not yet published. To document the efficacy and tolerance of CV8 we performed a clinical study. As the comparator in this randomized clinical trial atovaquone plus proguanil (Malarone) was chosen, a combination not in use in Viet Nam.
CV8 vs atovaquone-proguanil

**Methods**

**Patients and treatment**

This study was conducted in Binh Thuan, a mountainous province in southern Vietnam from April 2001 until August 2002. The study protocol was reviewed and approved by the scientific board of Cho Ray Hospital and the Vietnamese Ministry of Health. All patients who presented with fever at a primary health care facility were evaluated for eligibility. Inclusion criteria were uncomplicated falciparum malaria with parasitaemia of at least 1000/μl and age >16 years. Exclusion criteria included pregnancy, lactation, complicated malaria, inability to take oral medication, known allergy to study drugs and verbal confirmation of the intake of artemisinin derivatives in the previous 24 h, mefloquine, tetracycline or doxycycline in the previous 7 days or quinine in the previous 12 h.

Written informed consent was obtained from all participating patients. After informed consent the treatment regimen was allocated by drawing an envelope with a computer-generated randomization code. The codes were allocated in randomized blocks of 10 with a 1:1 ratio. A total of 90 patients per treatment arm were thought to be sufficient to detect with statistical significance a 50% reduction of a recrudescence rate of 25–30%, values observed in previous studies with artemisinin combinations in this area. Patients received two tablets of CV8 (each containing dihydroartemisinin 32 mg + piperaquine phosphate 320 mg + trimethoprim 90 mg + primaquine phosphate 5 mg, CV8) at t = 0, 8, 24 and 48 h (CV8 regimen) or four tablets of atovaquone 250 mg + proguanil hydrochloride 100 mg (Malarone regimen) at t = 0, 24 and 48 h. When vomiting occurred within 1 hour after intake of drugs, a full second dose of the study medication was given.

CV8 tablets were obtained from Central Pharmaceutical factory 26, Ho Chi Minh City, Vietnam. Malarone tablets were kindly donated by Glaxo Wellcome Inc., UK.

**Patient follow-up**

All patients were admitted to the health facilities where intake of medication was supervised. Vital signs were recorded every 8 h and a complete physical examination was performed every day. Any sign or symptom was recorded and assessed to its possibility of being drug related. A full blood count was performed before patient inclusion (day 0) and on day 2. The parasitaemia was counted every 8 h until three
negative smears had been obtained. Blood smears were repeated on days 7, 14, 21 and 28. The parasite density was expressed as the number of parasites per micro litre of blood. Gametocytes were recorded but not enumerated and the slides of t = 0 or 8 h, regardless of amount, were taken as the baseline of gametocytes. All blood smears were retained and re-examined by an experienced technician of the department of parasitology of Cho Ray Hospital in Ho Chi Minh City. His results were taken as gold standard in case of conflicting results with the study sites. 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.

Cure was defined as disappearance of symptoms of malaria including normalization of temperature and disappearance of parasites. 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 14 to 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 parasitaemia to not <25% of the initial value, assessed at 48 h after initiation of therapy.

Treatment failure and recrudescence were treated according to the local routine. For R2 or R3 responses this usually comprises intravenous administration of quinine or artesunate followed by mefloquine orally. Recrudescence was usually treated with oral artesunate plus mefloquine.

The patient data were analysed with the statistical package SPSS (v. 11; SPSS Inc., Chicago, IL, USA). Outcome was expressed as the proportion of patients who completed the 28 days of follow-up. Patients who, upon review of the blood slides, appeared to be erroneously included, were excluded from the analysis of efficacy. Patients who withdrew from the study before any endpoint was reached and patients who were lost to follow-up after initial cure were analysed, on an intention to treat basis, with survival analysis and extreme case scenarios. 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.
CV8 vs atovaquone-proguanil

The time course of the parasite count was fitted in a non-linear mixed effect population model as described previously [8]. Different models were generated with maximized log-likelihood estimation. They were compared using analysis of variance and the Bayesian information criterion. The variables sex, bodyweight and regimen were introduced in the model as fixed effects to investigate whether this improved the goodness-of-fit. The final population model was used to estimate the parameters per individual by restricted maximum log-likelihood estimation (REML). Statistical significance was accepted when $P < 0.05$.

