Infection (2018) 46:867–870
https://doi.org/10.1007/s15010-018-1174-9

CASE REPORT

Dihydroartemisinin–piperaquine treatment failure in uncomplicated *Plasmodium falciparum* malaria case imported from Ethiopia

Gianluca Russo[1] · Mariangela L'Episcopia[2] · Michela Menegon[2] · Samaly Santos Souza[3] · Bruna Ghyslaine Djeunang Dongho[1] · Vincenzo Vullo[1] · Naomi W. Lucchi[3] · Carlo Severini[2]

Received: 30 April 2018 / Accepted: 4 July 2018 / Published online: 6 July 2018

Abstract
Dihydroartemisinin–piperaquine (DHA–PPQ) is the artemisinin combination therapy that was recently introduced for the treatment of *Plasmodium falciparum* uncomplicated malaria, but emerging resistance in South-East Asia is threatening its use. This report describes a case of DHA–PPQ treatment failure in uncomplicated malaria occurring in an immigrant living in Italy, after a travel to Ethiopia. Thirty days after malaria recovery following DHA–PPQ therapy, the patient had malaria recrudescence. According to the genotyping analysis, the same *P. falciparum* was responsible for both episodes. Thus, it seems important to consider possible malaria recrudescence occurring after DHA–PPQ therapy in patients from African countries

Keywords Dihydroartemisinin · Piperaquine · Malaria recrudescence · Failure · Ethiopia

Background

Dihydroartemisinin–piperaquine (DHA–PPQ) is one of the five artemisinin combination therapy (ACTs) regimens recommended by World Health Organization (WHO) for uncomplicated malaria [1]. For decades, the South-East Asian region (mainly the Thailand–Cambodian border) has been the epicentre for the emergence of *Plasmodium falciparum* multidrug resistance, including that related to DHA–PPQ which was recognized in Cambodia and rapidly spread throughout the country (25% in 2010, 46% in 2014) [2].

According to studies performed between 2002 and 2010 (mainly in African countries) comparing clinical efficacy of DHA–PPQ with artemether–lumefantrine (AL), DHA–PPQ showed a lower treatment failure rate and a longer prophylactic effect on new infections [3] possibly because the half-life of PPQ (2–3 weeks) is longer than that of lumefantrine (LUM) (4.5 days). Furthermore, recent studies conducted in Burkina Faso [4], Kenya [5], Sudan [6] and Angola [7] confirmed the high efficacy of DHA–PPQ regimen, and showed very rare cases of recrudescence within 28 days. Currently, although some evidence of slow parasite clearance in patients treated with AL for uncomplicated malaria has been reported [6, 8], there is no confirmation of artemisinin resistance spread in Africa [9]. The present study describes a potential DHA–PPQ treatment failure in an Ethiopian immigrant living in Italy who developed uncomplicated malaria after visiting relatives and friends in her country of origin.

Case report

Clinical description

A 39-year-old Ethiopian woman (living in Italy) was admitted at the Infectious Disease Unit of Policlinico Umberto I in Rome (Italy) on 29th September 2016 because of a 4-day history of fever (up to 39 °C), headache, arthromyalgia, malaise, nausea, and diarrhoea. Symptoms appeared 10 days after returning from a trip to Ethiopia

✉ Gianluca Russo
gianluca.russo@uniroma1.it

[1] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

[2] Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena Rome 299, 00161 Rome, Italy

[3] Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Malaria Branch, 1600 Clifton Rd, Atlanta, GA 30333, USA

