Quinine-Resistant Malaria in Traveler Returning from Senegal, 2007

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We describe clinical and parasitologic features of in vivo and in vitro Plasmodium falciparum resistance to quinine in a nonimmune traveler who returned to France from Senegal in 2007 with severe imported malaria. Clinical quinine failure was associated with a 50% inhibitory concentration of 829 nmol/L. Increased vigilance is required during treatment follow-up.

Resistance of Plasmodium falciparum to antimalarial drugs is one of the most worrisome problems in tropical medicine. Quinine remains the first-line antimalarial option for treatment of patients with complicated malaria in Europe and Africa. However, emergence of quinine resistance has been sparsely documented (1). Maximizing the efficacy and longevity of quinine as a drug to control malaria will critically depend on pursuing intensive research into identifying in vitro markers and implementing active in vitro and in vivo surveillance programs such as those supported by the World Antimalarial Resistance Network. Such molecular markers are needed to monitor temporal trends in parasite susceptibility (2). We report quinine-resistant P. falciparum malaria in a patient who returned to France from Senegal.

The Patient

A 17-year-old white man from France spent ≈2 months (April and most of May) in Dielmo, Senegal, where malaria is highly endemic and shows intense perennial transmission (3). He did not use antimalarial prophylaxis or protection against mosquitoes. After returning to France, he was admitted to the Bordeaux University Hospital Cen-
ter on May 27, 2007 (day 0). The patient had P. falciparum parasitemia level of 7% and a 2-day history of fever, myalgia, vomiting, and rapid deterioration of consciousness into an arousable coma. A diagnosis of severe malaria with cerebral involvement was confirmed.

Intravenous quinine formiate (loading dose 17 mg/kg) was administered, followed by a maintenance dose (8.3 mg/kg 3×/day for 7 days). The patient was afebrile on day 3, and his thin and thick blood films became negative for P. falciparum on day 6. He was discharged from the hospital on day 7. However, on day 26, he relapsed and had fever and vomiting. He was hospitalized again on day 27 with a core temperature of 40°C, deterioration of consciousness, and a P. falciparum parasitemia level of 4%. He received the same regimen of quinine formiate plus intravenous clindamycin (10 mg 3×/day) for 7 days. His serum quinine level (free and bound drug assayed by high performance liquid chromatography) taken immediately before the fourth drug dose was low (7 mg/L). The patient was then given quinine (10 mg/kg 3×/day from day 30 through day 34). Serum quinine levels then increased and fever cleared within 72 hours. However, a blood smear was positive on day 34.

Because of the treatment failure with quinine and clindamycin, the patient was treated with oral co-artemether (20 mg artemether and 120 mg lumefantrine, given as 4 tablets, followed by 4 tablets after 8 hours, and 4 tablets 2×/ day for 2 days; total = 24 tablets). Parasitic clearance was observed within 48 hours. Blood smears and results of a PCR for P. falciparum were negative from day 36 through day 62. No further recrudescence occurred over the next 12 months.

The isotopic microdrug susceptibility tests used have been described (4). The chloroquine-susceptible 3D7 P. falciparum clone (Africa) and the chloroquine-resistant W2 clone (Indochina), after 2 rounds of sorbitol synchronization, were used as controls. The 50% inhibitory concentration (IC50) values for 12 antimalarial drugs for the study isolate and these 2 controls are shown in the Table. The isolate and these 2 controls are shown in the Table. The strain isolated on day 27 showed reduced susceptibility to quinine (IC50 829 nmol/L, threshold 800 nmol/L) and chloroquine (472 nmol/L, threshold 100 nmol/L). The IC50 for clindamycin was 39 μmol/L (the in vitro resistance cutoff value was not determined). The isolate was susceptible to all other antimalarial drugs tested. Phenotypes and genotypes were assessed only for parasites obtained on day 27.

