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In-vivo resistance of *Plasmodium falciparum* to chloroquine and amodiaquine in South Cameroon and age-related efficacy of the drugs

Chloroquine resistance of *Plasmodium falciparum* (CRPF) was first observed in Cameroon in 1985, in the south–west of the country, a region which is hyperendemic for malaria (Sansonetti *et al.*, 1985). Although such resistance has since spread rapidly throughout the country (Louis *et al.*, 1992; Basco *et al.*, 1993), chloroquine still remains the most widely used antimalarial drug. Most of the information collected by the local Ministry of Health on CRPF is biased because it comes from studies in schoolchildren, in whom the immune response is likely to complement the action of drugs, by clearing parasites from the blood (Targett, 1992). Studies must also be conducted among other groups, particularly of children aged <5 years and pregnant women, if (1) the public-health problem posed by CRPF is to be fully addressed and (2) control measures and treatment policies are to be effective. A study to assess how the efficacy of chloroquine and amodiaquine varied with the age of the subject treated was therefore conducted in Ebolowa, a town with about 35 000 inhabitants, in South Cameroon, 160 km south of Yaounde. The region is characterised by an equatorial climate. Although rain falls all year round (with an annual total of about 1700 mm), it is heaviest between September and December and between April and June. This pattern allows sites suitable for the breeding of anopheline mosquitoes to persist throughout each year and transmission of *Plasmodium* spp. is perennial in the region. Health services in Ebolowa are provided by one state, provincial hospital (Ekombitie Hospital), one missionary hospital (EPC Enongal Hospital) and state or missionary health centres. Oral chloroquine is the current first-line treatment for uncomplicated malaria, whereas intramuscular or intravenous injections of quinine are used for severe malaria. Most (59.8%) of the families of the subjects investigated declared that they used drugs for the prevention of malaria, most (95%) of these using chloroquine. However, only 16% of the mothers interviewed gave a dose of chloroquine to their children which was large enough to be considered effective even against non-resistant malaria.

Overall, 250 infants (12–24 months), 120 young children (36–60 months) and 600 schoolchildren (5–15 years) were screened for malaria by examination of Giemsa-stained blood smears. The schoolchildren, from one district of Ebolowa, were screened in April 1995 and the younger children, from Ebolowa or its surroundings, were screened monthly between September and December 1994. The 192 children enrolled for further study were the asymptomatic subjects who had >1000 *P. falciparum* parasites/μl blood but had not taken any treatment within 3 days of the initial examination. Each enrolled child was given a total of 25 mg chloroquine or amodiaquine/kg over 3 days (10, 10 and 5 mg/kg on days 0, 1 and 2, respectively). Each dose was administered as tablets (of 100 mg chloroquine or 200 mg amodiaquine) under the supervision of a team member. The initial aim was to give chloroquine to three of every four schoolchildren as they enrolled and to two of every three of the younger children (the other children receiving amodiaquine). However, as 23 children were lost to follow-up, the final chloroquine:amodiaquine ratios were slightly different. The in-vivo response of the *P. falciparum* parasites in each subject was assessed using a slightly modified 7-day test (Bruce-Chwatt, 1986). Thick, Giemsa-stained blood smears were prepared from each subject on days 3 and 7. Parasitaemias were then estimated by counting the parasites/200 leucocytes (positives) or 1000 leucocytes (negatives)

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TABLE

Chloroquine and amodiaquine resistance by subject age and by level of resistance on day 7 after initiation of treatment

	Treatment			
	Chloroquine		*Amodiaquine*	
Age (years)	1–4	5–15	1–4	5–15
No. of subjects	49	65	35	20
Mean and (S.D.) of ages (years)	2.2 (2.0)	8.8 (4.2)	2.5 (2.4)	8.4 (4.5)
Mean parasitaemia (and 95% CI) on day 0 (parasites/μl blood)	6137 (4425–8527)	2306 (1890–2814)	6067 (3879–9251)	3555 (2218–5807)
RESISTANCE (% of subjects)				
S	45.8	49.2	85.7	95.0
RI/RII	45.8	50.8	14.3	5.0
RIII	8.4	0	0	0

CI, Confidence interval.

and assuming that each subject had 8000 leucocytes/μl blood. All blood smears were read by one microscopist who was unaware of the drug used to treat each child.

