In vitro susceptibility of *Plasmodium falciparum* to monodesethylamodiaquine, quinine, mefloquine and halofantrine in Abidjan (Côte d'Ivoire)

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Abstract

Background: Malaria is the primary cause of hospitalization in Côte d'Ivoire. Early treatment is one of the strategies to control this illness. However, the spread of resistance of *Plasmodium falciparum* to antimalarial drugs can seriously compromise this strategy.

Objectives: The aim of this study was to assess the *in vitro* susceptibility of *P. falciparum* to monodesethylamodiaquine and aminoalcohols in Abidjan (Côte d'Ivoire).

Methods: We assessed the in vitro susceptibility of isolates collected from patients with uncomplicated malaria by using the WHO optical microtest technique.

Results: The proportions of resistance to monodesethylamodiaquine, méfloquine and halofantrine were 12.5%, 15.6% and 25.9%, respectively. For quinine, none of isolates showed evidence of *in vitro* resistance. However, two isolates (6.1%) had IC_{50} values above 300 nM. The IC_{50} of each drug was positively and significantly correlated to that of the other three drugs, and the correlation was higher between halofantrine and mefloquine.

Conclusions: Our results showed that the *in vitro* chloroquine resistance reported in previous studies has been extended to other antimalarial drugs investigated in this study except for quinine. Therefore, it is necessary to implement a long-term monitoring system of antimalarial drug resistance.

Key words: *in vitro* test, *Plasmodium falciparum*, monodesethylamodiaquine, quinine, mefloquine, halofantrine, Abidjan (Côte d'Ivoire).

African Health Sciences 2010; 10(2): 111 - 116

Introduction

Malaria still remains a major public health problem in sub-Saharan African countries[1]. Several strategies, such as rapid diagnosis and appropriate treatment, have been recommended to control malaria. However, faced with the growing inefficiency of monotherapies, as in most countries, the Ministry of Public Health in Côte d'Ivoire has adopted a novel strategy based on the use of drug combinations including artemisinin derivatives (artemisinin-based combination therapy, i.e ACT). Quinine is reserved for curative treatment in case of treatment failure of ACTs or severe and complicated malaria. Sulfadoxine-pyrimethanine (SP) is used to prevent malaria in pregnant women [2, 3]. Unifortunately, these recommendations are not always followed by drug prescriptors [4], thereby increasing the probability of selection and spread of drug-resistant *Plasmodium falciparum* strains.

Because of the presence of mefloquine and amodiaquine in some ACTs, it is necessary to assess the susceptibility of *P. falciparum* to these antimalarial drugs by *in vitro* and/or *in vivo* tests.

Moreover, a decrease in the sensitivity of *P. falciparum* to quinine has been reported in Southeast Asia, East Africa and South America [5, 6, 7]. As quinine has been used for decades to treat severe and complicated

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malaria until now, resistance to quinine could lead to a public health disaster. It is therefore necessary to implement an improved program for monitoring drug-resistant malaria in order to plan and adopt appropriate strategies to control this disease. Several methods can be used to evaluate the susceptibility of *P. falciparum* to antimalarial drugs. Laboratory tools, such as *in vitro* drug sensitivity assays, can provide an early warning to orient therapeutic efficacy studies and antimalarial treatment policy [8]. Furthermore, cultivation of clinical isolates and measurement of their susceptibility to antimalarial compounds *in vitro* remove host-related variables, such as patients' compliance, nutritional status, immune status, re-infection and pharmacokinetics, thereby providing a powerful technique for detecting the emergence of drug-resistant parasites [9]. The aims of the present study were: a) to assess the *in vitro* susceptibility of clinical isolates of *P. falciparum* to monodesethylamodiaquine, quinine, mefloquine, and halofantrine, and b) analyse the potential for cross-resistance between these drugs.

Methods

Study area

The study was carried out between February 2006 and February 2007 in the district of Abobo, situated in the north of Abidjan (the economic capital city). In this area, malaria is hyperendemic with seasonal transmission. The most common vectors are *Anopheles gambiae ss* and *A. funestus*.

