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DRUG SUSCEPTIBILITY OF Plasmodium Falciparum IN THE WESTERN AMAZON REGION, STATE OF ACRE, BRAZIL

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SUMMARY

Field studies in the western Amazon region (state of Acre, Brazil) indicate that the 4-aminoquinolines, as well as the combined regimen with sulfadoxine-pyrimethamine, can no longer be recomended for the treatment and prophylaxis of **P. falciparum** infections in this region. Quinine remains an effective drug when used correctly. However, compliance problems arise due to the often occurring side-effects during a ten day regimen. Prospects of overcoming these constraints by combining a short course of quinine with other drugs are limited, because of the lack of suitable partner compounds. For this reason quinine/ clindamycin appears to be a more practical therapy of **P. falciparum** malaria. In vitro data from this study suggest that mefloquine is another effective alternative for the treatment of falciparum malaria in this Amazon region.

KEY WORDS: Plasmodium falciparum; Drug resistance; Acre

INTRODUCTION

The great increase in migration towards the Amazon region (states and territories of Para, Rondonia, Amazonas, Mato Grosso, Acre, Amapa and Roraima) and the shifts of populations within these states have led to a considerable rise in the transmission of malaria. For example, during the 1980's, the population of Rondonia state where the majority of immigrants settled, doubled from 570,000 to 1040,000 inhabitants [7]. In the state of Acre a similar strong population increase could be observed. The population increase in the states of Rondonia and Acre was largely in response to the agricultural settlement project of the *National Institut for Land Reform,* which supported the start of more than 30.000 new medium size farms in less than 10 years. On the other hand the migration to the state Para was mainly attracted by the gold prospect and mining operations[7].

As a consequence, the cases of malaria, covering the period from 1981-1985, were paralleled by the migration towards the Amazon region and doubled during that period[7].

Resistance of **Plasmodium falciparum** to antimalarial drugs has been reported in South America since 1961[21,27]. Resistance to chloroquine was first observed in Colombia and Brazil [21,27], and is now common in many endemic areas of the Americas[3, 6, 10, 17, 22, 29].

In 1972, resistance to sulfadoxine-pyrimethamine was first reported in Brazil and

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later on in Colombia[2, 11]. Until the late 1980's there was a lack of sufficient information concerning the resistance of **P. falciparum** to amodiaquine, quinine and mefloquine[10, 17, 29] on the South American continent. Meanwhile multidrug resistance of **P. falciparum** has become a major public health concern, especially in the Amazon region of Brazil where a high incidence of malaria disease (i.e. 10.120 cases in 1985 in the state of Acre) and a high prevalence of up to 15% (1985) is common[7]. Until 1987 in Acre, the north westernmost state of Brazil, only one report on the in vitro drug susceptibility of **P. falciparum** existed.

The present study is concerned with in vivo and in vitro drug resistance of **P. facilparum** strains to common antimalarial chemotherapeutics in the state of Acre, Brazil. Furthermore, combination therapies were evaluated for their value as antimalarial regimens in this Brazilian Amazon region.

MATERIAL AND METHODS

Location of the study

The study took place in Rio Branco, Acre, Brazil. Rio Branco is situated at longitude 68° west and latitude 10° south in the Amazonas rain forest near the Bolivian and Peruvian border.

Patients

All patients attending the Superintendencia de Campanhas de Saúde Pública (SUCAM) outpatient clinic who fulfilled the following criteria were admitted to the in vivo studies: a) they had a parasitologically proven monoinfection with **P. falciparum**; b) they had not received antimalarial therapy within the preceding seven days; c) they could remain under complete observation for 48 hours after clearance of the parasites from peripheral blood; d) they could remain in the Rio Branco municipality, an area considered to be without malaria transmission and e) they were older than 14 years. Patients were excluded if they had a severe clinical illness requiring hospitalization, if they had chronic diarrhoea, or if they were pregnant.

A total of 159 patients were admitted to the studies. Of these 44 were further subdivided into the non-immune and the semi-immune. They were classified as non-immune if they had had two or less previous attacks of malaria. Those who had had malaria more than three times in the past two years were classified as semi-immune.

