High prevalence of mefloquine-resistant falciparum malaria in eastern Thailand

A.L. Fontanet,1 B.D. Johnston,2 A.M. Walker,1 W. Rooney,3 K. Thimasarn,4 D. Sturchler,5 M. Macdonald,6 M. Hours,6 & D.F. Wirth1

In order to assess the risk and predictors of mefloquine resistance we monitored a cohort of 113 patients in eastern Thailand who had been treated for uncomplicated falciparum malaria with a single dose of 15 mg/kg of the drug and followed up for 42 days. The overall treatment failure rate at day 42 was 59.1% (95% confidence interval (CI) = 50%, 68%) with only 2.7% of the patients being lost to follow-up. There were 6.4% RIII, 20.9% RII, 31.8% RI, and 40.9% sensitive responses, based on a modified WHO classification.

A low haemoglobin level on the day of treatment and diarrhoea during the first two days after treatment were independent predictors of treatment failure. These findings remained statistically significant in a Cox proportional hazards model, after controlling for other baseline characteristics and adverse effects. Although a history of digestive disorders prior to treatment was associated with diarrhoea on day 2 (P = 0.024), it was in itself not a predictor of treatment failure (adjusted hazard ratio = 1.16; 95% CI = 0.35, 2.14).

A total of 60 patients with an R response were hospitalized for 7 days to receive supervised treatment with quinine–tetracycline. Only three had a positive thick smear for asexual forms of Plasmodium falciparum 14 days later, and quinine–tetracycline therefore remains a good alternative treatment for mefloquine-resistant falciparum malaria.

Introduction

Since 1984 mefloquine has been used to treat uncomplicated falciparum malaria in Thailand because of its activity against multidrug-resistant falciparum strains and its efficacy in a single dose. The cure rates obtained with mefloquine in the initial field trials of the drug were close to 100% (1–4). Recent studies have revealed, however, that these rates have declined to 70% in eastern and western Thailand (5, 6).

Mefloquine was initially given in combination with sulfadoxine and pyrimethamine, which permitted its use at a dose of 15 mg/kg body weight (7). However, in July 1990, treatment centres dealing with Khmer displaced persons along the eastern border of Thailand switched from use of the triple combination to that of mefloquine alone.

Since 1990 a dramatic increase in the incidence of malaria has been reported by the United Nations Border Relief Operations (UNBRO) involved in supervising the Khmer displaced persons camps (see Fig. 1). This increase may be related to the greater mobility and exposure to malaria-infested areas of the population living in and around the camps caused by the changing military and political situations. Emergence of mefloquine resistance may also have

Fig. 1. Monthly incidence of malaria (all types) in Site 8, 1988–91. (Source: UNBRO)
contributed to this phenomenon, and treatment failures have been reported by the medical staff responsible for refugee care. We therefore initiated an in vivo test of mefloquine efficacy to estimate the drug failure rate at the current dose of 15 mg/kg.

Patients and methods

Study site

The study was carried out in Site 8, a Khmer displaced persons camp on the Thai–Cambodian border. The camp has a population of 45 000, 40% of whom are under 10 years of age; it is also used as a referral hospital for patients for Cambodia. According to data from the outpatient department and the hospital, malaria is the leading cause of morbidity and mortality among the camp’s inhabitants. The monthly malarial incidence typically varies between 50 and 2000 cases, depending on the season. Most of the cases (90%) are due to Plasmodium falciparum, with the remainder being P. vivax infections. Uncomplicated falciparum malaria cases are treated with 15 mg/kg of mefloquine (Lariam, Roche) in a single dose; severe cases are treated with intravenous quinine for up to 7 days, which is replaced with oral quinine and tetracycline when the patient is able to swallow tablets.

Mosquito collections made over the period 1983–85 showed that areas close to the camp were heavily infested with both of the region’s malaria vectors—Anopheles dirus and A. minimus. In Site 8 itself it was very rare to encounter either vector in the mosquito light-trap collections until recently, when an occasional A. dirus was collected and A. minimus became much commoner.

Study procedure

Inclusion of patients. Patients treated with mefloquine for uncomplicated falciparum malaria at the outpatient department of the camp in August and September 1991 were eligible for inclusion in the study. The following were contraindications for mefloquine treatment: cerebral symptoms; haemoglobin level <7 g/dl; high parasitaemia (>10 parasites per thick film field); and pregnancy. Treatment consisted of administration of 15 mg/kg of mefloquine in a single dose for patients weighing <40 kg; those whose weight was >40 kg were given three tablets (750 mg) in a single dose.

