Efficacy and effectiveness of dihydroartemisinin-piperaquine versus artesunate-mefloquine in falciparum malaria: an open-label randomised comparison

Frank Smithuis, Moe Kyaw Kyaw, Ohn Phe, Khin Zarli Aye, Lhin Htet, Marion Barends, Niklas Lindegardh, Thida Singtoroj, Elizabeth Ashley, Saw Lwin, Kasia Stepniewska, Nicholas J White

Summary

Background **Artemisinin-based combinations are judged the best treatments for multidrug-resistant *Plasmodium falciparum* malaria. Artesunate-mefloquine is widely recommended in southeast Asia, but its high cost and tolerability profile remain obstacles to widespread deployment. To assess whether dihydroartemisinin-piperaquine is a suitable alternative to artesunate-mefloquine, we compared the safety, tolerability, efficacy, and effectiveness of the two regimens for the treatment of uncomplicated falciparum in western Myanmar (Burma).**

Methods **We did an open randomised comparison of 3-day regimens of artesunate-mefloquine (12/25 mg/kg) versus dihydroartemisinin-piperaquine (6·3/50 mg/kg) for the treatment of children aged 1 year or older and in adults with uncomplicated falciparum malaria in Rakhine State, western Myanmar. Within each group, patients were randomly assigned supervised or non-supervised treatment. The primary endpoint was the PCR-confirmed parasitological failure rate by day 42. Failure rates at day 42 were estimated by Kaplan-Meier survival analysis. This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN27914471.**

Findings **Of 652 patients enrolled, 327 were assigned dihydroartemisinin-piperaquine (156 supervised and 171 not supervised), and 325 artesunate-mefloquine (162 and 163, respectively). 16 patients were lost to follow-up, and one patient died 22 days after receiving dihydroartemisinin-piperaquine. Recrudescent parasitaemias were confirmed in only two patients; the day 42 failure rate was 0·6% (95% CI 0·2–2·5) for dihydroartemisinin-piperaquine and 0 (0–1·2) for artesunate-mefloquine. Whole-blood piperaquine concentrations at day 7 were similar for patients with observed and non-observed dihydroartemisinin-piperaquine treatment. Gametocytaemia developed more frequently in patients who had received dihydroartemisinin-piperaquine than in those on artesunate-mefloquine: day 7, 18 (10%) of 188 versus five (2%) of 218; relative risk 4·2 (1·6–11·0) p=0·011.**

Interpretation **Dihydroartemisinin-piperaquine is a highly efficacious and inexpensive treatment of multidrug-resistant falciparum malaria and is well tolerated by all age groups. The effectiveness of the unsupervised treatment, as in the usual context of use, equalled its supervised efficacy, indicating good adherence without supervision. Dihydroartemisinin-piperaquine is a good alternative to artesunate-mefloquine.**

Lancet 2006; 367: 2075–85

See Comment page 2037

Médecins Sans Frontières (Holland), Yangon, Myanmar (F Smithuis MD, M K Kyaw MBBS, O Phe BSc, K Z Aye MBBS, L Htet MBBS); **Shoklo Malaria Research Unit, Mae Sot, Thailand** (M Barends PhD, N Lindegardh PhD, E Ashley MBBS, K Stepniewska PhD); **Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand** (M Barends, T Singtoroj PhD, E Ashley, K Stepniewska, Prof N J White MD); **Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, UK** (N Lindegardh, E Ashley, Prof N J White); **and Vector Borne Disease Control, Department of Health, Myanmar** (S Lwin MBBS)

Correspondence to:
Prof N J White, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand
nickw@tropmedres.ac

Introduction

Resistance to antimalarial drugs has increased rapidly nearly everywhere in the tropics, and southeast Asia still has the most drug-resistant falciparum malaria in the world.[1–3] In Myanmar (Burma), chloroquine has been the first-line treatment for falciparum malaria until recently. Studies in 1995 and 1998 by Médecins Sans Frontières (Holland), with the support of the Department of Health and the Vector Borne Disease Control Department, in the western and northern parts of the country showed very high rates of resistance to chloroquine and sulfadoxine-pyrimethamine, the recommended treatments at the time. Mefloquine monotherapy (15 mg/kg) had failure rates of 7% in Rakhine State, in the west of the country,[3] and 20% in Kachin State, in the north.[4] Thus, there was resistance to all available monotherapies. Combination therapy that includes an artemisinin derivative is now the preferred first-line treatment for malaria.

In Myanmar, a 3-day treatment of mefloquine and artesunate was highly efficacious, even when the treatment was not fully supervised.[5] In September, 2002, the Myanmar Department of Health changed the national protocol for first-line treatment of uncomplicated falciparum malaria to a 3-day artesunate-mefloquine treatment (artesunate 12 mg/kg and mefloquine 25 mg/kg). This change was an important step forward but there are several obstacles to implementing the policy. First, the high price of artesunate-mefloquine (around US$3 for one adult treatment) means that this regimen is not accessible to most people with malaria, who are therefore still treated with chloroquine. Second, adverse effects are common after mefloquine, and although most side-effects are not serious, they can lead to poor adherence with multiple-dose regimens. Third, mefloquine and artesunate are not yet formulated in one tablet, so patients can take only the artesunate, thereby compromising efficacy and encouraging resistance.

Two old drugs, dihydroartemisinin and piperaquine, have been formulated in a new combination treatment. Several studies in southeast Asia indicate that this

combination is a safe and highly efficacious treatment for uncomplicated multidrug-resistant falciparum malaria in adults and children.[6–9] Compared with artesunate-mefloquine, dihydroartemisinin-piperaquine has several potential advantages. First, it is cheaper (around US$1·50 for an adult treatment), which might improve access to effective antimalarial treatment for poor populations. Second, available data suggest that dihydroartemisinin-piperaquine is better tolerated than is artesunate-mefloquine.[7] Third, the combination treatment is formulated in one tablet, which makes it easier to take, and should increase adherence and thereby limit the chance for selection of resistant parasites. Until recently, dihydroartemisinin-piperaquine was produced in the People's Republic of China and Vietnam, but the available products did not meet internationally accepted standards for good manufacturing practices.

In Myanmar, as in most countries, intake of antimalarial drugs is generally not supervised. Furthermore, treatment is unlikely to be supervised in the foreseeable future, with the exception of the first dose, which can be given in the health clinic. Highly efficacious treatments may not be effective if adherence to the prescribed treatment is poor. Adherence is related to the adverse-effect profile. To assess this issue and inform policy we did a four-way randomised trial to compare the safety, tolerability, efficacy, and effectiveness of dihydroartemisinin-piperaquine with that of artesunate-mefloquine for the treatment of uncomplicated falciparum malaria in adults and children in western Myanmar.

Methods

Study area and population

The study was done in two village-tracts, Dabhine and Mingan, which are about 10 km apart, in the township of Sittwe along the coast of the Bay of Bengal, in Rakhine State, western Myanmar. In this region, malaria transmission is seasonal (May–January) with peaks during the post-monsoon (November–January) and sometimes in the early monsoon (May–June) periods. The transmission intensity is geographically heterogeneous, varying from low to high over short distances. *Plasmodium falciparum* causes around 80% of the malaria infections that present to the clinics.