Isolates of P. falciparum

Patients aged between 2 to 45 years presenting signs and symptoms of uncomplicated malaria were recruited at El Rapha and Anokoua Kouté, two health centers of Abobo area. Informed consent was obtained from the patients or guardian accompanying the sick children. The study was approved by the Ethics Committee of the Ivorian National Institute of Public Health (NIPH).

Venous blood samples were collected in EDTA-coated Vacutainer® tubes (Terumo Europe N.V., Leuven, Belgium) before treatment. They were transported at 4°C to NIPH within 6 h, if the parasitemia was at least 4,000 asexual parasites/µl of blood.

Parasitized erythrocytes were washed three times in RPMI 1640 medium (Invitrogen, UK), and Giemsa-stained thin blood smears were examined under the microscope to determine the parasite density and confirm the *Plasmodium* species (*P. falciparum* monoinfections).

Samples with parasitemia ranging from 0.1% to 0.25% were used directly to test drug susceptibility. If parasitemia exceeded 0.25%, infected erythrocytes were diluted to this parasitemia range with uninfected erythrocytes. Patients were treated with amodiaquine-artesunate or artemether-lumefantrine according to the recommended national therapeutic regimens.

Drugs

The test compounds were obtained from the following sources: monodesethylamodiaquine (TDR/World Health Organization [WHO] Drug Discovery Research), quinine chlorhydrate (Sanofi-Aventis, Antony, France), mefloquine hydrochloride (Roche, Mannheim, Germany), and halofantrine (Glaxo Smith Kline, Evreux, France). Stock solutions of each drug were prepared in 70% methanol. Twofold serial dilutions were prepared in RPMI 1640 medium and distributed in triplicate into 96-well culture plates.

In vitro assay

The WHO microtest technique was used to measure the inhibition of schizont maturation by microscopy [10]. Washed infected erythrocytes were suspended in RPMI 1640 with 10% human serum, 25 mM HEPES, and 25 mM $NaHCO_3$ at a hematocrit of 1.5%. Fifty microliters of the blood-medium mixture were distributed into each well of the predosed 96-well tissue culture plates and incubated at 37°C in candle jars for 42 h according to standard methodology. Final concentrations were ranged from 3.125 to 400 nM for monodesethylamodiaquine and mefloquine, from 12.5 to 1600 nM for quinine and from 0.25 to 32 nM for halofantrine. After incubation period, parasites were harvested and Giemsa stained thick blood films were prepared. The number of schizonts, defined as schizonts with more than 3 nuclei, was counted per 200 asexual parasites. Isolates with less than 20% of schizonts in drug-free control well were excluded. The results were expressed as 50% inhibitory concentration values (IC_{50}).

The cut-off values for in vitro resistance to monodesethylamodiaquine, quinine, mefloquine, halofantrine were fixed at 60 nM [11], 800 nM [12], 30 nM [13], 6 nM [14] respectively.

Statistical analysis

The IC_{50} values were determined by nonlinear regression analysis of the plot of logarithm of concentration against growth inhibition. Data were adapted to fit the logprobit model (Excel; Microsoft, Redmond, WA). The in vitro response was expressed as the geometric mean IC_{50} values with 95% confidence intervals.

The degree of correlation between different antimalarial drugs was estimated by the Spearman correlation coefficient (rho) and the coefficient of determination (r^2). The level of significance was set at 0.05.

Results

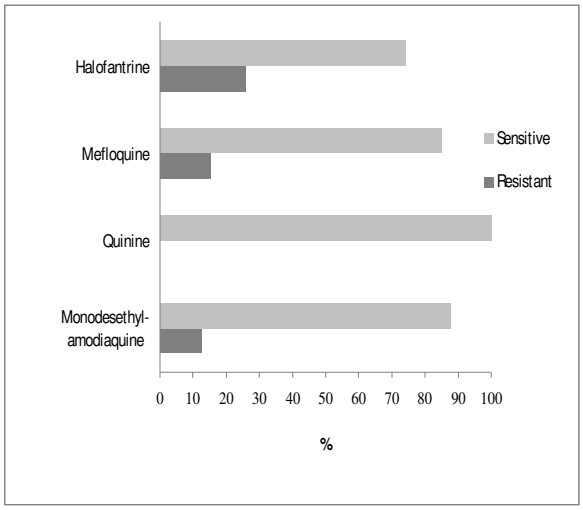
Forty three isolates of *P. falciparum* were collected. In this study, asexual parasite densities ranged from 0.1% to 13.5%. The following proportions of isolates were successfully cultured for each drug tested: 74.4% (32/43) for monodesethylamodiaquine, 76.7% (33/43) for quinine, 76.7% (33/43) for mefloquine and 62.8% (27/43) for halofantrine. The geometric mean IC_{50}s of four antimalarial drugs tested are summarized in Table 1.

