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Comparison of the susceptibility of *falciparum* malaria to mefloquine–sulphadoxine–pyrimethamine and chloroquine in Nigeria

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Summary

In Nigeria chloroquine remains the drug of choice for the treatment of *falciparum* malaria since chloroquine resistance is not yet a problem. Nevertheless, in view of the rapid spread of multi-resistant *Plasmodium falciparum* in Africa, it is desirable to test alternative drugs for efficacy and safety. To this end we undertook a comparative controlled trial of the new triple combination, mefloquine–sulphadoxine–pyrimethamine (MSP, Fansimef®, Hoffman-La Roche, Switzerland) with chloroquine in a group of Nigerian children with symptomatic *falciparum* malaria. Our results showed that Fansimef was a rapidly acting blood schizontocide against the Nigerian strain of *P. falciparum*, and was well tolerated. In particular, sinus bradycardia, which was frequently observed with Fansimef in the trials conducted in Zambia, was not seen in any of the Nigerian patients.

Résumé

La chloroquine est le médicament encore couramment utilisé au Nigéria pour le traitement de la malaria *falciparum* car la résistance à la chloroquine ne constitue jusqu'à présent aucun problème. Pourtant, quand on considère la diffusion rapide en Afrique du multirésistant *Plasmodium falciparum*, la vérification de l'efficacité et la sécurité d'autres médicaments qui pourraient la remplacer devient souhaitable. Dans ce but, nous avons développé une épreuve contrôlée de comparaison de la nouvelle et triple combinaison méfloquine–sulphadoxine–pyriméthamine (MSP, Fansimef®) et de la chloroquine dans un groupe d'enfants nigérians avec des symptômes de malaria *falciparum*. Les résultats ont montré que le Fansimef est un schizontozide du sang d'action rapide contre la forme nigériane du *P. falciparum* et qu'il est tres bien toléré. Notamment, la sinus bradycardia qui avait été fréquemment constatée dans les expériences conduites en Zambie avec le Fansimef n'a pas été observée chez aucun des patients nigérians.

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Introduction

The appearance and rapid spread of resistance by *Plasmodium falciparum* to chloroquine in several parts of the world has stimulated the search for newer and more effective drugs for the treatment of *falciparum* malaria resistant to current standard therapy. One such drug is mefloquine, which was developed and introduced into therapy because preliminary tests indicated that it holds out promise of activity against chloroquine- and multi-resistant *P. falciparum* [1]. In extensive studies in South-East Asia, South America and Zambia in Southern Africa, both mefloquine and its fixed dosage combination with sulphadoxine and pyrimethamine have lived up to their early promise of being safe, rapidly acting blood schizontocides active against multi-resistant *P. falciparum* [2–4]. In Nigeria, resistance to chloroquine is not yet a problem, but, that notwithstanding, it is desirable to evaluate other antimalarials for activity against *P. falciparum* in the area. In this study, we have compared, in children of primary school age, the *in-vivo* susceptibility of *P. falciparum* to chloroquine and mefloquine–sulpha-

doxine-pyrimethamine (MSP, Fansimef®) using the World Health Organization extended field test [1].

Patients and methods

Children between 6 years and 10 years of age attending four schools in Ijaye, a village about 50 km to the north of Ibadan, were examined for malaria parasites. Parasitaemia was determined by microscopic examination, under oil immersion, of a Giemsa-stained thick blood film. Parasite density was determined by counting the number of asexual forms of the parasite against 1000 white blood cells (WBC). In infections with *P. falciparum* in which there was gametocytaemia, the gametocyte density was determined by counting against at least 2000 WBC. Normal WBC count was assumed to be 8000/mm^3. A minimum of 200 oil immersion fields were examined for each subject. The size of the spleen was recorded according to Hackett's classification [5].

Seventy of these children with parasitaemia and fever were allocated randomly into two treatment groups. One group was treated with approximately 25 mg/kg chloroquine given in divided doses over a period of 3 days. Commercially available chloroquine tablets (Resochin, Bayer, FRG) were used. The second group was treated with MSP in a single dose. The amount of MSP (M 125 mg, S 250 mg, P 12.5 mg per tablet) received according to weight was as follows:

10–20 kg weight = 1 tablet;
21–30 kg weight = 1.5 tablets;
31–45 kg weight = 2 tablets.

The drugs were administered under the personal supervision of a physician. Every child admitted into the trial was examined on the day that drug treatment commenced (day 0) and subsequently every day for 7 days. After the first week, examination was done once a week for up to 4 weeks. The examination consisted of weight and height measurement (done only on day 0), oral temperature measurement, palpation of the abdomen for spleen and liver enlargement, pulse rate and blood pressure measurements, examination of blood for malaria parasites, and completion of a checklist of symptoms, which included vomiting, diarrhoea, headache, itching and dizziness. In addition, blood was taken from each subject on days 0, 4, 7, 14, 21 and 28 for the determination of packed cell volume (PCV) and white cell count (WBC).

Although the drugs administered were not identical in appearance, observer bias was avoided by dividing up the tasks of treatment, clinical examination, parasitological and haematological tests among various members of the team such that no one person performed more than one task. Furthermore, results of treatment were not discussed among the members of the team until the end of the study.