In 1994, Médecins Sans Frontières (Holland) started to support a malaria control programme in Rakhine State in cooperation with the Vector Borne Disease Control Department. The programme focuses on early diagnosis and treatment. Since 1996, all patients with falciparum malaria accessing this programme have been treated with a combination of mefloquine and artesunate. The programme supports 30 fixed clinics and three mobile malaria clinics, which visit remote areas by boat. In 2003, the programme provided a diagnostic test to 300 000 patients, of whom around 150 000 had falciparum malaria confirmed and received a 3-day combination treatment with artesunate and mefloquine (artesunate 12 mg/kg+mefloquine 25 mg/kg). Our prospective study was done between November, 2003, and April, 2004.

If patients had fever (axillary temperature ≥37·5º C) or a history of fever within 48 h, a blood smear was prepared. Patients with confirmed falciparum malaria were invited to take part in the study if they had more than 500 but fewer than 100 000 asexual parasites per µL, no clear signs or symptoms of other diseases, and no signs of severe or complicated malaria. Children younger than 1 year, pregnant women, patients with severe malaria,[10] patients with a history of taking mefloquine in the previous 2 months or any other antimalarial drugs in the previous 48 h, and patients with a history of psychiatric diseases were excluded from the study. Patients with mixed infection (*P falciparum* with *P vivax, P malariae,* or both) were included. Patients who met the inclusion criteria were enrolled in the study only after fully informed consent was obtained from them or their carer. The protocol was approved by the Myanmar Department of Health and by the Médecins Sans Frontières ethics review board.

Procedures

Patients were stratified prospectively into three age groups (1–4 years, 5–14 years, and older than 14 years) and allocated randomly to the four treatment regimens. At both study locations three boxes were prepared, one for each of the three age groups, by an administrator who was otherwise not connected with the study. In each box, 40 unmarked and sealed opaque envelopes were deposited. Each envelope contained a card that described the treatment assignment, and each treatment allocation had an equal number of cards (ten). Each new patient (or his or her carer) was asked to take one of the envelopes from the box for their age group. Treatment was then dispensed in accordance with the treatment allocation in the envelope. Whenever a box became empty, another 40 envelopes were put in that box.

All patients were examined clinically. A gametocyte count was done, blood was taken for measurement of the haemoglobin concentration, and a blood spot was taken on filter paper for PCR examination. Each PCR filter paper was dried, put in an individual plastic bag, and kept in an airtight box with silica gel.

This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN27914471.

Antimalarial drug regimens

The patients were randomly allocated to four treatment groups. The first group was assigned supervised dihydroartemisinin-piperaquine (Artekin, Holleykin Pharmaceuticals, Guangzhou, China). The drug was given in a dose of 2·1 mg/kg dihydroartemisinin and 16·8 mg/kg piperaquine once daily for 3 days. Patients took every dose under supervision in the clinic. The number of pills was rounded off to the nearest half tablet. The usual adult dose was three tablets per day. The second group was assigned unsupervised dihydroartemisinin-piperaquine in the

same doses as the first group. The first dose was supervised, but the treatments on days 1 and 2 were self-administered at home. Group three was assigned 3-day treatment with mefloquine (Lariam, Hoffman-La Roche, Basel, Switzerland) 25 mg base/kg single dose on day 0, and artesunate (Guilin Pharmaceutical Factory, Guilin, China) 4 mg/kg on days 0, 1, and 2. Every dose was given under supervision in the clinic. The number of pills was rounded off to the nearest quarter of a tablet. The usual adult dose was five tablets of mefloquine in one dose, and four tablets of artesunate per day. The fourth group was assigned the same drugs and the same doses as group three, but with the first dose supervised and the treatments on day 1 and day 2 self-administered.

The differences in drug formulations meant that masking of treatment at the point of administration was not possible. For the drugs that were to be self-administered in treatment groups 2 and 4 on days 1 and 2, two stapled small plastic bags were provided, marked 1 and 2, with the medication for the next 2 days. The importance of taking these drugs, even if symptoms had already subsided, was explained twice. All other antimalarial drug doses were given under the supervision of a treatment observer for 1 h. For children, tablets were crushed and syrup was added. If the patient vomited within 30 min, the full dose was repeated. Patients who vomited between 30 min and 60 min after drug administration were given half the initial dose. If the patient vomited the drugs twice, he or she was withdrawn from the study. For patients who vomited after 1 h, no repeat treatment was given and the patient remained in the study. Patients from treatment groups 1 and 3 were asked to return to the clinic on the next 2 days for supervised doses. Patients in these groups who missed doses in the mornings of days 1 and 2 were traced within 24 h, and the drugs were given under direct supervision. Patients from the unsupervised treatment groups (2 and 4) were asked to come back if they vomited within 1 h of drug administration.

Patients were requested to come back routinely on days 1 and 2 (only for the supervised treatment groups 1 and 3) and on days 7, 14, 21, 28, 35, and 42 after starting treatment (for all treatment groups) and at any other time when they felt unwell or thought they had fever. At each routine visit, a blood film was taken for the identification of malaria parasites, a gametocyte count was done, and a questionnaire was completed (for patients 4 years of age or older) to document side-effects. On day 7, the questionnaire for side-effects was detailed retrospectively for each day of the week (days 3–7 for groups 1 and 3, and days 1–7 for groups 2 and 4). Additionally, one blood sample (100 µL taken by micropipette) was kept on a filter paper on day 7, which was used later for the measurement of the blood concentration of piperaquine. On day 28, a blood sample was obtained for repeat haemoglobin measurement.

Repeat treatment was provided to patients who deteriorated clinically at any time, and those who returned on days 2–6 with patent *P falciparum* parasitaemia of more than 25% of the count on day 0. Patients who had persistent parasitaemia on days 2–6 but less than 25% of the initial parasite count and fever (but no clinical deterioration) were not defined as having treatment failure. These patients were followed up closely to ensure that no further deterioration took place. Repeat treatment was also provided to patients with *P falciparum* parasites on day 7 or any subsequent day.

For patients treated previously with dihydroartemisinin-piperaquine the repeat treatment was with artesunate-mefloquine if there was no contraindication, and for patients who received artesunate-mefloquine previously it was dihydroartemisinin-piperaquine. Mode of administration (supervised or not) was the same as for the initial treatment. Patients with treatment failure were followed up in the same way as those whose first treatments had failed.

From all patients whose treatment failed, additional blood was obtained on a filter paper for PCR genotyping. Patients with a positive blood smear for *P vivax*, *P ovale*, or *P malariae* were not defined as having treatment failure. They were given chloroquine (25 mg base per kg during 3 days) and follow-up was continued.

