

Delayed Onset of *Plasmodium falciparum* Malaria after Doxycycline Prophylaxis in a Soldier Returning from the Central African Republic

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More than 97% of imported *Plasmodium falciparum* malaria infections in France in 2014 were diagnosed within 2 months after returning from an area of endemicity (B. Pradines, personal data). The onset of malaria symptoms occurred a median of 6 days after arrival in France (25th to 75th percentile, 2 to 11). Most of the delayed malaria cases appeared in patients with partial immunity in the absence of antimalarial prophylaxis (1, 2). Doxycycline at 100 mg, given 1 day before travel to an area with endemic malaria and administered daily during travel and for 4 weeks after return from an area of endemicity, is currently a recommended chemoprophylactic regimen for travelers visiting malaria-endemic areas with a high prevalence of chloroquine or multidrug resistance (3). Causes of prophylactic and clinical failures of doxycycline against *P. falciparum* are both inadequate doses and poor patient compliance due to simply forgetting and side effects/safety concerns (4). However, resistance can also explain failures of prophylaxis. Here, a case of *P. falciparum* malaria in a French soldier is reported. He was diagnosed 3 months after his last stay in an area of endemicity and after correct intake of chemoprophylaxis by doxycycline (self-report of compliance).

A 36-year-old French soldier without a previous medical history was admitted to Laveran Military Hospital in Marseille, France, in January 2015 with a high-grade fever (temperature, 39°C). Three months previously (November 2014), he had returned from a 4-month peacekeeping mission in the Central African Republic and had stayed in metropolitan France since then. He had been compliant in taking doxycycline prophylaxis daily during the mission until the end of the 4th week after his return home. He used appropriate personal protective measures against mosquito bites, including bed nets and mosquito repellent, during the mission. He did not take medications that could interact with doxycycline. The diagnosis of uncomplicated *P. falciparum* malaria was made on the basis of a thick smear and a quantitative buffy coat (QBC) malaria test completed by a thin smear for determination of parasitemia (0.7%). No other infection was documented. He was successfully treated with atovaquone-proguanil (1,000 mg and 400 mg, respectively, per day for 3 days) (parasitemia of <0.01% at day 3).

The delayed malaria presentation despite appropriate prophylaxis and the absence of reexposure suggested a 3-month period of subclinical and latent *P. falciparum* infection. A decreased susceptibility to doxycycline was investigated, particularly because in 2014 a fatal case of cerebral malaria due to a *P. falciparum* isolate resistant to doxycycline occurred in a French soldier in the Central

African Republic (expected plasmatic concentration of doxycycline with predictive molecular markers of *in vitro* resistance, i.e., two copies of *pfmdt* and *pftetQ* and two PfTetQ KYNNNN motif repeats in the isolate) (5).

Indeed, to date, two studies suggest that copy numbers of >1 of a TetQ GTPase family gene, *pftetQ*, and a metabolic drug transporter gene, *pfmdt*, are potential molecular markers of decreased *in vitro* susceptibility to doxycycline in African isolates (6, 7). In addition, isolates with <3 PfTetQ KYNNNN motif repeats are associated with *in vitro* reduced susceptibility to doxycycline and a significantly higher probability of having a half-maximal inhibitory concentration (IC₅₀) above the doxycycline resistance threshold of 35 μM (odds ratio of 15) (6, 8).

In our patient, the *in vitro* susceptibility to doxycycline and other antimalarial drugs was successfully evaluated by using the 72-hour histidine-rich protein 2 test under controlled atmospheric conditions that consisted of 10% O₂, 5% CO₂, and 85% N₂ (9). *In vitro*, the isolate was susceptible to doxycycline (8.7 ± 0.6 μM [standard deviation], *n* = 4) (10) and the majority of antimalarial drugs (Table 1). The IC₅₀ of mefloquine was relatively high compared with values from previous studies in Africa. The incubation time is one of the conditions that interferes significantly with the IC₅₀s for doxycycline. The IC₅₀s decrease by a factor of 10 to 100 upon prolonged exposure to doxycycline (11, 12). However, the current time of incubation ranges from 48 h to 72 h for all of the studies that evaluated the *ex vivo* or *in vitro* susceptibility of *P. falciparum* isolates to doxycycline. The number of copies of the *pftetQ* and *pfmdt* genes was evaluated relative to the single-copy gene *pfβtubulin* as previously described (6, 7). The genotyping of *pftetQ* sequence polymorphisms was performed by a conventional method as previously described (5, 8). The sample had only one copy of the *pftetQ* and *pfmdt* genes; one PfTetQ KYNNNN motif repeat was also present but was not associated with an IC₅₀ of >35 μM as previously shown (8). In this case, the single copy of *pftetQ*

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TABLE 1 *In vitro* susceptibility to standard antimalarial drugs of the *Plasmodium falciparum* isolate in comparison with the *P. falciparum* W2 clone tested with the same plate batch

Drug	IC ₅₀ for ^a :		Isolate/W2 IC ₅₀ ratio	Resistance cutoff
	Isolate	W2 clone		
Doxycycline	8.7 μM	10.2 μM	0.85	35 μM
Chloroquine	24.7 nM	423 nM	0.06	100 nM
Quinine	35.4 nM	350 nM	0.10	800 nM
Desethylamodiaquine	20.8 nM	110 nM	0.19	80 nM
Lumefantrine	0.6 nM	0.6 nM	1.00	150 nM
Mefloquine	64.0 nM	26.9 nM	2.38	30 nM
Piperazine	26.4 nM	57.3 nM	0.46	135 nM
Pyronaridine	20.7 nM	32.7 nM	0.63	60 nM
Dihydroartemisinin	1.5 nM	1.9 nM	0.79	10.5 nM
Artesunate	1.8 nM	1.6 nM	1.13	10.5 nM

^a Values are averages from 4 independent experiments.

and *pfmdt* suggested *in vitro* susceptibility of *P. falciparum* to doxycycline, and the single PfTetQ KYNNNN motif repeat was not predictive of *in vitro* resistance to doxycycline. The activity of doxycycline was demonstrated to be partially effective on the liver forms of *P. falciparum* (13). These findings justify the recommendation of the currently approved doxycycline regimen (i.e., 100 mg daily for 4 weeks after returning from an area of endemicity). However, it was not sufficient in this case. This prophylactic failure with doxycycline seems to be due not to inadequate doses, poor patient compliance, or resistance but to much longer parasite liver-stage development.

Thus, we report a delayed doxycycline prophylaxis failure in a nonimmune compliant patient with a doxycycline-susceptible *P. falciparum* isolate from the Central African Republic.

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We declare that we have no competing interests.

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