Therapeutic Efficacy and Effects of Artesunate-Mefloquine and Mefloquine Alone on Malaria-Associated Anemia in Children with Uncomplicated Plasmodium falciparum Malaria in Southwest Nigeria

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Abstract. The treatment efficacy and effects of artemesunate-mefloquine (AMQ) and mefloquine (MQ) on malaria-associated anemia (MAA) were evaluated in 342 children ≤ 10 years of age with uncomplicated Plasmodium falciparum malaria randomized to receive either drug/drug combination. All children recovered clinically. Fever clearance times were similar. Parasite clearance was significantly faster with AMQ (mean ± SD = 1.4 ± 0.6 days, 95% confidence interval [CI] = 1.3–1.5, P < 0.0001), but polymerase chain reaction–corrected cure rates were similar (97% versus 94%). Gametocyte carriage rates and the drug-attributable fall in hematocrit were significantly lower with AMQ (mean ± SD = 4.8 ± 3.8%, 95% CI = 3.6–6.0, P = 0.03), but the rates of resolution of MAA were similar. Both regimens were well tolerated. AMQ clears parasitemia and reduces gametocyte carriage more rapidly and causes lesser fall in hematocrit than MQ, but both regimens are effective treatment of uncomplicated P. falciparum malaria in Nigerian children.

INTRODUCTION

The development and spread of drug resistance in Plasmodium falciparum to many antimalarials is of global concern and a major public health problem in malaria-endemic countries in Africa. As part of the strategy to combat the spread of antimalarial drug resistance globally, the World Health Organization recommended the use of artemisinin-based combination therapies (ACTs) because they quickly reduce the burden of parasitemia and gametocytemia and the chance of drug resistance. Artemesunate-mefloquine (AMQ) combination is not considered a viable option for use as first-line therapy in Africa because of intense transmission. However AMQ, co-packaged as well as fixed dose formulations, is readily available and used in Africa. Despite high efficacy and increasing use of ACTs in Africa, many individuals still use antimalarial monotherapies because they are inexpensive and readily available, and the parasite has remained largely sensitive to, for example, mefloquine (MQ), despite the reported innate resistance to this drug in some areas of West Africa. Additionally, the inverse relationship in the sensitivity of P. falciparum to MQ and chloroquine (CQ) in West Africa has further renewed interest in the use of MQ, because high-grade CQ resistance is common in the sub-region.

Virtually all of the studies comparing AMQ with MQ have been conducted in areas of low transmission. There is no reported study of the efficacy of AMQ versus MQ in African children living in areas of intense transmission despite the availability and ready use of artesunate and MQ in these areas. It is also unclear whether MQ-based ACTs have superior efficacy to MQ alone in Africa, making it imperative to evaluate such regimens in sub-Saharan Africa. Such a study is essential as it may, in the future, influence policy and management of drug resistance in the community.

In this study, we report the tolerability, antimalarial treatment efficacy, effects on gametocyte carriage, and malaria-associated anemia of AMQ and MQ in children ≤ 10 years of age with acute, symptomatic, uncomplicated, P. falciparum malaria.

MATERIALS AND METHODS

Study area. The study was carried out in Ibadan, southwest Nigeria, from July 2007 to August 2008. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season from April to October. In vitro and in vivo MQ resistance of 14% and 0%, respectively, and in vitro reduced susceptibility to artemisinin (the parent drug from which artesunate is derived) in 5% of P. falciparum isolates in the area in the 1990s have been reported, but there are no current estimates of failure rates.

Patients, treatment, and follow-up. Patients were eligible to join the study if they were ≤ 10 years of age, had symptoms compatible with acute uncomplicated malaria such as anorexia, vomiting, or abdominal discomfort with or without diarrhea, with P. falciparum parasitemia > 2,000 asexual forms/μL, a body (axillary) temperature > 37.4°C or history of fever in the 24–48 hours preceding presentation, absence of other concomitant illness, no history of antimalarial use in the 2 weeks preceding presentation, negative urine tests for antimalarial drugs (Dill-Glazko and lignin for 4-aminoquinolines and sulfonamides, respectively), and written informed consent given by parents or guardians. Patients with severe malaria, severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. The study protocol was reviewed and approved by the Ethics Committee of The Ministry of Health, Ibadan. The disease history, taken by the attending physician, was recorded by asking patients or their parents/guardians when the present symptomatic period started and was followed by a full physical examination by the same physician.