Results

During the study period 165 patients were included in the study, 84 in the CV8 arm and 81 in the Malarone arm. Tolerance to the study medication was good. In the CV8 group one patient complained about a dry mouth and dermal itch without visible abnormalities. Another patient suffered from a headache. In the Malarone group one patient complained of itch, also without visible abnormalities, and of diarrhoea. Two patients vomited soon after intake of the first dose of Malarone and then withdrew from the study. These effects were rated as possibly related to the study medication but they can also be attributed to malaria. Four patients, two in both groups, were excluded from the analysis of efficacy because upon review the baseline blood slide showed $P. \text{vivax}$, either with or without $P. \text{falciparum}$. This leaves 161 cases for analysis of efficacy. All patients, except the two who vomited after taking Malarone were followed up for 28 days and 92 of them even for 56 days. 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.

The parasites disappeared in all patients of both groups with a similar elimination rate (Figure 1). The treatment results are shown in Table 2. Both regimens offered rapid parasite clearance and defervescence. The two subjects who withdrew were analysed in best and worst case scenarios. Recrudescence was treated successfully with artesunate plus mefloquine. The proportional cumulative parasite clearance and recurrence is shown in Figure 2. There was no significant difference between the two regimens ($P > 0.1$; OR = 0.74, 95% C.I.: 0.30–1.22). Age, sex and body weight did not affect the hazard function.
Table 1: Characteristics of patients with uncomplicated falciparum malaria treated with CV8 or Malarone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV8</td>
</tr>
<tr>
<td>Number of patients included</td>
<td>82</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>9/73</td>
</tr>
<tr>
<td>Age [years, median (range)]</td>
<td>27.0 (16–73)</td>
</tr>
<tr>
<td>Weight [kg, mean (95% CI)]</td>
<td>51.9 (51.1–52.7)</td>
</tr>
</tbody>
</table>

Figure 1: Time course of the geometric mean parasite count after treatment with CV8 or Malarone for uncomplicated falciparum malaria. Error bars indicate the 95% confidence interval. At lower parasite counts the geometric mean is artificially raised because of the decreasing number of remaining positive cases. The straight line indicates the mean population estimate of the parasite clearance curve and is a better reflection of the mean.

The non-linear mixed effects population model of the time-course of the parasitaemia revealed that a mono-exponential model with a single elimination constant, yielded the best description of the decline of the parasite count. In this model the estimate of the parasite count at \( t = 0 \text{h} \), \( P_{0est} \), may be different from the
CV8 vs atovaquone-proguanil

observed parasite count at enrolment, P₀obs. Introduction of a time lag between t = 0 and 8 h gave no improvement of the model. There were no significant effects of sex, bodyweight or treatment regimen on the goodness-of-fit. The geometric mean parasite densities and overall mean fitted elimination curve is shown in Figure 1. In the lower ranges of the parasite count the geometric mean of the observed values deviates from the fitted line because of its artificial distortion when some but not all blood smears become negative. The elimination constant was used to calculate the time needed to reduce P₀est by 50% (PC50) or by 90% (PC90). The mean values of these parameters are shown in Table 2. There was no significant difference of parasite elimination dynamics between patients with radical cure or recrudescence (data not shown).

