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First Case of Emergence of Atovaquone Resistance in *Plasmodium falciparum* during Second-Line Atovaquone-Proguanil Treatment in South America ▽

The atovaquone-proguanil combination (Malarone) has been introduced in French Guiana for prophylaxis and second-line treatment for *Plasmodium falciparum* malaria in 2002. We report here a treatment failure in a patient who was given a second-line atovaquone-proguanil treatment. A nonimmune *P. falciparum* patient was infected during a 5-day visit without prophylaxis in Maripasoula, a region of malaria endemicity, and while residing in a malaria-free area, the patient experienced three malaria episodes on day 0 (treated with halofantrine [Halfan]), day 25 (treated with atovaquone-proguanil), and day 49 (treated with quinine-doxycline). All treatments were well tolerated. Plasma atovaquone concentration, measured 1 day after atovaquone-proguanil administration, was 1.45 μg/ml, indicating adequate drug absorption (8).

In vitro susceptibility tests, performed on blood samples from day 0 and day 49 (no blood sample was collected before atovaquone-proguanil administration), showed an increased 50% inhibitory concentration (IC_{50}) value for atovaquone on day 49 compared to that on day 0 (Table 1). Genotyping of the parasites from days 0, 26, and 49, using four microsatellite loci (C4M69, 7A11, C4M79, and Pf2802) (1), *msp2*, and *glurp* (6), showed identical alleles for each locus in the three samples. The sequencing of *cyt b*, the atovaquone target (4, 9, 11), showed a wild-type 268 codon in the day 0 and day 26 samples and a 268S mutation in the day 49 sample. The sequencing of *Pfdhfr-ts* (the target of proguanil) (3) showed the same C50R N51I S108N triple mutant in the three samples, which furthermore had an increased copy number (two gene copies). In brief, the parasite genotypes of the three episodes were indistinguishable for all loci except *cyt b*. These data indicate that the second and third episodes were recrudescences due to successive treatment failures.

The day 49 parasites presented elevated IC_{50} levels for atovaquone and a 268S mutant Cyt *b*, a mutation consistently associated with in vitro resistance to atovaquone and therapeutic failures (2, 4, 5, 8, 10). The resistance mutation was undetected in both pretreatment samples, indicating the emergence of resistant parasites during the course of the atovaquone-proguanil treatment. The presence of the 268S *cyt b* mutation was associated with a significantly lower IC_{50} level for atovaquone than for some atovaquone-proguanil treatment failures associated with the same mutation (2, 8). This result may suggest, in light of lack of *cyt b* mutation in an atovaquone-proguanil failure (12), additional targets for atovaquone-proguanil. However, a similar moderate increase in IC_{50} has been reported for recrudescent isolates from Thai patients treated with atovaquone-proguanil (7). Both in Thailand and in French Guiana, therapeutic failure may involve reduced susceptibility to both proguanil and atovaquone. In vitro resistance to cycloguanil, the major metabolite of proguanil, is very high in French Guiana (close to 100% prevalence in 2000 with a mean IC_{50} of >4,100 nM). The mutant *Pfdhfr-ts* and *cyt b* genotypes observed in our atovaquone-proguanil therapeutic failure are consistent with combined resistance to both proguanil and atovaquone.

The observation of this first indigenous case of atovaquone-proguanil treatment failure in South America, due to drug resistance, shows the need for increased vigilance in the follow-up of patients treated with atovaquone-proguanil and in the reinforced surveillance of the parasite population.

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TABLE 1. In vitro drug susceptibility profiles of the day 0 and day 49 parasites[a]

Sample	Date of collection	Parasitemia	IC_{50} (nmol/liter) for drug					
			Chloroquine	Quinine	Mefloquine	Halofantrine	b-Artemether	Atovaquone
Day 0	14 February 2005	2	116	83	14.2	8.7	1.3	1.6
Day 49	04 April 2005	1	111	364	21	3.4	2.7	20.5

[a] Drug assays were performed at a 1% hematocrit over a 48-h culture period using the ^{3}H-hypoxanthine incorporation microtest (6). Each isolate was tested once in duplicate against serial twofold dilutions of antimalarials over the following concentration ranges: 5,120 to 5 nM for chloroquine (chloroquine diphosphate; Sigma; catalog no. C6628), 6,400 to 6.2 nM for quinine (quinine sulfate; Sigma; catalog no. Q1878), 1,024 to 1 nM for mefloquine (Hoffman-La Roche, Inc.), 25.6 to 0.025 nM for halofantrine (GlaxoSmithKline, Inc.), and 200 to 0.19 nM for artemether (Novartis Pharma, Inc.) and atovaquone (GlaxoSmithKline, Inc.). The IC_{50} was calculated using probit/log regression of the percent growth inhibition for each drug.

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