Values are expressed in the text and tables as means ± standard deviation. Statistical comparisons between the two treatment groups were made using Student's *t*-test, and probability (*P*) values of 0.05 and above were not regarded as significant.

Results

Sixty-two of the initial 70 patients completed the trial. Of the eight who did not, one was withdrawn because he was found to have sickle cell anaemia (HbSS). Two others were withdrawn because they had mixed *P. falciparum* and *P. malariae* infection. Five patients were withdrawn because of irregular attendance at follow-up.

Of the 62 who completed the trial, 30 were in the chloroquine treatment group and 32 in the Fansimef® treatment group.

Parasitological response

The initial parasite density in the chloroquine (CQ) group was between 143,000 and 45,000 per cubic mm (mean 5692 ± 10,494.9/mm^3) whilst in the MSP group it was between 80,000 and 12,000 per cubic mm (mean 1779.2 ± 2115.4/mm^3). *Plasmodium falciparum* was the only species. Gametocytes of *P. falciparum* were present in four subjects in the CQ group and six in the MSP group, giving an overall gametocyte rate of 15.9%.

The parasitological response to treatment was evaluated by determining the parasite rates during treatment and comparing this with the rate before treatment, which is taken as 100%. This is summarised in Table 1. In the CQ group

Table 1. Parasite rates in two groups of children given therapeutic doses of chloroquine or Fansimef

	Day of examination										
	0	1	2	3	4	5	6	7	14	21	28
CQ group (n = 30)											
No. positive	30	21	5	1	1	0	0	0	0	1	1
%	100	70	16.7	3.3	3.3	0	0	0	0	3.3	3.3
MSP group (n = 32)											
No. positive	33	24	6	4	1	1	1	1	1	0	3
%	100	72.7	18.2	12.1	3.0	3.0	3.0	3.0	3.0	0	9.1

Mean clearance time: CQ group = 1.9 ± 0.87 days; MSP group = 2.1 ± 1.01 days (excluding the RI responder).

the parasite rate fell to zero by day 5 and stayed there until days 21 and 28 when one subject developed parasitaemia again. This was thought to be a re-infection. A similar re-infection rate has been observed in some of our earlier *in-vivo* tests with chloroquine [6–8]. In the MSP group (Table 1) the parasite rate did not disappear completely, only briefly on day 21 due to one patient who showed an RI type of response [1]. The parasite densities per mm^3 in this subject were 1300, 720, 0, 0, 0, 47, 23, 48, 5, respectively, on days 0, 1, 2, 3, 4, 5, 6, 7 and 14. The mean parasite clearance time in the CQ group was 1.93 ± 0.87 days and in the MSP group (excluding the non-responder) it was 2.06 ± 1.01 days (Table 1). The parasitaemia in three subjects on MSP on day 28 was thought to be a reinfection.

The parasite rate does not provide a good indication of the actual degree of parasitaemia in the experimental groups. This is best indicated by the parasite density. However, in view of the wide interindividual variation in parasite counts and the wide difference in the mean pretreatment parasite densities between the two groups, the use of mean parasite densities to compare response with treatment in the two groups might be confusing. The Parasite Density Index (PDI) [9] was therefore used as a convenient arbitrary method of comparing the parasitological response between the two groups. In order to determine the PDI, the parasite densities per mm^3 were divided into 10 classes as shown in Table 2. The frequencies of individual infections found in each class were multiplied by the number of the corresponding class and the results added. The sum obtained divided by the number of subjects examined (including the negative findings) gave the PDI. The PDI for the CQ group fell to zero within the first week but that of the MSP group stayed above zero during that period, a reflection of the patient who responded at the RI level [1].

The gametocyte density was generally low. It was 400/mm^3 in one patient and between 4/mm^3 and 48/mm^3 in the remaining nine subjects with gametocytaemia. The gametocytes persisted for up to 14 days in the CQ group and 28 days in the MSP group.

Effect on other clinical/laboratory parameters

The pretreatment temperature was 37.8 ± 0.49°C (range 37.5–39.6°C) in the CQ group and 37.7 ± 0.15°C (range 37.5–38.1°C) in the MSP group. The mean fever clearance time in the CQ group was 2.7 ± 1.09 days (range 1–5 days), whilst in the MSP group it was 2.7 ± 1.05 days (range 1–4 days); these were not significantly different.

