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In vitro EVALUATION OF QUINIDINE SENSITIVITY IN BRAZILIAN *Plasmodium falciparum* ISOLATES: COMPARATIVE ANALYSIS TO QUININE AND CHLOROQUINE

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SUMMARY

Falciparum malaria represents a serious and an increasing world public health problem due to the acquired parasite's resistance to the most available drugs. In some endemic areas, quinidine, a diastereoisomer of the antimalarial quinine, has been employed for replacing the latter. In order to evaluate the use of quinidine as an alternative to the increasing loss of quinine effectiveness in Brazilian *P. falciparum* strains, as has been observed in the Amazon area, we have assayed quinidine, quinine and chloroquine. The *in vitro* microtechnique was employed. All isolates showed to be highly resistant to chloroquine. Resistance to quinine was not noted although high MIC (minimal inhibitory concentration) values have been observed. These data corroborate the decreasing sensitivity to quinine in strains from Brazil. Quinidine showed IC_{50} from 0.053 to 4.577 µmol/L of blood while IC_{50} from 0.053 to 8.132 µmol/L of blood was estimated for quinine. Moreover, clearance of the parasitemia was observed in concentrations lower than that used for quinidine in antiarrhythmic therapy, confirming our previous data. The results were similar to African isolate.

KEYWORDS: Quinidine; Malaria; Quinine; Chloroquine; *P. falciparum;* Antimalarial resistance

INTRODUCTION

Current malaria chemotherapy was considered to start in the middle of the XVII century with the jesuit's discovery of the folkloric use of *Cinchona* bark as a febrifuge by South American indians. However, *Artemisia annua* was already known by Chinese population since the beginning of the Christian era[13], although the pharmacological studies have started in the 70's .

After its introduction in Europe, *Cinchona* tree bark powder begun to be extensively employed in the treatment of fevers. In 1820, two alkaloids, quinine (Fig. 1) and cinchonine, were isolated but the antimalarial effect was attributed only to quinine. The pure alkaloid replaced the *Cinchona* bark and quinine became the only drug in the therapy and prophylaxis of malaria[38].

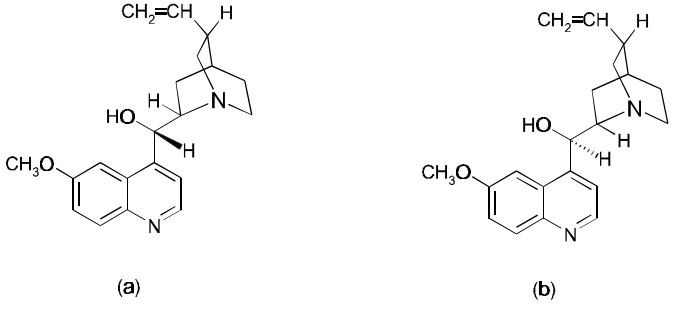

Fig. 1 - Quinine (a) and quinidine (b).

The unavailability of quinine during World Wars I and II led to the advent of the synthetic drugs, from which chloroquine stood out. Considered to be the ideal antimalarial, chloroquine showed to be less toxic, more effective and better to be administered than quinine, therefore, substituting it[36].

Plasmodium falciparum strains resistance to chloroquine and to the second-line therapy sulfadoxine-pyrimethamine since the 60's led to the re-introduction of quinine. This alkaloid has been specially used to treat severe or complicated infections and chloroquine or mefloquine resistant malaria. In the later ones, the combination therapy has been recommended for maintaining the antimalarial effectiveness. For instance, tetracycline has been used in combinations with quinine[40]. Quinine resistant strains were identified in the early stages of the drug usage. Lately, a continuous decreasing in parasite sensitivity has been noted along different endemic areas[8,24,27]. This has increased the interest in using quinidine (Fig. 1), the dextro-rotatory diastereoisomer of quinine, as an alternative therapy.

