Early treatment failure during treatment of *Plasmodium falciparum* malaria with atovaquone-proguanil in the Republic of Ivory Coast

Nathalie Wurtz1,2*, Aurélie Pascual1,2, Adeline Marin-Jauffre1, Housem Bouchiba1, Nicolas Benoit2,3, Marc Desbordes2,3, Maryse Martelloni2,3, Vincent Pommier de Santi4, Georges Richa5, Nicolas Taudon2,3, Bruno Pradines1,2 and Sébastien Briolant1

**Abstract**

The increased spread of drug-resistant malaria highlights the need for alternative drugs for treatment and chemoprophylaxis. The combination of atovaquone-proguanil (Malarone®) has shown high efficacy against *Plasmodium falciparum* with only mild side-effects. Treatment failures have been attributed to suboptimal dosages or to parasite resistance resulting from a point mutation in the cytochrome b gene. In this paper, a case of early treatment failure was reported in a patient treated with atovaquone-proguanil; this failure was not associated with a mutation in the parasite cytochrome b gene, with impaired drug bioavailability, or with re-infection.

**Keywords:** Malaria, *Plasmodium falciparum*, Malarone®, Atovaquone-proguanil, Cytochrome b, Resistance, Clinical failure, in vitro, Anti-malarial drug

**Background**

Increasing reports of drug-resistant *Plasmodium falciparum* throughout the world have forced changes in both prevention and treatment. Atovaquone-proguanil (A-P, Malarone®, GlaxoSmithKline Inc) is one of the common first-line agent for the prophylaxis [1-3] and treatment [2,4,5] of falciparum malaria in France and causes only mild side-effects. Since the introduction of the A-P combination, several cases of treatment failure have been observed in travellers returning from Africa [6-14]. Treatment failures have been attributed to suboptimal dosage or impaired bioavailability, re-infection or to a point mutation in the cytochrome b gene (*pfcytb*) [4,9,15]. In this paper, a case of A-P treatment failure in a military employee stationed in the Republic of Ivory Coast was reported; this treatment failure was not due to low plasma levels of the drug, mutations in the *pfcytb* gene, or re-infection.

**Case presentation**

The patient was a 45-year-old military employee deployed to Abidjan in the Republic of Ivory Coast (Port Bouet camp). He had been stationed in Abidjan for six months and had repeatedly forgotten to take the prescribed anti-malarial chemoprophylaxis drug, doxycycline. He presented general malaise and headaches on 27 September, 2011, and was treated by self-medication with paracetamol. Two days later (29 September, 2011 (day 0)), the patient consulted the medical centre of the camp, where he presented headache, myalgias, chills and fever with a temperature of 39.7°C. A blood smear examination revealed *P. falciparum* at a parasitaemia of 0.74%, and a rapid diagnosis test confirmed *Plasmodium* infection. The patient was hospitalized and immediately treated with the standard treatment of Malarone®, four 250 mg tablets daily on day 0, day 1 and day 2, associated with paracetamol and the continuation of chemoprophylaxis with doxycycline. The tablets were taken with fatty food and were not taken with fatty food and were...
Methods