(Adama city, Centre region) during which no malaria prophylaxis was taken. Physical examination at admission was normal and the patient's weight was 56.5 kg. Blood tests showed normal white blood cell (WBC) count (3840/µL), slight anaemia (haemoglobin 11.9 g/dL), thrombocytopenia (48,000/µL), increased aspartate aminotransferase (AST 183 IU/L), alanine aminotransferase (ALT 125 IU/L), lactate dehydrogenase (LDH 595 IU/L) and C-reactive protein (CRP 99,000 µg/L). Microscopic urine exam showed micro-hematuria (131/µL); creatinine was normal. Markers for hepatitis A, B, and C were negative. Peripheral blood films showed asexual forms of *P. falciparum* on microscopic exam with an estimated parasitaemia of 3%. Moreover, a routine X-ray showed a small pulmonary infiltrate. The patient was treated with DHA–PPQ 320–40 mg, three tablets/day for three consecutive days, plus levofloxacin (750 mg/day/7 days). Symptoms disappeared after the second dose of DHA–PPQ, and the parasitaemia dropped to < 1%. On the 4th day, the patient was discharged. A follow-up blood microscopic exam and chest X-ray were planned, however, the patient did not return for follow-up. Thirty days after treatment, a recurrence of symptoms (fever, malaise, nausea and vomit) was reported and, 2 days later the patient was readmitted to our hospital. No additional travel abroad or contact with persons with recent journey to malaria-endemic countries was reported. Physical examination was normal. The blood tests showed normal WBCs count (4900/µL), anaemia (haemoglobin 10 g/dL), thrombocytopenia (106,000/µL), increased LDH (453 IU/L) and CRP (70,000 µg/L); liver enzymes, creatinine, and microscopic urine exam were normal. Chest X-ray and abdomen ultrasound examination were normal. Microscopic diagnosis showed asexual forms of *P. falciparum* (parasitaemia of 1%). Treatment with i.v. quinine hydrochloride (QN) was started given that the patient was unable to take any medication orally. Two days after, the drug was shifted to 1-day oral mefloquine (MFQ) (total dose: 1500 mg). After 1 day, symptoms disappeared and the parasitaemia dropped to < 0.1%. After two negative blood microscopic exams, performed 24 and 48 h after stopping antimalarial therapy, the patient was discharged. At 1- and 2-month follow-up visit, all blood tests were normal, and blood microscopic exams remained negative.

Molecular analysis

Total genomic DNA was extracted using PureLink Genomic DNA Kits-Invitrogen, from 200 µL of whole blood samples collected from the patient at both hospital admittances (pre-treatment and 30 days post-treatment). To determine if the two malaria infections were caused by the same parasite isolates (recrudescence), genotyping of the pre-treatment and 30 days post-treatment *P. falciparum* isolate(s) was performed by amplification of three polymorphic markers, allowing the detection of multiclonal *P. falciparum* infections: the merozoite surface protein 1 (*Pfmsp1*) and merozoite surface protein 2 (*Pfmsp2*), as previously described [10], and glutamate-rich protein (*Pfglurp*) genes as described by Viriyakosol et al. [11]. In addition, seven neutral microsatellite markers were also analysed. Genetic characterization confirmed the presence of the same parasite isolates in both the pre-treatment and 30 days post-treatment samples implying that the second infection was a recrudescence (Table 1).

Sanger sequencing of known genetic point mutations associated with various antimalarial drug resistance (artemisinin: *P. falciparum Kelch 13* gene, *PfK13*; chloroquine: *P. falciparum* chloroquine resistance transporter gene, *Pfcrt;* amodiaquine: *P. falciparum* multidrug resistance 1 gene, *Pfmdr1;* sulphadoxine–pyremithamine: *P. falciparum* dihydropteroate synthase and dihydrofolate reductase genes, *Pfdhps* and *Pfdhfr*, respectively; and atovaquone: *P. falciparum* cytochrome B, *PfCytB*) was performed as previously described [12]. In addition, the copy number of *P. falciparum plasmepsin 2* (*PfPM2*) gene, a known marker of piperaquine resistance was investigated using a PET-PCR based assay as described by Souza et al. [13]. The two sequential *P. falciparum* isolates had a single copy of *PfPM2* gene. No mutations associated with artemisinin resistance (*PfK13* gene) and atovaquone resistance (*PfCytB*) were detected; however, previously described mutations in the *Pfcrt, Pfdhps, Pfmdr1* and *Pfdhfr* genes were observed (Table 2).