To distinguish recrudescences from new infections, parasite genotypes were established on admission and in cases of recurrent parasitaemia. Parasite genotyping was done with PCR for variable blocks within *MSP1, MSP2,* and *GLURP* as described previously.[11] If a definitive statement could not be made, the outcome was recorded as indeterminate. Treatment failure rates were calculated for patients with proven recrudescences only and for patients with recrudescences or indeterminate PCR outcomes. The primary outcome measure was the rate of recrudescence confirmed with PCR.

Piperaquine whole-blood concentrations were measured by solid-phase extraction and liquid chromatography.[12] The blood spots were cut into pieces and extracted with perchloric acid, phosphate buffer pH 2 containing internal standard, and acetonitrile for 1 h. The supernatants were loaded onto an MPC solid-phase extraction column (3M Empore, Bracknell, UK); the eluates were evaporated, reconstituted, and injected into a liquid chromatography system with a Chromolith performance column (VWR International, Darmstadt, Germany) and a mobile phase containing acetonitrile-phosphate buffer pH 2·5 0·1 mol/L (8:92 by volume) at a flow rate of 3 mL/min. The total precision for all quality controls during the analysis was 11·5% at 0·2 µg/mL, 10·9% at 0·75 µg/mL, and 7·4% at 2·5 µg/mL.

Statistical analysis

This trial was designed as a non-inferiority study. With a sample size of 160 patients in the four study groups, a cure rate of 95% with either drug regimen could be estimated

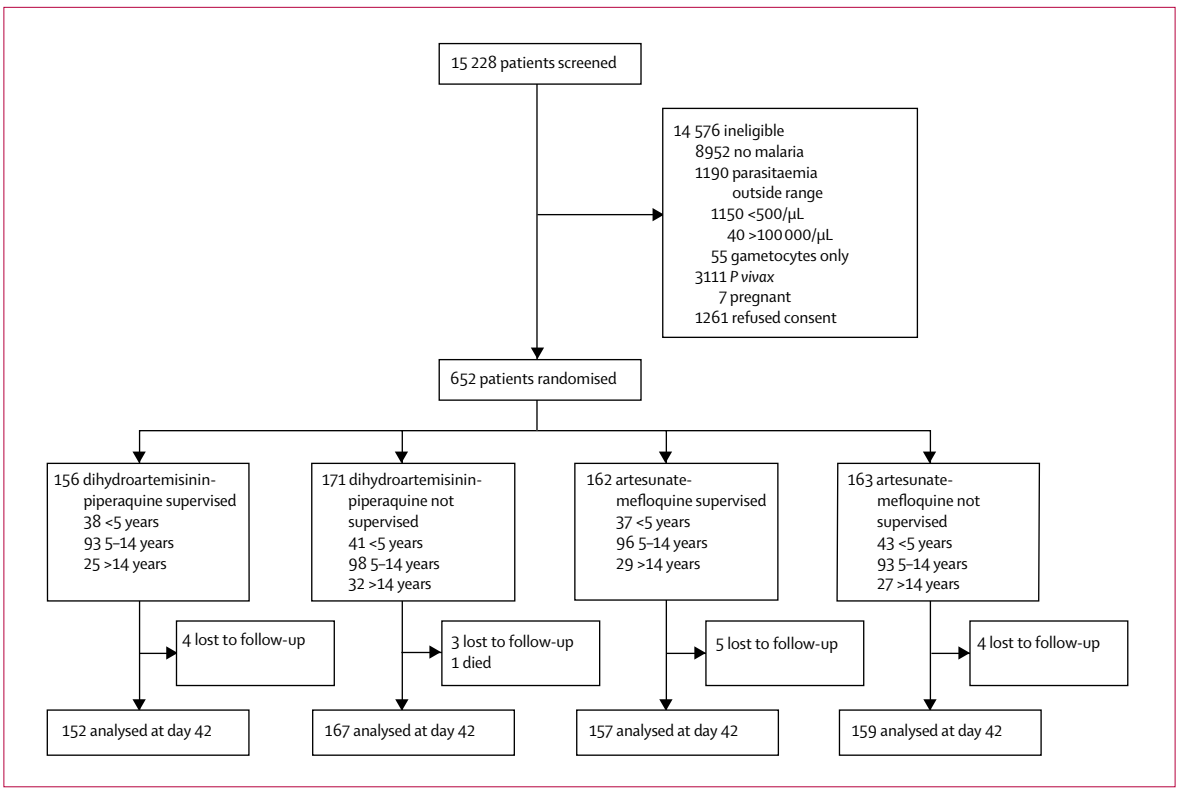

Figure 1: **Trial profile**

with 5% precision, and a maximum allowable inferiority of 10% for dihydroartemisinin-piperaquine compared with artesunate-mefloquine could be detected with 90% power and 95% confidence, taking into account a withdrawal rate of up to 20%. Data were analysed with SPSS for Windows version 11.5, STATA version 9, and EpiInfo version 6. Continuous data were analysed by Student's *t* test or ANOVA, and proportions of categorical data were compared by χ^2 test with Yates' correction or a two-tailed Fisher's exact test. Comparisons between treatment groups were done in a two-way factor analysis: ANOVA for continuous data and multiple logistic regression model for categorical data. The effect of the treatment on recurrence of falciparum malaria was summarised by the risk difference. Effects of the treatment on other secondary outcomes were summarised by relative risks.

As a measure of transmission potential, we calculated the person-gametocyte-weeks, which were defined as the number of weeks in which blood slides were positive for gametocytes during the first 2 weeks of follow-up after treatment divided by the number of weeks followed up, and were expressed per 1000 person-weeks.[13] Failure rates were estimated by survival analysis with the Kaplan-Meier method. Patients who did not complete follow-up or had a new infection were censored at the time they were last seen.

CIs around failure rate estimates were calculated by Greenwood's formula. When failure rates were zero, CIs were calculated by the exact binomial method with the effective sample sizes.[14] Equivalence between treatment groups was assessed by calculating the differences between failure rates and the CIs around these differences. CIs were based on the effective sample sizes as above and calculated with the Score method.[15]

Recurrences that were indeterminate on the PCR analysis were treated firstly as censored observations and secondly as failures (to describe the worst-case scenario).

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between November, 2003, and February, 2004, 15 228 patients were screened, but 14 576 were not eligible (figure 1). 1261 refused consent, mostly because they lived far from the clinic and because part of the study period was during Ramadan, which made it difficult for patients to comply with the follow-up requirements.

652 people met the inclusion criteria and were recruited for the study. Of them, 156 were assigned dihydroartemisinin-piperaquine (supervised), 171 dihydroartemisinin-piperaquine (home), 162 artesunate-

mefloquine (supervised), and 163 artesunate-mefloquine (home). 399 patients were recruited in Dabhine and 253 in Mingan. Baseline characteristics were comparable across groups (table 1), with the exception of gametocytaemia on admission.