For the main statistical analysis, the subjects were split into two age-groups: young (1–4 years); and old (5–15 years). Differences in the frequencies of each level of resistance (S, RI/RII or RIII) with age-group were analysed by χ^2 test. [Those found aparasitaemic on day 7 were considered to be infected with sensitive (S) strains of the parasite.] Geometric mean parasitaemias were compared by analysis of variance after log-transformation. Values of $P < 0.05$ were considered significant.

The prevalence of infection with CRPF (see Table) was high in both age-groups ($> 50\%$) but an RIII response to chloroquine was only observed in four children, all of them young. Of those given chloroquine, most (32; 69.6%) of the young children had positive blood smears on day 3, compared with just 14 (29.6%) of the old children ($P < 0.0001$), and the mean (and range) of the parasitaemias at this time was also higher in the young children [963 (537–1729) *v.* 174 (83–384) parasite/μl]. Although 32 of the old children given chloroquine had negative blood smears on day 7 (and were therefore considered S responses), 11 (41%) of 27 of them re-tested on day 14 were then found positive.

The treatment efficacy of amodiaquine on day 7 was $> 85\%$ in both age-groups (see Table) and no RIII responses to this drug were observed. Among those given amodiaquine, the proportions of each age-group which were smear-negative on day 3 were similar [30/35 (85.7%) of the young children *v.* 19/20 (95%) of the old]. Nineteen of the old children given amodiaquine were smear-negative on day 7; only one of the 16 of these re-tested on day 14 was then smear-positive.

None of the subjects (including those lost to follow-up who were seen later) developed any symptoms requiring health care during the follow-up period

The overall CRPF prevalence, in children aged 1–15 years, was 52.8%. The CRPF in the asymptomatic schoolchildren of Ebolowa (50.8%) is considerably higher than Mulder *et al.* (1994) found in asymptomatic schoolchildren in Edea (31.5%), also located in South Cameroon. However, the difference may perhaps be the result of excluding those with low parasitaemias (≤ 1000 parasites/μl) from the present study, as no such threshold was applied in Edea.

The efficacy of antimalarial treatment is modified by the immune status of the host (Björkmann and Phillips-Howard, 1990; Baird *et al.*, 1991; Targett, 1992). Premunition develops progressively in young children; rapidly in some, less quickly in others, depending on the level of endemicity prevailing in the area. Very young children may be considered non-immune individuals. Although in-vivo drug resistance did not vary with the age of the subjects in the present study, CRPF was present in most (65.5%) of the 29 subjects aged 1–2 years who were given chloroquine, and RIII responses were only observed in the younger subjects. The old children cleared their parasitaemias faster than the young children, perhaps reflecting premunition in the former.

Given the high CRPF prevalence encountered in Ebolowa, the continued use of chloroquine as the first-line treatment for malarial infections might seem surprising. However, chloroquine may remain an effective symptomatic treatment in areas where high CRPF rates prevail, and its use may still greatly reduce the risk of severe malaria developing (Müller *et al.*, 1996). The consequences of being a chronic carrier of *P. falciparum*, however, remain unknown and continued use of chloroquine may permit the existing RIII strains to spread and make the situation more serious. Children with highly resistant parasites are at an increased risk of developing severe malaria (Brewster and Greenwood, 1993). The results of re-testing children who were apparently infected with sensitive parasites 7 days after the 7-day test are interesting as they indicate delayed resistance, particularly in the older children. Most studies of drug resistance are carried out in older (school-age) children. It seems advisable to extend these studies to the target risk groups, such as young children. Carrying out in-vivo tests among asymptomatic young children or infants may be a simple means of studying the level of drug resistance of *P. falciparum* more precisely and of anticipating the evolution of resistance to drugs among the whole population more accurately.

Amodiaquine remains a fairly effective treatment of falciparum malaria in children of all age groups in South Cameroon (present study) and in The Gambia (Müller *et al.*, 1996). Although amodiaquine is not recommended as a curative treatment by the World Health Organization (1990), because of the risk of agranulocytosis (Hatton *et al.*, 1986) and hepatitis (Neftel *et al.*, 1986), its risk:benefit ratios for prevention and treatment are quite different (Phillips-Howard and West, 1990). To deal with the evolution of drug resistance in Central Africa, therapeutic policies need to be reassessed and amodiaquine needs to be considered as an alternative to chloroquine as the first-line treatment.

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