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Rapid clearance of *Plasmodium falciparum* hyperparasitaemia after oral amodiaquine treatment in patients with uncomplicated malaria

Mathieu Ndounga [a,b], Leonardo K. Basco [a,*]

[a] *Unité de Recherche "Paludologie Afro-tropicale", Institut de Recherche pour le Développement (IRD), Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), B.P. 288 Yaoundé, Cameroon*
[b] *Centre d'Etudes sur les Ressources Végétales, Délégation Générale à la Recherche Scientifique et Technique, B.P. 1249, Brazzaville, Congo*

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Abstract

Amodiaquine is one of the possible alternative therapeutic options to treat chloroquine-resistant *Plasmodium falciparum* infections in Africa. In this study, its efficacy to treat patients with acute uncomplicated falciparum malaria and high parasitaemia ($\geq 5\%$ of infected erythrocytes in the peripheral blood) was assessed by using the standard protocol developed by the World Health Organization. The mean pre-treatment parasitaemia was 10.8% (range, 5.0–35%). All 32 patients who completed the study (four lost to follow-up) had an adequate clinical and parasitological response on day 14. As compared with the pre-treatment parasitaemia, the mean parasitaemia decreased by 97.8% on day 2 (two patients with negative smears) and 99.97% on day 3 (22 patients with negative smears; the other ten patients had low residual parasitaemia ranging from 24 to 400 asexual parasites/μl). Our results suggest that, in areas of stable transmission in Africa, hyperparasitaemic patients with uncomplicated malaria can be safely treated with oral amodiaquine provided that their clinical and parasitological responses are closely monitored for the first 3 days and controlled on day 7 and 14.

Keywords: *Plasmodium falciparum*; Drug treatment; Drug resistance; Chloroquine

1. Introduction

At present, many African countries are facing the difficult choice of possibly changing the first-line drug for uncomplicated malaria, i.e. chloroquine in many countries in West and Central Africa, as chloroquine becomes less effective in treating *Plasmodium falciparum* infections. Amodiaquine, a Mannich-base derivative containing a 4-aminoquinoline moeity, is recognized as one of the effective alternative antimalarial drugs to treat chloroquine-resistant *P. falciparum* infections in areas where the degree of chloroquine resistance has not yet attained a high level, as in most of the

* Corresponding author. Present address: OCEAC, B.P. 288, Yaounde, Cameroon. Tel.: +237-223-2232; fax: +237-223-0061.
E-mail addresses: lkbasco@yahoo.fr, leonardo.basco@ibaic.u-psud.fr (L.K. Basco).

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African continent (Olliaro et al., 1996). Although the drug is no longer recommended for chemoprophylaxis of temporary visitors to endemic countries (World Health Organization, 1990), considerable clinical experience has been gained with amodiaquine therapy in several African countries.

In Cameroon, amodiaquine is the officially recommended first-line drug for uncomplicated falciparum malaria in non-pregnant patients. Its clinical efficacy has been demonstrated in several recent studies in both children and adults (Ringwald et al., 2000; Basco et al., 2002). The efficacy of amodiaquine administered as monotherapy can be further enhanced by amodiaquine–sulfadoxine–pyrimethamine combination therapy (Staedke et al., 2001; Basco et al., 2002). There is less clinical experience with the use of amodiaquine (or other antimalarial drugs) to treat patients with high parasitaemia but clinically uncomplicated falciparum malaria. One of the reasons is related to the fact that malaria is often treated either on the basis of a presumptive clinical diagnosis or after a semi-quantitative determination of parasite density in most dispensaries in Africa. Our retrospective analysis of blood smears from patients with uncomplicated *P. falciparum* malaria and successfully treated with oral amodiaquine in the past has shown that some of these patients had high pre-treatment parasitaemia (unpublished data). To confirm this observation and evaluate the efficacy of oral amodiaquine to clear hyperparasitaemia in patients with uncomplicated malaria, we have conducted the present prospective study in a dispensary where approximately 10 000 microscopy-confirmed cases of malaria are treated annually with amodiaquine since 1993.