We concurrently screened blood samples for resistance-associated point mutations. A sequence containing the ms4760 microsatellite was amplified as described (5). The observed ms4760–18 profile was composed of 2 DNNND repeats and 2 DDDNHNDHNN repeats. Genotyping of the P. falciparum chloroquine resistance transporter (Pfcrt) gene, which encodes a transport protein...
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Table. In vitro susceptibility of 3 Plasmodium falciparum isolates to 12 antimalarial drugs, France*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Study isolate</th>
<th>3D7</th>
<th>W2</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>829 nmol/L</td>
<td>157 nmol/L</td>
<td>574 nmol/L</td>
<td>&gt;800 nmol/L</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>472 nmol/L</td>
<td>21 nmol/L</td>
<td>392 nmol/L</td>
<td>&gt;100 nmol/L</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>10.4 nmol/L</td>
<td>49.3 nmol/L</td>
<td>39.3 nmol/L</td>
<td>&gt;30 nmol/L</td>
</tr>
<tr>
<td>Lumeonatrine</td>
<td>19 nmol/L</td>
<td>29 nmol/L</td>
<td>35 nmol/L</td>
<td>&gt;150 nmol/L</td>
</tr>
<tr>
<td>Monodesethylamodiaque</td>
<td>47 nmol/L</td>
<td>17 nmol/L</td>
<td>162 nmol/L</td>
<td>&gt;80 nmol/L</td>
</tr>
<tr>
<td>Dihydroartemisinin</td>
<td>1.1 nmol/L</td>
<td>2.5 nmol/L</td>
<td>3.0 nmol/L</td>
<td>&gt;10.5 nmol/L</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>13.3 nmol/L</td>
<td>4.1 nmol/L</td>
<td>3.6 nmol/L</td>
<td>&gt;350 nmol/L</td>
</tr>
<tr>
<td>Cycloguanil</td>
<td>70 nmol/L</td>
<td>&lt;10 nmol/L</td>
<td>191 nmol/L</td>
<td>&gt;500 nmol/L</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>354 nmol/L</td>
<td>&lt;50 nmol/L</td>
<td>9139 nmol/L</td>
<td>&gt;2,000 nmol/L</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12.8 μmol/L</td>
<td>10.5 μmol/L</td>
<td>13.5 μmol/L</td>
<td>&gt;35 μmol/L</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>24 μmol/L</td>
<td>48 μmol/L</td>
<td>39 μmol/L</td>
<td>ND</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>39 μmol/L</td>
<td>108 μmol/L</td>
<td>126 μmol/L</td>
<td>ND</td>
</tr>
</tbody>
</table>

*P. falciparum strains 3D7 and W2 were used as controls. ND, not determined.

involved in chloroquine resistance (K76T), and the dihydropteroate synthase gene, which encodes the sulfadoxine target (A437G), identified the resistant allele in our isolate (6). There was no mutation in codon 268, which encodes the atovaquone target (4). The isolate had only 1 copy of the P. falciparum multidrug resistance (Pfdmrd1) gene and a mutation in codon 184, which suggested in vitro susceptibility to mefloquine (7). Amplification of DNA from parasites obtained on day 0 and preserved on fixed and stained thin blood films by a modification of the procedure of Edoh et al. (8) was not successful.

Conclusions

Quinine remains a reliable treatment for patients with complicated or severe P. falciparum malaria outside southern Africa. Clinical failure with quinine used alone or in combination with clindamycin is common in Africa. In our case-patient, a correlation between the results of the in vivo and in vitro assessments was demonstrated at day 27. Because of the lack of reliable data on the correlation between quinine IC₅₀ and clinical failure, arbitrary IC₅₀ cutoff values were chosen for in vitro quinine resistance (300 nmol/L, 500 nmol/L, or 800 nmol/L) (9).

Quinine resistance appears to share common characteristics with chloroquine resistance. It is associated with mutations in the pfmdr1 (10) and pfcr (11) genes. Nevertheless, the mechanism of quinine resistance is still unknown. In addition to the pfmdr1 and pfcr genes, other genetic polymorphisms such as microsatellite length variations in the P. falciparum sodium/hydrogen exchanger (pfne-1) gene (5) and mutations in the P. falciparum multidrug resistance protein gene may contribute to quinine resistance (12).

We report an association of clinical failure of quinine treatment with an IC₅₀ of 829 nmol/L, a mutation in codon 76 of the pfcr gene, and an ms4760–18 profile for pfne-1 composed of 2 DNNND repeats. Isolates of P. falciparum with ≥2 DNNND repeats may be associated with reduced susceptibility to quinine. Henry et al. (5) reported that 2 DNNND repeats were associated with quinine IC₅₀ values ranging from 300 nmol/L to 700 nmol/L, and that 3 repeats were associated with an IC₅₀ >600 nmol/L. However, the 3 strains with IC₅₀ >800 nmol/L had ≥2 DNNND repeats (6). Our results are consistent with these data.

P. falciparum resistance levels may differ depending on malaria transmission and drug pressure. Data from Senegal are fragmentary and were obtained by in vitro susceptibility studies conducted with isolates reported to have decreased in vitro susceptibility to quinine (6). Our patient had traveled to Dielmo, Senegal, where in vitro surveillance of antimalarial drug susceptibility has been conducted since 1996. During 1996–2005, the overall prevalence of isolates with IC₅₀ >800 nmol/L for quinine was <6%: 1% in 1996, 4% in 1997, 0% in 1998, 6% in 1999, and 0% in 2005 (13). Quinine was used for 96.4% of the treatments administered in Dielmo during 1990–1995 (14). This drug has since been replaced by chloroquine, sulfadoxine-pyrimethamine, and artesiminin-based combination therapies.

We report a patient with clinical failure associated quinine resistance in a traveler to Senegal. Our results are consistent with those of a recent review of the Uganda Malaria Surveillance Project that reported a higher risk for selecting quinine-resistant parasites associated with a 7-day quinine treatment course (15). Thus, resistance to quinine should be monitored in West Africa. Although such clinical failure of therapy is rare, increased vigilance is required during treatment follow-up, and surveillance of the parasite population should also be increased.

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References


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