Case report

Malarone treatment failure and in vitro confirmation of resistance of Plasmodium falciparum isolate from Lagos, Nigeria

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Abstract

We report the first in vitro and genetic confirmation of Malarone® (GlaxoSmithKline; atovaquone and proguanil hydrochloride) resistance in Plasmodium falciparum acquired in Africa. On presenting with malaria two weeks after returning from a 4-week visit to Lagos, Nigeria without prophylaxis, a male patient was given a standard 3-day treatment course of Malarone®. Twenty-eight days later the parasitaemia recrudesced. Parasites were cultured from the blood and the isolate (NGATV01) was shown to be resistant to atovaquone and the antifolate pyrimethamine. The cytochrome b gene of isolate NGATV01 showed a single mutation, Tyr268Asn which has not been seen previously.

Introduction

Increasing reports of drug-resistant P. falciparum throughout the world have forced changes in both prevention and treatment. Malarone® (GlaxoSmithKline; atovaquone and proguanil hydrochloride) is a recently introduced new drug combination for the treatment [1,2] and prophylaxis [3,4] of falciparum malaria. We report the first in vitro and genetic confirmation of Malarone® resistance in a case of P. falciparum acquired in Africa.

Case Report

A forty-five year old Nigerian male, resident in the UK, presented with a fever and 1.5% P. falciparum parasitaemia two weeks after returning from a 4-week visit to Lagos, Nigeria without taking prophylaxis. The patient was given a standard 3-day treatment course of Malarone®, four tablets daily (one tablet is equivalent to 250 mg of atovaquone and 100 mg of proguanil hydrochloride) with food which he tolerated well without vomiting and was later discharged. Twenty-eight days later, his malaria symptoms returned. After a further five days the patient was readmitted to hospital with a parasitaemia of less than 1%. A blood sample taken at this point was placed into culture. The patient was successfully treated with quinine 600 mg three times per day for three days followed by doxycycline 100 mg per day for seven days.

Drug sensitivity assays were performed at 1% parasitaemia and 1% haematocrit using tritiated hypoxanthine uptake as a measure of parasite viability [5] and the isolate (NGATV01) was shown to be resistant to atovaquone (Table 1). The NGATV01 isolate was also resistant to the antifolate pyrimethamine. The standard laboratory strain K1 was assayed as above and exhibited resistance to both
Table 1: In vitro sensitivity of isolate NGATV01 and strain K1 to standard antimalarial drugs with standard deviations (nmol/L).

| Drug             | NGATV01 Mean IC₅₀ ± SD | K1 Mean IC₅₀ ± SD | Resistance Cut-off |
|------------------|------------------------|-------------------|--------------------|
| Chloroquine      | 9.54 ± 1.18            | 133.29 ± 30.12    | 100                |
| Mefloquine       | 24.14 ± 5.20           | 8.55 ± 0.29       | 20                 |
| Pyrimethamine    | 16012.80 ± 2643.55     | 8082.84 ± 1202.69 | 100                |
| Atovaquone       | 1888.15 ± 106.65       | 2.41 ± 1.01       | 20                 |
| Proguanil        | 4205.50 ± 716.99       | 10239.94 ± 843.51 | not determined     |
| Dihydroartemisin | 2.39 ± 0.07            | 1.26 ± 0.46       | not determined     |

Drug assay was performed at 1% parasitaemia and 1% haematocrit. Experiment was repeated twice in duplicate. * Cut-off points for resistance as previously reported [16,17,6]

Figure 1
Sequence analysis of P. falciparum CYT b gene from isolate NGATV01 showing codons 70 to 309. Residue 268 highlighted shows the change from tyrosine (Y) to asparagine (N) compared to atovaquone-sensitive strain K1 and the change to serine (S) in the atovaquone-resistant strain TM93-C1088 [6].
chloroquine and pyrimethamine. The DNA of NGATV01 was extracted and the cytochrome b coding region of mitochondrial DNA (mtDNA) sequenced [6] in both directions together with DNA samples from *P. falciparum* control strains. The sequence showed a change from TAT to AAT in codon 268 (Figure 1), specifying a change from tyrosine (Tyr) to asparagine (Asn): Y268N. A different mutation in this codon leading to serine was reported earlier in a sample (TM93-C1088) from an atovaquone and pyrimethamine treatment failure in a Thai patient [6].

**Discussion**

The target of atovaquone, CYT b, plays an important role in electron transport during mitochondrial respiration. It is thought that the drug, an analogue of coenzyme Q (ubiquinone), interrupts electron transport and leads to loss of the mitochondrial membrane potential [7,8]. Tyr268 is a conserved bulky hydrophobic contact of the drug in the Q_{o} II region of the ubiquinol oxidation site. Substitution of the less bulky Asn268 should affect the fit and binding of the drug (Figure 2).

