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In vivo chloroquine resistance and prevalence of the *pfcrt* codon 76 mutation in *Plasmodium falciparum* isolates from the Republic of Congo

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Abstract

Chloroquine (CQ) resistance in *Plasmodium falciparum* has been particularly associated with mutations in the *pfcrt* gene. The present study was carried out in the malaria hyperendemic town of Brazzaville (Republic of Congo, Central Africa) where CQ is still recommended and used as a first-line drug for *P. falciparum* malaria. We assessed the efficacy of CQ in vivo, and the association between *pfcrt* mutation at codon 76 and treatment outcome in 50 children with uncomplicated malaria. The failure rate on day 28 was 95.7% and the *pfcrt* K76T mutation was present in 100% of isolates. No variation in the multiplicity of infection was observed in pre- and post-treatment isolates. In further 87 isolates from uncomplicated patients not treated with CQ, the mutation was detected in 98.5% of isolates. This study confirms the high level of in vivo resistance to CQ and shows the high prevalence of *pfcrt* K76T mutation in the Republic of Congo.

Keywords: *P. falciparum*; Chloroquine; Malaria; Congo; *Pfcrt*

1. Introduction

Chloroquine (CQ) has been considered as the first-line drug for the prevention and therapy of malaria. In 1950s resistance of *Plasmodium falciparum* strains to this antimalarial was reported from Southeast Asia, South America and sub-Saharan Africa. However, CQ remains the most affordable and widely used antimalarial for many parts of endemic areas in Africa (Trape, 2001; Warhurst, 2001).

Chloroquine resistance (CQR) in *P. falciparum* is conferred by mutations in the parasite *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene, which encodes a putative transporter localized in the digestive

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vacuole (Sidhu et al., 2002). The substitution from lysine (K) to threonine (T) at amino acid 76 (K76T) in the *pfcrt* protein appears to be a primary genetic mechanism conferring resistance to CQ (Fidock et al., 2000).

Direct evidence for in vivo selection for mutant *pfcrt* has been shown in patients originated from different countries like Cameroon (Basco et al., 2002), Mali (Djimde et al., 2001a), Mauritania (Jelinek et al., 2002) and Gabon (Binder et al., 2002), whereas the results on in vitro evaluation of resistance to CQ are more controversial. Several in vitro studies using field isolates showed high but imperfect association between chloroquine response and the key *pfcrt* mutation (Babiker et al., 2001; Basco and Ringwald, 2001a; Thomas et al., 2002). In vitro resistance of *P. falciparum* strains has been shown to be higher in isolates from patients with severe malaria than those with uncomplicated disease in Nigeria (Olumese et al., 2002). Thus, this codon has been considered as a highly reliable genetic marker for the epidemiologic monitoring of chloroquine resistance (Djimde et al., 2001b).

The detection of high-grade resistance to chloroquine led to a change of national drug policy and the use of sulfadoxine–pyrimethamine (S/P) and/or amodiaquine (AQ) as first-line therapy for uncomplicated *P. falciparum* malaria in Western Africa (Djimde et al., 2004), Eastern Africa (Kublin et al., 2003; Shretta et al., 2000), and Central Africa (Basco et al., 2002; Kremsner et al., 1993, 1994; Winkler et al., 1994). To our knowledge, only one published study (Nsimba et al., 2004) reported the high level of in vivo resistance to CQ in the Republic of Congo. In this work, the level of resistance to CQ was evaluated at 70% using the Lot Quality Standard test of WHO and considering 26 patients with uncomplicated malaria and originated from different parts of Brazzaville, the capital of Congo. As this city is divided into different districts with different levels of transmission (Carme et al., 1993), it seemed important to provide additional data from this area where 30% of the Congolese population is concentrated.

To achieve this goal we conducted a study in a southern district of Brazzaville. Children with diagnosed malaria were treated with CQ which remains the first-line drug for the treatment of uncomplicated malaria in the country. These children were followed up for 28 days and as additional investigation tool, the genotyping of *pfcrt* mutation K76T in pre- and post-treatment *P. falciparum* isolates was also done. As a secondary objective, the genetic diversity and multiplicity of infections of *P. falciparum* isolates from patients were investigated.

2. Materials and methods

2.1. Study site

This study was carried out in Brazzaville (Republic of Congo). In this urban area, malaria is highly endemic, stable with a perennial transmission (Trape et al., 1985). Malaria is primarily due to *P. falciparum*. An entomological inoculation rate (EIR) of 22.5 infective bites/person/year was reported (Trape and Zoulani, 1987). Between 1993 and 2002, armed conflicts occurred in the Republic of Congo. During this period, the populations left the city and went to surrounding rural Southern areas where malaria transmission is higher with EIR estimated at one infected bite per person per night in 1985 (Trape et al., 1985). The current study was approved by the Ministry of Public Health of the Republic of Congo.

2.2. Enrolment of patients

At the Centre de Santé Intégré (CSI) of Terinkyo Hospital in the south of Brazzaville, children aged 6–60 months presented the following criteria: axillary temperature $\geq 37.5\,^{\circ}C$ measured with an electronic thermometer, parasite density between 2000 and 200,000 μl^{-1} of blood, absence of severe malaria symptoms, or febrile conditions caused by other diseases than malaria and ability to come for the stipulated follow up visits were recruited from February to July 2003. Informed consent was obtained from parents or guardians. The recruited children were treated with CQ tablets (Cinpharm, Cameroon, Lot 7.090), according to WHO protocol based on the assessment of therapeutic efficacy of antimalarial drugs for uncomplicated *falciparum* malaria. Each dose of chloroquine (25 mg/kg of body weight over a three-day period; 10 mg/kg the first day and the second day, and 5 mg/kg the third day). After the administration of the treatment, the children were observed during 30 min for an eventual vomiting