Immediately after treatment, the informed consent to participate in the study was obtained from the patients with the help of a translator. Patients were recruited for the study if they agreed to remain available for follow-up for 42 days and if they were willing to have further blood samples taken. The patients had just received therapy when their consent for follow-up was sought and were aware that their subsequent care did not depend on participating in the study. This study design was approved by the ethics committees of both UNBRO and Harvard School of Public Health.

All the thick smears were examined by an expert microscopist from the Thai Malaria Division, and patients were retained for the study if they had >1600 asexual forms of P. falciparum per μl of blood and no mixed falciparum and vivax infections. Finally, patients were included in the study only if they had had no history of treatment with mefloquine or quinine during the previous month.

Routine clinical information was collected while the patients were observed for 1 hour after treatment, in order to record accurately whether or not they vomited.

Follow-up. Study subjects were asked to return to the outpatient department on days 2, 3, 7, 14, 21, 28 and 42. At each visit any malaria symptoms and side-effects were recorded, and a thick blood smear was stained with Giemsa. Patients were told to report to the outpatient department if they experienced any fever or symptoms of malaria outside the regular follow-up days.

Treatment responses were labelled RI, RII, RIII or sensitive, using a slightly modified WHO classification (RI: all patients with a negative slide on day 7, but which became positive again for asexual forms of P. falciparum between day 8 and day 42; RII: patients with a slide still positive for asexual forms of P. falciparum on day 7; RIII: patients whose parasitaemia on day 2 had decreased by <75% of that on day 0; and sensitive: patients who remained free of parasitaemia after day 6). R is used subsequently in this article to indicate either an RI, RII or RIII response. Any positive slide on day 7 was confirmed by examination of a new slide on day 9. Patients with an R response were considered to be symptomatic if they had either a recorded temperature of >37.5°C or complained of the reappearance of at least two of the following symptoms: fever, headache, and joint pain. All patients with R responses were treated orally in hospital for 7 days with quinine (30 mg/kg/day) and tetracycline (25 mg/kg/day). A final thick smear was performed 14 days after the first dose of quinine had been administered to confirm the cure. Patients with an RIII response were treated on day 2 only if they were symptomatic at that time; otherwise, a daily smear was taken until the parasitaemia cleared. Patients with an RII response were treated only if they were symptomatic or if the control slide at day 9 was positive.

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A total of 5 ml of venous blood was collected from adult patients who had an R response before day 14, after their further consent had been obtained. Serum was separated from the blood by centrifugation, kept on ice, and stored at < 4 °C. A hand centrifuge was used and separation of the serum may therefore have been incomplete for samples from some patients. For each of these patients, a control was selected in the cohort from a patient of the same age (±5 years), sex, weight (±5 kg), day of treatment, and who had a negative smear. The permission of the control patient was obtained before 5 ml of venous blood was collected to determine the drug level in serum. The control patient’s status was maintained even if he/she exhibited an R response later in the follow-up. Serum levels of mefloquine were determined using high-performance liquid chromatography (HPLC).

**Statistical analysis.** Differences in proportion were analysed using $\chi^2$ and Fisher’s exact tests, and means were compared using Student’s $t$-test. A multivariate analysis was performed using linear regression, logistic regression, and Cox proportional hazards models.

**Results**

**Patients’ baseline characteristics**

A total of 113 patients were enrolled in the study in August and September 1991; their baseline characteristics are summarized in Table 1. The small number of children (8/113) in the cohort could have arisen because of the severity of their disease, leading to intravenous or oral treatment with quinine, and hence making them ineligible for the study.

A history of vomiting or diarrhoea prior to treatment was common (29.2% of patients) and was significantly associated with parasitaemias >100 000 asexual forms of *P. falciparum* per μl of blood ($P = 0.007$).

The relationship between haemoglobin level at admission and the other variables was examined using tabular analyses and linear regression procedures. Women and recent camp arrivals had lower haemoglobin levels than the other study subjects ($P = 0.002$ and $P = 0.01$, respectively). Although there was little association between haemoglobin level and the number of mefloquine treatments in the previous year, there was evidence that individuals who had received the drug within the previous 120 days had lower haemoglobin levels ($P = 0.016$).

**Adverse effects**

A total of 6 of the 113 patients (5%) vomited within 1 hour of receiving mefloquine; four were treated again because the drug appeared to have been regurgitated in the vomitus.

