Failure of atovaquone-proguanil malaria chemoprophylaxis in a traveler to Ghana

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Summary Clinical failure of Malarone™ chemoprophylaxis is extremely rare. We report a case of Plasmodium falciparum malaria in a returned traveler to Ghana who fully adhered to atovaquone-proguanil (Malarone™) chemoprophylaxis daily dosing, yet took the pills on an empty stomach. Screening of the P. falciparum isolate revealed triple codon mutation of Dhfr at positions 51, 59, and 108. Plasma drug levels of both atovaquone and proguanil revealed sub-therapeutic concentrations.

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1. Case report

A 28-year-old woman returned from a one-month trip to urban Ghana for the purpose of visiting friends and relatives (VFR), and was well until 5-days post-travel, at which time she developed fever. Prior to travel, she sought pre-travel medical advice, and was prescribed Malarone™ for malaria chemoprophylaxis. She filled her prescription locally in Canada, and took her Malarone™ everyday until her presentation to the emergency room, starting 1-day prior to travel, continuing each day during travel, and then taking her last dose within 24 h of admission to hospital. The patient took her Malarone™ each morning approximately 60 min before breakfast with water. She was born in Ghana

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and immigrated to Canada 20 years prior to her presentation. There was no past history of malaria.

On examination, the patient appeared unwell though not toxic. Temperature was 38.8°C with a heart rate of 104 bpm and BP of 119/60 mmHg. There was no rash or lymphadenopathy. Laboratory investigations revealed anemia and thrombocytopenia, with a hemoglobin of 113 g/L (normal 120–140 g/L), a WBC count of $4.4 \times 10^9$/L (normal 4–11 $\times 10^9$/L), and a platelet count of 88 $\times 10^9$/L (normal 150–400 $\times 10^9$/L). Hepatic transaminases were normal, although bilirubin was elevated at 36 umol/L (normal <20 umol/L). Creatinine revealed normal renal function.

Thick and thin blood films for malaria were positive for Plasmodium falciparum at a parasitemia of 3%. Rapid diagnostic test (RDT) for malaria was also positive (BinaxNOW; Inverness Professional Medical Diagnostics, Scarborough, ME). Due to the history of Malaroné™ prophylaxis and the absence of stigmata of severe or complicated malaria, the patient was started on treatment with oral quinine and doxycycline. The following day, the patient reported subjective improvement in symptoms, however, repeat thin smear revealed parasitemia of 6.5%. Malaria thin films on day 3 and prior to discharge revealed an expected reduction in parasitemia to <0.1%. She completed her course of quinine and doxycycline uneventfully, and was discharged home 5 days after admission. Recovery was complete, and there was no recrudescence of P. falciparum parasitemia. At telephone follow-up 2 months following treatment, the patient remained well.

Given the history of P. falciparum malaria despite complete Malaroné™ adherence, we undertook gene sequencing of the parasite to look for genetic markers of atovaquone-proguanil resistance, and we obtained plasma drug levels of both atovaquone and proguanil within 24-h and 72-h of her final dose of Malaroné™.

2. Methods

2.1. Polymerase chain reaction and gene sequencing

DNA extraction and quantitative real time PCR (qPCR) were conducted to confirm species as previously described [1,2]. Single-nucleotide polymorphism (SNP) analysis for cytochrome b (cytb) codon 268 and dihydrofolate reductase (dhfr) codons 16, 50, 51, 59, 108 and 164 were performed by Sanger sequencing (ABI 3130xl) and Pyrosequencing (Qiagen PyroMark Q24) [3]. In addition to sequencing of cytb 268 and dhfr, SNP analysis for drug resistance markers on ATPase 6 codons 623 and 769; chloroquine resistance transporter ( crt) 72, 74, 75 and 76; dihydropteroate synthase (dhpS) 436, 437, 540, 581, 613; and multiple resistance gene (mdr1) 86, 184, 1034, 1042, and 1246 was also performed. Sanger sequencing and pyrosequencing primers were as described in Appendix 1 (Supplementary file). Pfdmrd1 copy number was analyzed as described [4,5]. Two independent qPCR runs were conducted with triplicate samples. The clinical sample copy number is expressed relative to 3D7 control.