Discussion

Resistance to artemisinin and its partner drugs is a serious threat to malaria control programs [8, 14]. Reliable molecular markers for artemisinin resistance (mutations

Table 1 Neutral microsatellites analysis for the *P. falciparum* isolates from patient samples (first and second malaria episodes)

Microsatellite markers	TA1	PolyA	PFPK2	TA109	2490	C2M34	C3069
September 29, 2016 sample	169	146	183	175	85	236	124
November 3, 2016 sample	169	146	183	175	85	236	124

Each number represents the size of the microsatellite repeat for each of the 7 markers

Table 2 Analysis of the molecular markers of *P. falciparum* associated with drug resistance

Marker		*Pfcrt* [a]				*Pfmdr1* [a]					*PfK13* [b]	*Pfdhfr* [c]			*Pfdhps* [c]					*PfCytB* [d]
codons		74	75	76	97	86	184	1034	1042	1246	580	51	59	108	436	437	540	581	613	268
Wild type		M	N	K	H	N	Y	S	N	D	C	N	C	S	S	A	K	A	A	Y
		atg	aat	aaa	cac	aat	tat	agt	aat	gat	tgt	aat	tgt	agc	tct	gct	aaa	gcg	gcc	tat
Mutant type		**I**	**E**	**T**	**Y**	**Y**	**F**	**C**	**D**	**Y**	**Y**	**I**	**R**	**N**	**A/F**	**G**	**E**	**G**	**S/T**	**S**
		att	**gaa**	**aca**	**tac**	**tat**	**ttt**	**tgt**	**gat**	**tat**	**tat**	**att**	**cgt**	**aac**	**gct/ttt**	**ggt**	**gaa**	**ggg**	**tcc/acc**	**tct**
Present work	Sample September 29, 2016	**I**	**E**	**T**	H	N	**F**	S	N	D	C	N	**R**	**N**	S	A	**E**	A	A	Y
		att	**gaa**	**aca**	cac	aat	**ttt**	agt	aat	gat	tgt	aat	**cgt**	**aac**	tct	gct	**gaa**	gcg	gcc	tat
	Sample November 3, 2016	**I**	**E**	**T**	H	N	**F**	S	N	D	C	N	**R**	**N**	S	A	**E**	A	A	Y
		att	**gaa**	**aca**	cac	aat	**ttt**	agt	aat	gat	tgt	aat	**cgt**	**aac**	tct	gct	**gaa**	gcg	gcc	tat
***Gobbi, 2016* [12]**	Sample November 27, 2014	**I**	**E**	**T**	H	N	Y	S	N	**Y**	C	N	**R**	**N**	S	**G**	**E**	A	A	Y
		att	**gaa**	**aca**	cac	aat	tat	agt	aat	**tat**	tgt	aat	**cgt**	**aac**	tct	**ggt**	**gaa**	gcg	gcc	tat
	Sample January 7, 2015	**I**	**E**	**T**	H	N	Y	S	N	**Y**	C	N	**R**	**N**	S	**G**	**E**	A	A	Y
		att	**gaa**	**aca**	cac	aat	tat	agt	aat	**tat**	tgt	aat	**cgt**	**aac**	tct	**ggt**	**gaa**	gcg	gcc	tat

[a]Mutations in *Pfcrt* and *Pfmdr1* gene are associated to some quinoline-based antimalarial resistance (e.g., chloroquine)

[b]*PfK13* is the molecular markers for artemisinin resistance

[c]Mutations in *Pfdhfr*/*Pfdhps* genes are responsible for *P. falciparum* resistance to antifolate-cycloguanil

[d]Polymorphisms in *PfCytB* gene were associated to atovaquone resistance

in the propeller domain of *PfK13* gene) and piperaquine resistance (multiplication of the plasmepsin 2–3 gene) have been observed in *P. falciparum* circulating in South-East Asia [15, 16]. Although some mutations in the *PfK13* gene and slow clearing parasites in patients treated with AL for uncomplicated malaria have been reported in several African countries [6, 8, 17], there is no confirmation of artemisinin resistance in Africa [9]. Resistance to PPQ has also not been confirmed in any African parasites. In this study, we report on *P. falciparum* recrudescence, 30 days post-treatment with DHA–PPQ, of a traveller returning from Ethiopia after visiting friends or relatives (VFR). No known mutations in the *PfK13* gene were observed nor did we observe an increase in the *P. falciparum* plasmepsin-2 gene, associated with PPQ resistance, implying that resistant parasites harbouring these known polymorphisms were not responsible for the recrudescence. There may be other explanations for this DHA–PPQ treatment failure such as sub-therapeutic drug exposure due to lack of efficient drug absorption by the patient or suboptimal DHA–PPQ formulation, or other unknown parasites or host genetic factors. Malabsorption due to the underlined medical reason was ruled out according to the anamnestic and clinical presentation of the patient. Moreover, DHA–PPQ was administered directly by the health personnel who ensured that the fasting condition necessary for the intake of the drug was properly carried out. Pharmacokinetic data was not collected for this patient and therefore the drug-related factors cannot be ruled out. However, the DHA–PPQ formulation used to treat the patient, Eurartesim®, is a drug regularly distributed in Italy, and currently, AIFA (the Italian competent authority for drugs) has not reported any concerns about the drug's quality. Furthermore, considering that levofloxacin is primarily excreted unchanged in the urine and its metabolism is minimal, the concurrent administration with DHA–PPQ will not result in an under-exposure of the antimalarial drug. To date, there is no evidence of different pharmacogenetic/pharmacokinetic profiles of DHA–PPQ in different ethnic populations [18], although individual absorption rates would differ.