Quinidine had its antimalarial activity despised in function of quinine. Recognized as a potent antiarrhythmic agent, quinidine has been mostly employed in the cardiovascular therapy since the 20's[29]. Nevertheless, *in vitro*, *in vivo* and clinical studies have reported quinidine as an effective

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or even superior to quinine as an antimalarial agent[5,7,11,21,22,24,25,32,33,39]. For a long time, quinidine has been considered by the Centers for Disease Control (CDC/Drug Service) in USA[14,15] to be the drug of choice in the treatment of complicated *P. falciparum* infection. Since the 80's it has shown to be useful for treating pregnant women with severe *falciparum* malaria[25], since abortifacient properties are alleged to quinine[40]. Moreover, quinidine, available worldwide, has increasingly been the only useful therapy for severe and complicated malaria since USA stopped the manufacture of quinine[25].

With the purpose of facing the challenge of quinine effectiveness decrease in Brazilian *P. falciparum* strains[1,2,8,9,16,26,34,41], we evaluated the *in vitro* sensitivity of isolates from North Brazilian States to quinidine in comparison to quinine and chloroquine. We were based on previous studies when quinidine showed to exert antimalarial effect in concentrations many times inferior than those used in the cardiovascular therapy[23].

MATERIAL AND METHODS

Drugs - The drugs used were quinidine bisulphate dihydrate (Asta Médica Ltda), quinine sulphate (Central de Medicamentos, CEME) and chloroquine diphosphate (Fundação para o Remédio Popular, Furp).

Isolates - Six isolates of *P. falciparum* were employed: Isolate 1 – 5,760 parasites/mm^3; Isolate 2 – 65,280 parasites/mm^3; Isolate 3 – 3,240 parasites/mm^3 and Isolate 4 – 65,000 parasites/mm^3. All these isolates were assayed immediately after blood venum puncture. Isolates 1-3 were obtained from patients infected in Brazilian Northern region and Isolate 4 from a patient infected in Angola. These people had not been submitted to any antimalarial treatment for the previous 28 days[10]. Blood samples were collected after formal consent from patients. Isolate 5 (SUCEN S20-87) was obtained from a patient infected in Rondonia, Brazil, in 1987. Isolate 6 (Uganda Palo Alto) was used as a reference for chloroquine sensitivity. Isolates 5 and 6 were assayed after synchronization of 10% ring forms parasitemia.

Drug sensitivity assay - The biological assay was the *in vitro* microtechnique[28]. 96 flat-bottom wells microplates contained quinidine (at the same concentrations range than quinine), quinine and chloroquine were titrated (Table 1). Each series included the usual antimalarial therapeutic concentrations of quinine (1.14 µmol/L of blood) and chloroquine (6.4 µmol/L of blood) as the medium of the twofold serial dilutions. This procedure was adopted in analogy to the test for the assessment of *P. falciparum* response to antimalarial drugs as stated by WHO[10].

The infected blood was diluted in culture medium to a 10% haematocrit. The plates were incubated at 37 °C for 24-48 hours (depending on schizont maturation) using the candle jar method[37]. The number of schizonts with three or more nuclei was determined in 200 parasites in thick blood smear stained with Giemsa.

Schizonts growth at 1.6 µmol/L of blood for chloroquine and 51.2 µmol/L of blood for quinine were considered as threshold for *in vitro P. falciparum* resistance[24]. The minimal inhibitory concentration (MIC), defined as the lowest concentration that completely inhibited schizont formation, was determined.

Statistical analysis – The parasitemia rate (number of parasites in the different wells in relation to the control) in function of the concentration was analyzed throughout the linear logistic model[17] for quinine and quinidine. According to this, coincident, parallel, concurrent (1 intercept) and concurrent (2 intercepts) lines were fitted. The comparisons were made with regard to the simplest model, coincident lines. The goodness-of-fit statistic and the corresponding p-value were computed to assess the adequacy of the model. A significance level of 10% was adopted. Then, the medium inhibitory concentration (IC_{50}) and the correspondent confidence interval (95%) were estimated for each drug.