Drug regimens

In a first trial of in vivo drug testing, patients were randomized into three treatment groups according to an age-dependent randomization schedule. Group A (29 patients) received 25 mg of amodiaquine base-kg given in three doses: 10 mg/kg at admission and 7.5 mg/kg 24 and 48 hours later. Group QSP (40 patients) received 15 mg of quinine sulfate/kg every 12 hours, for a total of six doses, plus sulfadoxine (500 mg)-pyrimethamine (25 mg) tablets, two tablets initially and one tablet 24 hours later. Group QC (46 patients) received 15 mg of quinine sulfate/kg every 12 hours, for a total of six doses, plus 10 mg of clindamycin/kg every 12 hours, for a total of six doses.

In a second in vivo drug testing trial we decided to investigate the efficacy and tolerability of a malaria treatment consisting out of 5 mg clindamycin/kg twice a day for five days.

Clinical and parasitological examination

The Clinical-follow up included a daily assessment of symptoms until all symptoms were absent for 48 hours. In addition, for two hours after administraton of the drug, patients were observed for the occurence of vomiting. Thick and thin blood smears were prepared daily for seven days or until the smears were free of parasites for 48 hours; thereafter, smears were prepared weekly until day 28 after admission. A patient was considered cured if the thick blood smear became negative before day 7 and remained free of parasites until day 28. Grade I resistance was defined as the disappearance of parasites from peripheral blood within the first week, followed by recrudescence at day 7, day 14, day 21, or day 28. Grade II resistance (RII) was defined as the persistence of asexual parasites until day 7 and a reduction of parasitaemia of up to 25% of the initial parasite count during the first 48 hours of treatment. Grade III resistance (RIII) was defined as an up to 75% reduction of parasitaemia during the initial 48 hours of treatment with the persistence of parasites until day 7, or as an up to 75% reduction of parasitaemia during the initial 48 hours, accompanied by clinical deterioration at day 2 and 3.

In vitro studies of drug sensitivity

In the first in vitro drug testing trial, blood samples from 161 patients were investigated. All patients included in this study had a monoinfection with **P. falciparum**, the parasitaemia being > 500 and < 90 000 asexual parasites per µl of blood. The patients denied having taken any antimalarial medication during the preceding four weeks.

In the second in vitro drug testing trial, the sensitivity of **P. falciparum** to chloroquine and mefloquine was evaluated in blood samples of two different epidemiological strata regarding human habitat and ecology, as well as parasite exposure to chloroquine. One stratum was along the main road BR 364 between Rio Branco and Porto Velho (stratum E). The population in this area consisted mainly of recent immigrants from the large cities of southern Brazil, who had ready access to antimalarial drugs due to several drug dispensaries located along the main road. The other stratum was along the River Acre, a confluent of the River Purus (stratum R). In this riverine area, access to medical facilities and antimalarial drugs was quite limited. Both areas were separated by a wide band of virtually uninhabited dense jungle and there was little contact between the populations of the two strata. 78 patients, 60 in the stratum E and 18 in the stratum R, respectively, were included in the study with regard to the above mentioned criteria.

In vitro test for drug sensitivity of *P.falciparum*

For the in vitro tests the Rieckmann micro-technique[25] was applied according to the standard procedure, with microtitration plates and culture media provided by the World Health Organization (WHO). The test was performed as previously described. The results were statistically evaluated using log-dose response probit analysis[12].

RESULTS

Results of in vivo studies of drug sensitivity of *P. falciparum.*

The results pertaining to amodiaquine therapy and the combination therapy of quinine/sulfadoxine-pyrimethamine and quinine/clindamycin upon day 28 are summarized in table 1. The amodiaquine therapy revealed only 1 cure response out of 25 patients, with 6 patients showing RI resistance, 7 RII resistance, and 11 RIII resistance, respectively. More positive results were obtained with the QSP regimen, under which 9 of 30 patients were cured, whereas 19 patients showed RI resistance and 2 RII resistance. The best results were obtained with the QC regimen. Using this combination therapy, 36 of 40 patients were cured and only 4 showed RI resistance following treatment[18].