Interestingly, a similar case of DHA–PPQ treatment failure was reported in Verona, Italy, in 2014 [12], in which an Italian woman also returning from a travel to Ethiopia (Omo River Valley, Southwest region) had a malaria recrudescence 35 days post DHA–PPQ therapy. In this case, pharmacokinetic data excluded under-exposure to DHA–PPQ as the cause of treatment failure, whereas genetic analysis of these *P. falciparum* isolates showed mutations in the *Pfmdr1, Pfcrt, Pfdhfr* and *Pfdhps* genes [12] (Table 1). Analysis of these genes in the *P. falciparum* isolates from our patient revealed similar point mutations (Table 1). This is consistent with what is commonly observed in Africa where previously used antimalarials such as chloroquine, sulphadoxine–pyrimethimine, and amodiaquine led to selection of these known polymorphisms, which are now common in circulation. *Pfmdr1* mutations and/or amplification has been proposed to confer resistance to artemisinin partner drugs such as mefloquine (MFQ) and lumefantrine (LUM)

[14]. In addition, *Pfmdr1* 86N/184F mutations together with *Pfcrt* CVIET (codons 72–76) and *Pfcrt*101F haplotype are associated with a slight decrease in PPQ susceptibility in field isolates [14, 19].

The main limitation of this study is the lack of pharmacokinetic data related to DHA–PPQ. Notwithstanding that we cannot rule out the possibility of pharmacological factors in this case report, this study, together with the previously published case report of recrudescence post DHA–PPQ treatment [12] raises the possibility of a potential DHA–PPQ failure in an Ethiopian isolate. We cannot rule out the possibility that other unknown factors, e.g., mutations in other known or unknown genes are involved. Multicopy occurrence of the *PfPM2* was shown to be a molecular marker associated with PPQ resistance in Cambodia [16]; however, the Ethiopian *P. falciparum* isolates in our study had only one copy of the *PfPM2*.

