

## Short-Course Artesunate Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Gabon

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**Artesunate is one of the most important antimalarial agents available, since it is effective against parasites that have developed resistance to conventional antimalarials in sub-Saharan Africa. Antimalarial combination chemotherapies with artesunate (4 mg/kg of body weight once daily for 3 days) as one partner have been proposed. However, the efficacy of a 3-day course of artesunate alone has never been evaluated in individuals in Africa (which has 90% of the worldwide malaria burden) living in regions of hyperendemicity, where a considerable degree of immunity might substantially enhance the efficacy of short courses of artesunate compared to those in regions where the levels of endemicity are low. This lack of information does not permit a systematic assessment of the value of artesunate-based combination chemotherapies in Africa. Therefore, we studied the efficacy and safety of a 3-day course of artesunate (4 mg/kg of body weight, orally, once daily) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Gabonese patients aged 4 to 15 years ( $n = 50$ ). Artesunate was well tolerated, and no severe adverse event was reported. Parasite elimination was rapid and was achieved in all patients within  $\leq 72$  h (geometric mean time to elimination, 34 h). The PCR-corrected cure rate by day 14 was 92% (46 of 50 patients), but it dropped to 72% (36 of 50 patients) by day 28. We conclude that a 3-day course of artesunate fails to achieve sufficiently high cure rates for uncomplicated falciparum malaria in Gabonese children.**

Sub-Saharan Africa still awaits a safe and effective replacement for chloroquine treatment, as drug-resistant *Plasmodium falciparum* continues to spread over the continent (12, 15, 22). Two other cheap antimalarials are widely available and in use, namely, sulfadoxine-pyrimethamine (S/P) and amodiaquine. World Health Organization (WHO)-coordinated studies evaluating the efficacies and safety of these drugs in combination with artesunate have recently been completed (1, 23). The rationale underlying such combination chemotherapies is to cure infections responding inadequately to monotherapy and possibly to prevent or to delay the development of resistance to these alternative drugs (25). The demonstration of improvements in the cure rates achieved with a combination compared to those achieved with each of the agents used alone thus constitutes the most important single parameter that can be used to justify the use of combination chemotherapies for the treatment of uncomplicated malaria and provides the basis for informed decisions on drug treatment policy. This has been the case for the combination of artesunate and mefloquine in Thailand, where those treated with the combination fared considerably better than those treated with either artesunate or mefloquine alone (11, 17).

So far, published data have failed to demonstrate unequivocally similar beneficial effects of artesunate-based combina-

tion chemotherapies in Africa. In The Gambia, a 3-day course of artesunate combined with S/P achieved a cure rate of 98% on day 14, but the high degree of efficacy of S/P (97%) alone precluded the demonstration of a significant improvement in the cure rate by use of the combination (23). Our own data from a double-blind, randomized, placebo-controlled study evaluating treatment efficacy and safety in 220 pediatric patients aged 1 to 10 years with falciparum malaria showed that the cure rate achieved with amodiaquine treatment can be significantly improved when amodiaquine is combined with artesunate (1). However, since data from studies comparing this combination therapy with artesunate monotherapy are not available, uncertainty remains as to whether this higher degree of efficacy is exclusively attributable to artesunate alone.

Monotherapy with artesunate for 3 days achieves only low cure rates of 20 to 54% on day 28 in nonimmune populations in Thailand and China (5, 10). Five to 7 days of artesunate therapy alone is required as the optimal curative treatment regimen in this region (25).

The minimal effective duration of treatment with artesunate monotherapy has never been evaluated in Africa. Published studies from Tanzania and Nigeria only evaluated regimens with durations of at least 5 days, with cure rates exceeding 80% (2, 6, 8). The relevance of a single case report that raised the possibility of parasite resistance to artesunate after early and late treatment failures, which occurred following the use of 5-day treatment regimens in Sierra Leone, is not clear (19).