RESULTS

The *in vitro* sensitivity of *P. falciparum* isolates to quinidine and the classical antimalarials quinine and chloroquine is shown in Table 1. Based on MIC, all parasites showed to be resistant to chloroquine, except the Isolate 6. Different susceptibility levels were observed to quinidine and quinine. No resistance to quinine was detected.

Table 1
In vitro sensitivity of *P. falciparum* isolates to chloroquine, quinine and quinidine

Isolate	Geographical Origin	MIC (µmol/L of blood)		
		Chloroquine	Quinine	Quinidine
1	Para/Brazil	ID	25.6	12.8
2	Para/Brazil	ID	6.4	6.4
3	Amazonas/ Brazil	6.4	12.8	6.4
4	Angola	6.4	6.4	25.6
5(SUCEN S 20-87)	Rondonia/ Brazil	6.4	6.4	6.4
6 (Palo Alto)	Uganda	1.14	6.4	12.8

ID = impossible to determine (growth in the higher concentration of drug, 6.4 µmol/L of blood); MIC = minimal inhibitory concentration

The results of the statistical analysis are depicted in Tables 2 and 3 and in Figure 2. For Isolate 1 the best-selected model was the concurrent lines (1 intercept). As the concentration increases, the parasitemia rate decrease is higher for quinidine than for quinine, leading to a lower quinidine IC_{50} (Table 3). For Isolate 2 parallel lines was the best-fitted model, with the quinine parasitemia rate and consequent IC_{50} smaller than those of quinidine. Even though none of the proposed models was well fitted for Isolate 3, the concurrent lines model (2 intercepts) indicates that for small and moderate concentrations quinine has a smaller parasitemia rate while an opposite tendency appeares in larger concentrations. Equal IC_{50} values were estimated. For Isolate 4, the concurrent lines model (2 intercepts) was the selected model. From the fitted lines, small concentrations of quinidine cause lower parasitemia rates while an opposite behavior is observed in high concentrations. Quinidine at 50% inhibition showed to be a worse antiplasmodial agent than quinine. For Isolate 5, coincident lines was the selected model and a common fitted line as well as estimated IC_{50} values were obtained. For Isolate 6, concurrent lines (1 intercept) was the best-fitted model. In this

Table 2
Results of the fitted linear logistic models for quinine and quinidine

Isolate	Model	Goodness-of-fit statistic	Degrees of freedom	p-value
1	Coincident lines	40.669	8	0.000
	Parallel lines	9.537	7	0.216
	Concurrent lines (1 intercept)	7.721	7	0.358
	Concurrent lines (2 intercepts)	6.352	6	0.385
2	Coincident lines	40.673	8	0.000
	Parallel lines	6.467	7	0.486
	Concurrent lines (1 intercept)	21.963	7	0.002
	Concurrent lines (2 intercepts)	4.053	6	0.669
3	Coincident lines	99.730	8	0.000
	Parallel lines	32.179	7	0.000
	Concurrent lines (1 intercept)	77.032	7	0.000
	Concurrent lines (2 intercepts)	18.507	6	0.005
4	Coincident lines	42.640	8	0.000
	Parallel lines	36.756	7	0.000
	Concurrent lines (1 intercept)	17.098	7	0.017
	Concurrent lines (2 intercepts)	9.588	6	0.143
5	Coincident lines	9.750	8	0.283
	Parallel lines	9.741	7	0.203
	Concurrent lines (1 intercept)	9.707	7	0.206
	Concurrent lines (2 intercepts)	9.388	6	0.153
6	Coincident lines	32.364	8	0.000
	Parallel lines	9.368	7	0.227
	Concurrent lines (1 intercept)	7.124	7	0.416
	Concurrent lines (2 intercepts)	5.734	6	0.454

Table 3
Estimated median inhibitory concentration (IC_{50}) and the confidence interval concentration of quinine and quinidine

