A Randomized Trial of Artesunate-Mefloquine versus Artemether-Lumefantrine for Treatment of Uncomplicated Plasmodium falciparum Malaria in Mali

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Abstract. The choice of appropriate artemisinin-based combination therapy depends on several factors (cost, efficacy, safety, reinfection rate, and simplicity of administration). In this study, we tested the hypothesis that artemesin-mefloquine (Artequin™) is as efficacious as artemether-lumefantrine (Coartem®) in treatment of uncomplicated Plasmodium falciparum malaria. The study was carried out from August 2004 through February 2005 in Kambila, Mali. Subjects with weights ≥ 10 kg and uncomplicated malaria were enrolled. Artesunate-mefloquine was given once a day for three days and artemether/lumefantrine twice a day for three days. A total of 470 (235 in each arm) patients were enrolled. The unadjusted 28-day cure rate was higher in artemesinate-mefloquine arm than in the artemether-lumefantrine arm (79.7% versus 67.8%; P < 0.004). After correction for re-infection, the 28-day cure rates were similar in the two groups (96.04% versus 96.93%). Artesunate-mefloquine is well-tolerated and is as effective as artemether-lumefantrine for the treatment of P. falciparum malaria. Artesunate-mefloquine also prevented more new infections.

INTRODUCTION

Several studies have been conducted or are currently under way to evaluate artemisinin-based combination malaria therapy. Studies using artemether-lumefantrine (AL), artesunate-amodiaquine, artesunate-sulfadoxine-pyrimethamine, artesunate-sulfadoxine-pyrazine-pyrimethamine, or artesunate-mefloquine have shown that the combination therapies are safe and effective.1–5 The World Health Organization (WHO) has recommended the use of artemesinin-based combination therapies and called for their evaluation in various malaria-endemic areas. Although artesunate-mefloquine has been widely studied in Asia, data are limited in malaria-endemic areas in Africa.6–8 Also, the artemisinin-based antimalarial combination therapies differ in price, efficacy, prevention of reinfection, side effects, and mode of administration. In the long term, the choice of artemisinin-based combination therapy in a specific malaria-endemic area needs to take into account the above criteria.

We investigated whether artesunate-mefloquine (AS + MEF) was efficacious and safe when compared with AL. Because mefloquine has not been used widely as an antimalarial drug in malaria-endemic areas of Africa, it is expected that the combination of this drug with artesunate will result in a highly efficacious treatment for P. falciparum malaria. Also, because of the long half-life of mefloquine,9,10 we believed that combination drug could be a potential candidate for intermittent preventive treatments in children or pregnant women. AL was used as a comparator because WHO has listed this drug as an essential antimalarial drug.

METHODS

Study site. The study was conducted in Kambila, Mali. Kambila is a peri-urban village of Kati located 25 km from Bamako with a population of approximately 1,500. Malaria in this area is hyper-endemic and transmission is highly seasonal. Plasmodium falciparum is the predominant infecting species, accounting for more than 95% of malaria cases.

Study population. Persons weighing ≥ 10 kg and ≥ 1 of age who came to the local health center during the study period (August 2004 through January 2005) were included in the study if they satisfied the following criteria: weighed ≥ 10 kg, had a P. falciparum parasite density between 2,000 and 200,000/μL, had an axillary temperature ≥ 37.5°C, were a resident of the study site, and could take oral medication. Persons were excluded if they had symptoms or signs of severe malaria, had a serious underlying disease, had an allergy to one or more study drugs, had used any component of the study drugs within 28 days of enrollment, or were pregnant (detected either clinically or with a urine ß-human chorionic gonadotropin test).

Each participant or participant’s guardian gave fully informed written consent prior to enrollment. The protocol was reviewed and approved by the ethical committee of the Faculty of Medicine, Pharmacy and Odonto-Stomatolgy, University of Bamako.

Procedures. Enrolled patients were randomly assigned to receive either AS + MEF (Artequin™; Mepha Ltd., Basel, Switzerland) or AL (Coartem®, Novartis, Basel, Switzerland). A bloc randomization code with treatment arm was computer generated by the study statistician not involved in patients’ enrollment or outcomes assessment. Study codes were sealed in individual opaque and sequentially numbered envelopes. The enrolled patient was assigned a treatment according to the envelope content. Although this was an open, randomized, clinical trial, thick blood smear slide readers were kept blinded to the treatment arm until the end of the study; this was conducted to minimize assessment bias because malaria parasite count was the key outcome used to define treatment failure.

