Short Report

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

Leonardo K. Basco and Pascal Ringwald

Istitut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) and Laboratoire de Recherches sur le Parasite de l’Afrique (LAF 502, Organisation de Coordination pour la Lutte contre les Épidémies en Afrique Centrale (OCEAC), B.P. 288, Yaoundé, Cameroon

Keywords: malaria, Plasmodium falciparum, pfmdr1 mutation, chloroquine sensitivity

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 (Asn-86 → 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., 1996). However, contradictory results were reported in Sudan (AWAD EL WEM 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 animalarial drugs. The patients were followed for 14 d. Parasitological response was evaluated using the revised World Health Organization classification (WHO, 1990; 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 PT-100 (MJ Research, Watertown, Massachusetts, USA) under the following conditions: approximately 200 ng of DNA, 15 pmol of primers 5'-AACAGA-AAAAAGATGTTACA CCTCAG-3' (sense) and 5'-ACACAAACAATATTACCGG-3' (antisense), buffer (50 mM KCl, 10 mM Tris, pH 8.4), 1.5 mM MgCl2, 200 μM dNTP (deoxynucleotide triphosphates), and 1 unit of Taq DNA polymerase in a 50 μL reaction at 94°C x 5 min for the first cycle and 1 min in subsequent cycles, 50°C x 5 min for the first cycle and 1 min in subsequent cycles, and 72°C x 3 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 NsiI (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 +2 digested 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 (47.5%) is similar to previous clinical data in Yaoundé (LOUIC 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 the pfmdr1 gene were applicable in vivo, patients infected with Tyr-86 type parasites would not be expected to respond to chloroquine.

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 observation to this position is 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 at Yaoundé, unlike, however, unlikely that chloroquine was not adequately absorbed at the standard dose, were carriers of isolates with the mutant allele.
 dose regimen. The third and most likely possibility is that a single point mutation in the pfmdrl gene does not confer resistance in vitro 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 pfmdrl 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 pfmdrl gene mutation and response in vivo to chloroquine. It may be concluded that determination of the pfmdrl 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.

Acknowledgements

We are grateful to Sister Solange and her nursing and laboratory staff at the Nlongkak Catholic Missionary Dispensary for their invaluable assistance. This investigation was supported in part by a grant from AUPELF-UREF.

References


Received 25 July 1996; accepted for publication 10 October 1996