Drug	Isolate	Estimated IC_{50} (μmol/L of blood)	95% Confidence Interval
Quinine	1	8.132	5.330 ; 10.934
	2	1.522	0.207 ; 2.836
	3	0.053	0.000 ; 0.145
	4	2.078	0.596 ; 3.560
	5	2.203	1.002 ; 3.404
	6	0.585	0.025 ; 1.145
Quinidine	1	4.577	1.690 ; 7.463
	2	3.569	0.741 ; 6.396
	3	0.053	0.000 ; 8.460
	4	3.132	2.799 ; 3.466
	5	2.203	1.002 ; 3.404
	6	1.674	1.374 ; 1.974

case, the parasitemia rate of quinine was smaller than the quinidine and the difference increases with the concentration. These results corroborate the estimated IC_{50} values (Table 3).

DISCUSSION

The loss in *P. falciparum* sensitivity to quinine in Brazil dated from the beginning of the XX century. A continuous decrease has been reported in special since the 80's when quinine replaced chloroquine and sulfadoxine-pyrimethamine in malaria resistant therapy. Then, tetracycline began to be co-administered with quinine in order to overcome the resistance problem and to reduce its inherent toxicity[2,8,9]. Quinine is still considered a useful drug for treating non-complicated *falciparum* malaria[8]. Nevertheless, a gradual increase in the time to clear the parasitemia followed by an elevation in recurrence frequency were observed in patients from the Eastern Amazon, where quinine was employed during the quadriennial 1983-1994[26]. Moreover, decrease in quinine sensitivity was also evidenced in two recent reports performed with Brazilian samples from Mato Grosso State, Amazon area[16,41].

In our study, different susceptibility levels were observed among the isolates in relation to quinine and quinidine. Resistance to quinine was not detected since no schizont maturation was observed at 51.2 μmol/L of blood[24]. Nevertheless, decreasing in *P. falciparum* susceptibility to quinine was confirmed, in particular, with Isolate 1 (the highest IC_{50}) and with Isolates 5 and 6, collected for a long time and much more sensitive to quinine. These data corroborated recent evaluations[16,26,41], showing a unquestionable and worryingly loss of quinine effectiveness in Brazilian strains. Interestingly, quinine was more

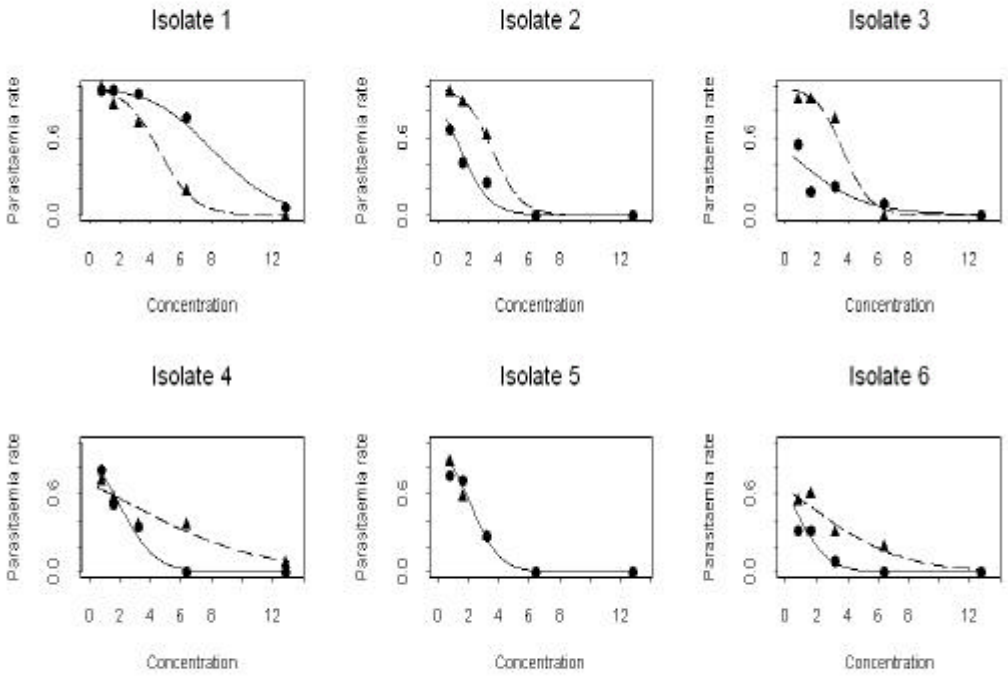

Fig. 2 - Observed parasitaemia rates for quinine (•) and quinidine (▲) and fitted linear logistic models for quinine (—) and quinidine (– –).