While these clinical studies have generally demonstrated the high cure rate of at least a 5-day regimen of artesunate alone

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in Africa, there are several reasons to study shorter regimens in Africa and to question the applicability of the results obtained from regions of hypoendemicity in Southeast Asia (where the efficacies of short courses of artesunate are low) to those obtained from areas of hyperendemicity in sub-Saharan Africa. Most importantly, a considerable degree of parasite-specific immunity in Africans living in regions of endemicity might substantially enhance the efficacy of a short course of artesunate. Moreover, artesunate is already marketed in many African countries as monotherapy, but compliance with the recommended 5- to 7-day regimen is very poor (personal observations). Because of the rapid effect of artesunate, artesunate treatment courses are likely to be discontinued after 3 days, when patients become free of symptoms. These considerations lead to the conclusion that the efficacy of a shorter artesunate regimen needs to be determined in African countries. We studied the efficacy of a 3-day course of artesunate for the treatment of uncomplicated *P. falciparum* malaria in children aged 4 to 15 years in Gabon to address these issues.

#### MATERIALS AND METHODS

The study took place in Lambaréné, Gabon, an area where *P. falciparum* malaria is hyperendemic and transmission is perennial (20, 26). The study protocol was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné, Gabon. Written informed consent was obtained from the parent or guardian of each child.

This study was designed with a sample size of 50 children to obtain an estimate of the cure rate (estimated to be 80%) within a 95% binomial confidence interval (CI) of less than 25%. Between January 2001 and April 2001 children between the ages of 4 and 15 years (inclusive) who attended the outpatient clinic of the Albert Schweitzer Hospital for symptoms suggestive of malaria were included if they met the following criteria: (i) they had uncomplicated *P. falciparum* infection with an asexual parasitemia level between 1,000 and 200,000 parasites/ $\mu$ l, (ii) they had a temperature of  $>38.5^{\circ}\text{C}$  or a history of fever in the preceding 24 h, and (iii) a parent or guardian had provided written informed consent. Exclusion criteria were (i) prior adequate treatment with antimalarial drugs for the present attack; (ii) not being able to drink, sit, or stand; (iii) vomiting more than twice within the preceding 24 h; (iv) at least one convulsion within the preceding 24 h; (v) hemoglobin concentration  $<8$  g/dl; (vi) severe underlying disease (cardiac, renal, or hepatic disease; malnutrition; known human immunodeficiency virus infection; concomitant disease); and (vii) a history of allergy to artemisinins.

Patients received artesunate (50-mg tablets; Sanofi, Gentilly, France) at 4 mg/kg of body weight once daily for 3 days (the dose was rounded to the nearest half tablet). Treatment was given under the direct supervision of a study physician. In the case of vomiting within 1 h after the first administration, the full dose was readministered. Patients who vomited the study drug more than once were withdrawn from the study and treated appropriately. Daily visits to determine the clinical, parasitological, and laboratory parameters were scheduled on days 1, 2, and 3 or until parasite clearance and improvement of symptoms. Patients were again evaluated on days 7, 14, and 28 or as clinically indicated. The primary efficacy end point was the PCR-corrected cure rate by day 28 (see genotyping). Failures were defined as early (the development of danger signs or severe malaria up to 7 days after the beginning of treatment, a parasitemia level  $>25\%$  of that at admission at any time between day 2 and day 6 of the study, or continuous parasitemia until day 7) or late (any parasitemia after initial clearance without fulfilling the criteria for early treatment failure). The secondary end points were the PCR-corrected cure rate by day 14, the mean parasite and fever clearance times, and the incidence of adverse events until day 7. An adverse event was defined as an unexpected change to the baseline situation, whether or not it was associated with the study drug. A Giemsa-stained thick blood film was prepared on each visit, and the level of parasitemia with the asexual stage per microliter was assessed by using the Lambaréné method (18). At the baseline and on day 28 or in the case of premature withdrawal from the study, a venous blood sample (1.2 ml) for laboratory analysis was placed into an EDTA-coated tube (Sarstedt, Nümbrecht, Germany). Hemoglobin concentrations, percent hematocrit, and leukocyte and platelet counts were determined by the QBC technique (Becton Dickinson, Paramus, N.J.). MSA-2 genotyping was performed to distinguish recrudescences from new infections by comparing matched pairs of para-

TABLE 1. Demographic, clinical, and laboratory characteristics at baseline<sup>a</sup>