All drugs were manufactured according to current Good Manufacturing Practice. AL was packaged in fixed-dose combination tablets, each containing 20 mg of artemether and 120 mg of lumefantrine. They were administered according to
body weight (5–14 kg, one tablet; 15–24 kg, two tablets; 25–34 kg, three tablets; ≥ 35 kg, four tablets) in six consecutive doses: one dose was administered at enrollment, one dose 8 hours later, and then two doses on the second day after initiation of treatment. The AS + MEF treatment was supplied as two separate tablets in a single blister pack: a tablet of mefloquine and a tablet of artesunate. One dose was administered daily for three days. Dosage was determined by weight, with different blister packs for different weight arms. For a weight ≥ 31 kg, each blister contained three 200-mg tablets of artesunate plus three 250-mg tablets of mefloquine. For a weight of 15–30 kg, each blister contained three 100-mg tablets of artesunate plus three 125-mg tablets of mefloquine. For a weight of 10–14 kg, artesunate has been given at a dose of 4 mg/kg and mefloquine at a dose of 5 mg/kg.

For young children in both treatment arms, tablets were crushed and mixed with water. All drug doses were administered in the health center by a physician. A full dose was re-administered if a participant vomited the study drugs within 30 minutes of initial administration.

Study participants were examined in the study clinic 1, 2, 3, 7, 14, 21, and 28 days after enrollment or at any time if they did not feel well. A finger skin puncture was used to obtain blood for a thick blood smear and a filter paper dot (for future parasite DNA extraction) at each follow-up visit (except on day 1 when blood was used only for a filter paper dot).

FIGURE 1. Trial profile.

997 patients screened for fever probably due to malaria

527 Not eligible

470 Volunteers randomized

235 Randomly assigned AS+MEF

235 Randomly assigned AL

1 violation of inclusion criteria

1 lost to follow-up

2 lost to follow-up

1 missed more than one scheduled visit

232 Completed the study

232 Completed the study
Study participants or their guardians were asked about drug consumption since the last visit to the clinic. Persons for whom treatment failed were treated according to the treatment guidelines of the Malian National Malaria Control Program (quinine in case of failure to artesinin-based combination malaria therapy).

Giemsa-stained thick blood smears were read by experienced microscopists who were blinded to treatment allocation. Parasite densities were calculated by counting the number of asexual \textit{P. falciparum} parasites until 300 leukocytes were observed and then converting that to parasites per microliter of blood, assuming an average leukocyte count of 7,500/\mu L. For quality control, 10\% of the slides were selected at random and re-read by another microscopist who was unaware of the results of the first reading.

A complete blood count plus serum alanine aminotransferase (ALT) and serum creatinine assessments were performed among the first enrolled on approximately 20\% of the total participants at baseline and 14 days after initiation of treatment. Tests were also performed if clinically indicated or if a significant abnormality was detected on day 14. In addition, hemoglobin levels were measured using a Hemocue (Angelholm, Sweden) machine at baseline, on day 14, and on day 28.

For participants with recurrent parasitemia after day 7, paired polymerase chain reaction (PCR) blots (from day 0 and the day of parasitemia recurrence) were analyzed for parasite merozoite surface proteins (MSP-1 and MSP-2) and microsatellite CA1 to distinguish between reinfection and recrudescence.\textsuperscript{12–15} DNA was obtained from the day 0 and failure day samples. Initial DNA was extracted using the methanol method.\textsuperscript{1} Samples that failed to yield interpretable results were re-extracted using a Qiagen Kit (Qiagen, Valencia, CA) according to the manufacturers instructions. We compared the day 0 and failure day alleles of MSP-1, MSP-2, and microsatellite CA1 gene loci.\textsuperscript{3}

The PCR was performed using the following primers pairs

\begin{itemize}
  \item MSP1: O1 = 5'-CACATGAAAGTTAACAAGATTC-TG-TC-3' and O2 = 5'-TACAGCTTTAATTCTTGTGACG-3'
  \item N1 = 5'-GACGTATTTAGCAGTTATGG-3' and N2 = 5'-GATGGAAAGTTATTCGACG-3'
  \item MSP2: S3: 5'-GAAGATTTAAACCATCAG-3' and S2: 5'-GAGGTATGTTGTGCCTCCACAG-3'
  \item S1: 5'-GAGTGATAGAAGGAGAAGTATG-3' and S4: 5'-CTAGAACCATTGACATGTG-3'
  \item MICROSATELLITE Ca1: Ca1-1L: 5'-CAATGCTGCTCCACAG-3' for PCR2
  \item Ca1-R: 5'-CAAACTGATATG-3'
\end{itemize}

One to two microliters of the product of that amplification was used for another 30 cycles of PCR. Each PCR was performed with 1 \mu M of each primer, 200 \mu M of each dNTP, and 1.5–3 mM MgCl\textsubscript{2}.