Fifteen of the 30 subjects (50%) in the CQ group had pretreatment splenomegaly; average enlarged spleen size [5] was 1.7. Eighteen of the 33 subjects (54.6%) in the MSP group had pretreatment splenomegaly; average enlarged spleen size [5] was 1.7. The overall pre-

Table 2. Frequency distribution of densities of *P. falciparum* trophozoites found in 63 patients before treatment

	Parasite densities per cu.mm of blood									
Class	100 1	101–200 2	201–500 3	501–1000 4	1001–2000 5	2001–5000 6	5001–10,000 7	10,001–20,000 8	20,001–50,000 9	50,001 10
CQ group										
Frequency	0	1	0	8	10	4	3	2	2	0
%	0	3.3	0	26.7	33.3	13.3	10	6.7	6.7	0
MSP group										
Frequency	2	1	2	8	13	5	1	1	0	0
%	6.1	3.0	6.1	24.2	39.4	15.2	3.0	3.0	0	0

Parasite Density Index: CQ group = 5.4; MSP group = 4.6.

treatment spleen rate and average enlarged spleen (AES) for the two groups combined were 52.4% and 1.7, respectively. Both drugs produced a fall in the spleen rate — to 36.7 and 36.4, respectively, for chloroquine and MSP. There was no change in AES. The difference between the mean duration of parasitaemia in patients with splenomegaly and in those without splenomegaly in each treatment group was not significant.

The packed cell volume ranged between 31% and 42% in all but one of the patients. The only exception was an 8-year-old girl in the CQ group who had a much lower PCV of 26% before treatment. Packed cell volume was not significantly altered in any patient during the follow-up. There was an initial rise in the WBC in both groups but this fell back to values around the pretreatment level by the end of 1 week.

Discussion

Malaria is holoendemic in Nigeria; the parasite rate at one single examination in most rural areas is over 80% and in most cities over 50% [9]. The parasite species responsible for acute malaria is *P. falciparum* in over 95% of cases [10]. The drug of choice for the treatment of acute malaria in this country is chloroquine, which has occupied this position since the late 1950s. *In-vivo* and *in-vitro* tests for susceptibility of *P. falciparum* to chloroquine in Nigeria in recent years have failed to detect the presence of resistance to the drug [6–8]. The present study confirms the earlier ones and is particularly reassuring in view of recent reports of confirmed *in-vivo* and *in-vitro* resistance to chloroquine in neighbouring Cameroon Republic [11].

The obvious alternative to chloroquine in the event of resistance should be sulphadoxine–pyrimethamine or amodiaquine, both of which have been shown in other countries to retain activity against *P. falciparum* after the parasites have become substantially resistant to chloroquine [12,13]. Both of these drugs are widely distributed and used for malaria in Nigeria, making the simultaneous appearance of chloroquine- and multi-resistance in the country a very real possibility. So far, mefloquine has not been used in the country and thus this study provides baseline data against which future behavior of the drug in the country may be measured. Mefloquine–sulphadoxine–pyrimethamine rather than mefloquine itself was evaluated in this study since it is the combined preparation with sulphadoxine–pyrimethamine that might ultimately be promoted for deployment as an antimalarial drug in endemic areas [1].

Our study shows Fansimef to be a rapidly acting blood schizontocide. Thirty-two out of the 33 patients treated with the drug showed full sensitivity to the drug, whilst one responded at what appeared to be the RI level. This patient did not vomit the drug but, since blood levels were not determined, it was not possible to rule out inadequate absorption of the drug in the patient. The pretreatment parasite density in the single treatment failure was only 1300/mm^3, therefore treatment failure could not be attributed to unusually heavy parasitaemia. However, in the absence of blood MSP concentration data, there must remain some doubt as to whether this is a true RI responder. Mefloquine is a quinoline–methanol, like quinine, but the possibility of cross-resistance with quinine does not arise in this case since quinine has not been used as an antimalarial drug in this country since the early 1950s. Therefore, our results suggest that although mefloquine is a very active blood schizontocide against the *P. falciparum* strains in this country, there may be a small level of primary resistance to the drug in the community. Such primary resistance to mefloquine has been found in other studies and was, in fact, part of the stimulus for developing the combined preparation with sulphadoxine–pyrimethamine as a way of preventing the selection of such resistant strains by treatment with mefloquine alone [14, 15].

The interpretation of the preceding response as a possible RI was based on the WHO classification of responses of *P. falciparum* to chloroquine [1], and it may not be exactly applicable to slower and longer acting drugs. Our patient had very low parasitaemia on days 5, 6, 7 and 14, which subsequently cleared spontaneously. Tin *et al.* [16] similarly found that patients treated with MSP occasionally showed parasitaemia on day 6 or 7 but were subsequently found cleared of parasites without further treatment. Delayed clearance until days 7–12 has also recently been observed with quinine, quinoline–methanol like mefloquine [17]. These observations suggest possible inappropriateness of the chloroquine response grading

for mefloquine and its combinations.

Mefloquine – sulphadoxine – pyrimethamine was well tolerated in our study and so can be considered safe for field use at doses comparable to the one used in this study.

The results of our study compare favourably with the results of similar studies in other countries. The parasite clearance time by MSP in this study, 2.1 days, is less than the 3.2 days obtained in Thai adults [2], and the fever clearance time of 2.7 days by MSP in this study is almost identical to the 2.3 days obtained in the Thai study [2]. Mefloquine–sulphadoxine–pyrimethamine appears to have been better tolerated in our study than in some of the earlier studies.

In conclusion, MSP appears to be an active blood schizontocide against *P. falciparum* in Nigeria. However, it would be unwise to allow its uncontrolled distribution and use in the country if it is to be protected for as long as possible from the danger of resistance.

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