Travel Medicine and Infectious Disease (2015) 13, 89–93

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

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Received 14 December 2014; received in revised form 18 December 2014; accepted 23 December 2014
Available online 31 December 2014

KEYWORDS
Drug resistance;
Malabsorption;
Malaria;
Malarone;
Plasmodium falciparum

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.

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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http://dx.doi.org/10.1016/j.tmaid.2014.12.010
1477-8939/

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 Malarone™ 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 Malarone™ 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 Malarone™.

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). Pfmdr1 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 Malarone™, 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 Malarone™), 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. *Pfmdr1* 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 *Pfmdr1* 1246. *Pfmdr1* copy number relative to 3D7 was 0.99. Interestingly, *Pfcrt* 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 *Pfcrt* 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]. Malarone™ 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