2.2. Plasma drug concentration assessment

Plasma concentration of both atovaquone and proguanil was performed by the Analytical Facility for Bioactive Molecules of The Centre for the Study of Complex Childhood Diseases, The Hospital for Sick Children, Toronto, Canada on EDTA blood drawn on day 1 and day 3 of illness (corresponding to 3% and <0.1% P. falciparum parasitemia), using HPLC-UV analysis and liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described in Appendix 1 (Supplementary file).

3. Results

qPCR confirmed isolated infection with P. falciparum. Parasitemia and plasma concentrations of atovaquone and proguanil on days 1 and 3 of illness are summarized in Table 1. On day 1 of illness, less than 24-h from her last dose of Malaroné™, plasma concentration of atovaquone was 2 ng/mL, and plasma concentration of proguanil was 1.3 ng/mL. By day 3 of illness (72-h from last dose of Malaroné™), plasma concentrations of atovaquone and proguanil fell to 1.3 ng/mL and 0.7 ng/mL, respectively (Table 1).

Sequencing of the P. falciparum parasite's cytochrome b and dhfr genes revealed point mutations only at dhfr positions 51, 59, and 108, with corresponding amino acid substitutions (Table 2). P. falciparum cytochrome b was wild type at position 268, and was also wild type at dhfr positions 16, 50, and 164 (Table 2). Sequencing of ATPase 6 revealed wild type P. falciparum at positions 623 and 769. Single point mutations were noted at dhpS positions 436 and 437, though the parasite was wild type at dhpS 540, 581, and 613. Pfdmrd1 sequence was wild type at positions 86, 1034, 1042, and 1246, and mutant at position 184. By pyrosequencing, a small population (7%) of mutant haplotype was noted at Pfdmrd1 1246. Pfdmrd1 copy number relative to 3D7 was 0.99. Interestingly, Pfcr was wild type at positions 72, 74, 75, and 76 by Sanger sequencing, revealing probable chloroquine susceptibility of this strain acquired in sub-Saharan Africa. Of note, however, was the presence of a small population (13%) of mutant haplotype at Pfcr position 72 by pyrosequencing (Cys 72 Ser), the clinical relevance of which is unknown.

4. Discussion

Malaria remains the top specific cause of fever in the returned traveler [6,7]. Every year, travelers returning from the tropics die of P. falciparum malaria [8], yet, malaria is preventable with adherence to well-tolerated chemoprophylaxis, and insect precautions [9]. Malaroné™ is a fixed drug combination of atovaquone and proguanil, which inhibit parasite mitochondrial electron transport at the cytochrome b complex and dihydrofolate reductase (DHFR), respectively [10]. Atovaquone has very low aqueous solubility and absorption is therefore poor unless the drug is taken with food, and in particular, a fatty meal [10]. Ingestion of a fatty meal along with atovaquone...
translates into a 5-fold increase in maximum plasma concentration of the drug, compared to ingestion with water alone or fasting [10]. Our patient’s plasma drug concentrations of atovaquone were in the single-digit nanogram per mL range, which is sub-therapeutic by 1000-fold. Typical therapeutic concentrations of atovaquone are in the 10–15 μg per mL range [11]. Her serum levels of proguanil were also sub-therapeutic by 100-fold, as typical therapeutic values are on the order of just under 1 μg per mL [11].