2. Patients and methods

2.1. Patient enrolment

The initial clinical and laboratory examinations were conducted by the dispensary staff as part of the routine procedures. Initial screening consisted of (i) clinical evaluation and diagnosis of uncomplicated malaria with no danger signs and (ii) microscopic examination of Giemsa-stained thick blood smear and semi-quantitative determination of parasite density by counting the number of asexual parasites per microscope fields in thick films. The presence of >10 asexual parasites per field (denoted as $++++$) usually indicates a high parasite density ≥ 3–4%. If the dispensary prescriptor judged that amodiaquine is the most appropriate treatment for a given patient with $++++$ parasitaemia, a second fingerpricked blood examination was performed, after informed consent was obtained, to determine haematocrit and count asexual parasites against 20 000 erythrocytes in a Giemsa-stained thin smear. Patients with acute uncomplicated falciparum malaria, high parasitaemia ($\geq 5\%$), and haematocrit $>15\%$ were enrolled. In this study, ''hyperparasitaemia'' was defined as the presence of $\geq 5\%$ infected erythrocytes in the day 0 thin blood smear (Warrell et al., 1982, 1990). Pregnant women and patients with clinically evident concomitant infectious diseases or signs and symptoms of severe and complicated malaria were excluded (Warrell et al., 1990). The study was approved by the Cameroonian Ministry of Public Health and Cameroonian National Ethics Committee.

2.2. Treatment and follow-up visits

Oral amodiaquine (total dose of 30 mg base/kg body weight in three equally divided daily doses) was administered under supervision either as liquid suspension (2 mg/ml) or 200 mg tablets. After each daily dose, patients were observed for at least 30 min. In addition, oral paracetamol was administered (50 mg/kg body weight/day in three divided doses) for fever and/or headache. Patients were followed on an out-patient basis on days 1, 2, 3, 7, and 14. During each visit, clinical and parasitological responses were evaluated. The number of asexual parasites was counted against 1000 leukocytes in Giemsa-stained thick blood smear. Parasite density was expressed as the number of asexual parasites per μl of blood by assuming a mean, normal leukocyte count of 8000 per μl of blood.

2.3. Clinical and parasitological responses

The standard World Health Organization (WHO) protocol was adopted to monitor the clinical and parasitological responses. In the original version of the WHO protocol, upper (100 000 asexual parasites/μl of blood) and lower (2000 asexual parasites/μl of blood) pre-treatment parasite densities are set as one of the inclusion criteria for the evaluation of antimalarial therapeutic efficacy (World Health Organization, 1996). The 2002 version of the WHO protocol has revised the upper limit to 200 000 asexual parasites/μl of blood in high transmission areas (World Health Organization, 2002). The clinical and parasitological response was classified as early treatment failure, late treatment failure, late parasitological failure, or adequate clinical and parasitological response. Since parasite density was measured every 24 h in our study, 'parasite clearance time' may yield a poor estimate of the rapidity of parasite clearance, especially when the sample size is small. The efficacy of amodiaquine to clear hyperparasitaemia was measured by the proportion of negative smears on days 2 and 3 and the percentage of decrease in parasite density, as compared with the pre-treatment parasitaemia, on each follow-up day.

3. Results

Of approximately 10 000 smear-positive patients consulting annually at the dispensary, a small proportion of patients fulfilled the inclusion criteria. A total of 36 patients were enrolled during a 7-week period in 2000–2001 at the Nlongkak Catholic missionary dispensary, Yaoundé. The mean pre-treatment parasitaemia was 10.8% (range, 5.0–35%; geometric mean, 133 000 asexual parasites/μl). The pre-treatment clinical features are summarized in Table 1. Of 36 enrolled patients, four were lost to follow-up (three patients on day 2 [after two of three daily doses] and one patient on day 7). There was no aggravating sign during the last visit of these patients. On day 1, the parasitaemia decreased by 53% (from the initial density of 61 600 asexual parasites/μl to 28 700 asexual parasites/μl), 66% (81 000 asexual parasites/μl to 27 500 asexual parasites/μl), and 98% (from 220 000 asexual parasites/μl to 3840 asexual parasites/μl) after the first dose in patients who were lost to follow-up on day 2. The second dose administered to these patients under supervision before being lost to follow-up is expected to further decrease their parasitaemia. These three patients (with pre-treatment rectal temperature of 36.6, 40.0 and 38.2 °C) were afebrile (36.7, 36.6 and 37.5 °C, respectively) at the time of the visit on day 1. The fourth patient who was lost to follow-up on day 7 was afebrile on days 1, 2, and 3 (pre-treatment rectal temperature, 38.7 °C), and the pre-treatment parasitaemia of 600 000 asexual parasites/μl decreased to 110 000 asexual parasites/μl on day 1, 1280 asexual parasites/μl on day 2, and 130 asexual parasites/μl on day 3.