On day 2, dizziness was the commonest complaint of the patients (79%). Vomiting and diarrhoea occurred almost equally frequently (20% and 25%, resp.) and both were significantly associated with a history of digestive disorders prior to treatment ($P < 0.001$ and $P = 0.024$, resp.) (Table 2). There was no association between the dose of mefloquine received by the patients (mean, 14.32 ± 1.92 mg/kg) and the occurrence of side-effects, even after controlling for age, sex, and initial parasitaemia using a logistic regression model.

**Table 1: Baseline characteristics of the 113 study subjects**

| No. of males | 95 |
| No. of females | 18 |
| **No. in age group:** | |
| ≤14 years | 8 |
| ≥15 years | 105 |
| Mean age (years) | 32 ± 11.3 |
| **No. of documented mefloquine treatments in past year:** | |
| 0 | 53/105 (50)$^b$ |
| 1–2 | 38/106 (36) |
| 3 | 15/106 (14) |
| No information | 7 |
| **No. with history of vomiting or diarrhoea prior to treatment** | |
| Mean haemoglobin level (g/dl) | 10.8 ± 1.6 |
| Geometric mean parasitaemia (per μl) | 33 000 |
| Mean mefloquine dose (mg/kg) | 14.32 ± 1.92 |

$^a$ Shown in the right-hand column are the number of subjects.

$^b$ Figures in parentheses are percentages.

**Table 2: Association between digestive disorders before and on day 2 after treatment with mefloquine**

<table>
<thead>
<tr>
<th>Digestive disorders</th>
<th>Vomiting on day 2:$^a$</th>
<th>Diarrhoea on day 2:$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td>Total</td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>86</td>
</tr>
</tbody>
</table>

$^a$ $P = 0.001$ ($\chi^2$ test).

$^b$ $P = 0.024$ ($\chi^2$ test).
Mefloquine failure

Only 3 of the 113 patients (2.7%) did not complete the follow-up. Information provided by their families or neighbours indicated that they were not symptomatic when they left the cohort. These patients were excluded from subsequent analyses.

The overall parasitological failure rate among the remaining patients was 59% (65/110) (95% confidence interval (CI) = 50, 68%). On the basis of a modified WHO classification, there were 7 RII (6.4%), 23 RII (20.9%), 35 RI (31.8%), and 45 sensitive cases (40.9%). Over the whole follow-up period, 34 of the 43 patients (79%) with a positive slide on day 3 showed an R response. A total of 35 of the 66 patients (53%) with a negative slide on day 3 were sensitive cases. All the patients with a positive slide on day 7 were checked again on day 9 and, with the exception of two, remained positive (25/27).

A total of 35 of 56 patients (63%) with an R response were symptomatic when treatment with quinine was initiated. The levels of parasitaemia were not significantly different for symptomatic and asymptomatic patients.

We analysed the following baseline characteristics and recorded side-effects as predictors of an R response using a Cox proportional hazards model: age; gender; number of malaria episodes in the past year; number of documented mefloquine treatments in the past year; number of days since the last mefloquine treatment; history of recent arrival in the camp; history of a trip outside the camp during the past month; number of days of illness prior to the consultation; history of digestive disorders prior to treatment; fever at the consultation; parasitaemia on day 0; haemoglobin level on day 0; mefloquine dose; diarrhoea on day 2; vomiting on day 2; and dizziness on day 2.

There were two independent and important predictors of an R response: the haemoglobin level at day 0; and diarrhoea in the first two days after treatment with mefloquine. The probability of resistance decreased with increasing haemoglobin levels and was higher for persons who experienced diarrhoea on day 2 (21/27 = 78%) than for those who did not (42/80 = 53%). Table 3 shows the results of a Cox proportional hazards model that incorporates these two predictors together with age, which had a non-significant protective effect.

Patients who had digestive disorders before treatment with mefloquine did not appear to have a higher risk of an R response. The hazard ratio associated with prior digestive disorders, estimated using a model that incorporated the predictors listed in Table 3, with a term for prior digestive troubles, was 1.16 (95% CI: 0.35, 2.14).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Adjusted hazard ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.98</td>
</tr>
<tr>
<td>Haemoglobin level on day 0 (g/dl)</td>
<td>0.80c</td>
</tr>
<tr>
<td>Diarrhoea on day 2</td>
<td>1.92d</td>
</tr>
</tbody>
</table>

* For age and haemoglobin level the adjusted hazard ratio estimates the amount by which the hazard of failure is multiplied for an increase of one unit in these variables.