One 11-year-old child died during the study. He had received dihydroartemisinin-piperaquine (not supervised). During the follow-up visits on days 7 and 14, he had no fever and the blood smears were negative for malaria parasites. On the evening of day 20, the child developed fever. The next morning he had generalised seizures and his parents took him to the local hospital. The hospital physician noted decreased consciousness but no signs of meningism. A blood smear for malaria parasites was negative. The physician treated for cerebral malaria and bacterial meningitis with a loading dose of quinine, intravenous penicillin, and chloramphenicol. Nevertheless, the child died the next day. No autopsy was done.

16 other patients (2·5%) did not complete the 42 day follow-up period (figure 1). These patients were included in the data analysis for the period they participated.

Early responses to treatment could be measured only in the supervised-treatment groups, who returned to the clinic on days 1 and 2. Of these patients, the proportions whose parasitaemia had cleared after 1 day were 53% (82 of 155) after dihydroartemisinin-piperaquine and 45% (73 of 161) after artesunate-mefloquine (relative risk 1·17 [95% CI 0·93–1·46], p=0·22). After 2 days the proportions of patients who had cleared parasitaemia were 97% (151 of 155) after dihydroartemisinin-piperaquine and 94% (150 of 159) after artesunate-mefloquine, respectively. Of the remaining nine infections in the latter group, eight were *P falciparum* and one *P vivax*.

At baseline, 44% of patients in each supervised group were febrile (table 1). 1 day after treatment these proportions had dropped to 3% in both treatment groups (four of 155 and four of 161, respectively). All patients had clearance of their parasites by day 7.

Only seven patients had reappearance of parasites within 42 days, one after artesunate-mefloquine (supervised) and six after dihydroartemisinin-piperaquine (four supervised, and two unsupervised). The relative risk for recurrent parasitaemia for dihydroartemisinin-piperaquine versus artesunate-mefloquine was 5·96 (0·72–49·26, p=0·12). Two patients presented with fever, whereas the other five were asymptomatic. Of the seven patients with recurrent parasitaemias, all were aged between 3 years and 6 years. Parasite genotyping was done on paired samples. In three patients a new genotype was confirmed, indicating a new infection (all 28 days after supervised dihydroartemisinin-piperaquine). In two patients, recrudescent parasitaemias were confirmed (one patient 14 days after dihydroartemisinin-piperaquine [home] and one patient 28 days after dihydroartemisinin-piperaquine [supervised]). Of the two remaining patients, the PCR results were indeterminate (one patient 28 days after dihydroartemisinin-piperaquine [supervised] and one patient 35 days after artesunate-mefloquine [supervised]). If recurrences of parasitaemias that were indeterminate on the PCR analysis were treated as censored findings, the failure rate was 0·6% (95% CI 0·2–2·5) for dihydroartemisinin-piperaquine and 0 (0–1·2) for artesunate-mefloquine, and the difference between the two treatment groups was 0·63% (–0·66 to 2·28, p=0·16). However, if we assume that the PCR-indeterminate recurrences were treatment failures (the worst-case scenario), the failure rate was 0·9% (0·3–2·9) for dihydroartemisinin-piperaquine and 0·3% (0–2·2) for artesunate-mefloquine, and the difference between the two treatment groups was 0·63% (–0·94 to 2·46). The four treatment groups were therefore regarded as therapeutically equivalent because all CI for differences were well within the 10% limits.

At baseline, 87 patients had mixed infections with *P vivax* (85 children and two adults). On day 7, all *P vivax* infections had cleared but 97 patients had presumed

	Dihydroartemisinin-piperaquine (supervised)	Dihydroartemisinin-piperaquine (home)	Artesunate-mefloquine (supervised)	Artesunate-mefloquine (home)
Number of patients	156	171	162	163
Women	79 (51%)	83 (49%)	72 (44%)	79 (49%)
Age groups (years)				
<5	38 (24%)	41 (24%)	37 (23%)	43 (26%)
5–14	93 (60%)	98 (57%)	96 (59%)	93 (57%)
>14	25 (16%)	32 (19%)	29 (18%)	27 (17%)
Axillary temperature ≥37·5°C	69 (44%)	79 (46%)	72 (44%)	69 (43%)
Haemoglobin g/L (SD)	94 (22)	92 (22)	95 (21)	95 (22)
Anaemic*	92 (59%)	113 (66%)	91 (56%)	95 (59%)
Geometric mean parasite count/µL (range)	8128 (627–91 741)	9593 (585–99 502)	7663 (560–90 480)	8365 (600–96 792)
Mixed infections	21 (14%)	19 (11%)	23 (14%)	24 (15%)
Gametocytaemia on admission	70 (45%)	67 (39%)	58 (36%)	45 (28%)

Data are number (%) unless stated otherwise. *Haemoglobin concentration <100 g/L.

Table 1: **Clinical and laboratory variables at baseline**

	P falciparum				*P vivax*
	Cumulative confirmed recrudescence and reappearances of parasitaemia with indeterminate PCR results			New infections	Reappearances
	Day 14	Day 28	Day 42		Day 7–42
Dihydroartemisinin-piperaquine (supervised)					
Age 0–14 years	0/130	1/129 (1%)*	1 /129 (1%)*	3	22/129 (17%)
Age >14 years	0/24	0/24	0/23	0	0/23
Total	0/154	1 /153 (1%)*	1 /152 (1%)*	3	22/152 (15%)
Dihydroartemisinin-piperaquine (home)					
Age 0–14 years	1/138 (1%)*	2 /137 (2%)†	2/137 (2%)†	0	18/137 (13%)
Age >14 years	0/31	0/31	0/30	0	0/30
Total	1/169 (1%)*	2 /168 (1%)†	2/167 (1%)†	0	18/168 (11%)
Artesunate-mefloquine (supervised)					
Age 0–14 years	0/129	0/129	1/129 (1%)‡	0	25/129 (19%)
Age >14 years	0/29	0/28	0/28	0	0/28
Total	0/158	0/157	1/157 (1%)‡	0	25/157 (16%)
Artesunate-mefloquine (home)					
Age 0–14 years	0/135	0/134	0/134	0	32/134 (24%)
Age >14 years	0/25	0/25	0/25	0	0/25
Total	0/160	0/159	0/159	0	32/159 (20%)

*One confirmed recrudescence. †One confirmed recrudescence and one indeterminate PCR result. ‡One indeterminate PCR result.

Table 2: **Parasitological responses to antimalarial treatment**

relapses of this species during follow-up (three patients had two vivax episodes; table 2). The median time to appearance of *P vivax* malaria was 42 days for dihydroartemisinin-piperaquine (supervised), 39 days for dihydroartemisinin-piperaquine (home), and 35 days for both artesunate-mefloquine groups. Patients who received artesunate-mefloquine had more vivax infections during follow-up (57 of 316) than patients who received dihydroartemisinin-piperaquine (40 of 319), but this difference was not significant (relative risk 0·70 [0·48–1·01], p=0·07). Children younger than 5 years were more likely to have vivax malaria during follow-up (61 of 154, 40%) than older children (36 of 375, 10%) or adults (none of 106; p<0·0001). Of the 87 patients who had mixed infections at baseline, 22 (25%) had relapses of vivax malaria during follow-up, whereas 75 (13%) of 565 patients who did not have mixed infections at day 0 had subsequent relapses (relative risk 1·86 [95% CI 1·22–2·83], p=0·007).