Table 1: The geometric mean IC_{50s} of four antimalarial drugs tested against wild isolates of *P. falciparum* in Abidjan

Drugs	No. of isolates tested	Geometric Mean (nM) IC_{50}	95% Confidence interval (nM)	Range (nM)	
				Minimum	Maximum
Monodesethylamodiaquine	32	13.3	12 – 14.6	2.04	269
Quinine	33	60.6	59.6 – 61.7	4.31	486
Mefloquine	33	7.45	6.41 – 8.49	1.11	64.2
Halofantrine	27	1.64	0.93 – 2.35	0.17	20.4

Four (12.5%) isolates were resistant to monodesethylamodiaquine, and five (15.6%) monodesethylamodiaquine-sensitive isolates showed IC_{50} up to 25 nM. Five (15.2%) and seven (25.9%) isolates showed *in vitro* resistance to mefloquine and halofantrine, respectively. For each antimalarial drug, there were three sensitive isolates which showed borderline sensitivity (i.e IC_{50} > 20 nM but < 30 nM for mefloquine and > 4 nM but < 6 nM for halofantrine). There was no resistance to quinine (Figure 1). Two isolates (6.1%) presented quinine IC_{50} up to 300 nM.

Figure 1: Rate of in *vitro* resistance of *Plasmodium falciparum*



Concerning cross-resistance, one isolate was resistant *in vitro* to monodesethylamodiaquine, mefloquine and halofantrine, three isolates were resistant to monodesethylamodiaquine and halofantrine, and additional three isolates were resistant *in vitro* to halofantrine and mefloquine.

The IC_{50} of each drug was positively and significantly correlated to that of the other three drugs, and correlation was highest between halofantrine and mefloquine. Mefloquine and quinine IC_{50}s were weakly correlated (Table 2).

Table 2: Correlation between the IC_{50s} values of the four antimalarial drugs tested

Drug pair	No. of isolates tested	*r*	r^2	*p**
Monodesethylamodiaquine - Halofantrine	27	0.52	0.27	0.0003
Monodesethylamodiaquine - Quinine	32	0.56	0.32	<0.0001
Monodesethylamodiaquine - Mefloquine	32	0.24	0.06	<0.0001
Halofantrine - Quinine	27	0.32	0.10	< 0.0001
Halofantrine -Mefloquine	27	0.67	0.46	0.0039
Mefloquine - Quinine	33	0.16	0.03	< 0.0001

*Student Fisher test

Discussion

In our study, the proportion of monodesethylamodiaquine-resistant isolates was higher than that described in previous studies in Africa, which was between 2 and 7% [15,16,17]. Our result can be explained by the high rate of chloroquine resistance in Abidjan area[18,19] and the similar chemical structure between amodiaquine and chloroquine. In Cameroon, IC_{50} values ranging from 25.6 to 115 nM were reported for most of the isolates collected at the time of treatment failure with amodiaquine, indicating that the threshold for monodesethylamodiaquine resistance *in vitro* might be lower than the usual value of more or equal to 60 nM [20]. On this basis, we can say that the rate of decreased sensitivity to monodesethylamodiaquine (i.e IC_{50}s > 25 nM) was 28.1% in our study. If the increase in clinical resistance to amodiaquine is confirmed, this situation could compromise the current efficacy of ACT which contains amodiaquine. Indeed, while currently employed ACTs demonstrate excellent clinical efficacy, the history of antimalarial chemotherapy predicts that it is only a matter of time before parasite resistance emerges [21].