Enrolled patients were randomly assigned to receive AMQ or MQ. AMQ or MQ alone was given according to body weight: artesunate at a dose of 4 mg/kg daily at presentation (Day 0) and daily for a further 2 days (Days 1 and 2) and MQ at a dose of 25 mg/kg at presentation only.

All drugs were given orally as direct observed therapy by the physician. After drug administration, all patients waited
for at least 3 hours after to ensure the drug was not vom-
tited. If it was, the patient was excluded from the study. If
necessary, patients were provided with antipyretics (par-
acetamol tablets, 10–15 mg/kg every 8 hours for 24 hours).

The randomization was computer generated, and treatment
codes were sealed in individual envelopes. Patient evaluation
and follow-up after drug administration was performed by
another physician blinded to the drug treatment. The study
nurse obtained thick and thin blood films from each child
as soon as they came to the clinic. The slides were carefully
labeled with the patients’ codes and were air dried before
being stained.

Follow-up with clinical and parasitologic evaluation was
done daily on Days 1–7 and then on Days 14, 21, 28, 35, and
42. This consisted of enquiry about the patient’s well being,
presence or absence of initial presenting symptoms, presence
of additional symptoms, measurement of body temperature,
heart and respiratory rates, and taking a blood smear for the
quantification of parasitemia.

Side effects were defined as symptoms and signs that first
occurred or became worse after treatment was started. Any
new events occurring during treatment were also considered
as side effects.

Thick and thin blood films prepared from a finger prick
were stained with Giemsa and were examined by light micros-
copy under an oil-immersion objective, at ×1,000 magnifica-
tion, by two independent assessors who did not know the drug
treatment of the patient. A senior member of the study team
reviewed the slides if there was any disagreement between
the microscopists. In addition, the slides of every third child
enrolled in the study were reviewed by this senior member.

Parasitemia (asexual or sexual) in thick films was estimated
by counting asexual or sexual parasites relative to 1,000 leuko-
cytes, or 500 asexual or sexual forms, whichever occurred first.
From this figure, the parasite density was calculated assuming
a leukocyte count of 6,000/μL of blood.

Capillary blood collected before and during follow-up
was used to measure packed cell volume (PCV). PCVs were
measured using a microhematocrit tube and microcentrifuge
(Hawksley, Lancing, UK). Drug-attributable fall in hemat-
ocrit (DAFH) during treatment was defined as the difference
between patient’s hematocrit on Day 0 and Day 3 after start-
ing treatment.

Blood was spotted on filter papers on Days 0, 3, 7, 14, 21,
28, 35, and 42 and at the time of treatment failures for para-
site genotyping. Paired primary and post-treatment para-
sites were analyzed using parasite loci that exhibit repeated
numbers of polymorphisms to distinguish true treatment
failures from new infections. Briefly, Block 2 of merozoite
surface protein-1 (MSP-1) and Block 3 of merozoite sur-
face protein-2 (MSP-2) and region II of glutamine-rich pro-
tein (GLURP) were amplified by two rounds of polymerase
chain reaction (PCR) using primers and amplification condi-
tions described previously. Ten microrollers of the nested
PCR products was resolved by electrophoresis on a 2% aga-
rose gel and sized against a 100-bp molecular weight marker
(New England Biolabs, Beverly, MA). The banding pattern of
the post-treatment parasites was compared with matched pri-
mary parasites in each of the patients who had parasitemia
after treatment with either artesunate-mefloquine or melfo-
quine. Post-treatment and primary infection parasites show-
ing identical bands were considered as true treatment failure,
whereas non-identity in banding patterns was considered as
newly acquired infections.

Response to drug treatment was assessed using WHO 1973
criteria as follows: S, sensitive, clearance of parasitemia with-
out recurrence; R1 (mild resistance), parasitemia disappears
but reappears within 7–14 days; RII (moderate resistance),
decrease of parasitemia but no complete clearance from
peripheral blood; RIII (severe resistance), no pronounced
decrease or increase in parasitemia at 48 hours after treat-
ment. In those with sensitive or mild resistance, parasite clear-
ance time was defined as the time elapsing between drug
administration and absence of detectable parasitemia for at
least 48 hours. Times taken to clear 50% and 90% parasitemia
were calculated from the plot of decline in parasitemia ver-
sus time. Fever clearance time was defined as the time from
drug administration until the body temperature fell to or
< 37.4°C and remained so for 48 hours. Response to drug treat-
ment was also classified according to a modified version of the
WHO 14-day in vivo clinical classification system; because all
patients were not febrile at enrollment, a temperature < 37.5°C
was not an exclusion criterion for enrollment. The modifica-
tion also involved a follow up for 42 days in this area of intense
transmission. The clinical classification system consisted of the
following categories of response: adequate clinical and para-
sitologic response (ACPR), late parasitologic failure (LPF), late
clinical failure (LCF), and early treatment failure (ETF).