Patient characteristic	Value
No. of males/no. of females.....	27/23
Age (yr).....	8.7 (3.3)
Wt (kg).....	25.3 (9.4)
Temp ( $^{\circ}\text{C}$ ).....	38.0 (1.2)
No. (%) of patients with the following symptoms and signs <sup>b</sup> :	
Headache.....	32 (64)
Fatigue and weakness.....	25 (50)
Vomiting.....	20 (40)
Abdominal pain.....	18 (36)
Anorexia.....	18 (32)
Splenomegaly.....	5 (10)
Coughing.....	3 (6)
Hepatomegaly.....	1 (2)
Parasitological density (geometric mean no. of parasites/ $\mu$ l [range]).....	
	22,000 (2,100–200,000)
Hematology	
Hemoglobin concn (g/dl).....	10.7 (1.6)
Hematocrit (%).....	34 (4.9)
Leukocyte count ( $10^3/\mu$ l).....	8.2 (2.5)
Platelet count ( $10^3/\mu$ l).....	172 (81)

<sup>a</sup> Data are given as the mean (SD) unless indicated otherwise. Fifty patients were treated and evaluated.

<sup>b</sup> Total number of symptoms.

site isolates obtained on admission and on the day of reappearing parasitemia (9, 13). Results from genotyping were used to calculate PCR-corrected cure rates. We classified parasitemia as reinfection if all electrophoretically separated PCR product bands detected on the day of reappearing parasitemia were distinct from those detected on the day of admission (the samples were tested in parallel). The data were summarized by calculating arithmetic and geometric means (95% CIs) for continuous data. The difference in the mean ages across two categories (cure and failure) was assessed by the *t* test. All calculations were performed with the Stata software package (version 7.0; Stata Corporation Inc., College Station, Tex.).

#### RESULTS

Fifty children were treated with artesunate (4 mg/kg/day for 3 days). The outcomes for all 50 children were evaluable; and no protocol violations, exclusions, or losses to follow-up occurred. The baseline characteristics of the children are summarized in Table 1. All patients had uneventful recoveries. Parasite elimination was rapid and was achieved in all patients within  $\leq 72$  h. The geometric mean time to parasite clearance was 34 h (95% CI, 31 to 38 h). Fever was also rapidly cleared (tympenic temperature,  $<37.5^{\circ}\text{C}$ ), with a geometric mean clearance time of 24 h (95% CI, 22 to 26 h).

There were no early treatment failures, and parasitological treatment failures were observed from day 14 onward. The affected patients had no signs or symptoms suggestive of malaria at the time of detection of reappearing parasitemia. On day 14 the parasitological cure rate was 92% (46 of 50 patients; 95% CI, 81 to 98%), but by day 28 it fell to 62% (31 of 50 patients; 95% CI, 48 to 76%).

PCR analysis revealed that for 5 of 19 patients with reap-

pearing parasites, the parasites obtained at the baseline and at parasite reappearance had different MSA-2 genotypes; and the patients were therefore considered reinfected and classified as cured. These five reinfections occurred from day 21 to day 28. The PCR-corrected parasitological cure rate on day 28 was therefore 72% (36 of 50 patients; 95% CI, 59 to 85%).

Patients with treatment failures tended to be younger (mean age, 7.8 years; standard deviation [SD], 3.4 years) than the cured patients (mean age, 9.3 years; SD, 3.2 years) ( $P = 0.11$ ).

Artesunate was well tolerated, and no serious adverse event was recorded. A total of 18 clinical adverse events (which occurred in six patients up to day 3) were reported, and all of them were mild and indistinguishable from the symptoms of uncomplicated malaria. Vomiting ( $n = 4$  patients), abdominal pain ( $n = 3$ ), coughing ( $n = 2$ ), headache ( $n = 2$ ), fever ( $n = 2$ ), dizziness ( $n = 2$ ), pruritus ( $n = 2$ ), and diarrhea ( $n = 1$ ) were reported.

## DISCUSSION

This study confirmed the well-described characteristics of artesunate in the treatment of *P. falciparum* malaria: namely, the patients' excellent tolerability of the drug as well as rapid parasite and fever clearances. It also demonstrated that a 3-day course of artesunate achieves a high cure rate by day 14 (92%) in Gabon. However, by day 28 the PCR-adjusted cure rate declined to 72%.

