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Sensitivity of *Plasmodium falciparum* to chloroquine, amodiaquine and mefloquine in Ibadan, Nigeria

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Summary

The schizontocidal effect of chloroquine was compared to that of amodiaquine *in vivo* and mefloquine *in vitro*. In the in-vivo study, 32 patients were randomly given chloroquine whilst 29 received amodiaquine. The mean parasite clearance time was 2.5 days for chloroquine and 2.4 days for amodiaquine. These times were not significantly different. The cure rate in both groups up to day 14 was 100%. In the in-vitro study, three isolates of *Plasmodium falciparum* were compared for their sensitivity to chloroquine and mefloquine. In all three isolates schizogony was inhibited at a concentration of 0.8×10^{-6} mol/l of either drug. It was concluded that chloroquine is still an effective schizontocide and should remain the drug of choice for the treatment of *P. falciparum* in the Ibadan area.

Résumé

L'effet schizontocidale de la chloroquine a été comparé avec celui de l'amodiaquine *in vivo* et de la méfloquine *in vitro*. Pour l'étude *in vivo*, 32 patients ont été donnés de la chloroquine au hasard et 29 ont reçu de l'amodiaquine. Le temps moyen pour l'élimination des parasites a été de 2.5 jours pour la chloroquine et 2.4 pour l'amodiaquine. Les temps donnés n'ont pas différé d'une manière significative. La proportion des guérisons pour les deux groupes a été de 100% pour le 14e jour. Dans l'étude *in vitro*, trois types de *Plasmodium falciparum* ont été comparés pour leur sensitivité vis à vis la chloroquine et la méfloquine. Dans tous les trois cas la schizogonie a été arrêtée à une concentration de 0.8×10^{-6} mol/l de toutes les deux drogues. On est arrivé à la conclusion que la chloroquine est encore un schizontocide efficace et qu'elle doit continuer comme la drogue de choix pour le traitement de *P. falciparum* dans la zone d'Ibadan.

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Introduction

Although chloroquine-resistant malaria has been reported and documented in many parts of East and Central Africa, the West African sub-region has as yet to report a proven case of chloroquine-resistant malaria. Previous studies [1–4] have all reported full sensitivity of *Plasmodium falciparum* to standard anti-malarials.

These findings, although encouraging, need not lead to complacency in the sub-region, as chloroquine-resistant malaria once established in an area increases rapidly both in severity and in geographical spread [5].

Our objectives in this study were therefore: (i) to assess the current status of the sensitivity of *P. falciparum* to chloroquine; (ii) to compare the schizontocidal effect of chloroquine with that of amodiaquine and mefloquine.

Patients and methods

In-vivo tests

The study was conducted in the Unit of Clinical Pharmacology of the University College Hospital (UCH), Ibadan between August and October 1985. The patients were initially screened at the General Out-patients Department of the UCH and were included in the study if they satisfied the following criteria:

(i) a blood film positive for *P. falciparum* only;

(ii) no history of anti-malarial ingestion during the previous fortnight or a negative Dill-Glazko urine test [6] for aminoquinolines;
(iii) consent to participate in the trial, which was obtained from either parents or guardians.

Patients satisfying these criteria were treated randomly with either chloroquine or amodiaquine. The dosage of either drug was 25 mg/kg body weight spread over 3 days (10 mg/kg was given on day 0, 10 mg/kg on day 1 and 5 mg/kg on day 2). The standard WHO '7-day test' [7] extended to 14 days was applied, and patients were seen daily for parasitological and clinical evaluation.

The level of parasitaemia was determined by counting the number of parasites per 200 leucocytes. This figure was then multiplied by 40 to obtain the count per mm^3 of blood (the mean leucocyte count in the population is 8000 WBCs per mm^3).

The count so obtained was expressed in terms of the Parasite Density Index or PDI [8].

Two indices were used to assess the parasitological response: (i) the parasite clearance time — this was defined as the day number of the first of two consecutive days after initiation of therapy on which no asexual parasites were found in the blood; (ii) the cure rate, defined as the proportion of treated subjects who remained clear of parasites at the completion of the 14-day observation period.

In-vitro tests

The method used was based on the in-vitro microtechnique of Rieckman *et al.* [9]. *Plasmodium falciparum* were obtained from four patients, all of whom had parasite counts of over $1000/mm^3$ blood and were Dill-Glazko urine test negative.

The growth medium was obtained from the World Health Organization (WHO) microtest kit and consisted of RPMI 1640 medium, which was supplemented with 1 ml of 7.2% HEPES solution and 1 ml of 2.4% $NaHCO_3$.

Blood (2–3 ml) was taken from each patient and diluted 1/10 with medium. Using an Eppendorf pipette, 50 µl of the blood/medium solution were added to wells of a 96-well microtitre plate, obtained from the WHO microtest kit, and predosed to give final concentrations of 0, 1, 2, 4, 5, 7, 8, 16 and 32 pmol chloroquine/well, corresponding to 0, 0.2, 0.4, 0.8, 1.14, 1.6, 3.2 and 6.4 µmol/l of blood; and 0, 0.5, 1, 4, 5.7, 8, 16 and 32 pmol mefloquine/well corresponding to 0, 0.1, 0.2, 0.4, 0.8, 1.14, 1.6 and 3.2 µmol/l of blood. The microtitre plates were then placed in a candle jar and incubated at 37–38°C for 28 h. After incubation, thick smears were prepared from each well and a valid test was one in which more than 20 schizonts per 200 asexual forms grew in the control wells. The total number of schizonts in each well was expressed as a percentage of the control wells (three columns of the microtitre plate were used for each strain and the average count taken as the final number of schizonts).