Cure rates were defined as the percentages of patients
whose asexual parasitemia cleared from peripheral blood and
who were free of patent asexual parasitemia on Days 14, 21,
28, 35, and 42 of follow-up. The cure rates on Days 21–42 were
adjusted on the basis of the PCR genotyping results of paired
samples for patients with recurrent parasitemia after Day 14
of starting treatment.

Re-treatment of drug treatment failures. All patients failing
treatment (within 35 days) with artesunate-mefloquine or
mefloquine were retreated with these drug/drug combination
and were followed for 63 days. Patients were re-treated
whenever they became symptomatic (usually between 18
and 35 days after initial enrollment). Patients with profound
clinical (hyperpyrexia, oral fluid intolerance) and parasitologic
deterioration (> 20% increase in baseline parasitemia) during
follow-up were treated with parenteral quinine and were
regarded as treatment failures.

Data analysis. Sample size was calculated so that the
study would be able to detect a 14% absolute difference in
parasitologic failure “rate” between AMQ and MQ groups,
with 99% power and at a 5% significance level. The expected
treatment success rates were 99% for AMQ and 85%
for MQ on Days 28–42. The sample size was 147 patients in
each treatment arm. Data were analyzed using version 6 of
the Epi-Info software and the statistical program SPSS for
Windows version 10.01. Variables considered in the analysis
were related to the densities of P. falciparum gametocytes and
trophozoites. Proportions were compared by calculating χ²
with Yates correction or by Fisher exact or Mantel Haenszel
tests. Normally distributed, continuous data were compared
by Student t tests and analysis of variance (ANOVA). Data
not conforming to a normal distribution were compared by
the Mann-Whitney U tests and the Kruskal-Wallis tests
(or by Wilcoxon ranked sum test). All tests of significance,
except where specifically indicated, were two-tailed. P <
0.05 was taken to indicate significant differences. Data were
RESULTS

Patient characteristics. Between July 2007 and August 2008, 355 patients were recruited: 175 in the AMQ group and 180 in the MQ group (Figure 1). There were 102 children < 5 years old: 56 (32%) in the AMQ group and 46 (25%) in the MQ group. Overall, 328 patients (166 in the AMQ group and 162 in the MQ group) completed 42 days of follow-up; 13 children, 4 in AMQ and 9 in MQ, were excluded because of failure to meet all inclusion criteria. Three hundred forty-two children completed at least 21 days of follow-up. Twelve children, five in the AMQ group and seven in the MQ group, could not be evaluated because of protocol violation or relocation from study area. Overall results are for 342 children. The baseline characteristics were similar for both treatment groups (Table 1). However, mean hematocrit value was significantly lower in children enrolled in the AMQ treatment arm.

Fever and parasite clearance. Two hundred thirty-seven children were febrile at enrollment: 120 in the AMQ group and 117 in the MQ group. On the whole, 120 of the children treated with AMQ and 117 of those treated with MQ received paracetamol during the first 24 hours. By Day 1, fever cleared in 99 and 94 children, respectively. There was no significant difference in the proportion of patients in whom fever cleared by Day 1 ($\chi^2 = 0.07, P = 0.8$). By Day 2, 6 and 16 children were still febrile in the AMQ and MQ groups, respectively ($\chi^2 = 4.3, P = 0.03$). However, overall, fever clearance was similar in the two treatment groups (Table 2).

Compared with MQ, AMQ substantially accelerated the clearance of parasitemia. By Day 1, 11 children in the AMQ treatment arm and 67 children in the MQ treatment arm had not cleared their parasitemias. The difference in this proportion was significant ($\chi^2 = 50.2, P < 0.0001$). Times to clear 50%
and 90% parasitemia were significantly shorter, and overall, parasite clearance was significantly shorter in those treated with AMQ (1.4 ± 0.6 versus 2.1 ± 1.0 days, P < 0.0001; Table 2). No patient received rescue medication.

Response to both treatment regimens was not related to age: 1 of 56 and 7 of 115 < 5 and ≥5 year olds, respectively, treated with AMQ failed treatment by Day 28 (P = 0.27, Fisher exact test). Similarly, 5 of 46 and 8 of 125 < 5 and ≥5 year olds, respectively, treated with MQ failed treatment by Day 28 (P = 0.33, by Fisher exact test). All 171 patients treated with AMQ and 168 of 171 patients treated with MQ had adequate clinical and parasitologic response (ACPR) up to Day 14.