At baseline, 240 (37%) of 652 patients had patent gametocytaemia (table 1). The proportions with gametocytaemia differed between the treatment groups despite randomisation, with higher proportions of gametocytaemia in the dihydroartemisinin-piperaquine groups than in the artesunate-mefloquine groups (table 1). The gametocyte carrier rate was higher in young children (85 of 159, 54%) than in older children (131 of 380, 35%) or adults (24 of 113, 21%; p<0·0001; table 3). There was a strong association between anaemia and gametocyte carriage. The proportion with patent gametocytaemia at baseline was highest in patients with haemoglobin concentration less than 80 g/L (35 of 78, 45%); 25% (26 of 110) of patients with haemoglobin of 80–100 g/L, and 19% (24 of 128) of those with haemoglobin more than 100 g/L, had gametocytaemia at baseline. In a stepwise multiple regression model both haemoglobin concentration and age were independently correlated with gametocytaemia (haemoglobin p<0·0001, and age p=0·008)

In the weeks after treatment, the gametocyte carriage rate decreased gradually and gametocytes were cleared by day 21 for both artesunate-mefloquine groups, day 35 for the dihydroartemisinin-piperaquine (home) group, and day 42 for the dihydroartemisinin-piperaquine (supervised) group (table 4, figure 2). New gametocytaemia was also more common in the dihydroartemisinin-piperaquine groups than in the artesunate-mefloquine groups on day 7 (18 of 188 [10%] and five of 218 [2%], respectively; relative risk 4·17 [1·58–11·03]; p=0·011) and on day 14 (three of 168 and one of 212, respectively; 3·79 [0·40–36·07], p=0·12). Gametocyte carriage rates, measured as person-gametocyte-weeks per 1000 person-weeks, were significantly higher for the dihydroartemisinin-piperaquine groups than for the artesunate-mefloquine groups (table 4, p=0·003).

	<5 years	5–14 years	>14 years	p
Gametocytes (+) day 0	85/159 (54%)	131/380 (35%)	24/113 (21%)	<0·0001
Gametocytes (+) day 7	54/153 (35%)	101/377 (27%)	21/110 (19%)	0·003
New gametocyte appearances on day 7	3/150 (2%)	17/360 (5%)	3/107 (3%)	0·60
New gametocyte appearances on day 14	1/69 (1%)	3/230 (1%)	0/81 (0%)	0·37

χ^2 test for linear trend.

Table 3: **Relation between gametocytaemia and age**

	Dihydroartemisinin-piperaquine (supervised)	Dihydroartemisinin-piperaquine (home)	Artesunate-mefloquine (supervised)	Artesunate-mefloquine (home)	Relative risk (95% CI) for dihydroartemisinin-piperaquine (all) *vs* artesunate-mefloquine (all)	p
Positive for gametocytes						
Day 0	70/156 (45%)	67/171 (39%)	58/162 (36%)	45/163 (28%)	1·32 (1·08–1·62)	0·006
Day 7	58/153 (38%)	60/169 (36%)	33/158 (21%)	25/160 (16%)	2·01 (1·53–2·64)	<0·0001
Day 14	43/153 (28%)	41/165 (25%)	14/158 (9%)	3/160 (2%)	4·94 (3·00–8·13)*	<0·0001
Day 21	14/149 (9%)	12/167 (7%)	0/154	0/156	..	<0·0001†
Day 28	3/152 (2%)	3/166 (2%)	0/156	0/158	..	0·03†
New gametocyte appearances						
Day 7	7/84 (8%)	11/104 (11%)	3/102 (3%)	2/116 (2%)	4·17 (1·58–11·03)	0·011
Day 14	3/78 (4%)	0/90	1/99 (1%)	0/113	3·79 (0·40–36·07)	0·115
Person-gametocyte-weeks	330 (101/306)	302 (101/334)	149 (47/316)	88 (28/320)		0·003‡

p values are adjusted for supervision (home *vs* supervised) unless stated otherwise. *Effect of treatment differs for supervised and home groups (p=0·04). Relative risk 13·25 (4·19–41·93) for home groups and 3·17 (1·81–5·56) for supervised groups. †Not adjusted for supervision. p value is from χ^2 test with Yates' correction. ‡Calculated with the Score test.

Table 4: **Gametocyte responses to antimalarial treatment**

The mean haemoglobin concentration at baseline did not differ in the four treatment groups, and 25% of patients had severe anaemia (haemoglobin <80g/L; table 1). On day 28, this proportion had decreased to 10% (64 of 631). The mean increases in haemoglobin did not differ across the groups (p=0·36, table 5). Haemoglobin concentrations were lower in young children (mean 83 g/L) than in older children (93 g/L) and adults (112 g/L). The mean haemoglobin concentrations increased to 95 g/L, 102 g/L, and 113 g/L, respectively, for the three age groups by day 28. The increase in haemoglobin concentrations was significantly greater for the younger groups (p<0·0001).

Adverse effects were reported most frequently in the first week and were more commonly reported by patients in the supervised treatment groups than the non-supervised groups (table 6). All patients were observed for 1 h after treatment on day 0. Overall 15 patients (2·3%) vomited within that time. On day 1, treatment was monitored only for the supervised groups. In these groups, eight patients vomited within 1 h (four in each drug group). On day 2, only two patients vomited, both from the dihydroartemisinin-piperaquine group. The patients in the non-supervised groups came back for the first time on day 7. None reported vomiting within 1 h of taking the medicines on days 1 or 2. Thus, vomiting within 1 h was significantly more common in the supervised than in the unsupervised groups (ten of 635 observations *vs* none of 668, p=0·0007). Vomiting on day 0 within 1 h of drug administration was more common in young children (ten of 159) than in older children (four of 386) and adults (one of 113, p=0·001).

Dizziness was the most frequently reported complaint. In the supervised groups, the proportion of patients reporting dizziness within 24 h of taking the tablets was significantly smaller for the dihydroartemisinin-piperaquine group than for the artesunate-mefloquine group (47 of 133, 35% *vs* 72 of 151, 48%; relative risk 0·74 [0·56–0·98], p=0·05). There was a similar pattern for the dihydroartemisinin-piperaquine (home) and artesunate-mefloquine (home) groups (38 of 144, 26% *vs* 55 of 138, 40%; 0·66 [0·47–0·93], p=0·02). The next day the frequencies of dizziness had decreased. Dizziness was again reported less in the dihydroartemisinin-piperaquine groups than the artesunate-mefloquine groups but these differences were not significant: 18% (24 of 134) after supervised dihydroartemisinin-piperaquine and 27% (41 of 150) after supervised artesunate-mefloquine (0·66 [0·42–1·02], p=0·08); and 19% (28 of 144) after dihydroartemisinin-piperaquine treatment at home and 28% (38 of 138) after artesunate-mefloquine at home (0·71 [0·46–1·08], p=0·14). After day 2 the proportion reporting dizziness was 3% or less for all groups.