Documented chemoprophylactic failures of atovaquone-proguanil are exceptionally rare [12], and only a handful of cases of treatment failure due to parasite resistance have been described [10]. Resistance to atovaquone results from a single point-mutation of P. falciparum cytochrome b, which reduces binding affinity for atovaquone [10]. Resistance to proguanil results from stepwise single point mutations in the DHFR gene, which affect interruption of folate cofactor and DNA synthesis [10]. Several cases of atovaquone-proguanil treatment failure have been described in patients with wild type cytb P. falciparum parasites [10], which likely reflects malabsorption of at least the atovaquone component of Malarone™, or otherwise undocumented resistant genotypes. One limitation of some reported cases of atovaquone-proguanil failure is incomplete reporting of gene sequences which may be implicated in resistant phenotypes [10].

Our patient is unusual in that she had clear evidence of sub-therapeutic drug levels, and then acquired a P. falciparum infection, with dhfr triple codon mutation of the parasite. Although we cannot say definitively whether it was the sub-therapeutic drug concentrations or dhfr mutations that contributed to prophylactic failure, this case highlights the need for counseling around the proper dosing and administration of lipophilic drugs such as atovaquone. Our patient followed the entirety of her pre-travel recommendations correctly, but did not understand the need for co-administration of food with Malarone™; an easily remedied misunderstanding which led to hospital admission and potentially fatal P. falciparum infection.

Travelers traveling for the purpose of “visiting friends and relatives” (VFR) are over-represented among cases of malaria imported to North America and Europe, and this is typically related to a lack of chemoprophylaxis, rather than chemoprophylactic failure [7,9,13,14]. Our patient is unusual in that although she traveled for the purpose of VFR, she sought pre-travel advice, purchased her drug locally in Canada, and, by history, took it everyday as prescribed. This case underscores the need for prompt exclusion of malaria by thick and thin film microscopy and RDT in any febrile traveler returning from a malarious area, even in the context of appropriate chemoprophylaxis.

This case also highlights the ongoing emergence of drug resistant P. falciparum isolates, and high rates of dhfr and dhps mutation, which confer resistance to sulphadoxine-pyrimethamine (SP). After the 2005 introduction of SP for intermittent preventive therapy of malaria in pregnant Ghanian women, rates of dhfr and dhps mutation increased rapidly, with a 2010 quadruple-mutation rate approaching 50% of isolates [15]. The wild type haplotypes observed in ATPase 6; pfmdr1 positions 84, 1034, 1042 and 1246 with pfmdr1 copy number of 1 rendered this isolate susceptible to mefloquine and artemisinin anti-malarials as well.

Our case also supports that under circumstances of drug withdrawal at a population level, chloroquine resistant genotypes may revert back to wild type due to fitness costs [16–18], thus rendering chloroquine potentially efficacious [19]. In our patient, only a small proportion of parasites (13%) demonstrated a mutant genotype at Pfcr position 72 by pyrosequencing, a fraction of resistance and at a position that is unlikely to translate into clinical failure of chloroquine [16]. Chloroquine resistance in Ghana dates back to 1965 [20], with chloroquine-resistant P. falciparum isolates documented increasingly thereafter [21–24], up until artemisinin-based combination therapy replaced chloroquine nation-wide in 2004–05 [25]. Now, rates of chloroquine resistant P. falciparum in Ghana are approximately 59%, with predominant mutations of Pfcr position 76 [26]. “Susceptible” Pfcr 76 alleles have been found in 25–76% of P. falciparum isolates in Ghana as recently as 2010 [19]. Thus, with withdrawal of mass chloroquine chemoprophylaxis and formulaic treatment in many parts of Africa over a decade ago, chloroquine susceptibility may re-emerge in circulating strains of P. falciparum.

In summary, the experience of our patient highlights the need for appropriate pre-departure counseling around anti-malarial chemoprophylactic dosing and administration, and that malaria remains on the differential diagnosis of any febrile returned traveler from the tropics, particularly West Africa. As resistance to frequently used anti-malarials continues to expand among populations of P. falciparum, new efficacious treatments are needed.