All remaining 32 patients were followed until day 14 and responded with an adequate clinical

Table 1
Pre-treatment clinical and laboratory features

Characteristics	Mean values
Number of patients enrolled (male:female)	36 (19:17)
Mean age (years) (range)	2.7 (6 months–10-years-old)
Mean weight (kg) (±S.D.)	13.8±7.0
Mean number of days before seeking consultation (range)	3.4 (1–7)
Mean rectal temperature (°C) (±S.D., range)	
All patients (n = 36)	38.8±1.3 (36.2–40.7)
Only febrile patients at the time of consultation (n = 29)	39.4±0.8 (37.6–40.7)
Mean parasite density (%) (range)	10.8 (5.0–35)
Mean haematocrit (±S.D., range)	26.2±5.4 (17–39)

and parasitological response. All but two patients (37.6 and 38.7 °C) were afebrile on day 2. Only one patient (with 37.6 °C on day 2) had a low-grade fever (37.9 °C) and a negative smear on day 3 (subsequent smears were negative until day 14). Two patients with pre-treatment parasitaemia of 84 000 asexual parasites/μl and 375 000 asexual parasites/μl had a negative smear as early as on day 2 (and thereafter). By day 3, 22 of 32 patients (69%) had complete parasite clearance, while the other ten patients had low, residual parasitaemia ranging from 24 to 400 asexual parasites/μl, which was completely cleared by day 7.

The rapid parasite clearance after amodiaquine therapy is illustrated in Fig. 1. The mean percentages of parasitaemia, compared with the pre-treatment (day 0) parasitaemia, were 62.9% on day 1, 2.2% on day 2 (range, 0–16.2%), and 0.03% on day 3 (range, 0–0.4%). All patients with complete follow-up had negative smears on days 7 and 14 (0% parasitaemia). Six patients had increased parasitaemia, compared with the pre-treatment parasitaemia, on day 1. An improvement in haematocrit was observed on day 14, as compared with the mean day 0 values (26.2% on day 0 vs. 30.1% on day 14).

4. Discussion

Pharmacodynamics plays an important role in the host/parasite interaction and may be one of the