44 patients were admitted to the monotherapy study with clindamycin, 35 of which completed the 4 week study. The therapy response of the monotherapy with clindamycin is shown in table 2. In this study 34 males and 10 females with uncomplicated **P. falciparum** malaria were investigated. Their average age was 26 (range 16-50) years. Five days after initiation of treatment parasitaemia was absent in the majority of patients and at day 7 all patients had negative blood smears. Dividing patients into non-immune and semi-immune individuals did not reveal further significant differences, although the duration of symptoms and parasitaemia was slightly longer among non-immunes. All 35 patients continuing the study until day 28 were cured. The clindamycin treatment was well tolerated, with only slight side effects such as transient diarrhoea, headache, or nausea being observed, although these could not be clearly differentiated from symptoms intrinsic to **P. falciparum** infection[19].

Results of the in vitro studies of drug sensitivity of *P. falciparum*

Drug resistance was assumed if schizont maturation was observed at 1.14 µmol chloroquine/1, 0.4 µmol amodiaquine/1, 3.2 µmol mefloquine/1, and 51.2 µmol quinine/1, while resistance to sulfadoxine-pyrimethamine was assumed if there was less than 90% schizont inhibition at a concentration of 130 µmol sulfadoxine/1 and 1.63 µmol pyrimethamine/1 of blood. With regard to this WHO based definition of resistance, 84% of 83 isolates tested were chloroquine resistant. Furthermore, 73% of 89 isolates were amodiaquine resistant and a 92% resistance could be observed in 25 isolates grown with sulfadoxine-pyrimethamine. Resistance to quinine was encountered only in three isolates (3%), which still showed schizont maturation at 51.2 µmol/1. Data on the effective concentrations indicate a relatively low sensitivity to quinine (table 3). All **P. falciparum** strains were sensitive to mefloquine[17].

Results of the in vitro tests on chloroquine sensitivity of **P. falciparum** in the two different epidemiological strata showed a schizont maturation at 1.14 µmol/l for similar percentages of isolates in stratum E and in stratum R, respectively. In contrast, a significant difference of the inhibition of schizont maturation by chloroquine could be demonstrated by EC values between the two strata. EC_{50} values for chloroquine were 0.85 and 0.46 µmol/l and EC_{90} values were 2.81 and 1.25 µmol/l in strata E and R, respectively, whereas no differences were obtained for **P falciparum** susceptibility to mefloquine[16] (tables 4 and 5).

Table 1 - Response to the three drug regimens for treating falciparum malaria during 28 days of follow-up

	N°(%) of patients treated with		
Response	A	QSP	QC
Cure	1 (4)	9 (30)	36 (90)
Resistance			
RI	6 (24)	19 (63)	4 (10)
RII	7 (28)	2 (7)	0 (0)
RIII	11 (44)	0 (0)	0 (0)

A, amodiaquine; QSP, quinine/sulfadoxine-pyrimethamine; QC, quinine/clindamycin
RI, grade I resistance; RII, grade 2 resistance; RIII, grade 3 resistance

Table 2 - Number of patients presenting parasitaemia during the first week after onset of clindamycin therapy (n=35)

day	Median (parasites/ µl blood)	Parasitaemia cumulative number of patients without parasitaemia
0	2875	0
1	1590	0
2	2130	0
3	450	1
4	21	9
5	0	20
6	0	33
7	0	35
14	0	35
21	0	35
28	0	35

Table 3 - In vitro drug sensitivity of **P. falciparum** in Acre, Brazil, 1987: Effective concentrations (µmol/l) calculated by probit analysis for 50% (EC_{50}), 90% (EC_{90}), 99% (EC_{99}) inhibition

Drug tested	EC_{50}	EC_{90}	EC_{99}
Chloroquine	0.73	2.50	6.82
Amodiaquine	0.34	0.86	1.83
Mefloquine	0.27	0.52	0.91
Quinine	4.60	10.30	19.87