**Table 2**: Treatment response of *Plasmodium falciparum* infections treated with CV8 or Malarone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment regimen</th>
<th>P-value (95% CI of the difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV8 (n = 82)</td>
<td>Malarone (n = 79)</td>
</tr>
<tr>
<td>No. patients withdrawn</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>No. radical cure (worst/best scenario)</td>
<td>77 (94%)</td>
<td>73 [95%, (92%/95%)]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 [OR 0.84 (95% CI: 0.18, 3.81)]</td>
</tr>
<tr>
<td>No. recrudescences</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Early (&lt;14th day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late (≥14th day)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fever clearance [h, mean (95% CI)]</td>
<td>24.6 (22.3, 26.8)</td>
<td>23.5 (20.8, 26.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4 (-1.9, 4.8)</td>
</tr>
<tr>
<td>Parasite clearance [h, mean (95% CI)]</td>
<td>34.8 (30.9, 38.6)</td>
<td>34.5 (30.7, 38.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.947 (-5.1, 5.5)</td>
</tr>
<tr>
<td>P₀obs [/ll, GM (95% CI)]</td>
<td>19 392 (15000, 25072)</td>
<td>18 020 (14139, 22967)</td>
</tr>
<tr>
<td></td>
<td>0.681 (-4375, 9540)</td>
<td></td>
</tr>
<tr>
<td>P₀est [/ll, GM (95% CI)]</td>
<td>20 785 (16965, 25466)</td>
<td>19 390 (16051, 23421)</td>
</tr>
<tr>
<td></td>
<td>0.620 [-3621, 7949]</td>
<td></td>
</tr>
<tr>
<td>PC50[h, mean (95% CI)]</td>
<td>6.8 (6.2, 7.4)</td>
<td>6.5 (6.1, 6.9)</td>
</tr>
<tr>
<td></td>
<td>0.41 [-0.4, 1.0]</td>
<td></td>
</tr>
<tr>
<td>PC90[h, mean (95% CI)]</td>
<td>22.7 (20.7, 24.6)</td>
<td>21.6 (20.2, 23.1)</td>
</tr>
<tr>
<td></td>
<td>0.41 [-1.4, 3.4]</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; OR: odds ratio; GM: geometric mean. P₀: initial parasite count; P₀obs: population observed; P₀est: population estimate. Time to reduce the initial parasite count by 50% (PC50) or 90% (PC90).

Gametocytes were detected at baseline in 19/159 of the patients (11.9%), 8.5% in the CV8 group and 15.5% in the Malarone group. Gametocytes disappeared along with asexual parasites after administration of drugs, at similar rates in both regimens.
In the CV8 group 6.6% and in the Malarone group 4.6% of the patients developed new gametocytes during follow up (P > 0.05).

![Cumulative proportion of parasitaemic patients after treatment with CV8 or Malarone for uncomplicated falciparum malaria.](image)

**Figure 2:** Cumulative proportion of parasitaemic patients after treatment with CV8 or Malarone for uncomplicated falciparum malaria.

**Discussion**

The combination of dihydroartemisinin and piperaquine, with trimethoprim and primaquine, is well tolerated and very effective in the treatment of falciparum malaria in an area with multi-drug resistance. Malarone was equally effective. Both regimens induced rapid clinical recovery, parasite clearance and high cure rates.

Outpatient follow-up was excellent. Genotyping for distinguishing between recrudescence and re-infection was not available but because of the low transmission rates of malaria in Binh Thuan province and because every recrudescence occurred during the first 2 weeks, the re-appearance of parasites is most likely to be a true recrudescence \(^9\).

The rate of recrudescence was lower than with the low dose (10–15 mg/kg) mefloquine ACT regimens, commonly used in Binh Thuan prior to the introduction of CV8 \(^7\), and also lower than with artemisinin mono-therapy \(^2\). The improvement is probably achieved by the combination with piperaquine \(^10\). Gametocytaemia at baseline (12%) compares well with our previous studies but the low carrier rate and the low gametocyte densities preclude further conclusions on this topic \(^6,7\).

