

Susceptibility of *Plasmodium falciparum* strains to mefloquine in an urban area in Senegal

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A total of 47 nonimmune febrile patients from Pikine, Senegal, with >1000 *Plasmodium falciparum* asexual forms per μ l whole blood were given 12.5 mg per kg body weight of mefloquine in a single oral dose and were followed up daily until day 7 and also on day 14 of the study.

Seven of the patients who vomited, four who had 4-aminoquinolines in their blood, and five drop outs were excluded. Fever and parasitaemia were suppressed within four days until day fourteen in 29 of the 31 remaining patients, including 10 with *P. falciparum* strains that had a low sensitivity to mefloquine. Two failures were due to poor absorption of mefloquine. The presence of *P. falciparum* strains with low *in vitro* susceptibility to mefloquine did not affect, within 14 days, the clinical and parasitological efficacy of a single oral dose mefloquine regimen in patients who had received no previous antimalarial treatment and who did not have partial immune protection.

Introduction

Chloroquine-resistant *falciparum* malaria has spread all over West Africa, one of the last countries to be affected being Senegal (1). Mefloquine is now available in several West African countries, and the recommended oral therapy is a single dose of 12.5–15 mg per kg body weight (2). Recent *in vitro* studies have reported the presence of *Plasmodium falciparum* strains with reduced susceptibility to mefloquine in West Africa (3), in particular in Mali and Senegal (4), and, several mefloquine prophylaxis failures *in vivo* were observed in 1988 in travellers returning from West Africa (5). The present study was undertaken to determine the *in vitro* and *in vivo* effectiveness of mefloquine against *P. falciparum* strains in Senegal and to assess the *in vitro* sensitivity of these strains to chloroquine and halofantrine.

Materials and methods

Selection of patients. A total of 47 patients from Pikine, a suburb of Dakar, were enrolled in the

study. The patients were selected from those who presented voluntarily in November 1988 at outpatient clinics with fever or a history of fever during the previous 48 hours, without neurological signs. Only patients older than 1 year with *P. falciparum* parasite densities ≥ 1000 asexual forms per μ l of blood were included. The free and informed consent of the subjects or one of their parents was obtained.

Drug dosages. The Dill–Glazko urine test (6) and the day-0 level of total blood 4-aminoquinolines (obtained using high-performance liquid chromatography (HPLC)) (7) were determined for 38 patients. The day-2 total venous blood levels of mefloquine and of 4-carboxymefloquine (the major metabolite) were determined by HPLC (8) in 37 patients.

In vivo test. The patients received 50 mg or 250 mg tablets of mefloquine,^a corresponding to a total amount of 12.5 mg per kg in a single oral dose. The patients were observed for 1 hour after receiving the drug to monitor possible secondary effects or vomiting; patients who vomited were excluded from the study. The WHO 14-day extended test was successfully completed by 31 patients. A thick blood film was prepared and the temperature of the patients was recorded daily on days 0–7, and again on day 14. After staining with Giemsa, the number of asexual parasites per 1000 white blood cells was counted and the parasite density per μ l calculated, assuming a

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^a Lariam®, Roche, France.

mean of 8000 leukocytes per μl of blood. The patients in whom asexual forms persisted until day 7 were routinely treated with a standard oral regimen of chloroquine (25 mg per kg) over 3 days. Susceptibility was defined according to WHO criteria (9).

In vitro test. Samples of 5 ml of venous blood were collected at enrolment in anticoagulant acid citrate dextrose solution, stored at $+4\text{ }^\circ\text{C}$, and sent to Paris. The *in vitro* sensitivity of the *P. falciparum* strains to chloroquine, mefloquine, and halofantrine was evaluated with the semimicro test, using [^3H]-hypoxanthine to assess parasite growth (10). Twofold dilutions of the following drugs were used: chloroquine (12.5–1600 nmol.l^{-1}), mefloquine, (2.5 – 400 nmol.l^{-1}), and halofantrine (0.25–32 nmol.l^{-1}). Each assay was performed in triplicate. Within 5 days of sampling, the red blood cells were washed, introduced at 2.5% haematocrit to 0.7-ml wells containing a mixture of RPMI 1640 supplemented with 10% (v/v) normal human serum and various drug doses, and incubated for 42 hours at $37\text{ }^\circ\text{C}$ in a gaseous mixture consisting of 5% (v/v) oxygen, 5% (v/v) carbon dioxide, and 90% (v/v) nitrogen.^b A total of $1\ \mu\text{Ci}$ [^3H]-hypoxanthine was added to each well after 18 hours. Parasites were harvested on filters after 42 hours and isotopic incorporation was evaluated using a scintillation spectrophotometer. Parasite median inhibitory concentrations (IC_{50}) of $\geq 100\ \text{nmol.l}^{-1}$ of chloroquine, of $\geq 40\ \text{nmol.l}^{-1}$ of mefloquine, or of $\geq 30\ \text{nmol.l}^{-1}$ of halofantrine were taken to indicate resistance to chloroquine or low sensitivity to mefloquine or halofantrine.