Possible outcomes were 1) recrudescence, if the alleles of the pre-treatment and post-treatment samples are the same for MSP-1, MSP-2, and CA1; 2) reinfection, if the alleles of the pre-treatment and post-treatment samples are distinct for any of these three loci; 3) mixed recrudescence and reinfection, if similar alleles are found in the pre-treatment and post-treatment samples for all the markers as mentioned above, but with additional distinct alleles identified; and 4) indeterminate, if either or both the pre-treatment and post-treatment samples could not be amplified. Mixed recrudescent and re-infection cases were computed as recrudescent.

**Objectives.** The primary objective was to test the hypothesis that AS + MEF is as efficacious as AL in treatment for uncomplicated \textit{P. falciparum} malaria. The secondary objectives were to assess and compare the following outcomes in the two treatment arms: parasite and fever clearances, reinfection rate, gametocyte carriage rate, anemia correction rate, and clinical and biologic adverse events.

Therapeutic outcomes were classified according to the current WHO protocol.\textsuperscript{16} The primary endpoint was an adequate clinical and parasitologic response, or cure rate, after 14 days of follow-up. Secondary endpoints were incidence of adverse events (clinical and laboratory abnormalities), reinfection rate, anemia correction rate (anemia was defined as a hemoglobin level < 10 g/dL), fever (temperature ≥ 37.5°C), parasitemia clearance rates, and gametocyte carriage rates. Fever clearance was assessed on days 1, 2, and 3; parasite clearance was assessed on days 2 and 3; and gametocyte carriage was assessed on days 0, 3, 7, 14, 21, and 28. The anemia correction rate was determined by subtracting the anemia rate for day 28 from the anemia rate for day 0 for each treatment arm. An adverse event was defined as any sign, symptom, or abnormal laboratory value not present on day 0 but that occurred during follow-up, or one that was present on day 0 but became worse during follow-up. A serious adverse event was defined according to the International Conference on Harmonization (ICH E6, Glossary 1.50).

**Statistical analysis.** The required sample size was calculated using the assumption that the two study drugs were equally effective in terms of the primary endpoint, with a two-sided \( \alpha \) of 0.05 and a power of 80\%. The maximum accepted difference in efficacy between the two study treatments was set at 6\%. On the basis of a study from the Gambia\textsuperscript{17} in which a 93.3\% 14-day cure rate was reported for AL, a total of 470 infection cases were computed as recrudescent.

**Table 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AS + MEF (n = 235)</th>
<th>AL (n = 235)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (range)</td>
<td>7.4 (1–70)</td>
<td>7.1 (1–33)</td>
</tr>
<tr>
<td>% &lt; 5 years of age</td>
<td>35.74</td>
<td>35.32</td>
</tr>
<tr>
<td>% ≥ 5 years of age</td>
<td>64.26</td>
<td>64.68</td>
</tr>
<tr>
<td>% Male</td>
<td>47.2</td>
<td>53.6</td>
</tr>
<tr>
<td>Geometric mean parasite count/ \mu L (range)</td>
<td>18,159 (2,025–192,075, 18,357 (2,025–187,050)</td>
<td></td>
</tr>
<tr>
<td>Mean hemoglobin level, g/L (SD)</td>
<td>11.4 (1.5)</td>
<td>11.3 (1.3)</td>
</tr>
<tr>
<td>Mean leukocyte count × 1012/L (SD)</td>
<td>9.6 (4.2)</td>
<td>10.1 (3.6)</td>
</tr>
<tr>
<td>Mean ALT level, IU/L (SD)†</td>
<td>31.6 (43.6)</td>
<td>30.9 (22.2)</td>
</tr>
<tr>
<td>Mean creatinine level, \mu mol/L (SD)†</td>
<td>55.3 (6.9)</td>
<td>56.1 (12.6)</td>
</tr>
</tbody>
</table>

\* AS + MEF = artesunate-mefloquine; AL = artemether-lumefantrine; ALT = alanine aminotransferase.