All isolates tested in our study were sensitive to quinine, as in the previous in vitro susceptibility tests carried out in Côte d'Ivoire [18,22]. These data suggest that quinine still highly effective and confirm the choice to treat severe malaria or treatment failures with this drug. However, we must monitor quinine susceptibility of P. falciparum isolates because of the increasing use of quinine as presumptive treatment for uncomplicated malaria, often without respecting the recommended therapeutic protocol and dosage [23].

This raises the question as to whether drug pressure due to quinine use in urban areas selects parasites with decreased sensitivity to quinine [24]. In Senegal, the prevalence of *in vitro* resistance to quinine was 5% [25], while it was 3% in Comoros [17], 6% in Congo [15] and 8% in Rwanda [16] with intermediate susceptibility to quinine. In Guyana, a reduced *P. falciparum* sensitivity to quinine was observed in 6/14 isolates tested [26]. In Asia, where decreased *in vitro* susceptibility to quinine was first reported at the beginning of the 1980s in patients living near the Thai-Cambodia border [27] treatment failures with this drug occurred subsequently [5]. Thus, it is necessary to evaluate the therapeutic efficacy of quinine in patients.

Despite the uncommon use of mefloquine compared to other antimalarial drugs in Côte d'Ivoire, 15.2% of mefloquine-resistant isolates were observed in our study. The presence of isolates that are naturally less sensitive to mefloquine could partially explain this proportion of resistant isolates. In Senegal, where there was 13% of isolates with reduced susceptibility to mefloquine, prophylactic failures with this drug were previously described [25,28]. The same situation could exist in Côte d'Ivoire. Indeed, in this country, mefloquine is one of the drugs recommended to prevent malaria in non-immune populations such as tourists [3]. Elsewhere in Africa, in particular in Madagascar and Central African Republic, there were only 2% of *in vitro* resistance to mefloquine [29,30].

The prevalence of *in vitro* halofantrine resistance was the highest in our study. In 2002-2003, we found 3 / 11 (27.3%) isolates tested resistant in vitro to halofantrine [31]. The data reported in this current study indicate that *P. falciparum* susceptibility to halofantrine has been stable. From 1994 to 2005, there was an alert issued on halofantrine resistance in French Guiana with a peak of 66% of prevalence of resistance in isolates from 2000 [32]. In Burkina Faso, where the rate of *in vitro* resistance to halofantrine was 11.2%, the authors attributed this rate to the presence of isolates naturally resistant, as with mefloquine [23]. Indeed, we observed a strongly positive correlation between halofantine and mefloquine, more than with the other drugs. This positive correlation between two aminoalcohols may be partly explained by their similar chemical structure [15, 33, 34]. The correlation between monodesethylamodiaquine and aminoalcohols has been previously described[16,34].

A positive correlation between the IC_{50s} of two antimalarial drugs may suggest in vitro cross-resistance [35] although we did not observe cross-resistance with quinine in our study.

Conclusion

In conclusion, our results showed that the in vitro P. falciparum resistance already observed with chloroquine has extended to other antimalarial drugs investigated in this study except for quinine. For quinine, the presence of isolates with reduced susceptibility and correlation with other antimalarial drugs need further investigations.

Acknowledgments

We are grateful for the hospitality and generous collaboration of El Rapha and Anokoua Kouté Health centers. We also thank the patients, parents of the patients included in this study and Mr. Issiaka Bassinka for his technical help.