Results

In-vivo study

A total of 155 patients were screened during the study period. Seventy-six or 49% of the patients were positive for malaria, with 74 or 97% being positive for *P. falciparum*. The other two cases were due to *P. malariae*. Thirteen of the 74 patients did not satisfy the criteria and were excluded. The remaining sixty-one patients were randomly assigned to either the chloroquine or amodiaquine group.

The chloroquine group consisted of 14 boys and 18 girls with a mean age of 4.8 years (range 1–11), a mean weight of 15.1 kg (range 6–32), pretreatment parasite counts of 720 to 60,000 per mm^3 and a Parasite Density Index on day 0 of 8.0 (range 4–10). The amodiaquine group consisted of 14 boys and 15 girls with a mean age of 5.3 years (range 11 months–16 years), a mean weight of 15.6 kg (range 8–40), pretreatment parasite counts of 340 to 60,000 per mm^3 and a Parasite Density Index on day 0 of 8.0 (range 3–10). Using Student's *t*-test it was shown that there was no significant difference between the two groups in weight, age and initial parasitaemia ($P > 0.1$).

Parasitological response

The parasitological response in the two groups is shown in Table 1. Parasitaemia in both groups disappeared by day 4, with the chloroquine group having a mean parasite clearance time of 2.5 days, and the amodiaquine group 2.4 days. The rate of parasite clearance was not significantly different between the two groups.

Table 1. Parasitological response in 61 patients treated with chloroquine and amodiaquine, Ibadan, Nigeria, 1985

Treatment group	Parasite Density Index (PDI)									Parasite clearance time (days)	Cure rate
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14		
Chloroquine (n = 32)	8.0	7.2	1.7	0	0	0	0	0	0	2.5	100%
Amodiaquine (n = 29)	8.0	6.7	1.7	0.2	0	0	0	0	0	2.4	100%

Clinical response

Most patients responded very well to either drug and by the second or third day had achieved a normal temperature. One patient in the chloroquine group had a spike of 39.3°C on the fifth day, whereas two patients both on amodiaquine had temperatures of 39.6°C and 39.2°C, respectively, on the fourth day.

In all three patients extensive history and examination revealed no abnormalities, and repeat blood slides were negative. A diagnosis of viraemia was made in the three patients and they responded well to aspirin.

Three patients, all on chloroquine, reported itching after taking the drug. The itch was suppressed with a course of antihistamines. None of the patients treated with amodiaquine presented with an itch.

In-vitro study

The in-vitro test for one sample was not successful, whilst the remaining three all had more than 20 schizonts in the control wells (Table 2). In all strains no growth was obtained at a concentration of 4 pmol/well, corresponding to 0.8×10^{-6} mol/l of blood of either drug.

Discussion

The emergence of chloroquine-resistant malaria has rekindled interest in the chemotherapy of this very important disease. One area of approach has been the development of new antimalarial drugs and there has also been a resurgence of interest in old drugs.

Amodiaquine is, like chloroquine, a 4-aminoquinoline but, unlike the latter, it has been less commonly used. Recent studies, however, have demonstrated that in chloroquine-resistant malaria it has a greater effect than chloroquine [10, 11].

In our study it was demonstrated that, in the Ibadan locality, *P. falciparum* is still very sensitive to chloroquine. The finding confirms earlier results [1–4]. When amodiaquine was compared with chloroquine it was shown, in this study, that amodiaquine did not have any significant superiority in its schizontocidal effect against *P. falciparum*. This result is similar to that obtained by Walker *et al.* [4].

Mefloquine is a new anti-malarial drug and is a 4-quinoline methanol. It has recently been registered in combination with sulphadoxine and pyrimethamine under the trade name Fansimef® (Roche, Basle, Switzerland). The assessment of the pure substance is, however, very vital and this study compared the schizontocidal effect of mefloquine with that of chloroquine. It was shown in our study that all three strains of *P. falciparum* did not grow schizonts at a concentration of 0.8×10^{-6} mol/l of either drug.

In RI, resistance growth is obtained at a concentration of 1.5 or 2.0×10^{-6} mol/l of chloroquine, whilst in mefloquine-resistant strains growth is obtained at concentrations of $1.14–1.16 \times 10^{-6}$ mol/l [8]. Thus, all three strains showed marked sensitivity to both mefloquine and chloroquine.

The results of this study have demonstrated that *P. falciparum* continues to be sensitive to chloroquine in the Ibadan locality and, as shown in this study, should continue to be the

Table 2. In-vitro response of three strains of *Plasmodium falciparum* to chloroquine (CQ) and mefloquine (MQ), Ibadan, Nigeria, 1985

Isolate	No. schizonts per 200 asexual parasites in control well (mean of 2 values)	Schizonts in predosed wells as % of the no. in control well: Concentration of drugs (pmol/well) 0.5 (MQ)	1 CQ	1 MQ	2 CQ	2 MQ	4 and above CQ	4 and above MQ
I	32	47	0	21.2	0	6.2	0	0
II	53	49	28.3	18.8	7.7	1 8	0	0
IV	77	45.4	44.1	16 8	9	2.6	0	0

drug of choice in the treatment of *P. falciparum* malaria here. Continuous surveillance of sensitivity patterns in the whole West African subregion should, however, continue and this should be coupled with epidemiological studies of the emergence and spread of chloroquine-resistant malaria before the sub-region becomes engulfed with resistant strains.

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