When CV8 was launched in Viet Nam, based on Chinese experience, clinical experience with dihydroartemisinin was limited and much was extrapolated from the
CV8 vs atovaquone-proguanil

experience with other artemisinin drugs. The extensive experience with piperaquine in China, especially on Hainan Island, and the reported resistance after application of mono-therapy was the basis for combining piperaquine with other agents. Trimethoprim was added based on recent empiric findings but the rationale for this not yet convincing. This also applies to primaquine.

Dihydroartemisinin, an artemisinin derivative, is a poor water-and oil-soluble product, formulated for human use. It is the main active metabolite of artesunate, arteether and arteether in humans and not exempted from the potential neurotoxic effects of prolonged supra-therapeutic dosing of artemisinin derivatives \[11,12\]. Its intrinsic activity is comparable with that of artesunate or arteether \[11,13\]. The elimination half life is 1 h or less and oral bioavailability is slightly lower than artesunate \[14,15\]. Dihydroartemisinin was used in some studies in combination with mefloquine for uncomplicated malaria and as suppositories for severe malaria \[16-18\]. It is also marketed for mono-therapy in Asia and Africa. The recommended adult dose is 80 mg, given once daily, with an extra dose on day 1.

Piperaquine is a bis-quinoline (Figure 3). It was synthesized more than 30 years ago in France and originally designated as RP 13 338. Little is known from pre-clinical studies \[19,20\]. Piperaquine is active mainly on late stage trophozoites of P. berghei \[21\]. The mechanism of its antimalarial activity is similar to other 4-amino-quinolines, interference with the haem polymerization \[22,23\].

Preliminary results in humans show that the elimination half-life ranges from 17 to 25 days (M. A. Ashton, T. M. E. Davis, T.-Y. Hung, K. F. Ilett, M. B. Denis and D. Socheat, personal communication). Measurement of piperaquine in plasma by high-performance liquid chromatography with ultraviolet absorbance detection presented at Mekong Malaria Symposium, 10-13 December, Siem Reap, Cambodia, (abstract in Mekong Malaria Forum, issue no 10, December 2002; \[24\]). Possible side-effects of piperaquine include mild dizziness, vertigo, headache, listlessness, nausea, vomiting, abdominal discomfort, but also reversible leucopenia was infrequently reported; dyspnea and palpitations were also reported but not further specified \[25,26\].
Although efficacy in treating malaria was proven, piperaquine was not selected for further development in France \(^{[26]}\). The same compound however, was synthesized in 1966 in China. More than 4000 subjects on Hainan Island received four consecutive monthly dosages of piperaquine (adults 600 mg, children 350–400 mg), resulting in a significant reduction of malaria incidence and parasite carrier rates. Forty-three individuals (adults and children) with symptomatic falciparum malaria were treated with piperaquine (adult dose 1800 mg over 2 days). Parasites disappeared all within 72 h and early recrudescence did not occur. In 1979 piperaquine replaced chloroquine as the standard treatment of *P. falciparum* infections on Hainan island \(^{[27]}\). Since then varying rates of resistance were reported \(^{[27,28]}\). Piperaquine resistance can be induced relatively easily in the *P. berghei* ANKA mouse model \(^{[27]}\). In vitro studies with clinical isolates from Madagascar in 1983 and 1984 did not demonstrate cross resistance with chloroquine \(^{[29]}\).

The antimalarial properties of trimethoprim were studied in the 1960s \(^{[30]}\). It has weak antimalarial activity and is mainly applied in combination with sulphamethoxazole for treatment of bacterial infections, but this combination can also be used for the treatment of malaria. Cross resistance between trimethoprim and pyrimethamine has been observed and resistance to the latter is widely spread in Southeast Asia \(^{[31]}\).