References

1- World Health Organization. World malaria report 2008. Geneva, WHO. 2008; 215 p.
2- World Health Organization. Guidelines for the treatment of malaria. WHO, Geneva. 2006; 266 p.
3- Ministry of health of Côte d'Ivoire. National therapeutic plan for the treatment of malaria in Côte d'Ivoire. MH, Abidjan. 2005; 16 p.
4- Kiki-Barro CP, Konan FN, Yavo W et al. Antimalarial drug delivery in pharmacies in non-severe malaria treatment. A survey on the quality of the treatment: the case of Bouake (Côte d'Ivoire). *Sante*. 2004; 14(2): 75-79.
5- Pukrittayakamee S, Supanaranond W, Looareesuwan S, Vanijanonta S, White NJ. Quinine in severe falciparum malaria: evidence of declining efficacy in Thailand. *Trans R Soc Trop Med Hyg*. 1994; 88(3): 324-327.
6- Jelinek T, Schelbert P, Loscher T, Eichenlaub D. Quinine resistant falciparum malaria acquired in east Africa. *Trop Med Parasitol*. 1995; 46(1): 38-40.
7- Segurado AA, di Santi SM, Shiroma M. In vivo and in vitro Plasmodium falciparum resistance to chloroquine, amodiaquine and quinine in the Brazilian Amazon. *Rev Inst Med Trop Sao Paulo*. 1997; 39(2): 85-90.
8- Ringwald P, Sukwa T, Basco L K, Bloland P, Mendis K. Monitoring of drug-resistant malaria in Africa. *Lancet*. 2002; 360(9336): 875-876.
9- Ekland EH, Fidock DA. In vitro evaluations of antimalarial drugs and their relevance to clinical outcomes. *Int J Parasitol*. 2008; 38(7): 743-747.
10- Rieckmann KH. Visual in-vitro test for determining the drug sensitivity of Plasmodium falciparum. *Lancet*. 1982; 1(8285): 1333-1335.
11- Basco LK, Le Bras J. In vitro activity of monodesethylamodiaquine and amopyroquine against African isolates and clones of Plasmodium falciparum. *Am J Trop Med Hyg*. 1993; 48 (1): 120-125.
12- Basco LK, Le Bras J. In vitro susceptibility of Cambodian isolates of Plasmodium falciparum to halofantrine, pyronaridine and artemisinin derivatives. *Ann Trop Med*. 1994; 88(2):137-144.
13- Hatin I, Trape JF, Legros F, Bauchet J, Le Bras J. Susceptibility of Plasmodium falciparum strains to mefloquine in an urban area in Senegal. Bull WHO. 1992; 70(3): 363-367.
14- Basco, L. K. and Le Bras, J. In vitro activity of halofantrine and its relationship to other standard antimalarial drugs against African isolates and clones of Plasmodium falciparum. *Am J Trop Med. Hyg* 1992. 47(4):521-7.
15- Pradines B, Hovette P, Fusai T et al. Prevalence of in vitro resistance to eleven standard or new antimalarial drugs among Plasmodium falciparum isolates from Pointe-Noire, Republic of the Congo. J *Clin Microbiol*. 2006; 44(7): 2404-2408.
16- Tinto H, Rwagacondo C, Karema C et al., In-vitro susceptibility of Plasmodium falciparum tomonodesethylamodiaquine, dihydroartemisinin and quinine in an area of high chloroquine resistance in Rwanda. *Trans R Soc Trop Med Hyg*. 2006; 100(6): 509-514.
17- Parola P, Pradines B, Simon F et al. Antimalarial drug susceptibility and point mutations associated with drug resistance in 248 Plasmodium falciparum isolates imported from Comoros to Marseille, France in 2004 2006. *Am J Trop Med Hyg*. 2007; 77(3): 431-437.
18- Djaman J, Abouanou S, Basco L, Kone M. Limits of the efficacy of chloroquine and sulfadoxine-pyrimethamine in Northern Abidjan (Cote d'Ivoire): Combined in vivo and in vitro studies. *Sante*. 2004; 14(4): 205-209.
19- Bla KB, Yavo W, Ouattara L, Basco L, Djaman AJ. Influence of asexual parasite biomass on in vitro susceptibility of Plasmodiun falciparum to antimalarial drugs in Abidjan. *Afr J Biotechnol*. 2008; 7 (8): 936-940.
20- Basco LK, Ndounga M, Keundjian A, Ringwald P. Molecular epidemiology of malaria in Cameroon. IX. Characteristics of recrudescent and persistent Plasmodium falciparum infections after chloroquine or amodiaquine treatment in children. *Am J Trop Med Hyg*. 2002; 66(2): 117-123.
21- Chretien JP, Fukuda M, Noedl H. Improving surveillance for antimalarial drug resistance. *JAMA*. 2007; 297(20): 2278-2281.
22- Toure, AO, Kone LP, Jambou R et al. In vitro susceptibility of P. falciparum isolates from

Abidjan (Cote d'Ivoire) to quinine, artesunate and chloroquine. Sante 2008; 18(1): 43-47.