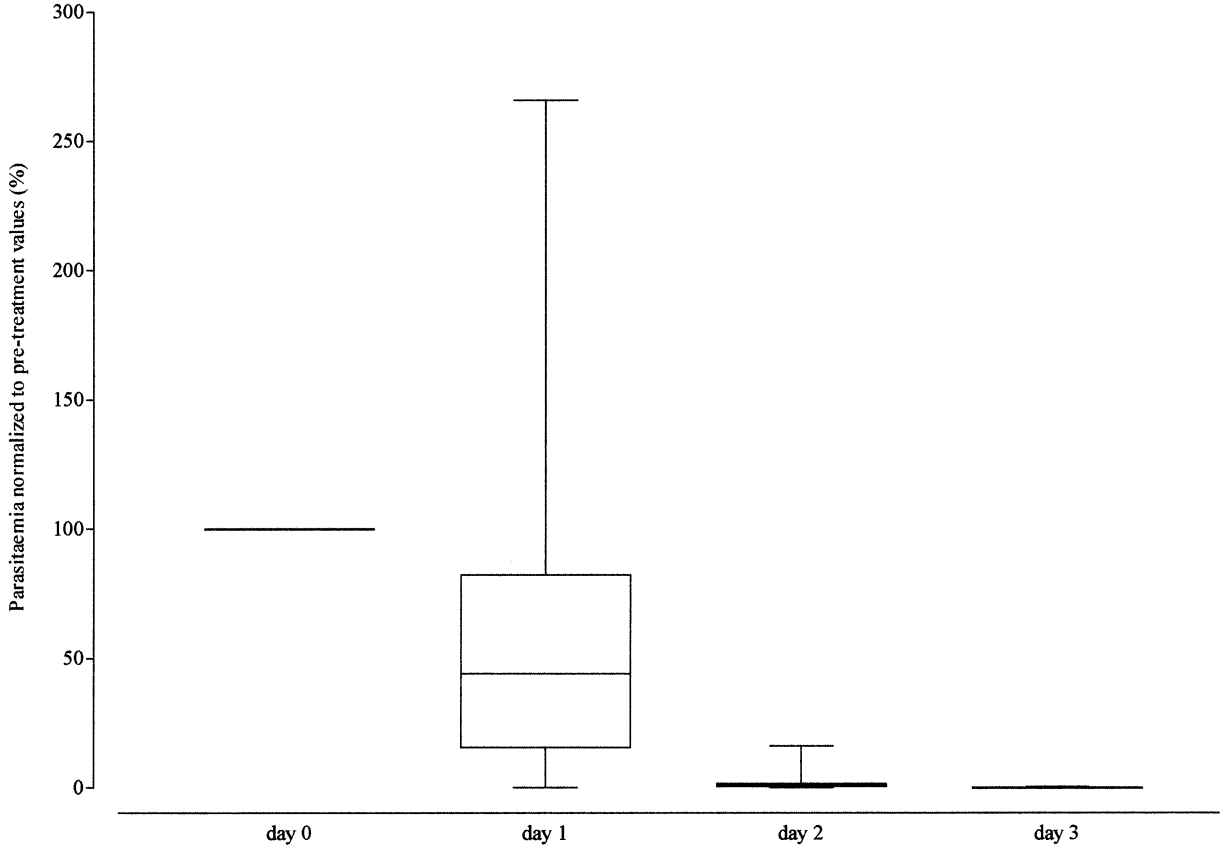

Fig. 1. Box-and-whiskers plot illustrating the parasitological response to amodiaquine in 32 hyperparasitaemic patients with acute uncomplicated falciparum malaria and complete follow-up. The horizontal line in the box corresponds to the median, top and bottom of the box correspond to the 75th and 25th percentiles, respectively, and the whiskers show the range. The large variation on day 1 is partly due to six cases with increasing parasitaemia (post-treatment parasite density > pre-treatment parasite density).

key elements that leads to a successful or inadequate antimalarial therapy, independently of the inherent antimalarial action of the drug (White, 1998). It may be conjectured that antimalarial drugs that are preferentially concentrated in malaria-infected erythrocytes, such as chloroquine and amodiaquine, lose part of their efficacy in hyperparasitaemic patients unless the blood concentration largely exceeds that of the usual minimal therapeutic concentration. Our data invalidate this hypothesis. Because a large majority of symptomatic patients with uncomplicated malaria present with parasitaemia below 1% at the time of consultation in endemic countries, the therapeutic efficacy of an antimalarial drug cannot be extrapolated to those presenting with hyper-parasitaemia unless clinical studies are conducted.

Hyperparasitaemia is not a fixed notion with a precise definition. The relation between parasite density and severity of malarial disease varies in different age groups and populations in different areas of transmission. In some patients, there is no direct correlation between peripheral parasitaemia and clinical severity. In Thailand, it has been suggested that parasitaemia ≥ 4–5% is associated with an increased risk of mortality and is a sign of severe malaria (Warrell et al., 1982; Luxemburger et al., 1997). Hyperparasitaemia has also been considered as one of the possible manifestations of severe and complicated malaria, depending on the endemic area (Warrell et al., 1990). Thus, understandably, most cases of hyperparasitaemia, often interpreted as a danger sign, especially in non-immune patients, are treated with either parenteral drugs in a hospital setting or rapidly-acting artemisinin derivatives (Luxemburger et al., 1997; Price et al., 1998).

As a consequence, clinical data on the efficacy of antimalarial drugs to clear hyperparasitaemia in uncomplicated malaria are relatively scarce in the literature. In a study conducted in eight Kenyan children with uncomplicated malaria, oral sulfadoxine–pyrimethamine was effective in clearing high parasitaemia (defined in that study as 10 000–100 000 asexual parasites/μl) at the time when resistance to this drug combination has not yet been fully documented in East Africa (Winstanley et al., 1992). In another study in Nigerian children, it has been reported that halofantrine and chloroquine were effective in curing two of three and one of two hyperparasitaemic patients, respectively (Falade et al., 1997). In our study, clinical data were obtained from a larger number of hyperparasitaemic patients with uncomplicated malaria. The rapid clearance of parasitaemia and fever with amodiaquine therapy was demonstrated in hyperparasitaemic patients without danger signs. The results of our study thus suggest that, even in a chloroquine-resistant region of sub-Saharan Africa, amodiaquine may still be a useful alternative in treating "semi-immune" hyperparasitaemic patients without signs of severe and complicated malaria.