Whole blood specimens from the first (day 0) and second (day 2) episodes of malaria were submitted to the reference laboratory for gene amplification by polymerase chain reaction (PCR), sequencing, genetic analysis and quantification of the plasma concentrations of drugs. No in vitro assays of the P. falciparum isolates could be performed. The DNA of both samples was extracted from blood samples using the QIAamp DNA Mini Kit according to the manufacturer’s recommendations (Qiagen, France). Confirmation of P. falciparum mono-infection was performed by real-time LightCycler® PCR (Roche, Meylan, France), as described elsewhere [17]. The pfcrts and dihydrofolate reductase (pfDHFR) genes were amplified by PCR and sequenced for both isolates to detect mutations associated with resistance to A-P, respectively, as described [15,18,19]. Molecular markers of resistance [19], such as pfcrts (chloroquine resistance transporter), pfndr1 (multidrug resistance 1 protein), pfsh1 (Na+/H+ exchanger 1), pfDHFR (dihydropterote synthetase), pfetQ (tetQ family GTPase) and pfmdt (metabolite/drug transporter) were also assessed. The single-nucleotide polymorphism and copy number assays for these different genes were previously described [15,20-22]. The parasite diversity between day 0 and day 2 was determined by genotyping the TRAP, 7A11, C4M79, P2802, and P2689 microsatellite loci; the highly polymorphic loci of the merozoite surface protein 1 and 2 antigen genes (mssp1-mssp2); and the highly polymorphic loci of the glutamate-rich protein gene (glurp) using fluorescent end-labelled nested PCR and restriction fragment length polymorphism analysis. The primer sequences, PCR conditions, and genotyping methods have been described elsewhere [23-25]. The drug absorption and compliance were estimated by quantification of the drug levels in the patient’s plasma; these assays were performed using a Waters Acquity UPLC instrument (Milford, MA, USA). Separation was carried out on an Acquity BEH C8 column (50 mm × 2.1 mm, 1.7 μm) maintained at 40°C. The mobile phase consisted of solvent A (0.5% acetic acid in purified water) and solvent B (acetonitrile). Two gradient programmes were used, one for the quantification of proguanil, cycloguanil and doxycycline and the second for the quantification of atovaquone. The flow rate was 0.8 mL/min. The injection volume and total run time were, respectively, 5 μL and 3 min. A purification step was performed before analysis using i) an OASIS® HLB SPE cartridge (Waters, Milford, MA, USA) for proguanil, cycloguanil and doxycycline and ii) protein precipitation by the addition of two volumes of an ACN/H2O/acetic acid solution (85:14:1, v/v/v).

Consent

Informed consent was not required because the sampling procedures and testing are part of the French national recommendations for the care and surveillance of malaria.

Results

As previously observed on the blood smear, P. falciparum is the only species detected by real-time PCR. The whole sequencing of the pfcrts gene, which encodes the target of atovaquone, revealed a wild-type P. falciparum isolate on day 0 and day 2 (M133, Y268). Moreover, no other point mutation was identified in the pfcrts gene. Genotyping of the whole pfDHFR gene showed that the three of the five major mutations (A16, N51I, C59R, S108N, I164) associated with proguanil (cycloguanil)/ pyrimethamine resistance were found in isolates from day 0 and day 2. Genotyping of the pfcrts gene (wild type, K76), the pfndr1 gene (N86, Y184F, S1034, N1042 and D1246) and the pfDHPS gene (S436A, A437G, S1034, N1042 and D1246) showed identical alleles. Only one copy each of pfndr1, pfetQ and pfmdt was found in each sample. The pfhshe1 microsatellite ms4760 exhibited profile 22 with one DNNND repeat and two DDDNHNDNHNN repeats. Genotyping of the parasites from days 0 and 2 using the microsatellite loci, msp1, msp2, and glurp showed that the alleles were identical for each locus in the two samples.

Ultra-high-performance liquid chromatography was performed on a blood sample withdrawn just before the third Malarone® administration. Thus, the results showed the residual levels of drugs just before the last intake. The atovaquone plasma concentration was 5.1 µg/mL, and the proguanil and doxycycline levels were, respectively, equal to 380 ng/mL and 509 ng/mL. The plasma concentration of cycloguanil was not determined due to the presence of an interfering signal.

Conclusion

Malarone®, a fixed-dose combination of A-P, is highly effective for the treatment and prophylaxis of multi-drug-
resistant *P. falciparum* malaria [1-5], and it is a useful agent due to its convenient mode of administration (oral), short treatment course (three days) and limited side effects. Atovaquone, a ubiquinone analogue that binds to CYTB of plasmodial mitochondria, exerts its action by inhibiting electron transfer in the respiratory chain [26,27]. The proguanil metabolite cycloguanil acts by inhibiting the parasite PDHFR protein, which is involved in pyrimidine biosynthesis, and the addition of proguanil leads to an enhancement of atovaquone's activity and reduces the chance of mutations arising in the mitochondrial DNA of the malaria parasite [28,29]. Since the introduction of the A-P combination, few cases of treatment failure have been identified in travellers returning from Africa [6-14]. Treatment failures have been generally attributed to suboptimal dosage, re-infections with a new parasite, or to a point mutation (Y268N, Y268S or Y268C) in the *pfcytb* gene [4,9,14,18]. However, several cases of clinical treatment failure were not associated with any known *pfcytb* mutation, the plasma drug concentrations were well within curative range, and re-infection was excluded [7,8,13]. In this paper, a case of early A-P treatment failure not associated with any known *pfcytb* mutation, could be classified as an early treatment failure. In particular, the genotype or an individual pharmacokinetics for proguanil was not possible.

