First Case of Emergence of Atovaquone-Proguanil (Malarone™) Resistance in

Plasmodium falciparum During Treatment in Traveller from Comoros

Malarone™ (GlaxoSmithKline) is now commonly used for the treatment and prophylaxis of falciparum malaria in France. We report here a treatment failure by Malarone™ in a patient who was infected during a 33-day visit without antimalarial prophylaxis in Comoros.

The patient presented fever ten days after the end of his trip and diagnosis of falciparum malaria was made. Treatment with Malarone™ was well tolerated. Isolated fever appeared 23 days after therapy in association with falciparum parasites. The patient was successfully treated with quinine.

In vitro susceptibility tests, performed on blood samples from day 0 and day 23 showed an increased inhibitory concentration (IC$_{50}$) value for atovaquone superior to a hundred-fold on day 23 compared to that on day 0 (Table 1). In addition, the IC$_{50}$ for cycloguanil was 18-fold increased.

The sequencing of cyt b gene coding the atovaquone target (12), showed a wild-type P. falciparum strain on day 0 and a Y268S mutation on day 23.

The genotyping of dhfr gene coding the proguanil target (5), showed a double mutant C59R and S108N on day 0, while a triple mutant N51I, C59R and S108N was observed on day 23. However, proguanil likely does not act by itself in Malarone™ but only facilitates the atovaquone activity (11).

The genotyping of pfcrt gene (wild type K76) coding a transport protein involved in chloroquine resistance and dhps gene (wild type S436, A437, K540, A581, A613) coding the sulfadoxine target (5), showed wild identical alleles.
The genotyping of the two isolates, using three of six microsatellite loci (7A11, Pf2802, C4M79, Pf2689, TRAP, and C4M69) (1), msp1 and msp2 (5) showed difference between day 0 and 23 (Table 1).

The day 23 parasites presented a high IC$_{50}$ for atovaquone associated with a Y268S mutant Cyt b. Since 2002, less than twenty cases of genetically confirmed clinical resistance to atovaquone-proguanil had been reported (2-4, 6, 7, 9, 13, 14). Clinical failures were associated with in vitro increased IC$_{50}$ for atovaquone between day 0 and the failure day only in five isolates (4, 7, 9). In some cases, the increased IC$_{50}$ was moderate (7, 8). An in vitro atovaquone threshold of 1900 nM was recommended to discriminate resistant isolates (10). Considering our result, this cut-off must be adjusted to $>350$ nM.

We were unable to detect Cyt b mutations on codon 268 and high IC$_{50}$ to atovaquone in the pre-treatment isolate. Reinfection was excluded because the patient was treated after returning to France. Atovaquone resistant strain was probably present in the initial isolate but in minority making it undetectable for classical genotyping methods and in vitro test. The isolate was polyclonal on day 0 and monoclonal on day 23.

This is the first observation of P. falciparum clinical failure to atovaquone-proguanil in a traveller from Comoros, an area where the in vitro prevalences of isolates with reduced susceptibility to classical antimalarial drugs were $<7\%$ (12). Although clinical failures of atovaquone-proguanil therapeutic remain rare in travellers, an increased vigilance is required during follow-up of their treatment, as well as surveillance of the parasite population should be reinforced.

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References


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Table 1. In vitro drug susceptibility profiles and changes in genotyping profiles of the day 0 and day 23 *P. falciparum* parasites

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parasitemia</th>
<th>IC₅₀ (in nM) for drug</th>
<th>Changes in <em>P. falciparum</em> genotyping profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CQ</td>
<td>QN</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.5%</td>
<td>32</td>
<td>206</td>
</tr>
<tr>
<td>Day 23</td>
<td>1.3%</td>
<td>62</td>
<td>652</td>
</tr>
</tbody>
</table>

Drug assays were performed at 1.5% hematocrit over a 60-h culture period using the ³H-hypoxanthine incorporation microtest (12). Each isolate was tested once in triplicate against serial dilutions of antimalarial drugs over the following concentration ranges: 5 to 3200 nM for chloroquine disphosphate (CQ, Sigma, Saint Louis, MO) and quinine hydrochloride (QN, Sigma), 3.2 to 400 nM for mefloquine (MQ, Hoffman-LaRoche, Bale, Switzerland), 1.56 to 1000 nM for monodesethylamodiaquine (MDAQ, World Health Organization, Geneva, Switzerland), 0.5 to 310 nM for lumefantrine (LMF, WHO), 10 to 20000 for cycloguanil (CYC, Zeneca Pharma, Reims, France), 50 to 40000 for pyrimethamine (PYR, Sigma) and 0.3 to 12480 for atovaquone (ATV, GlaxoSmithKline).