Results

4-Aminoquinoline and Dill–Glazko urine tests were performed on 38 of the 47 patients recruited for the study, 42 completed the *in vitro* test, the level of mefloquine was determined in 37 (age range, 1–40 years), and 35 were followed up until day 14 of the study.

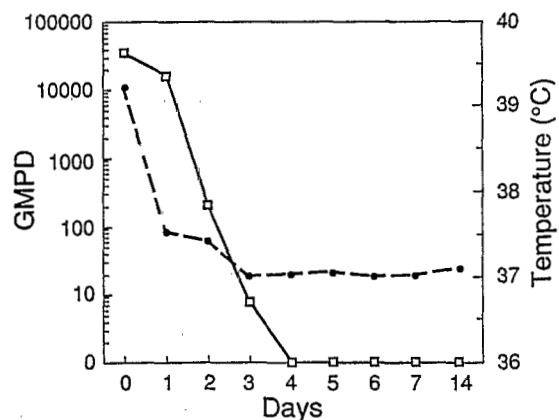
Previous 4-aminoquinoline intake. A total of 19 subjects had detectable 4-aminoquinoline in their blood (by HPLC). Levels of chloroquine plus desethylchloroquine of 17–140 nmol.l^{-1} were found in 14 patients, four had levels of 473–1948 nmol.l^{-1} , and one patient had 5228 nmol.l^{-1} of monodesethylamodiaquine in total blood. No correlation was found between the results of the HPLC and Dill–Glazko urine tests. Of 15 subjects with a positive urine test,

11 had a negative blood test. Also of 18 subjects with a positive urine test, 10 had detectable levels of 4-aminoquinoline in their blood.

In vivo tests. Seven of the nine patients who vomited on day 0 were withdrawn from the study on day 1 (including the patient who had monodesethylamodiaquine in his blood) and were given alternative treatment; five did not complete the test; and another four were omitted from the analysis because their total blood chloroquine plus desethylchloroquine was more than $150\ \text{nmol.l}^{-1}$ on day 0. A total of 31 tests were successful: parasitaemia and fever cleared in 29 patients within 4 days (Fig. 1); two patients who had an RII and an RIII response were discharged on day 14 and day 5, respectively, and then cured with a 25 mg per kg oral regimen of chloroquine (Table 1).

Blood mefloquine levels. The average mefloquine blood levels on day 2 were not different for the nine patients who reported that they had vomited 1–6 hours after swallowing the drug and for the 28 patients who did not report having vomited. For 29 subjects who had no recrudescence, the total blood mefloquine and 4-carboxymefloquine levels 48 hours after drug intake were $362\text{--}1719\ \text{ng.ml}^{-1}$ (average, $955 \pm 74\ \text{ng.ml}^{-1}$) and $50\text{--}1180\ \text{ng.ml}^{-1}$ (average, $407 \pm 49\ \text{ng.ml}^{-1}$), respectively, without significant variation with age or sex. For the RIII and RII cases, respectively, the total blood levels of mefloquine were $150\ \text{ng.ml}^{-1}$ and $180\ \text{ng.ml}^{-1}$ and of 4-carboxymefloquine, $140\ \text{ng.ml}^{-1}$ and $243\ \text{ng.ml}^{-1}$.

Fig. 1. Plot of the geometric mean parasite density (GMPD) expressed as the number of asexual forms per μl of blood (based on 8000 white blood cells per μl of blood) and the mean patient temperature during the study.



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^b Heraus incubator, Hanau, Germany.

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Susceptibility of *Plasmodium falciparum* to mefloquine in Senegal

Table 1: Data on the two mefloquine treatment failures

	Case 1	Case 2
Age	20 months	3.5 years
<i>No. of parasites per µl:</i>		
Day 0	64 000 (38.9) ^a	27 200 (38.5)
Day 1	5600 (37.2)	240 000 (37.2)
Day 2	—	24 000 (37.5)
Day 3	800 (36)	200 000 (37.5)
Day 4	400 (37)	20 000 (40.7)
Day 5	30 (36)	168 000 (37.9)
Day 6	10 (36.4)	
Day 14	160 (37.1)	
<i>Levels of mefloquine in blood (ng.ml⁻¹)</i>		
Day 2	180	150
Day 5	—	124
<i>Parasite strain IC₅₀ (nmol.l⁻¹)</i>		
Chloroquine	—	48
Mefloquine	—	32

^a Figures in parentheses are the patient's temperature in °C.