Before resorting to parenteral quinine, artemisinin derivatives, or possibly other new drugs available in pharmacies, oral amodiaquine therapy may yet be another therapeutic option for the treatment of hyperparasitaemic "semi-immune" patients. However, due to possible host differences in tolerating high parasitaemia and the ever-present potential risk of aggravation when diagnosis and treatment are delayed, the decision to prescribe amodiaquine in hyperparasitaemic patients without signs and symptoms of severe and complicated malaria at the time of consultation should be taken with caution and should accompany daily follow-up for the first 3–4 days to rule out aggravating clinical conditions. Likewise, prior clinical studies should be conducted to assess the current efficacy of amodiaquine, possibly in combination with sulfadoxine–pyrimethamine, in different epidemiological settings in Africa, before prescribing amodiaquine to hyperparasitaemic patients with uncomplicated malaria.

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References

Basco, L.K., Same-Ekobo, A., Foumane Ngane, V., Ndounga, M., Metoh, T., Ringwald, P., Soula, G., 2002. Therapeutic efficacy of sulfadoxine-pyrimethamine, amodiaquine, and sulfadoxine–pyrimethamine–amodiaquine combination for the treatment of uncomplicated *Plasmodium falciparum* malaria in young children in Cameroon. Bull. World Health Org. 80, 538–545.

Falade, C.O., Salako, L.A., Sowunmi, A., Oduola, A.M.J., Larcier, P., 1997. Comparative efficacy of halofantrine, chloroquine and sulfadoxine–pyrimethamine for treatment of acute uncomplicated falciparum malaria in Nigerian children. Trans. R. Soc. Trop. Med. Hyg. 91, 58–62.

Luxemburger, C., Ricci, F., Nosten, F., Raimond, D., Bathet, S., White, N., 1997. The epidemiology of severe malaria in an area of low transmission in Thailand. Trans. R. Soc. Trop. Med. Hyg. 91, 256–262.

Olliaro, P., Nevill, C., Le Bras, J., Ringwald, P., Mussano, P., Garner, P., Brasseur, P., 1996. Systematic review of amodiaquine treatment in uncomplicated malaria. Lancet 348, 1196–1201.

Price, R., Luxemburger, C., Van Vugt, M., Nosten, F., Kham, A., Simpson, J., Looareesuwan, S., Chongsuphajaisiddhi, T., White, N.J., 1998. Artesunate and mefloquine in the treatment of uncomplicated multidrug-resistant hyperparasitaemic falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 92, 207–211.

Ringwald, P., Keundjian, A., Same Ekobo, A., Basco, L.K., 2000. Chemoresistance of *Plasmodium falciparum* in the urban region of Yaounde, Cameroon. Part 2: Evaluation of the efficacy of amodiaquine and sulfadoxine–pyrimethamine combination in the treatment of uncomplicated *Plasmodium falciparum* malaria in Yaoundé, Cameroon. Trop. Med. Int. Health 5, 620–627.

Staedke, S.G., Kamya, M.R., Dorsey, G., Gasasira, A., Ndeezi, G., Charlebois, E.D., Rosenthal, P.J., 2001. Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomised trial. Lancet 358, 368–374.

Warrell, D.A., Looareesuwan, S., Warrell, M.J., Kasemsarn, P., Intraprasert, R., Bunnag, D., Harinasuta, T., 1982. Dexamethasone proves deleterious in cerebral malaria. A double blind trial in 100 comatose patients. New Engl. J. Med. 306, 313–319.

Warrell, D.A., Molyneux, M.E., Beales, P.F., 1990. Severe and complicated malaria. Trans. R. Soc. Trop. Med. Hyg. 84, 1–65.

White, N.J., 1998. Why is it that antimalarial drug treatments do not always work. Ann. Trop. Med. Parasitol. 92, 449–458.

Winstanley, P.A., Watkins, W.M., Newton, C.R.J.C., Nevill, C., Mberu, E., Warn, P.A., Waruiru, C.M., Mwangi, I.N., Warrell, D.A., Marsh, K., 1992. The disposition of oral and intramuscular pyrimethamine/sulphadoxine in Kenyan children with high parasitaemia but clinically non-severe falciparum malaria. Br. J. Clin. Pharmacol. 33, 143–148.

World Health Organization, 1990. Practical chemotherapy of malaria. Technical Report Series No. 805, WHO, Geneva.

World Health Organization, 1996. Assessment of therapeutic efficacy for uncomplicated falciparum malaria in areas with intense transmission. WHO/MAL/96.1077.

World Health Organization, 2002. Monitoring antimalarial drug resistance. Report of a WHO consultation, Geneva, Switzerland, 3–5 December 2001, WHO/CDS/RBM/2002.39.