effective against the African isolate, Isolate 4. However, opposite data have been obtained with isolates from Gabon[24] and Niger[35] and, also, out of the African continent, in Thailand[32]. In addition, all isolates showed to be highly resistant to chloroquine. MIC values were much higher than the considered threshold concentration, 1.6 µmol/L of blood[24].

We consider that as observed in former studies [5,7,11,21,22,24,25,32,33,39], quinidine shows a promising activity against Brazilian *P. falciparum* strains. Quinidine inhibitory effect was observed in concentrations lower (estimated IC_{50} ranged from 0.053 to 4.577 µmol/L of blood that corresponds to 0.0172 to 1.485 µg/mL) than those employed in antiarrhythmic therapy, 2-6 µg/mL[4]. These results are in accordance to our previous assay carried out with two North Brazilian *P. falciparum* isolates[23]. Although further *in vitro*, *in vivo*, and clinical studies should be conducted, the preliminary results here reported are indicative of the possibility of using quinidine in uncomplicated but, especially, in complicated and severe forms of *falciparum* malaria. This finding assumes greater importance with the increasing loss of quinine efficacy against Brazilian strains. Nevertheless, precautions have been recommended concerning to the intravenous dispensing of quinidine due to its inherent cardiotoxicity[6,25,40]. In addition, the exchange transfusion after quinidine administration has been recommended for rapidly reducing the parasitemia, lowering the mortality by severe *falciparum* malaria[20].

Notwithstanding, quinidine may also be used in combination with other antimalarial agents as well as with its stereoisomers. *Cinchona* alkaloid combinations have been successfully used for treating *falciparum* malaria in endemic areas[3,12,18,19,30,31]. Quinidine, quinine and cinchonine (Quinimax®) or even quinidine, quinine, cinchonine and chinchonidine have been employed by different administration routes for treating uncomplicated as well as complicated *falciparum* malaria. These combination regimens allow to reduce the individual doses of the alkaloids, decreasing their inherent toxicities.

RESUMO

Avaliação da sensibilidade *in vitro* à quinidina em isolados brasileiros de *Plasmodium falciparum*: análise comparativa à quinina e à cloroquina

Malária *falciparum* representa grave e crescente problema de saúde pública mundial, dada a resistência do parasito à maioria dos fármacos disponíveis. Em algumas áreas endêmicas, a quinidina, diastereoisômero do antimalárico quinina, vem sendo empregada em substituição a este último. Com o objetivo de avaliar o emprego da quinidina como alternativa à perda crescente de sensibilidade de cepas brasileiras de *P. falciparum* à quinina, como o observado na região Amazônica, realizamos ensaio comparativo entre quinidina, quinina e cloroquina. A técnica *in vitro* do microteste de sensibilidade foi utilizada. Todos os isolados mostraram-se altamente resistentes à cloroquina. Resistência à quinina não foi observada, embora altos valores de CMI (concentração mínima inibitória) tenham sido encontrados. Estes resultados corroboram o decréscimo de suscetibilidade de cepas brasileiras à quinina. Observou-se variação de IC_{50} de 0,053 a 4,577 µmol/L de sangue para a quinidina, enquanto para a quinina estimou-se IC_{50} de 0,053 a 8,132 µmol/L de sangue. Ademais, observou-se clareamento da parasitemia em concentrações inferiores à da quinidina quando empregada como fármaco antiarrítmico, confirmando estudo anterior por nós realizado. Resultados semelhantes foram encontrados em isolado oriundo da África.

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