Our study highlights the need to consider possible malaria recrudescence after DHA–PPQ therapy for patients coming from African countries like Ethiopia, for which a longer clinical follow-up seems necessary. It is important to determine the cause of these recrudescences associated with DHA–PPQ treatment in travellers returning from Ethiopia. To this end, it is worthwhile for clinicians presented with similar cases to preserve such parasite isolates to further investigate if the failure is associated with reduction in in vitro drug sensitivity. This will further help to investigate parasite genetic factors if there is any in vitro evidence to suggest potential resistance to one of the drugs.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. World Health Organization (WHO). Geneva, Switzerland. Guidelines for the treatment of malaria. 3rd edn. 2015. http://apps.who.int/iris/bitstream/handle/10665/162441/9789241549127_eng.pdf;jsessionid=2A0CF4ADEA9CB100157BB2844B2D7C13?sequence=1. Accessed 1 Mar 2018
2. Duru V, Witkowski B, Ménard D. *Plasmodium falciparum* resistance to artemisinin derivatives and piperaquine: a major challenge for malaria eradication in Cambodia. Am J Trop Med Hyg. 2016;95:1228–38.
3. Zani B, Gathu M, Donegan S, Olliaro PL, Sinclair D. Dihydroartemisinin–piperaquine for treating uncomplicated *Plasmodium falciparum* malaria. Cochrane Database Syst Rev. 2014;1:CD010927. https://doi.org/10.1002/14651858.CD010927.
4. Zongo I, Somé FA, Somda SA, Parikh S, Rouamba N, Rosenthal PJ, et al. Efficacy and day 7 plasma piperaquine concentrations in African children treated for uncomplicated malaria with dihydroartemisinin–piperaquine. PLoS One. 2014;9:e103200.
5. Agarwal A, McMorrow M, Onyango P, Otieno K, Odero C, Williamson J, et al. A randomized trial of artemether-lumefantrine and dihydroartemisinin–piperaquine in the treatment of uncomplicated malaria among children in western Kenya. Malar J. 2013;12:254.
6. Mohamed AO, Abdel Hamid MM, Mohamed OS, Elkando NS, Suliman A, Adam MA. Efficacies of DHA–PPQ and AS/SP in patients with uncomplicated *Plasmodium falciparum* malaria in an area of an unstable seasonal transmission in Sudan. Malar J. 2017;16:163. https://doi.org/10.1186/s12936-017-1817-9.
7. Plucinski MM, Talundzic E, Morton L, Dimbu PR, Macaia AP, Fortes F, et al. Efficacy of artemether–lumefantrine and dihydroartemisinin–piperaquine for treatment of uncomplicated malaria in children in Zaire and Uíge provinces, Angola. Antimicrob Agents Chemother. 2015;59:437–43.
8. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2014;371:411–23.
9. Haldar K, Bhattacharjee S, Safeukui I. Drug resistance in *Plasmodium*. Nat Rev Microbiol. 2018;16:156–70. https://doi.org/10.1038/nrmicro.2017.161.
10. Soulama I, Nébié I, Ouédraogo A, Gansane A, Diarra A, Tiono AB, et al. *Plasmodium falciparum* genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. Malar J. 2009;8:135.
11. Viriyakosol S, Siripoon N, Petcharapirat C, Petcharapirat P, Jarra W, Thaithong S, et al. Genotyping of *Plasmodium falciparum* isolates by the polymerase chain reaction and potential uses in epidemiological studies. Bull World Health Organ. 1995;73:85–95.
12. Gobbi F, Buonfrate D, Menegon M, Lunardi G, Angheben A, Severini C, et al. Failure of dihydroartemisinin–piperaquine treatment of uncomplicated *Plasmodium falciparum* malaria in a traveller coming from Ethiopia. Malar J. 2016;15:525.
13. Santos Souza S, L'Episcopia M, Menengon M, Russo G, Saracino A, Lo Caputo S, et al. Detection of piperaquine resistant marker plasmepsin 2 gene amplification using PET-PCR real time assay. 66th Annual Meeting of the American Society of Tropical Medicine and Hygiene; Baltimore, 2017:LB-5226 (Abstract).
14. Veiga MI, Dhingra SK, Henrich PP, Straimer J, Gnädig N, Uhlemann AC, et al. Globally prevalent PfMDR1 mutations modulate *Plasmodium falciparum* susceptibility to artemisinin-based combination therapies. Nat Commun. 2016;7:11553. https://doi.org/10.1038/ncomms11553.
15. Ménard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. N Engl J Med. 2016;374:2453–64.
16. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study. Lancet Infect Dis. 2017;17:174–83. https://doi.org/10.1016/S1473-3099(16)30415-7.
17. Lu F, Culleton R, Zhang M, Ramaprasad A, von Seidlein L, Zhou H, et al. Emergence of indigenous artemisinin-resistant *Plasmodium falciparum* in Africa. N Engl J Med. 2017;376:991–3. https://doi.org/10.1056/NEJMc1612765.
18. Sugiarto SR, Davis TME, Salman S. Pharmacokinetic considerations for use of artemisinin-based combination therapies against falciparum malaria in different ethnic populations. Expert Opin Drug Metab Toxicol. 2017;13:1115–33. https://doi.org/10.1080/17425255.2017.
19. Dhingra SK, Redhi D, Combrinck JM, Yeo T, Okombo J, Henrich PP, et al. A variant PfCRT isoform can contribute to *Plasmodium falciparum* resistance to the first-line partner drug Piperaquine. mBio. 2017. https://doi.org/10.1128/mBio.00303-17.