In the context of this case report, the genotype or an individual pharmacokinetics for proguanil was not possible.

In summary, this case represents the first observation of the clinical failure of A-P treatment for *P. falciparum* infection in a military in Ivory Coast that was not due to impaired drug bioavailability, resistance due to *pfcytb* mutations or re-infection with a new parasite. The absence of mutations in *pfcytb* suggests that alternative mechanisms may be involved in the resistance to this drug combination [7,8]. Indeed, resistance may be associated with either inhibition or alteration of key enzymes that are targets for anti-malarial drugs, or alteration of drug accumulation in the parasite that results from reduced uptake of the drug, increased efflux, or a combination of both processes [19].

For further analysis, the day 0 and day 2 *P. falciparum* isolates were investigated for point mutations in the *pfcytb* codon 268 [15], and this analysis revealed wild-type alleles in both isolates. Genotyping of the blood samples from day 0 and day 2 at microsatellite loci (TRAP, 7A11, CAM79, Pf2802, and Pf2689) and at the highly polymorphic loci of the *msp1* and *msp2* antigen genes and of the *glurp* gene were performed. The results showed that the two samples had the same molecular signature and complete homology, excluding the possibility of a re-infection, and these results were confirmed by genotypic analysis of resistance markers. Based on these results, the clinical and parasitological features of the patient between day 0 and day 2, and the WHO definition of treatment failure [16,36], this case could be classified as an early treatment failure.

Several other factors may also contribute to the emergence of A-P resistance, including hyperparasitaemia, rapid metabolism of proguanil or prior exposure to related drugs [37]. In this case, high parasitaemia was not observed (parasitaemia on day 0 = 0.74%; parasitaemia on day 2 = 1.3%). Caucasians are known to metabolize proguanil to cycloguanil relatively rapidly compared to other ethnic groups, leaving the parasites exposed to atovaquone alone for a longer period of time [31]. However, there was no clear evidence to implicate this mechanism as a factor in the emergence of A-P resistance in this case. Moreover, the metabolic status (i.e. poor/extensive) of the patient can’t be assumed with this only concentration versus time level. In the context of this case report, the genotype or an individual pharmacokinetics for proguanil was not possible.

In summary, this case represents the first observation of the clinical failure of A-P treatment for *P. falciparum* infection in a military in Ivory Coast that was not due to impaired drug bioavailability, resistance due to *pfcytb* mutations or re-infection with a new parasite. The absence of mutations in *pfcytb* suggests that alternative mechanisms may be involved in the resistance to this drug combination [7,8]. Indeed, resistance may be associated with either inhibition or alteration of key enzymes that are targets for anti-malarial drugs, or alteration of drug accumulation in the parasite that results from reduced uptake of the drug, increased efflux, or a combination of both processes [19].

Although clinical failure of A-P treatment is rare among travellers, increased vigilance is required during treatment and post-treatment, and the monitoring of the parasite population should be strengthened. Further research is required to gain a better understanding of the mechanisms involved in the clinical failure observed after treatment with Malarone®, one of the few available drugs used to treat infections with multidrug-resistant *P. falciparum* parasites.

**Competing interests**
The authors declare that they have no competing interests.

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**Author details**

1. Unité de Parasitologie - Unité de Recherche pour les Maladies Infectieuses et Tropicales Emergentes - UMR 6236, Institut de Recherche Biomédicale des Armées, Marseille, France.
2. Centre National de Référence du Paludisme, Marseille, France.
3. UMR MD3 Infections Parasitaires: Transmission, Physiopathologie et Thérapeutique, Aix-Marseille Université, Institut de Recherche Biomédicale des Armées, Marseille, France.
4. Centre d’Épidémiologie et de Santé Publique des Armées, Marseille, France.
5. Centre médical des armées de Nîmes Orange Laudun, antenne colonel De Chabritiers, Nîmes, France.

**Authors’ contributions**
GR, MD and VPS carried out diagnostic monitoring of the patient, collection of clinical and epidemiological data. NW, AP, AJ, HB and NB carried out the molecular genetic studies. NT and MM performed the quantification of...
plasmatic concentration of atovaquone-proguanil and cycloguanil. BP and SB conceived and coordinated the study. NW, SB, NT and BP drafted the manuscript. All authors read and approved the final manuscript.

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