**Gametocyte carriage.** Gametocytes were detected in the peripheral blood in 108 children (31%) from the two treatment groups (Table 3). The overall detection rate at enrollment was 12% and was not significantly different between the two treatment groups (P = 0.71). After treatment, the emergence of gametocytes was significantly less frequent in the artesunate-mefloquine group than in the mefloquine alone group (P = 0.004; Table 3). Post-treatment, gametocyte carriage was significantly higher than pre-treatment in the mefloquine alone group (P = 0.001) but not in the artesunate-mefloquine group (P = 0.87; Table 3). Analysis of daily gametocyte carriage rates showed that carriage was significantly lower from Days 2 to 7 in those treated with AMQ compared with those treated with MQ (P < 0.03 at all times).

**Rate of re-appearance of parasitemia.** There were 28 patients, 9 in the AMQ group and 19 in the MQ treatment group, in whom, after initial clearance, parasitemia reappeared between Days 7 or 14 and 42 (Table 2). The cumulative proportion of children with *P. falciparum* reappearance (recrudescence or re-infection) between 2 and 6 weeks after start of therapy was significantly lower with AMQ at 4 and 6 weeks (P < 0.05; Table 2). All of the patients were symptomatic within 10 days of reappearance of parasitemia.

**PCR findings.** Matched sample pairs collected before and after treatment from 27 of 28 patients in whom infections recurred after treatment with MQ and AMQ were successfully analyzed at MSP-1, MSP-2, and GLURP loci. Presence of different allelic families of MSP-1 and MSP-2 was often found in parasite DNA derived from a single patient, indicating a
polyclonal infection. Ten of 18 (56%) paired pre- and post-treatment samples obtained from patients who showed re-appearance of parasitemia after treatment with MQ showed identical allelic families of msp-1, msp-2, and glurp, indicating genuine treatment failures. Paired samples from the remaining eight patients (44%) showed different allelic families of msp-1, msp-2, and glurp and were classified as newly acquired infections after MQ treatment. Polymorphic loci of msp-1, msp-2, and glurp in paired pre- and post-treatment samples of patients who showed reoccurrence of infections after treatment with AMQ confirmed genuine recrudescent infections in five of nine patients, whereas four were classified as newly acquired infections after genotyping. Overall, the PCR-corrected cure rate was 97% for AMQ and 94% for MQ.

**Adverse events.** Overall, 44 children (32 boys and 12 girls), 20 in the AMQ group and 24 in the MQ group, reported at least one adverse event within the first week of starting treatment. There was no significant difference in the proportions of patients reporting adverse events in both treatment groups ($\chi^2 = 0.43, P = 0.51$). However, the proportion of male children reporting adverse events was significantly higher than that of female children ($\chi^2 = 8.91, P = 0.002$). No child reported more than two adverse events. Many of the children reporting adverse events were > 5 years of age (24 of 44). Table 4 is a summary of adverse events reported within the first week. Intravascular hemolysis (marked drop in hematocrit from 28% to 17% and hemoglobinuria) requiring hospitalization for 6 days occurred on Day 3 in a 6-year-old male child treated with AMQ. There were no other significant clinical findings in this patient. However, glucose-6-phosphate dehydrogenase (G6PD) status of balance or hearing impairment attributable to artesunate.

**Drug-attributable fall in hematocrit and resolution of anemia after treatment.** Hematocrit data were available in 245 children, 119 in the AMQ group and 126 in the MQ group, at enrollment. Hematocrit values were available in 191 children, 89 in the AMQ group and 102 in the MQ group, on both Days 0 and 3, and these were used for the assessment of DAFH. In these children, there was no change in hematocrit values between Days 0 and 3 in seven and nine children, respectively, in the AMQ and MQ groups. In 15 and 10 children in the AMQ and MQ groups, respectively, there was an increase in hematocrit values between days 0 and 3. In 66 and 83 children in the AMQ and MQ groups, respectively, there was a decrease in hematocrit values between days 0 and 3. There were no significant differences in these proportions between the two treatment groups. DAFH was significantly greater in MQ- than AMQ-treated children ($6.1 \pm 4.5$; 95% CI = 5.2–7.0 versus $4.8 \pm 3.9$; 95% CI = 3.6–6.0; $P = 0.03$; Figure 2). In general, the rates of rise in hematocrit in the two treatment groups were similar after Day 3 (Figure 2).

