Short Communication

Chloroquine-resistance molecular markers (Pfcrt T76 and Pfmdr-1 Y86) and amodiaquine resistance in Burkina Faso

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Summary

We investigated the relationship between the two main molecular markers for chloroquine resistance (Pfcrt T76 and Pfmdr-1 Y86) and the clinical efficacy of amodiaquine in Burkina Faso. Before treatment, the prevalence of Pfcrt T76, Pfmdr-1 Y86 or both mutations in the same infection was significantly higher in patients who experienced a recrudescence than in those who successfully responded to the treatment. Therefore, these two molecular markers could be useful in monitoring amodiaquine resistance, particularly in countries where this drug is used in combination with artesunate as first- or second-line treatment.

keywords malaria, Plasmodium falciparum, drug resistance, amodiaquine, Pfcrt, Pfmdr-1

Although chloroquine (CQ) and amodiaquine (AQ) are chemically similar; AQ might still be effective where CQ resistance is high (Gorissen et al. 2000; Staedke et al. 2001; Tinto et al. 2006). Nevertheless, several in vitro studies have shown cross-resistance between CQ and AQ or monodeshethyl-AQ, the active metabolite of AQ (Gonzalez et al. 2003; Tinto et al. 2006). This suggests that molecular markers linked to CQ resistance, such as Pf T76 and Pfmdr-1 Y86, might be useful for monitoring AQ resistance (Ochong et al. 2003). We investigated the relationship between the two main CQ resistance markers (Pfcrt T76 and Pfmdr-1 Y86) and the clinical efficacy of AQ in children aged 6 months to 15 years.

The study was conducted in Burkina Faso in two sites with differing intensity of malaria transmission; the north with an entomological inoculation rate (EIR) estimated at around 15–20 infecting bites/man/year and the centre with an EIR estimated to be around 50 to 60 infecting bites/man/year (T. Baldet, personal communication).

Children were treated and followed up according to the World Health Organization (WHO) 28-day in vivo test (WHO 2003). Outcomes were defined according to the WHO classification for monitoring antimalarial drug resistance (World Health Organization 2003). Blood samples for the molecular analysis were collected on filter paper (Whatman 3; Maidstone, England) at day 0 before treatment and at the time of recurrent parasitaemia. DNA was extracted using the Chelex-100 method (Plowe et al. 1995). Detection of Pfcrt T76 and Pfmdr-1 Y86 mutations was done by using PCR followed by sequence-specific restriction enzyme digestion (Djimde et al. 2001a). Nested PCR (Ranford-Cartwright et al. 1997) was adopted for the analysis of Msp1 and Msp2 to distinguish between recrudescence and new infection.

Outcome at day 28 was known for most patients enrolled (90%, 195/217). The PCR-corrected total treatment failure (TTF) was 6% (12/195) with no difference between the two sites (P = 0.6) nor age groups (<5 years vs. >5 years (P = 0.11)).

Before treatment, the prevalence of the Pfcrt T76, Pfmdr-1 Y86 or both mutations in the same infection was significantly higher among the TTF than among the adequate clinical and parasitological response (ACPR) samples (P = 0.03; P = 0.007; and P = 0.001, respectively) (Table 1). The prevalence of molecular markers did not differ between sites or age groups with the exception of Pfmdr-1 Y86, whose prevalence was significantly higher in children <5 years old (40%) than in the older group (21%) (P = 0.03).
The Pfcrt T76 mutation is the main determinant for CQ resistance (Djimdé et al. 2001a; Dorsey et al. 2001; Tinto et al. 2003). In our study, the Pfcrt T76 mutation was strongly associated with AQ resistance, a result consistent with earlier studies in Burkina Faso (Dokomajilar et al. 2006) and in other African countries (Ochong et al. 2003; Holmgren et al. 2006), confirming its primary role in determining aminoquinolones resistance. A study in Nigeria reported that none of the sample collected at the time of failure carried the wild-type Pfcrt K76 allele, indicating the critical role of the Pfcrt T76 mutation in determining AQ resistance (Happi et al. 2006).

The prevalence of Pfmdr-1 mutation was also significantly high in TTF than in ACPR samples, even if the relation was not as strong as for Pfcrt T76, a result consistent with earlier studies in Kenya and Nigeria, where AQ resistance is substantially higher than in Burkina Faso (Happi et al. 2006; Holmgren et al. 2006). However, a study in Sudan was unable to find an association (Ochong et al. 2003). In our study, the prevalence of both mutations Pfcrt T76 and Pfmdr-1 Y86 was higher in post-treatment than in pre-treatment samples, even if the difference was not statistically significant, possibly because of low AQ resistance. Indeed, in an earlier study carried out in South-Western Burkina Faso, where AQ resistance is slightly higher (9%), this difference was statistically significant (Dokomajilar et al. 2006).

From the results obtained in this study, the definition of an AQ Genotype Failure Index, similar to that established for CQ (Djimdé et al. 2001b; Tinto et al. 2005) would be extremely useful in countries where AQ is used in combination with artesunate as the first- or second-line treatment. However, we were unable to define that, possibly because of the limited number of failures we detected.

In conclusion, the two molecular markers of CQ resistance seem to be linked to AQ resistance as well and could be used for surveillance purposes. However, larger studies in areas of higher AQ resistance should be conducted for this purpose.

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References