In vitro tests. A total of 42 *in vitro* tests were performed; of these, 7 (19%) gave an invalid result (insufficient sample or bacterial contamination), while 35, 33, and 34, respectively, gave an acceptable result for chloroquine, mefloquine, and halofantrine susceptibility tests. Thirty-three strains of *P. falciparum* (94%) were sensitive to chloroquine

and two (6%) were resistant, the sensitive strains being inhibited at a mean IC₅₀ of 24.2 nmol.l⁻¹. Twenty-three strains (70%) were sensitive to mefloquine, while 10 (30%) had low sensitivity, the sensitive strains being inhibited at a mean IC₅₀ of 20.6 nmol.l⁻¹ (for mefloquine, 1 ng.ml⁻¹ = 2.17 nmol.l⁻¹). Twenty-three strains (68%) were sensitive to halofantrine and 11 (32%) had low sensitivity, the sensitive strains being inhibited at a mean IC₅₀ of 16.6 nmol.l⁻¹ (Fig. 2).

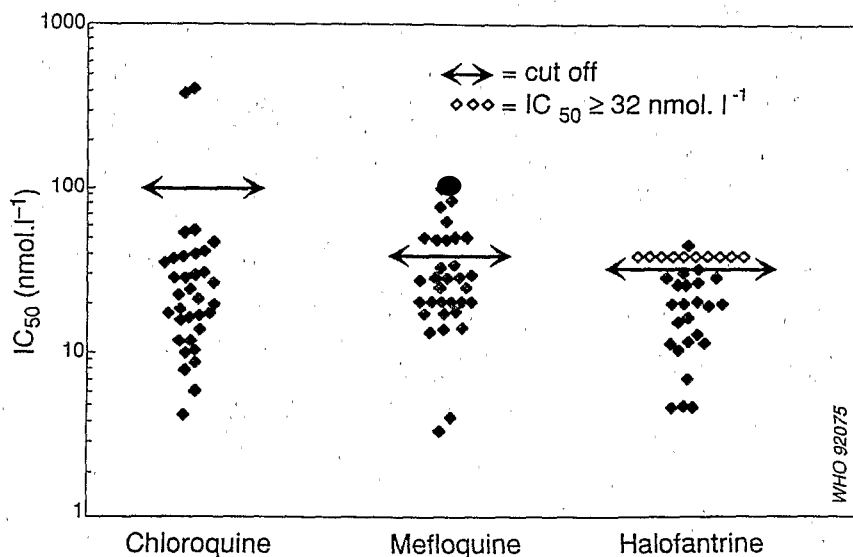
Of the 10 patients with low sensitivity *in vitro* to mefloquine, seven completed the *in vivo* test. The strains involved were suppressed without recrudescence on day 14 by the mefloquine regimen. There was no significant correlation between the response to mefloquine and chloroquine ($r = 0.04$; degrees of freedom = 32) or between that to halofantrine and chloroquine ($r = 0.06$; degrees of freedom = 33). Correlation between the IC₅₀ for mefloquine and halofantrine was highly significant ($r = 0.81$; degrees of freedom = 32).

Discussion

Mefloquine has been available since 1984 for the prophylactic treatment of visitors to Africa but has not yet been used by Senegal residents. Prophylactic failures of mefloquine were first demonstrated in West Africa a few months before the present study (5).

Pikine was chosen for the study since the seasonal transmission of malaria is low and malarial patients supposedly do not have partial immunity. Of

Fig. 2. Plots showing the range of IC₅₀ values in the *in vitro* tests for 35 *Plasmodium falciparum* isolates for chloroquine, mefloquine, and halofantrine. Strains above the cut-off value were resistant (chloroquine) or less sensitive (mefloquine and halofantrine).



the subjects, 34 stated that they had not left Pikine in the 2 months prior to the study. On the basis of the results of interviews or of the urine Dill-Glazko tests, previous antimalarial intake by the participants could not be ruled out. The successful *in vitro* growth of parasites from three subjects who stated that they had received an intramuscular injection of quinine between one day and seven days before the study demonstrates the lack of efficacy of this previous treatment, which was probably inadequate.

For all patients with mefloquine blood levels of $\geq 362 \text{ ng.ml}^{-1}$ on day 2, parasitaemia and fever cleared within 96 hours without recrudescence until day 14. These blood levels are approximately two-thirds of the mean, day-2 whole blood mefloquine concentration that has been reported for Thai malarial patients receiving 15 mg.kg^{-1} of mefloquine (11, 12).