Overall, 76 children were considered anemic (PCV < 30%) at presentation: 43 in the AMQ group and 33 in the MQ group. Ten of the 76 children with anemia were gametocyte carriers at presentation, whereas 18 of the 169 children without anemia were gametocyte carriers at presentation. The difference between the two proportions was not significant ($\chi^2 = 0.39, P = 0.53$). Table 5 is a summary of the rate of resolution of anemia after treatment in 66 children in whom complete data were available from Days 0 to 42. These rates were similar for both treatment groups.

**Re-treatment of treatment failures.** Of the 28 children with reappearance of parasitemia during follow-up, 7 each were retreated with AMQ or MQ. In all children, parasitemia cleared rapidly.

### Table 4

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>ARMO</th>
<th>MQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of children</td>
<td>171</td>
<td>171</td>
</tr>
<tr>
<td>Adverse event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Weakness</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Sleeplessness</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Icterus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intravascular hemolysis</td>
<td>1*</td>
<td>0</td>
</tr>
<tr>
<td>Excessive salivation</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Male child 6 years of age; G-6-PD status not determined; required hospitalization.

AMQ = artesunate-mefloquine; MQ = mefloquine.

### Table 5

<table>
<thead>
<tr>
<th>Resolution of malaria-associated anemia after treatment</th>
<th>AMQ</th>
<th>MO</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with PCV &lt; 30%</td>
<td>34</td>
<td>32</td>
<td>0.74</td>
</tr>
<tr>
<td>Mean PCV (%) and [range]</td>
<td>24.9[17–29]</td>
<td>25.2[18–29]</td>
<td></td>
</tr>
<tr>
<td>No of males (%)</td>
<td>20</td>
<td>16</td>
<td>0.50</td>
</tr>
<tr>
<td>Age (months)</td>
<td>58.8(1)</td>
<td>50</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>61.5 ± 35.4</td>
<td>67.9 ± 36.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12–120</td>
<td>10–132</td>
<td></td>
</tr>
<tr>
<td>Number &lt; 60 months</td>
<td>16</td>
<td>12</td>
<td>0.59</td>
</tr>
<tr>
<td>Parasite count (/μL)</td>
<td>46,183</td>
<td>51,399</td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>2,800–503,200</td>
<td>6,900–346,875</td>
<td></td>
</tr>
<tr>
<td>No. with PCV &lt; 30% at presentation (%)</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>No with PCV &lt; 30%</td>
<td>13</td>
<td>15</td>
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</tr>
<tr>
<td>Day 7</td>
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<td>Day 14</td>
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<td>Day 21</td>
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<td>Day 28</td>
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</tr>
<tr>
<td>Day 35</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher exact test.

AMQ = artesunate-mefloquine; MQ = mefloquine.
within 2 days and did not recur during another 42 days of follow-up. PCR analysis showed that these were re-infections.

**DISCUSSION**

Both AMQ and MQ proved to be effective treatments for acute, uncomplicated *P. falciparum* malaria in this endemic area where innate resistance to MQ was reported > 20 years ago. The insignificant decline in MQ efficacy compared with the findings 20 years ago and despite the reported innate resistance could be because of a number of reasons. First, as previously suggested, there may be a lack of predictive value of performance in vivo by in vitro data. Second, there may be a role of the inverse relationship in sensitivity in vitro in isolates of *P. falciparum* to MQ and CQ, because CQ resistance is currently high grade in the area. Third, until recently, the very limited use of MQ in the area since the early 1990s may have allowed restoration of sensitivity to isolates with reduced sensitivity in vitro. Thus, the efficacy of MQ in the first 2 weeks after starting treatment, in very young children, in this study is nearly similar to that in the same area almost 20 years ago. However, there was a significant decline in efficacy after 2 weeks, indicating MQ-resistant parasites are present in the area. In very young children from another endemic setting in West Africa, the efficacy of this drug has been compromised because of low blood levels after oral administration.

As anticipated, the results with AMQ were better than with MQ alone despite the relative absence of a significant decline in in vivo sensitivity to MQ. The initial rapid responses, characterized by significantly rapid clearance of asexual parasitemia with AMQ were clearly caused by the artemisinin derivative, artesunate. The latter ensured rapid recovery and reduced the risk of reappearance of parasitemia and prevented the risk of deterioration to severe malaria that may sometimes occur with slow-acting schizonticides. The artesunate in AMQ also characterized by significantly rapid clearance of asexual parasitemia in vivo in isolates of *P. falciparum* to MQ and CQ, because CQ resistance is currently high grade in the area.

**REFERENCES**

Mefloquine and Combination Antimalarial Treatment