Artesunate eliminates circulating parasites very rapidly, but due to the short half-life of its active metabolite (dihydroartemisinin) of less than an hour in uncomplicated falciparum malaria, its antiparasitic action is limited to the times that treatment is given (16). Therefore, it has been estimated that, given a rate of parasite reduction of  $10^4$  parasites per dose and per parasite cycle and the lack of an assisting immune response, artesunate needs to be administered over at least 5 days to reach radical parasitological cure (24). If parasite populations are not completely removed from the body, low-level replication persists until the parasite density again crosses the microscopic or clinical detection threshold and treatment failure occurs (classified as recrudescence infection). Consequently, the parasitological cure rates achieved by day 28 are potentially a more sensitive marker for the in vivo efficacy of an antimalarial drug compared to the cure rates achieved by day 14. This particularly holds true for drugs with long half-lives in plasma, but it also apparently applies, although to a lesser extent, to drugs with very short half-lives in plasma. Additionally, analysis of parasite genotypes by validated PCR protocols (9, 13) to distinguish between recrudescence and new infections serves to amplify the signal (recrudescence) against the background noise of new infections and as a result increases the specificity of the in vivo test. Nonetheless, the current WHO protocol (27) for in vivo antimalarial tests uses the PCR-uncorrected cure rate at day 14 as the primary parameter of efficacy. We defined the PCR-corrected cure rate at day 28 as the primary outcome measure to increase the sensitivity of the test as well as to generate appropriate data for a comparison of the different treatment regimens (artesunate alone versus the combination of artesunate and amodiaquine) in Gabon.

The decrease in the PCR-corrected cure rates from day 14

(92%) to day 28 (72%) in our study clearly showed the higher sensitivity of the in vivo test at day 28. We also conclude that a 3-day course of artesunate is not sufficiently effective for the treatment of uncomplicated falciparum malaria in patients aged 4 to 15 years in Gabon. However, our findings compare favorably with results obtained in Thailand, where courses of artesunate with durations of treatment of less than 4 days led to cure rates below 20% by day 28 (5). Results from in vitro studies demonstrate that isolates from Southeast Asia have lower levels of in vitro susceptibility to artesunate, with a 50% inhibitory concentration ( $IC_{50}$ ) of 1.6 ng/ml (4), whereas the  $IC_{50}$  for isolates from Africa was 0.5 ng/ml (3). However, the significance of this difference remains unclear, as concentrations in serum are far above these levels (7, 16). Another, more likely explanation is related to the modulatory effect of acquired immunity. Humoral immunity can control low-level parasitemia and therefore potentially prevent treatment failure (14). The extent of this effect depends on the patient's age and level of prior exposure to parasite infections; these, in turn, translate into important variations in parasitological cure rates. In this context, we consider our study cohort to have had acquired immunity and attribute the higher cure rates achieved by day 28 in Gabon compared to those achieved in Thailand to the effect of that acquired immunity. In addition, the delayed detection of infections that were not radically cured from day 14 onward may also be attributable to the effect of the level of immunity in our study population (24).

Although we are aware of the multiple limitations of historical comparisons, we nevertheless attempted to compare the cure rates achieved at day 28 with artesunate alone from this study to the results from a previously published WHO-coordinated randomized, double-blind, placebo-controlled study evaluating the combination of artesunate and amodiaquine versus amodiaquine alone (artesunate at 4 mg/kg of body weight once daily for 3 days and amodiaquine at 10 mg/kg once daily for 3 days, in which, from January 1999 until January 2000, 76 patients aged 4 to 10 years [inclusive] received this combination). This is justified because (i) artesunate was dosed identically in both studies and was provided by the same manufacturer; (ii) both studies followed similar protocols, including crucial inclusion and exclusion criteria, apart from a small difference in the age range of the study population (4 to 15 and 4 to 10 years, respectively); and (iii) patients were recruited from the same study site, where the transmission of *P. falciparum* is perennial and *P. falciparum* is hyperendemic (1, 20, 21, 26). The combination of artesunate and amodiaquine achieved a PCR-corrected cure rate at day 28 of 92% (58 of 63 patients), whereas artesunate alone achieved a PCR-corrected cure rate at day 28 of 72% (36 of 50 patients) ( $P = 0.005$  for the comparison by Pearson's chi-square test). Thus, the efficacy of the combination cannot be attributed to the effect of artesunate alone.

In summary, artesunate given once daily for 3 days is not sufficient to radically cure *P. falciparum* malaria. Therefore, the optimal artesunate treatment regimen for uncomplicated *P. falciparum* malaria in African children remains undefined. A historical comparison revealed that the addition of amodiaquine significantly improves the efficacy of treatment.

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