Oduola et al. have proposed an *in vitro* cut-off point for resistance to mefloquine of $\text{IC}_{50} = 26 \text{ nmol.l}^{-1}$ (12 ng.ml^{-1}). The median IC_{50} for African *P. falciparum* strains that we studied in 1983-89 was $12.3 \pm 13.6 \text{ nmol.l}^{-1}$ ($n = 762$) for mefloquine and $7.5 \pm 10.4 \text{ nmol.l}^{-1}$ ($n = 349$) for halofantrine (normal distribution). In the absence of a series of *in vitro* tests of mefloquine treatment failures, those strains with mefloquine $\text{IC}_{50}\text{s} \geq 40 \text{ nmol.l}^{-1}$ (mean $\text{IC}_{50} + 2\text{SD}$) may be considered to have low sensitivity. In our study, 10 strains of *P. falciparum* with mefloquine IC_{50}s of $45\text{--}110 \text{ nmol.l}^{-1}$ were cleared before day 14 by a single 12.5 mg per kg oral dose of mefloquine. The lowest sensitivity strains were isolated from two children (aged 2 years and 4 years) who cannot be considered to be semi-immune. These children had no detectable 4-aminoquinoline in samples of blood on day 0, an adequate mefloquine level on day 2 (684 ng.ml^{-1} and 745 ng.ml^{-1}), and were successfully cured.

The treatment failures were probably due to poor absorption of mefloquine (the RIII patient, who had a *P. falciparum* strain with a mefloquine IC_{50} of 32 nmol.l^{-1}) and to poor absorption and/or low mefloquine sensitivity of the strain involved (the RII patient, who failed the *in vitro* test). These two children did not vomit after taking the mefloquine. We were not able to observe late treatment failures since the follow-up period lasted only 14 days.

Strains of *P. falciparum* that have low sensitivity to mefloquine occur in areas where the drug has never been used and where most *P. falciparum* strains are still sensitive to chloroquine. Nevertheless, this low sensitivity to mefloquine still permits the standard mefloquine regimen to be effective. The highly significant correlation between the *in vitro* response of the *P. falciparum* strains to mefloquine and halofantrine limits the perspective of halofan-

trine as a replacement drug if mefloquine-resistant *P. falciparum* malaria emerges in West Africa.

Acknowledgements

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Résumé

Sensibilité des souches de *Plasmodium falciparum* à la méfloquine dans une zone urbaine du Sénégal

Afin d'évaluer l'efficacité curative de la méfloquine dans le paludisme à *P. falciparum* dans un pays d'Afrique de l'Ouest où des souches faiblement sensibles *in vitro* et des échecs prophylactiques à la méfloquine avaient été observés en 1987-1988, nous avons effectué une étude *in vivo* en novembre 1988 au Sénégal. Quarante-sept sujets fébriles se présentant aux dispensaires de Pikine, un faubourg de Dakar à transmission saisonnière faible, avec une parasitémie isolée supérieure à 1000 trophozoïtes de *P. falciparum*/ μl ont reçu une dose orale unique de $12,5 \text{ mg/kg}$ de méfloquine et ont été suivis quotidiennement jusqu'au 7ème jour et au 14ème jour. Sept sujets qui ont présenté des vomissements et 5 sujets qui ne sont pas revenus ont été exclus. Quatre patients présentaient une concentration d' amino-4-quinoléines dans le sang total supérieure à 150 nmol/l et ont également été exclus. La parasitémie et la fièvre ont disparu avant le 4ème jour chez 29 malades. Un échec RII et un échec RIII ont été observés. Quarante-huit heures après traitement, la méfloquinémie était de $955 \pm 74 \text{ ng/ml}$ dans 29 succès et 150 et 180 ng/ml dans 2 échecs. La culture *in vitro* a mis en évidence une faible sensibilité à la méfloquine de 10 souches sur 33 ($\text{CI}_{50} = 45$ à 110 nmol/l), une résistance à la chloroquine pour 2 souches sur 35 ($\text{CI}_{50} = 300$ et 500 nmol/l) et une faible sensibilité à l'halofantrine ($\text{CI}_{50} > 32 \text{ nmol/l}$) pour 11 isollements de *P. falciparum* sur 34. Les concentrations médianes inhibitrices d'halofantrine et de méfloquine pour 33 isollements de *P. falciparum* étaient fortement corrélées. Les 2 échecs étaient dus à un taux sanguin insuffisant de méfloquine et/ou à la faible sensibilité des souches plasmodiales. Des souches de *P. falciparum* faiblement sensibles à la méfloqui-

ne sont présentes dans cette région d'Afrique alors que ce médicament n'y est pas utilisé et que la plupart des souches restent sensibles à la chloroquine. Malgré cette faible sensibilité des parasites à la méfloquine, l'efficacité thérapeutique à dose unique de cette molécule est satisfaisante chez des sujets dépourvus d'immunité protectrice.

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