Short Report

pfmdr1 Gene mutation and clinical response to chloroquine in Yaoundé, Cameroon

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The genetic basis of resistance to chloroquine is still unclear. Previous studies have shown an association between chloroquine resistance *in vitro* and a single point mutation (asparagine [Asn]-86→ tyrosine [Tyr]-86) in the *Plasmodium falciparum* multi-drug resistance (*pfmdr1*) gene in parasites originating from various regions in Asia, Papua New Guinea and Africa (FOOTE *et al.*, 1990; BASCO *et al.*, 1995; COX-SINGH *et al.*, 1995; ADAGU *et al.*, 1996). However, contradictory results were reported in Sudan (AWAD EL KARIEM *et al.*, 1992). Chloroquine-resistant *P. falciparum* strains in South America were reported to be characterized by distinct triple mutations in the *pfmdr1* gene (FOOTE *et al.*, 1990). Since these studies were based on drug assays *in vitro*, we have evaluated whether codon 86 of the *pfmdr1* gene can predict chloroquine response *in vivo* in symptomatic Cameroonian patients.

The study was part of a randomized clinical trial comparing chloroquine (25 mg/kg in divided oral doses over 3 d) with pyronaridine in Yaoundé, Cameroon (RINGWALD *et al.*, 1996). All patients (37 adults, 3 children) had acute uncomplicated falciparum malaria and a negative urine test for antimalarial drugs. The patients were followed for 14 d. Parasitological response was evaluated using the revised World Health Organization classification (WHO, 1994; see Table, footnote a).

Parasite deoxyribonucleic acid (DNA) was extracted from venous blood samples obtained before treatment as described in our previous study (BASCO *et al.*, 1995). A polymerase chain reaction was performed using the thermal cycler PTC-100 (MJ Research, Watertown, Massachusetts, USA) under the following conditions: approximately 200 ng of DNA, 15 pmol of primers 5′-AGAGAAAAAAGATGGTAACCTCAG-3′ (sense) and 5′-ACCACAAACATAAATTAACGG-3′ (antisense), buffer (50 mM KCl, 10 mM Tris, pH 8·4), 1·5 mM $MgCl_2$, 200 µM dNTP (deoxynucleotide triphosphates), and 1 unit of *Taq* DNA polymerase in a 50 µL reaction at 94°C×5 min for the first cycle and 1 min in subsequent cycles, 50°C×5 min for the first cycle and 1 min in subsequent cycles, and 72°C×5 min for the first cycle and 2 min in subsequent cycles, for a total of 30 cycles. The amplified fragment (609 base pairs) of the *pfmdr1* gene was treated with restriction enzyme *Nsp*I (FREAN *et al.*, 1992). The quantity of the polymerase chain reaction products was adjusted to 2–4 µg to ensure complete enzymatic digestion. The presence of a mutant Tyr-86 codon is indicated by digestion of the polymerase chain reaction products into 2 fragments. Since the Asn-86 codon is not a restriction site, there is no digestion if the wild-type codon is present. If a parasite carries mixed alleles, 3 bands (one undigested fragment +2digested fragments) are observed after electrophoresis in an agarose gel. In the latter case, enzymatic digestion was repeated with an increased quantity of restriction enzyme for a longer incubation period to ensure that the undigested fragment originated from mixed alleles.

Of the 40 patients treated with chloroquine and whose blood samples were examined for the *pfmdr1* gene mutation, 19 had negative blood films on days 7 and 14 (parasitological response A; WHO, 1994). This cure rate (19/40; 48%) with chloroquine was consistent with previous clinical data in Yaoundé (LOUIS *et al.*, 1992). Twenty-one patients had a recrudescence or required alternative treatment due to worsening clinical condition on or before day 7 (parasitological responses B [n=17] and C [n=4; WHO, 1994). The relationship between codon 86 of the *pfmdr1* gene and the clinical response to chloroquine is summarized in the Table. Thirty-three of the 40 patients were carriers of isolates with the mutant allele Tyr-86. Parasite clearance (response A) was obtained in 4 of the 7 carriers of mixed or pure Asn-86 codons; the other 3 patients displayed response B. However, 15 patients also had parasite clearance (response A) despite the presence of parasites with the mutant allele. If the criteria of chloroquine resistance *in vitro* based on Tyr-86 of the *pfmdr1* gene were applicable *in vivo*, patients infected with Tyr-86 type parasites would not be expected to respond to chloroquine.

Table. *pfmdr1* Gene allele and parasitological response in Cameroonian patients with *P. falciparum* malaria treated with chloroquine

Parasitological response[a]	Codon 86 of the *pfmdr1* gene[b] Asparagine	Asparagine+ tyrosine	Tyrosine
A	1	3	15
B	2	1	14
C	0	0	4
Total	3	4	33

[a]Response A, negative blood film before day 3 or positive on day 3 (parasitaemia <25% of the pre-treatment parasitaemia) and negative thereafter; B, positive blood film on day 3 (<25% of pretreatment parasitaemia) and either positive film on day 7 or alternative treatment required between days 3 and 7; C, parasitaemia on day 3 >25% of the pre-treatment value or alternative treatment required on or before day 3 (WHO, 1994).
[b]According to FOOTE *et al.* (1990), the wild-type codon asparagine-86 is associated with chloroquine susceptibility *in vitro* in parasites from various geographical origins, except South America; the mutant codon tyrosine-86 is associated with chloroquine resistance *in vitro*.

The obvious discordance between the prediction based on allelic form of the *pfmdr1* gene and clinical response may be explained by several possibilities. Firstly, assays, *in vitro*, on which previous studies have been based, do not take into account the patients' immune response to the parasites. It may be that the 'booster effects' of the immune system enhance the schizontocidal effects of chloroquine, leading to parasite clearance despite the presence of drug-resistant parasites with Tyr-86. An objection to this possibility is the fact that Yaoundé is a hypoendemic urban area, where adults represent as many as one-third of symptomatic malaria patients. This implies that, contrary to the surrounding hyperendemic rural areas in southern Cameroon, the adults enrolled in this study were not well immunized against malaria. Secondly, although correct dosage and compliance were strictly observed (patients who vomited within 6 h after chloroquine administration were excluded from the study), pharmacokinetic data were not determined in this study. It is, however, unlikely that chloroquine was not adequately absorbed at the standard

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dose regimen. The third and most likely possibility is that a single point mutation in the *pfmdr1* gene does not confer resistance *in vivo* to chloroquine. Previous studies *in vitro* have not found a perfect correlation between the mutant allele and chloroquine resistance (FOOTE *et al.*, 1990; BASCO *et al.*, 1995). In other studies *in vitro* of multi-drug resistant parasites in south-east Asia, there was no association between the allele and chloroquine resistance (WILSON *et al.*, 1993; BASCO *et al.*, 1996). As suggested by WELLEMS *et al.* (1991), there may be another key chloroquine resistance gene which may, or may not, interact with the *pfmdr1* gene to produce the resistant phenotype.

Although the complex interactions of pharmacokinetics, pharmacodynamics, immune defence and drug susceptibility of the parasites were not analysed in this study, we failed to demonstrate a possible association between the *pfmdr1* gene mutation and response *in vivo* to chloroquine. It may be concluded that determination of the *pfmdr1* allele type probably does not provide useful clinical data that allow accurate prediction of the clinical outcome in patients who are treated with chloroquine in Africa.

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