23- Tinto H, Ouedraogo JB, Traore B, Coulibaly SO, Guiguemde TR. In vitro susceptibility of 232 isolates of Plasmodium falciparum to antimalarials in Burkina Faso (West Africa). *Bull Soc Pathol Exot*. 2001; 94: 188-191.

24- Randrianarivelojosia M, Randrianasolo L, Randriamanantena A, Ratsimbasoa A, Rakotoson JD. Susceptibility of Plasmodium falciparum to the drugs used to treat severe malaria (quinine) and to prevent malaria (mefloquine, cycloguanil) in Comoros Union and Madagascar. *S Afr Med J*. 2004; 94 (1): 47-51.

25- Henry M, Diallo I, Bordes J et al. Urban malaria in Dakar, Senegal: chemosusceptibility and genetic diversity of Plasmodium falciparum isolates. *Am J Trop Med Hyg*. 2006; 75(1): 146-151.

26- Best Plummer W, Pinto Pereira L. Diminished Plasmodium falciparum sensitivity to quinine exposure in vitro and in a sequential multi-drug regimen: A preliminary investigation in Guyana, South America. *Int J Infect Dis* 2008. 12(6): 27-31.

27-Giboda M, Denis MB. Response of Kampuchean strains of Plasmodium falciparum to antimalarials: in-vivo assessment of quinine and quinine plus tetracycline; multiple drug resistance in vitro. *J Trop Med Hyg*. 1988; 91(4): 205-211.

28- Gari-Toussaint M, Pradines B, Mondain V, Keundjian A, Dellamonica P, Le Fichoux Y . Senegal and malaria. True prophylactic failure of mefloquine. *Presse Med*. 2002; 31(24): 1136.

29- Randrianarivelojosia M, Ratsimbasoa A, Randrianasolo L, Randrianarijaona A, Jambou R. In-vitro sensitivity of Plasmodium falciparum to chloroquine, halofantrine, mefloquine and quinine in Madagascar. *East Afr Med J*. 2002; 79(5): 237-241.

30- Menard D, Djalle D, Manirakiza A et al. Drug-resistant malaria in Bangui, Central African Republic: an in vitro assessment. *Am J Trop Med Hyg*. 2005; 73(2): 239-243.

31- Yavo W. Sensibilité in vitro des isolats de Plasmodium falciparum à la chloroquine et à l'halofantrine à Abidjan. DEA biologie humaine tropicale. UFR Sciences Médicales, Université de Cocody-Abidjan. 2004; 36 p.

32- Legrand E, Volney B, Meynard JB, Mercereau-Puijalon O, Esterre P. In vitro monitoring of Plasmodium falciparum drug resistance in French Guiana: a synopsis of continuous assessment from 1994 to 2005. *Antimicrob Agents Chemother*. 2008; 52(1): 288-298.

33- Ouedraogo JB, Dutheil Y, Tinto H et al. In vitro sensitivity of Plasmodium falciparum to halofantrine compared with chloroquine, quinine and mefloquine in the region of Bobo-Dioulasso, Burkina Faso (West Africa). *Trop Med Int Health*. 1998; 3(5): 381-384.

34- Randrianarivelojosia M, Harisoa JL, Rabarijaona LP et al. In vitro sensitivity of Plasmodium falciparum to amodiaquine compared with other major antimalarials in Madagascar. *Parassitologia*. 2002; 44(3-4): 141-147.

35- Pradines B, Tall A, Ramiandrasoa F et al. In vitro activity of iron-binding compounds against Senegalese isolates of Plasmodium falciparum. *J Antimicrob Chemother*. 2006; 57(6): 1093-1099.