Using a similar model, we found that a positive malaria smear on day 3 was significantly associated with treatment failure after controlling for the other variables (adjusted hazard ratio = 2.22 (95% CI: 1.27, 3.87)).

Serum mefloquine concentrations

The serum mefloquine concentrations of 10 patients with an R response during the first 14 days of follow-up were compared with those of 10 controls, matched for age, sex, weight, and day of treatment. The mefloquine levels were almost identical for four of the pairs; for four pairs the levels were higher in the controls; and for two pairs were higher in the cases. Differences in mefloquine serum concentrations were much larger and in favour of the controls for the two pairs from whom blood was taken early in the follow-up period (day 3). A total of 10 of the 13 patients with recrudescence between day 7 and day 14 had a mefloquine level >500 ng/ml at recrudescence; the mean serum mefloquine concentrations of these 13 patients was 646 ng/ml.

For 26 patients there was no correlation between the haemoglobin level on day 0 and the mefloquine serum concentrations on day 3 to day 18.

Samples of blood were taken on day 3 from two patients who had diarrhoea on day 2. On day 3 the serum mefloquine concentrations for these patients were much lower than those of three patients who did not have diarrhoea (227 and 499 ng/ml compared with 1455, 1693, and 2922 ng/ml).

Subsequent course of patients showing resistance

Three of the seven RIII cases were symptomatic and were treated intravenously on day 3 with quinine. The remaining four cases were followed up without further therapy until recrudescence occurred (day 21 (two), day 23, and day 28).
A total of 60 patients were treated again orally with quinine (30 mg/kg/day) + tetracycline (25 mg/kg/day) for 7 days in hospital; a control malaria smear was obtained for all of them (14 days after the first day of quinine for 44 patients and between day 14 and day 42 for the others). Only three were positive for P. falciparum and one for P. vivax. Falciparum cases were re-treated with mefloquine (25 mg/kg).

Of the five patients who did not want to be hospitalized, three received a single dose of 25 mg/kg of mefloquine; their control smears on day 14 were negative. The other two were mistakenly given only 15 mg/kg of mefloquine; one of them who had a positive control smear was successfully treated with quinine–tetracycline over 7 days.

**Discussion**

Resistance against many widely used antimalarial drugs has begun to emerge in south-east Asia (8). *In vitro* resistance to mefloquine was described in eastern Thailand even prior to the introduction of the drug as a first-line treatment for falciparum malaria (9). However, the first trial of mefloquine on the Thai–Cambodian border reported an excellent cure rate (3). Only recently has the susceptibility of some falciparum strains in this area decreased to such an extent that even adequate mefloquine concentrations in blood are insufficient to clear parasitaemia (10). This finding, together with a recent increase in the number of malaria cases in some Khmer refugee camps, led us to initiate the *in vivo* test of mefloquine efficacy that we have reported in this article.

The overall risk of treatment failure in our follow-up of 110 patients was 59%. The proportion of patients who dropped out was only 2.7% and is insufficient to explain the high risk of failure as an artefact due to the elimination of healthy patients from the cohort (i.e., if healthy patients are lost to follow-up, the proportion of treatment failures is falsely increased). Some patients may have been reininfected during the follow-up; however, the early treatment failures, i.e., RII and RIII responses (which cannot be re-infections), accounted for a large proportion of the total. Such a high level of clinical resistance among adult males, whose immunity is likely to be greater than that of women and children, is particularly disturbing. Doses of 25 mg/kg of mefloquine, which are designed for the treatment of non-immunes, could be adopted for semi-immunes in areas of multiresistance to increase cure rates. A clinical trial of mefloquine at doses of 15 mg/kg and 25 mg/kg is in progress in the study area. However, we are concerned that patients with an R response had a mefloquine serum concentration > 500 ng/ml, i.e., a level that should be associated with successful treatment (11).

Digestive disorders prior to treatment were common and significantly associated with higher parasitaemia. Patients with such disorders were more likely to report vomiting or diarrhoea on day 2. However, they were not at increased risk of failure, as shown by the Cox proportional hazards model (adjusted hazard ratio = 1.16; 95% CI = 0.35, 2.14). This indicates that disease-induced diarrhoea may not decrease mefloquine absorption enough to impair the drug's efficacy but that drug-induced diarrhoea appears to do so. The mefloquine serum concentrations on day 3 for the two patients with diarrhoea were very low indeed.

