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Case report

Malarone treatment failure not associated with previously described mutations in the cytochrome b gene

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Abstract

Malarone® (atovaquone-proguanil) is an effective drug for the treatment and prophylaxis of multidrug-resistant falciparum malaria. However, first cases of resistance have been reported, which are associated with mutations at codon 268 of the parasite's cytochrome b gene. We report the first case of Malarone® treatment failure from Central Africa.

Drug concentration was well within curative range. Pre- and post-treatment *Plasmodium falciparum* isolates revealed codon 268 wild-type alleles, and no other mutations of the putative atovaquone-binding domain.

These findings illustrate the spread of atovaquone-proguanil-resistance in Africa and question the usefulness of codon 268 as the only target for the surveillance of its emergence.

Introduction

Resistance to antimalarial drugs poses an increasingly serious problem to public health worldwide. Since the late 1990s, the combination of atovaquone and proguanil is commercially available as a fixed antimalarial compound (Malarone®; GlaxoSmithKline). It is well tolerated and effective against multidrug-resistant *Plasmodium falciparum* isolates. Both atovaquone and proguanil have causal prophylactic activity against the hepatic stages of *P. falciparum* which enables prophylaxis to be stopped seven days after leaving a malaria-endemic area. For these reasons, Malarone is a frequently used prophylactic agent especially in short-term travellers [1].

In malaria parasites, atovaquone inhibits mitochondrial electron transport at the level of the cytochrome bc_1 complex and collapses the electropotential across the mitochondrial inner membrane [2]. Atovaquone as a single agent is associated with high recrudescence rates, but the combination with proguanil resulted in cure rates close to 100%. However, the probability of selecting for atovaquone-proguanil-resistant mutants has been estimated as one in 500 treatments [3].

Non-immune travellers who import malaria parasites into non-endemic countries are a suitable group for the surveillance of antimalarial drug-resistance. The first case of Malarone® resistance in Africa occurred in a traveller returning from Nigeria [4]. Analysis of the cytochrome b

gene revealed a point mutation at codon 268 (Tyr-268→Asn). For East Africa, the first case of confirmed Malarone® resistance was observed in a non-immune traveller to Kenya. That patient's isolate also revealed a mutation at codon 268, but tyrosine changed to serine (Tyr-268→Ser) [5]. These two codon 268 variants are within the region encoding the putative atovaquone-binding domain. This is the only genetic change in the parasite to-date which has been associated with Malarone® treatment failure *in vivo*. Therefore, these mutations are considered useful tools for the surveillance of resistance to Malarone® [6,7].

Case report

The case of a thirty-eight year old Congolese woman, a resident in Germany for 12 years, who returned from a four-week stay in Kinshasa with fever, chills, headache and myalgia, is reported. It was her first trip to Africa since she had settled in Germany and she took chloroquine for chemoprophylaxis. Blood smear examination revealed *P. falciparum* at a parasite density of 0.1% and an antigen capture test (Mala Quick, R-Biopharm, Germany) confirmed *P. falciparum* mono-infection.

The patient received a directly observed standard treatment course of Malarone® for three days, four tablets daily (one tablet is equivalent to 250 mg of atovaquone and 100 mg of proguanil hydrochloride). Tablets were taken with food and were well tolerated. No vomiting or diarrhoea occurred during hospitalization. High performance liquid chromatography (Shimatzu, Japan) on Day 2 of treatment confirmed a drug concentration of atovaquone above the required therapeutic plasma concentration (17.2 μg/mL) which excludes impaired bioavailability. Symptoms resolved and parasites were cleared after three days. Sixteen days after discharge from hospital, the patient presented at our clinic with fatigue and headache but no fever. A blood smear demonstrated once again *P. falciparum* mono-infection at an asexual parasite density of 0.01%. Re-treatment with a 6-dose regime of co-artemether (20 mg artemether and 120 mg lumefantrine) was successful.

For further analysis, the pre-treatment and the resistant *P. falciparum* isolates were investigated for point mutations of *cytochrome b* codon 268 by nested PCR and enzymatic restriction digest as recently described [6]. Analysis revealed wild-type alleles in both isolates. A 716 bp fragment of the cytochrome b gene containing the encoding region of the putative atovaquone-binding domain was amplified [7]. Sequencing (ABI, Applied Biosystem, MWG Biotech Ebersberg, Germany) revealed the absence of all mutations previously described to be involved in atovaquone-resistance *in vivo* and *in vitro* [8], no further variants were found.

Discussion

So far, investigations on Malarone resistance supported the association with the *cytochrome* b codon 268 mutations. In contrast, the late treatment failure in our patient in the absence of above described mutations suggests that other mechanisms might also be involved. The symptomatic reappearance of malaria parasites after therapy despite initial adequate drug concentrations confirms clinical resistance to this drug.

Recently, another case of Malarone® treatment failure not associated with *cytochrome b* mutations has been reported [9], in which parasites were not cleared on Day 2 of the treatment course. Therapy was changed to mefloquine and parasites were cleared on Day 4; Day 3 parasite counts were not presented. Considering the comparatively slow onset of parasite clearance following Malarone® treatment [10], the definition as an early treatment failure seems questionable in that case.

Conclusion

The value of *cytochrome b* codon 268 as a single molecular marker for the surveillance of Malarone® resistance should be reconsidered in the light of this finding. Further research is required for a better understanding of the mechanisms involved in the development of resistance to Malarone®, one of the few available drugs for multidrug-resistant falciparum malaria.

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