EMERGENCE OF ATOVAQUONE-PROGUANIL RESISTANCE DURING TREATMENT OF PLASMODIUM FALCIPARUM MALARIA ACQUIRED BY A NON-IMMUNE NORTH AMERICAN TRAVELLER TO WEST AFRICA

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Abstract. The importation of drug-resistant malaria is a growing public health problem in non-endemic countries. The combination of atovaquone and proguanil (Malarone™) has become established as an agent of choice to prevent and treat chloroquine-resistant Plasmodium falciparum malaria in travelers. We describe the first reported case in North America of genetically confirmed atovaquone/proguanil-resistant P. falciparum malaria. Polymerase chain reaction and sequence analysis of the primary and recrudescent isolates confirmed the acquisition of a point mutation (Tyr268Ser) in the cytochrome b gene of the recrudescent isolate known to confer high-level resistance to atovaquone. Suboptimal therapy may have played a contributory role in the emergence of resistance.

INTRODUCTION

The combination of atovaquone and proguanil (Malarone™; GlaxoSmithKline, Research Triangle Park, NC) is the latest drug to be approved by the Health Protection Branch of Health Canada and by the U.S. Food and Drug Administration for the treatment and prevention of chloroquine-resistant Plasmodium falciparum malaria in travelers. As with all antimalarial drugs, it is critical to identify the development and spread of resistance to monitor their efficacy and extend the effective lifespan of a drug. We report a case of atovaquone/proguanil (ATQ/PRO)−resistant P. falciparum malaria in a Canadian traveler to Africa.

CASE REPORT

A previously healthy 25-year-old non-pregnant Canadian woman lived for two and a half years in Freetown, Sierra Leone. She took daily pyrimethamine for malaria prophylaxis and had no past history of malaria. One day after returning to Canada, she developed fever, chills, sweats, and headache. Malaria was suspected and blood smears were performed two days after the onset of symptoms. Smears revealed P. falciparum malaria. Although quantification was not available through the regional tropical laboratory where she presented, she did not meet World Health Organization clinical criteria for severe malaria.1

Treatment was initiated with ATQ/PRO using two tablets (250 mg of ATQ/100 mg of PRO per tablet) twice a day for three days as outlined in a standard resource.2 Therapy was not directly observed. She developed thrombocytopenia and her attending physician advised her to discontinue treatment with two tablets remaining in the total course. She defervesced and malaria smears were negative by day six of treatment.

Fever recurred 19 days after therapy, and she was treated empirically with the same regimen of ATQ/PRO (two tablets twice a day for three days). A malaria smear was not done at that time. All doses were taken and symptoms resolved within a few days of completion. Fever recurred 15 days later, and blood smears performed at this time confirmed the presence of asexual stages of P. falciparum. Treatment consisted of oral quinine (600 mg three times a day) and doxycycline (100 mg twice a day) for seven days. Symptoms resolved and repeat malaria smears on days 7 and 28 post-treatment remained negative.

Blood specimens from the first and third episodes of malaria were submitted to a reference laboratory for amplification by polymerase chain reaction (PCR), sequence, and genetic fingerprint analysis. Unfortunately, quantification of parasitemia was not performed by the referring laboratory and additional specimens were not available. This study was reviewed and approved by the Institutional Review Board of the Toronto General Hospital. The DNA was extracted from the primary isolate at presentation and from the second recurrence using columns obtained from Qiagen (Chatsworth, CA).3 The gene encoding merozoite surface protein 1 (MSP-1) was amplified by a PCR and subjected to genetic fingerprinting with single-strand conformational polymorphism (SSCP) analysis as described.4 The MSP-1 gene fragment from the recurrent isolate had an identical SSCP fingerprint to the day 0 isolate, confirming true treatment failure.4 Parasite DNA was extracted from the malaria isolate at presentation and from the day of recurrence as above, and the cytochrome b and the dihydrofolate reductase (DHFR) genes were amplified by PCR and sequenced to detect mutations associated with resistance to atovaquone and proguanil.3,5