Nausea was also less frequently reported within 24 h of taking the tablets in the dihydroartemisinin-piperaquine (supervised) group than in the artesunate-mefloquine

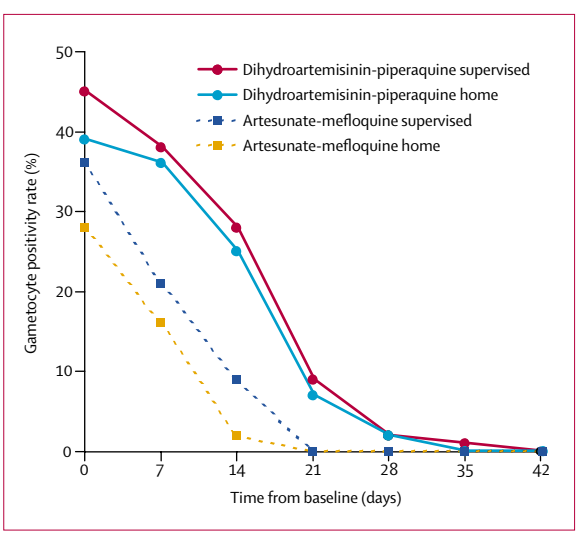

Figure 2: **Proportions of patients with patent gametocytaemia**

	Dihydroartemisinin-piperaquine (supervised)	Dihydroartemisinin-piperaquine (home)	Artesunate-mefloquine (supervised)	Artesunate-mefloquine (home)	Relative risk (95% CI) for dihydroartemisinin-piperaquine (all) versus artesunate-mefloquine (all)	p
All age groups						
Mean haemoglobin (g/L)						
Day 0	94	92	95	95	..	0·15
Day 28	104	101	103	102	..	0·65
Change	9·4	9·1	8·1	8·1	..	0·36
Proportion of people anaemic*						
Day 0	92/156 (59%)	113/171 (66%)	91/162 (56%)	95/162 (59%)	1·13 (0·99–1·28)	0·18
Day 28	56/152 (37%)	75/165 (46%)	59/156 (38%)	68/158 (43%)	1·02 (0·85–1·23)	0·85
Children <5 years						
Mean haemoglobin (g/L)						
Day 0	84	78	82	87	..	0·18
Day 28	96	89	95	99	..	0·06†
Change	12	10	13	12	..	0·46
Proportion of children anaemic*						
Day 0	27/38 (71%)	37/41 (90%)	30/37 (81%)	34/43 (79%)	1·04 (0·90–1·21)	0·85
Day 28	19/37 (51%)	29/39 (74%)	17/33 (52%)	22/43 (51%)	1·23 (0·93–1·63)	0·12

Continuous data were analysed by two-way ANOVA and proportions of categorical data were compared with multiple logistic regression model. p values adjusted for effect of supervision (supervised *vs* home). *Haemoglobin <100g/L. †Effect of the treatment differed for supervised group and home treated groups (p=0·03).

Table 5: **Haemoglobin values after treatment**

	Dihydroartemisinin-piperaquine (supervised)	Artesunate-mefloquine (supervised)	Relative risk (95% CI)	p	Dihydroartemisinin-piperaquine (home)	Artesunate-mefloquine (home)	Relative risk (95% CI)	p
Dizziness	60/134 (45%)	84/150 (56%)	0·8 (0·63–1·01)	0·08	44/144 (31%)	60/138 (44%)	0·7 (0·51–0·96)	0·03
Nausea	24/133 (18%)	43/149 (29%)	0·63 (0·40–0·97)	0·05	15/144 (10%)	22/138 (16%)	0·65 (0·35–1·21)	0·23
Anorexia	8/133 (6%)	14/149 (9%)	0·64 (0·28–1·48)	0·40	2/144 (1%)	5/138 (4%)	0·38 (0·08–1·94)	0·27
Diarrhoea	9/133 (7%)	7/149 (5%)	1·44 (0·55–3·76)	0·62	2/144 (1%)	2/138 (1%)	0·96 (0·14–6·71)	1·0
Itchiness	2/133 (2%)	0/149 (0%)	..	0·22	1/144 (1%)	0/138 (0%)	..	1·0
Abdominal pain	2/133 (2%)	4/149 (3%)	0·56 (0·10–3·01)	0·69	1/144 (1%)	1/138 (1%)	..	1·0
Fatigue	1/133 (1%)	5/149 (3%)	0·22 (0·03–1·89)	0·22	0/144 (0%)	1/138 (1%)	..	0·49
Vomiting day 0								
Within 30 min	2/144 (1%)	5/149 (3%)	0·41 (0·08–2·10)	0·45	4/165 (2%)	2/153 (1%)	1·67 (0·31–9·0)	0·69
30–60 min	0/144 (0%)	1/149 (1%)	..	1·0	1/165 (1%)	0/153	..	1·0
Vomiting day 1								
Within 30 min	0/156	1/162 (1%)	..	1·0	0/171	0/163	..	..
30–60 min	4/156 (3%)	3/162 (2%)	1·38 (0·31–6·09)	0·73	0/171	0/163	..	..
Vomiting day 2								
Within 30 min	1/155 (1%)	0/162	..	0·49	0/171	0/163	..	..
30–60 min	1/155 (1%)	0/162	..	0·49	0/171	0/163	..	..

χ^2 test with Yates' corrected p values.

Table 6: **Adverse effects within the first week after treatment**

(supervised) group (21 of 134, 16% *vs* 35 of 151, 23%; 0·68 [0·41–1·10], p=0·15). For the patients who took the treatment at home the proportions were 13 of 144 (9%) for dihydroartemisinin-piperaquine, and 19 of 138 (14%) for artesunate-mefloquine (0·71 [0·46–1·08]). The next day the proportion reporting nausea in the supervised groups had fallen to 2% (two of 132) after dihydroartemisinin-piperaquine and 13% (17 of 133) after artesunate-mefloquine (0·12 [0·03–0·50], p=0·0009) and in the home groups to 7% (ten of 134) after dihydroartemisinin-piperaquine and 7% (ten of 138) after artesunate-mefloquine (1·03 [0·44–2·39], p=0·87). On day 3, only one patient reported nausea. Adults reported significantly more dizziness (p<0·0001) and nausea (p=0·0002) than children in the first week.

