**PLASMODIUM FALCIPARUM IN VIVO RESISTANCE TO QUININE: DESCRIPTION OF TWO RIII RESPONSES IN FRENCH GUIANA**

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Abstract. The resistance of Plasmodium falciparum to antimalarial drugs is one of the most worrisome problems in tropical medicine, but few clinical studies or observations have described confirmed cases of therapeutic failure. We report two cases of in vivo P. falciparum resistance (RIII response) to quinine in French Guiana, an Amazonian focal zone in which multi-resistant malaria is endemic. Both patients presented with uncomplicated malaria and were initially treated with intravenous quinine. Although absorption was normal, the treatment was not effective and the patients still had fever and significant parasitemia three days after the onset of treatment (day 3). The addition of intravenous tetracycline completely resolved the parasitemia within approximately 96 hours. These clinical reports confirm the necessity to combine quinine with tetracycline in this area, as recommended by the recent French regional antimalarial policy.

**INTRODUCTION**

Only a small number of clinical studies or observations have described confirmed cases of therapeutic failure when a sufficient dose of antimalarial agent was given and when the active substance was correctly absorbed. There are two reasons for this: 1) the need to obtain precise and convincing parasitologic and pharmacologic data with regular follow-up over several days, and 2) the cautious attitude required so that treatment can be modified without delay in cases of suspected failure. Thus, the possibilities for confirmation are considerably limited. Most of the published data concern in vitro studies and report the effective concentration (EC₅₀) of antimalarial drugs against Plasmodium strains isolated from their natural environment (i.e., humans), which means that their indicative value is debatable. As a result, it is important to report even isolated observations when there is proof of resistance, especially resistance to a reference product such as quinine.

Only a few cases of clinical resistance to quinine monotherapy have been officially reported in South America. Most of these cases occurred in Brazil, and none have been reported in French Guiana. This French province has a population of 160,000 habitants (official data, March 1999) and is situated in the Amazon basin in South America. Plasmodium falciparum malaria is a worrisome public health problem in its inland regions: it is estimated that between 3,000 and 5,000 proven cases occur each year, mostly along the Maroni River on the border with Suriname and the Oyapock River on the border with Brazil. According to reliable in vitro drug-susceptibility surveillance data from the Pasteur Institute, multi-drug resistance with reduced susceptibility to quinine has become common in this country in recent years. However, no reports are available concerning in vivo susceptibility to antimalarial drugs.

We describe the clinical and parasitologic features of in vivo P. falciparum resistance to quinine monotherapy in two patients with uncomplicated malaria in French Guiana.

**CASE REPORTS**

**Case 1.** Patient 1, a 27-year-old white man, was admitted to a public referral hospital, the Hôpital André Rosemon, in Cayenne in December 1996 with a six-day history of fever, myalgia, nausea, and vomiting. He lived in a coastal area and had been to the Maroni River 10 days before the beginning of the symptoms. He had been taking preventive, self-administered medication (proguanil) for an undetermined period of time, and took curative self-administered medication for four days after the onset of the symptoms as recommended by regional guidelines (chloroquine, 600 mg per day). He had no underlying disorders, such as immunodeficiency, and had not undergone splenectomy. Upon admission, his core temperature was 39°C and 1% of his erythrocytes were infected with ring forms of P. falciparum. He presented no clinical or biologic signs of complicated malaria. He was given 580 mg (8 mg/kg) of intravenous quinine formiate three times per day according to the treatment recommendations that have been in place in French Guiana since 1990 (First Regional Consensus on Malaria in French Guiana, unpublished data). The intravenous route was chosen because of his vomiting. His level of parasitemia decreased to 0.3% after 24 hours (day 1), but then increased to 1.5% at 48 hours (day 2). Based on the plasma quinine concentration (16.5 mg/L) measured by high-performance liquid chromatography (HPLC) on day 3, the drug was absorbed normally. After three days of quinine therapy, the patient was still febrile and his parasitemia had not decreased, corresponding to 205% of the admission value on day 0. He was then given 200 mg of intravenous tetracycline per day for five days, which led to complete parasite clearance within 96 hours of starting therapy with both drugs. The patient developed lymphangitis caused by Staphylococcus aureus in his intravenous drip and his temperature remained elevated. He received oral antibiotics and became asymptomatic on day 10. He did not develop any malaria complications and was discharged on day 11. Blood smears remained negative for malaria parasites from day 7 to day 11. The in vitro antimalarial sensitivity assay (Pasteur Institute, Cayenne, French Guiana) was not successful.