The inverse relationship between haemoglobin level and the risk of treatment failure is interesting. We initially hypothesized that people infected with a resistant strain would develop anaemia through repetitive malaria attacks, and it appears that recent prior therapy with mefloquine was associated with lower haemoglobin levels. Alternatively, mefloquine may be more rapidly cleared from the blood of patients with anaemia since the drug is less able to fix on their red blood cells (12). It should be noted, however, that serum mefloquine concentrations did not correlate with initial haemoglobin levels for the 26 patients for whom both were measured; the hypothesized increased rates of mefloquine elimination in anaemic patients therefore remain speculative.

Quinine–tetracycline was still effective against mefloquine-resistant falciparum strains (there were only three recrudescences on day 14 among the 60 re-treated patients). However, at the time when they received quinine–tetracycline, some of the patients may still have had mefloquine in their blood, since its half-life is long. This should therefore not be considered as an evaluation of the effect of quinine–tetracycline alone on mefloquine-resistant strains. Mefloquine has been a convenient treatment for malaria in the refugee camps on the Thai–Cambodian border since its single-dose administration can easily be controlled. Over the past 5 years, high-grade resistance has developed, however, and other drug regimens are now urgently needed. Quinine–tetracycline remains a valuable alternative for the treatment of mefloquine-resistant falciparum malaria, but the more widespread use of quinine should not be allowed to jeopardize, by creating drug resistance, its intravenous use as a life-saving drug for severe malaria.

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Résumé

Forte prévalence du paludisme à falciparum résistant à la méfloquine dans l’est de la Thaïlande

Une cohorte de sujets traités par la méfloquine dans l’est de la Thaïlande a été suivie afin d’évaluer le risque et les facteurs prédicifs de résistance à ce médicament. A cette fin, nous avons traité 113 malades atteints de paludisme à falciparum sans complication avec 15 mg/kg de méfloquine en dose unique, et nous les avons suivis pendant 42 jours.

Au jour 42, le taux global d’échec était de 59,1% (intervalle de confiance (IC) à 95%: 50–68%), 2,7% seulement des malades ayant été perdus de vue. Selon une classification OMS modifiée, nous avons observé 6,4% de cas de résistance RIIL, 20,9% de résistance RII, 31,8% de résistance RI et 40,9% de sujets sensibles.

Au jour 2, les malades se plaignaient principalement de vertiges (79%) tandis que 20% souffraient de vomissements et 25% de diarrhée.

Deux variables indépendantes permettaient de prédire l’échec du traitement: un faible taux d’hémoglobine le jour du traitement, et la survenue d’une diarrhée dans les deux jours suivant l’administration du médicament. Ces observations ont conservé leur signification statistique dans un modèle de risque proportionnel de Cox après correction des autres paramètres de base et des effets indésirables. Pour expliquer la relation inverse entre le taux d’hémoglobine et le risque d’échec thérapeutique, nous avons supposé que les sujets infectés par une souche résistante de Plasmodium falciparum présentaient une anémie due à des crises répétées de paludisme. Bien que l’association soit faible entre le taux d’hémoglobine et le nombre de traitements par la méfloquine que les sujets avaient reçu au cours de l’année précédente, les malades les plus récemment traités semblaient avoir les taux d’hémoglobine les plus faibles. Nous avons également recherché s’il existait un taux accru d’élimination de la méfloquine chez les sujets anémiques. Mais les taux sériques de méfloquine n’étaient pas corrélés au taux initial d’hémoglobine chez les 26 sujets chez lesquels ces deux taux ont été mesurés. Des antécédents de troubles digestifs avant le traitement étaient associés à la survenue d’une diarrhée au jour 2 ($P = 0,024$), mais ne constituaient pas en eux-mêmes un facteur prédicif de l’échec du traitement (rapport de risque corrigé = 1,16; IC 95% = 0,35–2,14). Cela laisse à penser que la diarrhée due à une maladie pourrait ne pas réduire l’absorption de la méfloquine suffisamment pour nuire à son efficacité, alors que la diarrhée iatrogène pourrait le faire.

Au total, 60 malades présentant une résistance au médicament ont été hospitalisés pendant 7 jours et ont reçu un traitement par la quinine et une tétracycline. Seuls trois d’entre eux avaient un frottis en goutte épaissi positif pour les formes asexuées de P. falciparum au bout de 14 jours. Le traitement associant la quinine à la tétracycline reste une bonne alternative chez les sujets atteints de paludisme à falciparum résistant à la méfloquine dans la zone étudiée.

References