Other side-effects such as anorexia, diarrhoea, itchiness, abdominal pain, and fatigue were less common, and their occurrence was not linked to the specific treatment groups. In the dihydroartemisinin-piperaquine group, three patients reported mild itching; two on day 0 and

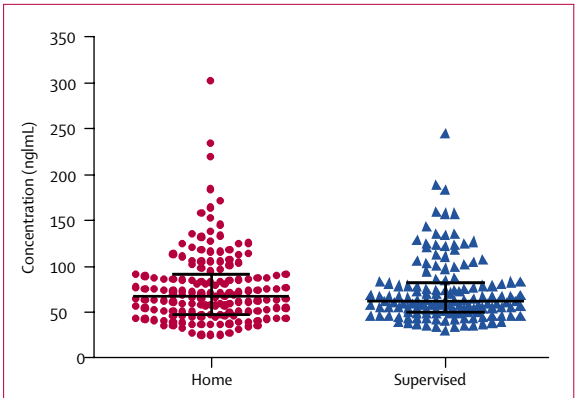

Figure 3: **Day 7 capillary whole-blood piperaquine concentrations in patients who took the day 2 and day 3 doses unsupervised at home, compared with those whose dose administration was supervised**
Bars show median and IQR.

one on day 7. The itching was mild, did not need treatment, and had disappeared at the next visit. No patient needed hospital admission for side-effects.

The median blood piperaquine concentration on day 7 was 63 ng/mL (IQR 49–86; n=299); 28 samples were unavailable because of instrument failure. The concentrations were similar for the supervised (median 62 ng/mL [50–82]; mean 72 ng/mL) and home treatment groups (median 66 ng/mL [47–90 ng/mL]; mean 74 ng/mL; p=0·66 for comparison of medians; figure 3). Six patients had piperaquine concentrations lower than the limit of quantification (25 ng/mL), of whom two had treatment failure (one recrudescent and one indeterminate). Five of six patients with piperaquine concentrations lower than the limit of quantification were in the unsupervised group. Blood concentrations below the limit of quantification were treated as zero and included in the analysis. The mean blood piperaquine concentration at day 7 was significantly higher in the 293 patients who were successfully treated than in six with recrudescence or reinfection (74 [SD 39] ng/mL *vs* 34 [31] ng/mL, p=0·013).

Discussion

Both artesunate-mefloquine and dihydroartemisinin-piperaquine were highly efficacious, effective, and well-tolerated treatments for uncomplicated falciparum malaria in western Myanmar. Similar high cure rates have been reported previously in studies in Vietnam[7] (56-day cure rate 98·7%), Cambodia[8] (28-day cure rate 96·9%), and Thailand[9] (63-day cure rate 96·1%). Under normal conditions, without supervision of dosing after the initial consultation, cure rates were almost 100%. Since the efficacy of incomplete regimens in these 3-day treatments is reduced, this rate suggests that nearly all patients completed their treatment as advised. In the case of artesunate-mefloquine, all the mefloquine and the first dose of artesunate were given under supervision on the first day, and in a previous study, this regimen (ie, 1-day treatment) was associated with a high (96·4%) cure rate.[5] But for dihydroartemisinin-piperaquine, a third of the dose (first-day treatment only) is certainly not enough to account for such high cure rates, so adherence was probably good. This assumption is lent support by the whole-blood piperaquine results, which indicated little difference between the supervised and non-supervised groups.

The artesunate-mefloquine combination, which has been the national protocol for falciparum malaria in Myanmar since 2002, has been used continuously since 1996 in this area. The treatment has retained a high cure rate, and the fact that there has been no selection of resistance evident in this region of western Myanmar over the past 7 years is encouraging. In Thailand, resistance to mefloquine monotherapy arose within 7 years, but resistance to mefloquine after deployment of the combination threatment has fallen.[16] These results, indicating good adherence to prescribed regimens and sustained high efficacy, augur well for more general deployment of these artemisinin-based combinations. Mefloquine is associated with predictable gastrointestinal and CNS adverse effects. In previous studies, tolerability and drug absorption were both increased by splitting the 25 mg/kg dose. In this study, the 3-day artesunate-mefloquine combination treatment was started with 25 mg/kg mefloquine on day 0, by contrast with a previous study we did in the same region in which mefloquine was divided over day 1 (15 mg/kg) and day 2 (10 mg/kg). This approach aimed to reduce the effects of poor adherence, which was thought to be more of a concern than reduced oral bioavailability. Indeed, cure rates were better in this study (no recrudescences in 159 patients) than in our earlier trial when mefloquine was taken over days 1 and 2 (seven recrudescences of 180; 4%; p=0·016). This finding corroborates our previous suggestion[5] that, if the treatment cannot be supervised, and the tablets are separate, the whole dose of mefloquine should be given on day 0 to ensure that it is taken, even though the drug's absorption and bioavailability might be less when taken as a single dose on day 0.[17]

Vomiting of antimalarial drugs is an important consideration in treatment. Early vomiting occurred in only 2% of patients treated on the first day with 25 mg/kg mefloquine. This proportion is lower than reported previously.[18] Dihydroartemisinin-piperaquine treatment was associated with a similarly low frequency of early vomiting (2%) but had significantly fewer other side-effects (dizziness and nausea), particularly in the first 2 days. Vomiting on the next 2 days of treatment in supervised patients was infrequent and was not a major drawback in this study. The patients who took their medicines at home did not report any vomiting within 1 h after drug intake at all whereas in the supervised group some patients had early vomiting, although this reaction was mainly after the first dose when febrile and ill. Thus, we think that some

patients in the non-supervised group might have vomited on days 1 and 2, but that this reaction was not reported. If so, and since they did not request extra medicines, they would have had an inadequate dose and cure rates might have been expected to fall. Alternatively, when partly recovered and in the familiar setting of home, vomiting might be less likely. Patients should be informed clearly that in case of vomiting, extra tablets should be obtained. This factor is particularly important with dihydroartemisinin-piperaquine, since a third of the dose (first day treatment only) is unlikely to be enough to cure the infection.

During follow-up, patients who received dihydroartemisinin-piperaquine had fewer vivax infections, though not significantly (p=0·07), but had significantly more gametocyte carriage and more new gametocyte appearances.

Although the proportion of gametocytaemia at baseline, by chance, was higher in the dihydroartemisinin-piperaquine group, the rate of new appearance of gametocytaemia was also significantly higher in this group than in the artesunate-mefloquine recipients. If the higher gametocyte carriage rate after dihydroartemisinin-piperaquine is confirmed, it would represent a public-health disadvantage because it would indicate increased transmissibility. The higher gametocyte carriage rate might result from the lower dose of artemisinin derivative used in the dihydroartemisinin-piperaquine combination. This effect should be balanced against the advantages of simplicity of dosing, very high efficacy and effectiveness, and an excellent adverse-effect profile. Vivax malaria commonly followed falciparum malaria; indeed *P vivax* was by far the most common cause of malaria during the 6-week follow-up. Mixed infections are much more frequent than recognised from admission blood smears.[19] The appearance of *P vivax* infection after an effective treatment presumably represents a relapse. These infections were particularly frequent (40%) in children under 5 years of age.[20] Both health providers and parents in this area need to be informed that there is a high risk of another malaria episode in young children, and parents should be encouraged to bring the children to the clinic if fever reappears.