Primaquine also has some activity against *P. falciparum* but the low dose in CV8 only affects gametocytes \(^{[32]}\). In Viet Nam, primaquine is traditionally used to eradicate gametocytes in patients treated for symptomatic falciparum malaria. However, there is no evidence that this reduces transmission at population level. It may cause adverse effects, notably haemolysis in G6PD deficient patients. In practice, CV8 has already been used extensively in Viet Nam and no severe adverse reactions
CV8 vs atovaquone-proguanil

such as haemolysis have been reported passively. The low dose of primaquine and the relatively low prevalence of G6PD deficiency in the population of southern Viet Nam may be responsible for this [33].

The efficacy of the dihydroartemisinin piperazine combination is probably not much enhanced by the addition of trimethoprim and primaquine. A non-comparative study with Artekin, a combination of only dihydroartemisinin (total adult dose 320 mg) and piperazine (similar total dose) yielded a comparable parasite clearance and cure rate [34]. CV8 is not indicated for treatment of P. vivax infections. There is not yet much need for an alternative to chloroquine and the primaquine content in CV8 is too low to eradicate hypnozoites.

Atovaquone plus proguanil, Malarone, is very effective, even against multi-drug resistant *P. falciparum* [35-38]. The power of this study was not aiming at comparing the efficacy of Malarone and CV8 but merely at showing that the recrudescence rate of the latter was lower than the unacceptably high rates of the regimes in use at that time. Interestingly, CV8 and malarone appeared to be equally effective with narrow confidence limits.

Atovaquone is a very active antimalarial compound but resistance develops rapidly during mono-therapy. It is therefore combined with the synergistic proguanil, in a fixed combination (Malarone) [39]. It is effective against *P. falciparum* in areas with multi-drug resistance. Malarone has been on the market for several years, but it is not used widely because of its high price. The variable bioavailability of the atovaquone component is of concern; it increases three to sixfold when administered with food [40]. However, intake of Malarone with either low- or high-fat food did not affect the outcome of malaria treatment [38]. In this study, patients did not always take Malarone with food. It is remarkable that the parasite clearance rate in this study, induced by Malarone, is equal to that of CV8. Artemisinin compounds are regarded to be the most rapid inducers of parasite clearance. On the basis of the very high intrinsic activity of atovaquone in vitro, high parasite clearance rates in vivo were expected but not yet confirmed, mainly because clearance rates are not routinely calculated in clinical trials. The absent association between the parasite clearance dynamics and treatment outcome, unlike previous studies, was surprising but probably caused by the low recrudescence rate, which precludes further conclusions on the mechanism of recrudescence.

What is the future of these two drug combinations? The low price and high
efficacy of dihydroartemisinin and piperaquine are very promising for use in developing countries. Especially the African nations are in need of a cheap and effective anti-malaria combination therapy, based on artemisinin drugs. The current market price of one course of CV8 in Viet Nam is approximately 1.3 US$. This price is still high for most African citizens but further development and large-scale production can reduce the price. Fortunately the World Health Organization has taken the lead in further developing these combinations. Pre-clinical studies with CV8 were planned and post-marketing surveillance of CV8 recently started in Viet Nam. Further initiatives were developed to study combinations similar to CV8 but leaving out primaquine, called Artekin I, or leaving out trimethoprim and primaquine, Artekin II\[19,34\]. These initiatives will also attempt to fill in the gaps in the basic knowledge about these drugs. Malarone, however, is a well-documented safe and effective antimalarial drug combination. It is used in industrialized countries for treatment of imported \textit{P. falciparum} infections and for chemo-prophylaxis for travellers. Its price, especially of the atovaquone component, is several times higher than that of CV8. Although there is some preferential price policy for developing countries, it will hardly be used.

This is the first formal comparative study with the combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine, CV8, against falciparum malaria. CV8 appears to be an effective and safe antimalarial drug combination in an area with multi-drug resistant parasites. This merits further studies with these compounds. The combination of atovaquone and proguanil proved to be an equally fast acting and effective combination for the treatment of multi-drug resistant falciparum malaria in Viet Nam.

References

CV8 vs atovaquone-proguanil


CV8 vs atovaquone-proguanil


