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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	K1	Resistance Cut-off*
	Mean IC_{50} ± SD	Mean IC_{50} ± SD	
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
Dihydroartemisinin	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]

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K1          SGWCFRYMHATGASLVFLLTYLHILRGLNYSYMYLPLSWISGLILFMIFIVTAFVGYVLP 109
TM93-C1088 SGWCFRYMHATGASLVFLLTYLHILRGLNYSYMYLPLSWISGLILFMIFIVTAFVGYVLP 109
NGATV01    SGWCFRYMHATGASLVFLLTYLHILRGLNYSYMYLPLSWISGLILFMIFIVTAFVGYVLP 109

K1          WGQMSYWGATVITNLLSSIPVAVIWICGGYTVSDPTIKRFFVLHFILPFIGLCIVFIHIF 179
TM93-C1088 WGQMSYWGATVITNLLSSIPVAVIWICGGYTVSDPTIKRFFVLHFILPFIGLCIVFIHIF 179
NGATV01    WGQMSYWGATVITNLLSSIPVAVIWICGGYTVSDPTIKRFFVLHFILPFIGLCIVFIHIF 179

K1          FLHLHGSTNPLGYDTALKIPFYPNLLSLDVKGFNNVIILFLIQSLFGIIPLSHPDNAIVV 249
TM93-C1088 FLHLHGSTNPLGYDTALKIPFYPNLLSLDVKGFNNVIILFLIQSLFGIIPLSHPDNAIVV 249
NGATV01    FLHLHGSTNPLGYDTALKIPFYPNLLSLDVKGFNNVIILFLIQSLFGIIPLSHPDNAIVV 249

K1          NTYVTPSQIVPEWYFLPFYAMLKTVPSKPAGLVIVLLSLQLLFLLAEQRSLTTIIQFKMI 309
TM93-C1088 NTYVTPSQIVPEWYFLPFSAMLKTVPSKPAGLVIVLLSLQLLFLLAEQRSLTTIIQFKMI 309
NGATV01    NTYVTPSQIVPEWYFLPFNAMLKTVPSKPAGLVIVLLSLQLLFLLAEQRSLTTIIQFKMI 309

K1          FGARD 314
TM93-C1088 FGARD 314
NGATV01    FGARD 314
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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].

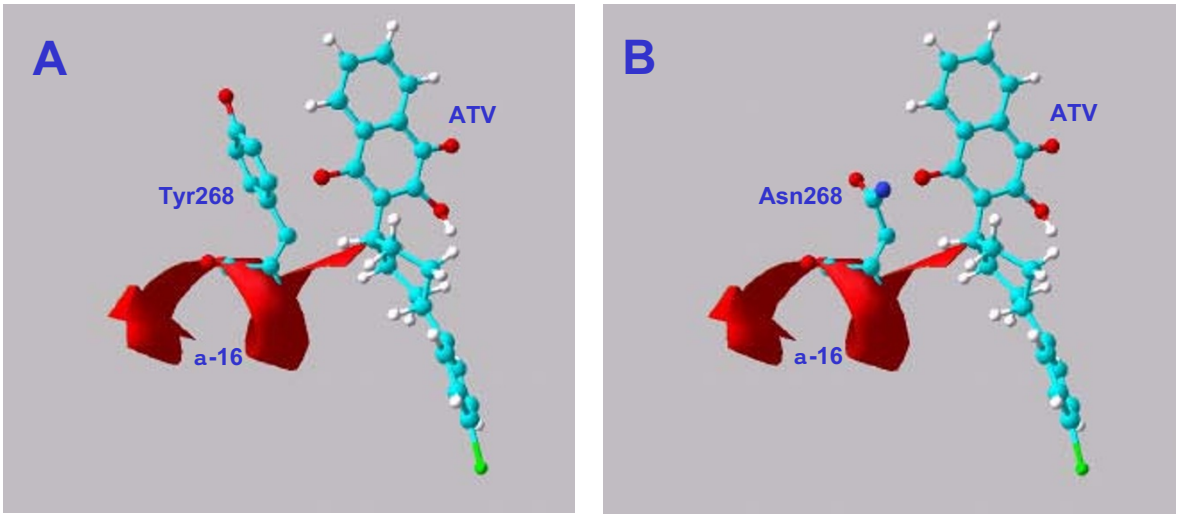

Figure 2
Atovaquone (ATV) in *P. falciparum* cytochrome *b* active site. **A**: Atovaquone built and docked using HyperChem release 6, in the active site of a model of *P. falciparum* cytochrome B. Homology model prepared using the structure of the chicken enzyme [14] with the aid of the SWISS-MODEL Protein Modelling Server and observed in the Swiss Model Viewer [15]. **B**: As A, with active site tyrosine268 replaced by asparagine.

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.

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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: **Malarone (atovaquone and 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, Bienzle 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: **Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique.** *Antimicrob Agents Chemother* 1979, **16:**710-718
6. Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann 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, Pudney 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, Morrisey 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, Ugulava N, Barquera B, Gennis R, Guergova-Kuras M, *et al*: **Pathways for proton release during ubihydroquinone 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 modelling for all.** *Trends Biochem Sci* 1999, **24:**364-367
16. Gay F, Bustos D, Traore B, Jardinel C, Southammavong 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, Ramiliarisoa 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