**Case 2.** Patient 2, a 20-year-old Amerindian man was admitted to the Hôpital André Rosemon in Cayenne in November 1996 with a four-day history of fever, vomiting, and coughing. He lived in the Oyapock region, an area in which malaria is endemic. He had no major illnesses, such as immunodeficiency, and had not undergone a splenectomy. Upon admission, his core temperature was 40.7°C and 6.1% of his erythrocytes were infected with asexual forms of P. falci-
*P. falciparum*. Traces of chloroquine were found in his blood. The liver was palpable 2 cm below the right costal margin. He presented with hepatic disorders and high levels of serum aspartate aminotransferase (111 units/L) and total bilirubin (80.5 µmol/L). He received 500 mg (8 mg/kg) of intravenous quinine formiate three times per day according to malaria guidelines in use since 1990 (First Regional Consensus on Malaria in French Guiana, unpublished data). The intravenous route was chosen because of his vomiting. His level of parasitemia decreased to 4.1% after 48 hours (day 2). Analysis by HPLC showed that the drug had been absorbed normally (17.1 mg/L on day 3). After three days of quinine therapy, the patient was still febrile and his parasitemia had increased, corresponding to 92% of that on day zero. The patient was also given 200 mg per day of intravenous tetracycline between days 3 and 8. This led to the complete clearance of fever and parasites within 60 and 117 hours, respectively, of starting therapy with both drugs. He remained well and was discharged from the hospital 10 days after presentation. Blood smears were negative on day 8 and day 9. An *in vitro* antimalarial sensitivity assay (Pasteur Institute, Cayenne, French Guiana) showed that the strain isolated had increased an EC50 for quinine (520 nmol/L), chloroquine (397 nmol/L), and mefloquine (30 nmol/l). These values were above the thresholds at which strains are considered to be resistant (500 nmol/L, 100 nmol/L, and 30 nmol/L, respectively).

**DISCUSSION**

Chloroquine- and mefloquine-resistant strains of *P. falciparum* and strains displaying reduced susceptibility to quinine are found in some focal areas in the Amazon basin that are considered to be malaria-endemic areas. Although the first clinical evidence of quinine-resistant strains was obtained in Brazil in 1910, further evidence of *in vivo* resistance to this molecule was obtained since the mid 1960s, especially in areas of Southeast Asia. However, few recent data are available for countries in the Amazon region. Most reports concern the *in vitro* sensitivity of *P. falciparum* to antimalarial drugs and seem to suggest that resistance is less frequent in the Amazon area than in Asia.

In 1990, the First Regional Consensus on Malaria Control in French Guiana (unpublished data) recommended that the first-line treatment of uncomplicated malaria should be a four-day oral regimen of chloroquine and that quinine monotherapy should be reserved as a second-line treatment. The *in vivo* and *in vitro* assessments carried out in 1995 showed strong and progressive changes in the responses of strains to antimalarial drugs: 66% of isolates were sensitive to chloroquine in 1987 compared with 3% in 1995 and 96% of isolates were sensitive to quinine in 1987 compared with 74% in 1995 (Venturin C, unpublished data). This led to the proposal of three options for the first-line treatment of uncomplicated malaria since 1996: 1) mefloquine, 2) halofantrine, or 3) oral quinine plus tetracycline because of the emerging resistance to quinine (Second Regional Consensus on Malaria Control in French Guiana, 1995, unpublished data). However, the *in vivo* quinine resistance of strains has not yet been reported.

We report two cases of resistance to parenteral quinine monotherapy in patients with uncomplicated *P. falciparum* malaria in which tetracycline was necessary for efficient treatment. The clinicians followed the official recommendations of the First Regional Consensus on Malaria Control in French Guiana, which prescribed quinine monotherapy for uncomplicated malaria. Although the quinine was correctly administered (intravenous route because of vomiting in the patients) and absorbed, at 48 hours the parasite densities (150%...
and 67% of admission values for patients 1 and 2, respectively) fulfilled the World Health Organization criteria for RIII drug resistance (> 25% of the admission value). An alternative treatment was introduced on day 3 and proved to be successful.

The apparent efficacy of treatment can be assessed by comparing the *P. falciparum* clearance within 24 and 48 hours of treatment, before and after the addition of tetracycline (Figure 1). For patient 2, the parasite clearance rate was twice as high following therapy with quinine and tetracycline than following quinine monotherapy. Furthermore, we detected a clear correlation between the results of the *in vivo* and *in vitro* assessment for patient 2. The *P. falciparum* strain isolated from this patient had a multi-drug-resistant profile that corresponded to those reported in the literature and by the Pasteur Institute, the National Reference Center for drug-resistant malaria⁵ in French Guiana.

These observations, which were made at the time when the regional antimalarial treatment policy was being changed, suggest that quinine combined with tetracycline is the most appropriate replacement for quinine monotherapy in French Guiana. Despite the weak and slow antimalarial activity of tetracycline, the aim of the association is to exploit the synergistic and additive potential of the two individual drugs, thus improving efficacy. Moreover, it should reduce the selection pressure on quinine and prolong its useful therapeutic life. This pharmacologic approach is identical to those in the other countries in the Amazon region such as Brazil and Peru.⁶ However, since the seven-day regimen can lead to low compliance, thus increasing the risk of selection of resistance, alternative three-day combination therapies (e.g., artemether-lumefantrine or artesunate-mefloquine) should be also considered. The artesunate-mefloquine combination has been adopted as the first-line regimen on the border of Peru and Brazil.⁶ These associations may improve the efficacy of treatment by reducing the development of multi-drug-resistant isolates and delaying the spread of resistant populations of *P. falciparum*.

The Third Consensus Conference on Malaria Control was held in French Guiana in October 2002 (unpublished data). Artemether-lumefantrine was strongly recommended as the first-line treatment for uncomplicated malaria, whereas atovaquone plus proguanil was the recommended as the second-line treatment. The recommended third-line treatment was oral quinine plus doxycycline. These new changes resulted more from a cautious and predictive attitude based on scientific principles, rather than being the result of current treatment failures.

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REFERENCES