Sequence analysis of the clinical isolate at presentation had a wild-type sequence of cytochrome b. However, the recrudescent isolate had a mutation at position 268 specifying a change from tyrosine to serine (Figure 1). Both isolates had mutations in the DHFR gene (S108N, C59R) associated with resistance to cycloguanil, the active metabolite of proguanil.3,5

DISCUSSION

Atovaquone/proguanil is a useful agent for the treatment of imported uncomplicated P. falciparum malaria due to its convenient route of administration (oral), short treatment course (three days), and attractive adverse-effect profile. Atovaquone causes parasite mitochondrial membrane collapse and inhibition of mitochondrial electron transport via the cytochrome b-c complex.6 Resistance can result from a single point mutation in plasmodial cytochrome b, resulting in reduced binding affinity of atovaquone.5
The pro-drug proguanil, rather than the major metabolite cycloguanil, appears to act synergistically with ATQ. Resistance to ATQ/PRO \textit{in vivo} has rarely been reported. As of April 2004, a total of five cases of genetically confirmed ATQ/PRO resistance had been reported, with all of these associated with single amino acid mutation at codon 268 of cytochrome b resulting in a change from tyrosine to serine (in four cases and the present case) or tyrosine to alanine (one case).

Several factors may contribute to emergence of ATQ or PRO resistance, including high parasite burdens, rapid metabolism of proguanil, exposure to suboptimal drug concentrations, or prior exposure to related drugs. In this case, a high parasitemia was not documented. Caucasians are known to metabolize proguanil to cycloguanil relatively fast compared with other ethnic groups, theoretically leaving longer time periods in which the parasites were exposed to ATQ alone. However, there was no clear evidence to implicate this mechanism as a factor in the emergence of ATQ/PRO resistance in this case.

Suboptimal drug levels may result from dosing errors, patient non-adherence, or malabsorption. Although there was no evidence of diarrhea, vomiting, or voluntary non-compliance in this case, the first two clinical episodes of malaria were treated with a divided dose of ATQ/PRO, which may have resulted in a reduced peak drug level and a reduced time above the minimum parasitidal concentration. Incomplete therapy with the first episode may also have been contributory.

Most clinical trials of ATQ/PRO treatment have used single daily dosing. One commonly used source recommends a divided dose regimen to reduce gastrointestinal adverse effects. A single open label study has been published showing clinical success using this approach in 12 patients, although unpublished pharmacokinetic data from the manufacturer (GlaxoSmithKline) also support efficacy when the drug is used in this manner.

This patient had been taking pyrimethamine for long-term prophylaxis while living in west Africa and had mutations observed in the DHFR gene of the \textit{P. falciparum} isolate. Pyrimethamine is not considered an effective drug for prevention, and like cycloguanil, it is a DHFR inhibitor. However it does not have the same synergistic effect in combination with atovaquone. It is unlikely that there is cross-over resistance to proguanil when used in combination with atovaquone. However once cytochrome b mutations at position 268 occur, then anti-malarial activity would be dependent on the anti-folate activity of cycloguanil and this would be expected to be compromised by the observed mutations in DHFR.

\begin{figure}
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\includegraphics[width=\textwidth]{figure1}
\caption{DNA sequence analysis of the \textit{Plasmodium falciparum} cytochrome b gene from the isolate at presentation (left panel) and at the time of recrudescence (right panel). Arrows indicate the development of a single nucleotide substitution in the recrudescent isolate encoding a tyrosine (TAT) to serine (TCT) amino acid change at position 268.}
\label{fig:sequence_analysis}
\end{figure}
Atovaquone/proguanil remains an effective treatment option for chloroquine-resistant *P. falciparum* malaria. It is not entirely clear what contributed to the emergence of resistance in this case, although incomplete therapy may have played a role. Given that there may not be a wide margin for dosing errors, particularly in the face of a large parasite load, clinicians should ensure close adherence to the complete treatment course of ATQ/PRO to increase the chance of successful treatment of *P. falciparum* malaria in a non-immune host. In addition, an effective alternative antimalarial therapy such as quinine plus doxycycline, or artemether/lumefantrine (if available) should be used to treat recrudescent malaria after clinical ATQ/PRO failure.7,8

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