Although the question of adherence to antimalarial drugs is much debated, there are no definitions or guidelines to interpret incomplete adherence and few studies that have tried to measure it. A recent review identified only 16 trials with widely differing methodologies.[21] The use of blood concentration measurements to assess adherence is facilitated by the availability of simple but sensitive validated filter-paper assay methods such as that for piperaquine. This method indicated generally excellent adherence to dihydroartemisinin-piperaquine in our study, although the day 7 whole-blood piperaquine concentrations for the patients with treatment failure were lower than those for successfully treated patients, suggesting that the terminal elimination phase of piperaquine is an important determinant of efficacy.[22] The fact that treatment success was high in this large study even when two-thirds of the treatment doses were not supervised is important because—in practice—most antimalarial treatments are taken at home. However, since the drugs were given free (whereas people generally have to pay), operational effectiveness might differ from that presented here. Education of patients and price subsidy are both likely to be important in ensuring good adherence to the prescribed regimens under normal conditions of use.[23]

In our setting, if the first dose is supervised and a clear instruction is provided to the patient or carer, the effectiveness of the treatment equals its observed efficacy. This study shows that both combination treatment regimens are effective, even when self-administered. Dihydroartemisinin-piperaquine is a good alternative for artesunate-mefloquine because it is less expensive, coformulated, and has fewer side-effects. Dihydroartemisinin-piperaquine has the potential to play an important part in future malaria management.

Contributors

F Smithuis initiated and coordinated the study. Saw Lwin oversaw the malaria programme. F Smithuis and N J White designed the study and wrote the first draft of this paper. K Stepniewska and E Ashley helped in the trial design and supported the statistical analysis. F Smithuis, M K Kyaw, and K Stepniewska analysed the data. M K Kyaw, K Z Aye, and L Htet recruited and followed up the patients. U Ohn Phe was responsible for microscopy, and M Barends the PCR analysis. N Lindegardh and T Singtoroj measured the piperaquine blood concentrations. All investigators and the coordinating committee reviewed and discussed the trial results.

Conflict of interest statement

N J White is chairman of the WHO malarial treatment guidelines committee. None of the other authors have any conflicts of interest.

Acknowledgments

We thank the staff of the clinics in Dabhine and Mingan and the staff of the Vector Borne Disease Control (Department of Health) for their support; Arjen Dondorp for his support with data analysis; and the clinical and laboratory staff of Médecins Sans Frontières (Holland) in Sittwe for their hard work. We also thank Piet Kager for critical reading of the manuscript. M Barends, K Stepniewska, N Lindegardh, T Singtoroj, E Ashley, and N J White are supported by The Wellcome Trust of Great Britain as part of the Wellcome Trust Mahidol University Oxford Tropical Medicine Research Programme. This study was financed by Médecins Sans Frontières (Holland).

References

1 Nosten F, ter Kuile F, Chongsuphajaisiddhi T, et al. Mefloquine-resistant falciparum malaria on the Thai-Burmese border. *Lancet* 1991; **337:** 1140–43.

2 Tin F. Malaria control in Myanmar. *Southeast Asian J Trop Med Public Health* 1992; **23** (suppl 4)**:** 51–54.

3 Smithuis FM, Monti F, Grundi M. *In vivo* sensitivity of *Plasmodium falciparum* to chloroquine, sulphadoxine-pyrimethamine, and mefloquine in Rakhine State, Western Myanmar. *Trans R Soc Trop Med Hyg* 1997; **91:** 468–72.

4 Smithuis F, Shahmanesh M, Kyaw MK, Savran O, Lwin S, White NJ. Comparison of chloroquine, sulfadoxine/pyrimethamine, mefloquine and mefloquine-artesunate for the treatment of falciparum malaria in Kachin State, North Myanmar. *Trop Med Int Health* 2004; **9:** 1184–90.

5 Smithuis F, van der Broek I, Katterman N, et al. Optimising operational use of artesunate-mefloquine: a randomised comparison of four treatment regimens. *Trans R Soc Trop Med Hyg* 2004; **98:** 182–92.

6 Denis MB, Davis TM, Hewitt S, et al. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. *Clin Infect Dis* 2002; **35:** 1469–76.

7 Hien TT, Dolecek C, Mai PP et al. Dihydroartemisinin-piperaquine against multidrug-resistant *Plasmodium falciparum* malaria in Vietnam: randomised clinical trial. *Lancet* 2004; **363:** 18–22.

8 Karunajeewa H, Lim C, Hung TY, et al. Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. *Br J Clin Pharmacol* 2004; **57:** 93–99.

9 Ashley EA, Krudsood S, Phaiphun L, et al. Randomized, controlled dose-optimization studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug-resistant falciparum malaria in Thailand. *J Infect Dis* 2004 **190:** 1773–82.

10 World Health Organization. Severe and falciparum malaria. *Trans R Soc Trop Med Hyg* 2000; **94** (suppl 1)**:** 1–90.

11 Brockman A, Paul RE, Anderson TJ, et al. Application of genetic markers to the identification of recrudescent *Plasmodium falciparum* infections on the northwestern border of Thailand. *Am J Trop Med Hyg* 1999; **60:** 14–21.

12 Malm M, Lindegårdh N, Bergqvist Y. Automated solid-phase extraction method for the determination of piperaquine in capillary blood applied onto sampling paper by liquid chromatography. *J Chromatogr B* 2004; **809:** 43–49.

13 Price RN, Nosten F, Luxemburger C, et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 1996; **347:** 1654–58.

14 Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: II, analysis and examples. *Br J Cancer* 1977; **35**: 1–39.

15 Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med* 1998; **17**: 873–90. http://www.cardiff.ac.uk/medicine/epidemiology_statistics/research/ statistics/ciproportion.xls (accessed November, 2005).

16 Nosten F, van Vugt M, Price R, et al. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 2000; **356:** 297–302.

17 Simpson JA, Price RN, ter Kuile FO, et al. Population pharmacokinetics of mefloquine in patients with acute falciparum malaria. *Clin Pharmacol Ther* 1999; **66:** 472–84.

18 ter Kuile FO, Nosten F, Luxemburger C, et al. Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3673 patients. *Bull World Health Organ* 1995; **73:** 631–42.

19 Mayxay M, Newton PN, Pukrittayakamee S, White NJ. Mixed species malaria infections in humans. *Trends Parasitol* 2004; **20:** 233–40.

20 Luxemburger C, Thwai KL, White NJ, et al. The epidemiology of malaria in a Karen population on the western border of Thailand. *Trans R Soc Trop Med Hyg* 1996; **90:** 105–11.

21 Yeung S, White NJ. How do patients use antimalarial drugs? A review of the evidence. *Trop Med Int Health* 2005; **10:** 121–38.

22 Hung TY, Davis TM, Ilett KF, et al. Population pharmacokinetics of piperaquine in adults and children with uncomplicated falciparum or vivax malaria. *Br J Clin Pharmacol* 2004; **57:** 253–62.

23 Institute of Medicine. Saving lives, buying time: the economics of malaria drugs in an age of resistance. Washington DC: National Academies Press, 2004.