† Biochemical tests and hemograms were performed among the first 20\% of the participants.
The intention-to-treat analysis included all randomized participants except for protocol violation cases, which were excluded from intention-to-treat analysis. The lost to follow-up cases were included into the intention-to-treat analysis. The per protocol analysis excluded the lost to follow-up cases.

Data were double-entered, validated using Microsoft Access (Microsoft Corporation, Redmond, WA), and analyzed with STATA version 8.2 (STATA Corporation, College Station, TX). For the baseline and the primary endpoint, binary data were analyzed by estimation of differences in proportions with corresponding 95% confidence intervals (CIs). Exact 95% CIs were calculated when the proportions were close to zero or one; otherwise, normal estimations were used. For secondary endpoints, the chi-square test or Fisher exact test were used as appropriate to compare categorical variables. Student’s unpaired $t$-test was used to assess differences between groups in mean for continuous variables. The McNemar paired chi-square test or Fisher exact test was used as appropriate to compare categorical variables. Student’s unpaired $t$-test was used to assess differences between groups in mean for continuous variables. The McNemar paired chi-square test was used to compare the prevalence of anemia before and after treatment in each treatment arm. A $P$ value (two-sided) < 0.05 was considered statistically significant.

RESULTS

Participant characteristics. Four hundred seventy of 997 patients who were screened for fever were enrolled. The screening failure cases (527) were subjects who had a fever (temperature $\geq 37.5^\circ C$) on day of enrollment but did not meet the other inclusion criteria (see study population in the Methods section).

Figure 1 contains detailed enrollment and follow-up information. Of the 470 enrolled participants, 235 were randomized to receive AS + MEF and 235 to receive AL. Of the 470, 9 (1.9%) withdrew or were lost to follow-up. Reasons for withdrawal included withdrawal of consent (one in the AS + MEF arm), receiving the wrong treatment drug on day 0 (one in the AL arm who mistakenly received one dose of AS + MEF), violation of inclusion criteria (one in the AS + MEF arm), and subjects who missed more than one scheduled visit (three in the AS + MEF arm and three in the AL arm). As shown in Table 1, the participants in the two treatment arms had similar demographic, clinical, and laboratory characteristics at enrollment.

Cure rates. No early treatment failures were observed in either treatment arm. As shown in Table 2, the 14-day per protocol cure (adequate clinical and parasitologic response) rates before adjusting for cases of reinfection were 99.6% (229 of 230) and 98.7% (228 of 231) for participants receiving AS + MEF or AL, respectively, was similar ($P = 0.3$). When adjusted for cases of reinfection, the 14-day cure rate for both arms was 100%.

As shown in Table 3, the per protocol 28-day cure rates were 79.6% (183 of 230) and 67.2% (156 of 231) for participants receiving AS + MEF or AL, respectively ($P < 0.003$). After adjusting for cases of reinfection, the 28-day cure rates were 96% (218 of 227) and 96.9% (221 of 228) for those receiving AS + MEF or AL, respectively. This difference between treatment arms was no longer significant ($P = 0.6$). Twenty-eight-day reinfection rates were 15.4% (35 of 227) and 29.9% (66 of 228) for participants receiving AS + MEF or AL, respectively ($P < 0.001$). Clinical and parasitologic responses at day 28 (intention-to-treat analysis) are shown in Table 4.

Fever, parasite clearance, and gametocyte carriage. As shown in Figure 2, the proportion of participants without fever was similar on days 2 and 3. However, on day 1, the fever clearance rate was higher in the AS + MEF arm (95.7%, 224 of 234) than in the AL arm (85.9%, 201 of 234) ($P = 0.001$).

Both treatments resulted in rapid clearance of parasites (Figure 3). The proportion of participants who were aparasitemic was similar between the two treatment arms: 34.8% (81 of 233) and 32.1% (75 of 234) for those receiving AS MEF or AL, respectively, on day 1 ($P = 0.5$). On day 2, this proportion was 97.4% (227 of 233) and 97.9% (229 of 234) for those receiving AS + MEF or AL, respectively ($P = 0.8$). No parasite carriers were found on day 3 in any of the treatment arms.

Gametocyte carriage rates decreased gradually from day 0 to day 28 (Figure 4) in both arms. One subject still had gametocyte on day 28 in AS + MEF arm, and no gametocytes were found in AL arm that day. No statistically significant differences were found in any of the treatment arms.
differences were detected between the two arms at any follow-up day ($P > 0.5$).