Table 1  Clinical and parasitologic parameters in a patient with Plasmodium falciparum malaria who failed atovaquone-proguanil chemoprophylaxis.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Parasitemia (by thin film microscopy)</th>
<th>Expected plasma drug concentration</th>
<th>Plasma drug concentration(^a), atovaquone</th>
<th>Plasma drug concentration(^b), proguanil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 of illness</td>
<td>3%</td>
<td>Atovaquone: 11.5 μg/mL; Proguanil: 0.509 μg/mL</td>
<td>2 ng/mL (0.002 μg/mL)</td>
<td>1.3 ng/mL (0.0013 μg/mL)</td>
</tr>
<tr>
<td>Day 3 of illness</td>
<td>&lt;0.1%</td>
<td>Atovaquone(^c): 9.43 μg/mL; Proguanil(^c): 0.102 μg/mL (102 ng/mL)</td>
<td>1.3 ng/mL (0.0013 μg/mL)</td>
<td>0.7 ng/mL (0.0007 μg/mL)</td>
</tr>
</tbody>
</table>

\(^a\) By LC-MS/MS; limit of detection for UV-HPLC is 100 ng/mL.
\(^b\) Half-life of atovaquone is 59 h, and that of proguanil is 14.5 h [11].

\(^c\) Concentration of the drug, compared to ingestion with water alone or fasting [10].
<table>
<thead>
<tr>
<th>Drug</th>
<th>Atovaquone, Cytb position 268 underlined (positions 214–305 shown)</th>
<th>Proguanil, Dhfr positions 16, 50, 51, 59, 108, and 164 underlined (positions 12–180 shown)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type sequence</td>
<td>ATATGTTATAATTTTATTTCTAATACAAAGTTTATTTGGAATTATACCTTTATCACATCCTGATAATGCTATCGTAGTAAATACATATGTTACTCCATCTCAAATTGTACCTGAATGGTACTTTCTACCATTTTATCATTACAATTATTATTCTTATTAGCAGAACAAAAGTTTAACAACTATAATTCAATTATGCCATATGTGCA</td>
<td>ATGTTAAAAACTGTTCCAAGTAAACCAGCTGGTTTAGTAATTGTATTATTATCATTACAATTATTATTCTTATTAGCAGAACAAAAGTTTAACAACTATAATTCAATTATGCCATATGTGCA</td>
</tr>
<tr>
<td>Isolate sequence</td>
<td>CTATTAAGTCTTGATGTTAAAGGATTTAATAATGTTATAATTTTATTTCTAATACAAAGTTTATTTGGAATTATACCTTTATCACATCCTGATAATGCTATCGTAGTAAATACATATGTTACTCCATCTCAAATTGTACCTGAATGGTACTTTCTACCATTTTATCATTACAATTATTATTCTTATTAGCAGAACAAAAGTTTAACAACTATAATTCAATTATGCCATATGTGCA</td>
<td>CTATTAAGTCTTGATGTTAAAGGATTTAATAATGTTATAATTTTATTTCTAATACAAAGTTTATTTGGAATTATACCTTTATCACATCCTGATAATGCTATCGTAGTAAATACATATGTTACTCCATCTCAAATTGTACCTGAATGGTACTTTCTACCATTTTATCATTACAATTATTATTCTTATTAGCAGAACAAAAGTTTAACAACTATAATTCAATTATGCCATATGTGCA</td>
</tr>
</tbody>
</table>

**Table 2.** Gene sequences of *P. falciparum* isolate (day 1) at targets known to confer resistance to atovaquone and proguanil.

- **Codon:** Tyr 268 Tyr (wild type)
- **Isolate sequence:** CTATTAAGTCTTGATGTTAAAGGATTTAATAATGTTATAATTTTATTTCTAATACAAAGTTTATTTGGAATTATACCTTTATCACATCCTGATAATGCTATCGTAGTAAATACATATGTTACTCCATCTCAAATTGTACCTGAATGGTACTTTCTACCATTTTATCATTACAATTATTATTCTTATTAGCAGAACAAAAGTTTAACAACTATAATTCAATTATGCCATATGTGCA

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Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tmaid.2014.12.010.

References