Resistance rapidly emerges when atovaquone is used alone [9]. It has been hypothesised that the mode of action of the drug might contribute to the rapid appearance of resistant parasites. During a stage in its interaction with the site when the drug is partially oxidised, the semiquinone formed would be capable of forming reactive oxygen species (ROS) capable of acting as local mutagens during replication of the mtDNA. Proguanil is believed to speed the loss of the membrane potential, and ensure that replication of DNA stops before mutagenesis can occur [10].

**Conclusions**

This is an unusual example of resistance detected during a single course of Malarone® on only a moderate parasitaemia. The atovaquone/proguanil combination has not been widely used yet in West Africa so it is unlikely that the patient was initially infected with an atovaquone-resistant strain. The presence of multidrug-resistant strains such as this example raises concern about the recent move to consider using Malarone as first-line therapy in Africa [11]. The case questions the potential useful life of this combination, especially as atovaquone may persist alone in plasma for up to 6 weeks after treatment [12]. It appears that the synergistic interaction with proguanil is not seen in atovaquone-resistant mutants [13], and higher resistance levels are achievable.

**Acknowledgements**

We thank Dr. Watcharee Chokejindachai and Dr. Jill Curtis of the London School of Hygiene and Tropical Medicine for technical advice and encour-
agement. Quinton Fivelman was supported by the Association of Commonwealth Universities and David Warhurst thanks UK PHLS for financial support.

References
1. Llanos-Cuentas A, Campos P, Clendenes M, Canfield CJ, Hutchinson DB: Atovaquone and proguanil hydrochloride compared with chloroquine or pyrimethamine/sulfadoxine for treatment of acute Plasmodium falciparum malaria in Peru. Braz J Infect Dis 2001, 5:67-72
2. Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson DB: Atovaquone (atovaquone-proguanil hydrochloride): a review of its clinical development for treatment of malaria. Malarone Clinical Trials Study Group. Am J Trop Med Hyg 1999, 60:533-541
3. Overbosch D, Schilthuis H, Bienzeis U, Behrens RH, Kain KC, Clarke PD, et al. Atovaquone-proguanil versus Mefloquine for Malaria Prophylaxis in Nonimmune Travellers: Results from a Randomized, Double-Blind Study. Clin Infect Dis 2001, 33:1015-1021
4. Hogh B, Clarke PD, Camus D, Nothdurft HD, Overbosch D, Gunther M, et al. Atovaquone-proguanil versus chloroquine-proguanil for malaria prophylaxis in non-immune travellers: a randomised, double-blind study. Malarone International Study Team. Lancet 2000, 356:1888-1894
5. Desjardins R, Canfield C, Haynes J, Chulay J: Mutations in Plasmodium falciparum cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob Agents Chemother 1999, 43:701-718
6. Korscinzky M, Chen N, Kotzeck A, Sieckmann K, Cheng Q: Mutations in Plasmodium falciparum cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. Antimicrob Agents Chemother 2000, 44:2100-2108
7. Fry M, Judney M: Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4'-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). Biochem Pharmacol 1992, 43:1545-1553
8. Srivastava IK, Rottenberg H, Vaidya AB: Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. J Biol Chem 1997, 272:3961-3966
9. Looareesuwan S, Viravan C, Webster HK, Kyle DE, Hutchinson DB, Canfield CJ: Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. Am J Trop Med Hyg 1996, 54:62-66
10. Vaidya AB, Mather MW: Atovaquone resistance in malaria parasites. Drug Resist Updat 2000, 3:283-287
11. Shretta R, Brugha R, Robb A, Snow RW: Sustainability, affordability, and equity of corporate drug donations: the case of Malarone. Lancet 2000, 355:1718-1720
12. Butcher GA, Mendoza J, Sinden RE: Inhibition of the mosquito transmission of Plasmodium berghei by Malarone (atovaquone-proguanil). Ann Trop Med Parasitol 2000, 94:429-436
13. Srivastava IK, Morrissey JM, Darrouzet E, Daldal F, Vaidya AB: Resistance mutations reveal the atovaquone-binding domain of cytochrome b in malaria parasites. Mol Microbiol 1999, 33:704-711
14. Crofts AR, Hong S, Uglavila N, Barquera B, Gennis R, Guergova-Kušanas M, et al. Pathways for proton release during ubiquinone oxidation by the bc(1) complex. Proc Natl Acad Sci U S A 1999, 96:10021-10026
15. Guex N, Diemand A, Peitsch MC: Protein modeling for all. Trends Biochem Sci 1999, 24:364-367
16. Day F, Bustos D, Traore B, Jardinel C, Southamavorsong M, Ciceron L, et al: In vitro response of Plasmodium falciparum to atovaquone and correlation with other antimalarials: comparison between African and Asian strains. Am J Trop Med Hyg 1997, 56:315-317
17. Basco LK, Ramilarioso O, Le Bras J: In vitro activity of pyrimethamine, cycloguanil, and other antimalarial drugs against African isolates and clones of Plasmodium falciparum. Am J Trop Med Hyg 1994, 50:193-199