**Anemia.** In the AS + MEF arm, 24 (11.3%) of 213 participants had anemia on day 0 compared with 10 (4.7%) of 213 on day 28. For the AL arm, 27 (14.0%) of 193 and 10 (5.2%) of 193 had anemia on day 0 and day 28, respectively. Using the McNemar paired chi-square test, we found that both treatments significantly lowered the prevalence of anemia 28 days after treatment initiation ($P < 0.0001$).

**Adverse events.** AS + MEF and AL were well tolerated in the treatment arms. As shown in Table 5, the proportion of reporting any symptom or sign within the first week after treatment was statistically similar between the arms ($P > 0.05$), except for vomiting reported and the total number of symptoms or signs, which were significantly higher in AS + MEF arm (12 [5.1%] of 234 and 65 [27.8%] of 234) compared with the AL arm (4 [1.7%] of 234 and 43 [18.4%] of 234 ($P = 0.04$, and 0.02).

One (1.5%) of 68 participants in the AL arm had mild and transient elevation of the ALT level on day 14. This abnormal value was not associated with clinical illness and resolved spontaneously by day 28.

**DISCUSSION**

This study demonstrates that both treatments are highly efficacious in treating uncomplicated *P. falciparum* malaria in Kambah, Mali. The PCR-corrected 14-day cure rates were 100% in both arms. The PCR-corrected 28-day cure rates 96.04% in the AS + MEF arm and 96.93% in the AL arm ($P < 0.6$).

The high cure rates we found for AS + MEF were expected because of the high efficacy of artesunate, non-documented resistance of *P. falciparum* to mefloquine in Mali, as in most west African countries in general, and potentially because of the rare use of mefloquine as an antimalarial drug. The advantage of a prolonged period of protection against new *P. falciparum* infections (longer half-life of mefloquine of approximately three weeks) has been weighed against the suspected increase in the risk for the selection of drug-resistant isolates in areas of high malaria transmission. More recently, however, the benefit of reduced rates of reinfection and relapse caused by long-acting antimalarial drugs has been emphasized (Van Vught M and others, unpublished data). The debate on this risk/benefit analysis is still ongoing and needs

![Figure 2. Proportion of participants with fever during the first three days of treatment. This figure appears in color at www.ajtmh.org.](image1)

![Figure 3. Proportion of participants with asexual *Plasmodium falciparum* parasitemia during the first three days of treatment. This figure appears in color at www.ajtmh.org.](image2)
further epidemiologic evidence for areas of both low and high endemicity.

In our study, AS + MEF showed an additional benefit of preventing new infections compared with AL; the 28-day re-infection rates were 15.4% and 29.9% (P < 0.001). Differences in elimination half-life may explain the advantage of mefloquine over AL (3–6 days) in preventing reinfection. Both treatments were similar regarding the secondary endpoints of parasite clearance rate, fever clearance rate (except for day 1 when the fever clearance was greater in the AS + MEF arm), gametocytes carriage rate, and clinical (except for vomiting and total adverse events, which were more prevalent in AS + MEF arm) and laboratory adverse events.

Administered in a single dose, a three-day course of AS + MEF is as effective and well tolerated (except for vomiting and total adverse events rate slightly higher in AS + MEF arm) as a six-dose course (twice a day for three days) as AL and total adverse events rate slightly higher in AS + MEF arm. Both treatments were similar regarding the secondary end-points of parasite clearance rate, fever clearance rate (except for day 1 when the fever clearance was greater in the AS + MEF arm), gametocytes carriage rate, and clinical (except for vomiting and total adverse events, which were more prevalent in AS + MEF arm) and laboratory adverse events.

Administered in a single dose, a three-day course of AS + MEF is as effective and well tolerated (except for vomiting and total adverse events) as slightly higher in AS + MEF arm than in the AL arm. A study in Thailand using AS + MEF with a slightly higher dose of mefloquine during the three-day course of AS + MEF showed that the frequency of drug-related adverse events was low (<10% for vomiting) and comparable to that for AL. However, adverse events such as anxiety and insomnia has been reported to be higher with an AS + MEF regimen than with dihydroartemisinin-piperaquine in a study conducted in Peru. In this study in Peru, the frequency of other adverse events was higher in both treatment groups than in the treatment groups in our study. Studies in other Africa malaria-endemic areas with different transmission patterns and different levels of Plasmodium falciparum resistance are needed to assess the efficacy and safety of AS + MEF.

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