Table 4 - Sensitivity of **Plasmodium falciparum** to chloroquine in 'stratum E' (60 isolates) and in 'stratum R' (17 isolates) of Acre State, Brazil, 1987

	Inhibition of schizont maturation	
Drug concentration 10^{-6}mol/l	Stratum E %	Stratum R %
0.2	6.20	11.77
0.4	25.82	40.78
0.8	45.49	81.51
1.14	58.33	85.19
1.6	71.62	93.35
3.2	94.10	98.96
6.4	99.84	99.28

Table 5 - Sensitivity of **Plasmodium falciparum** to mefloquine in 'stratum E' (60 isolates) and in 'stratum R' (17 isolates) of Acre State, Brazil, 1987.

	Inhibition of schizont maturation	
Drug concentration 10^{-6}mol/l	Stratum E %	Stratum R %
0.1	3.19	1.45
0.2	28.65	35.32
0.4	70.71	81.25
0.8	99.10	99.56
1.14	99.95	100.00
1.6	99.98	100.00
3.2	100.00	100.00

DISCUSSION

Drug resistance of **P. falciparum** to chloroquine was first reported in 1981 in the state of

Acre, Brazil[24]. REYES demonstrated that 7 of 8 **P. falciparum** isolates were resistant to chloroquine in vitro, 2 isolates were RII resistant, and 5 isolates were RIII resistant, respectively[24].

Our results confirmed these findings and showed **P. falciparum** resistance to chloroquine in 84% of the tested isolates. Furthermore, amodiaquine, the drug usually recommended for falciparum malaria treatment in that region, failed to induce maturation inhibition of **P. falciparum** in vitro at 0.4 µmol/l in 73% of the isolates[17]. The in vivo results of amodiaquine efficacy reflected the in vitro findings[15, 18, 20]. Only 1 of 25 patients was cured using the recommended doses of 25 mg/kg over 48 hours. In summary we have demonstrated a widespread resistance of **P. falciparum** to the 4-aminoquinolines chloroquine and amodiaquine in the western region of the Brazilian Amazon.

Furthermore, chloroquine resistance appears to be caused by drug pressure, as indicated by the comparison of in vitro drug studies of chloroquine sensitivity in two epidemiological strata. The stratum in which chloroquine had been extensively used showed **P. falciparum** isolates with a higher level of resistance to chloroquine than in the stratum where chloroquine was rarely used[16].

Additionally, the in vitro data allow a prediction of resistance to sulfadoxine/pyrimethamine in all **P. falciparum** infections tested. After a single dose treatment with sulfadoxine/pyrimethamine, maximum plasma concentrations between 0.8 and 1.63 µmol pyrimethamine per liter of blood are reached[30]. If the later concentration is taken as the threshold level for in vitro resistance, then 92% of the cases investigated were resistant.

Moreover, 3 of 86 isolates were resistant to quinine in vitro, whereas mefloquine showed a 100% inhibition of schizont maturation at a concentration of 3.2 µmol/l[17], although in other part of Brazil data concerning in vivo and in vitro resistance to mefloquine and a combination of mefloquine, sulfadoxine, and pyrimethamine were reported[4, 28]. Meanwhile others have also demonstrated in vitro resistance to mefloquine also in the state of Acre[8].

The observed drug resistance could be partly overcome by the use of oral short-term regimens of quinine combination therapy with sulfadoxine-pyrimethamine, or clindamycin, respectively. Although most of the isolates were susceptible to quinine in vitro, treatment with quinine is not practical in an area where the population is highly dispersed and where there is limited access to medical facilities, since the drug must be administered on ten consecutive days. We therefore decided to investigate a combination of sulfadoxine-pyrimethamine with quinine in a three day schedule. The results revealed a 70% failure rate with the combination therapy in vivo despite a 92% in vitro resistance of **P. falciparum** isolates to sulfadoxine-pyrimethamine. However, the treatment of falciparum malaria with clindamycin in combination with quinine in a three day regimen showed a cure rate of 90%[18]. Clinical trials with clindamycin in **P. falciparum** malaria given in a relatively high dose (20-40 mg/kg/day) yielded promising results in studies undertaken in the Philippines and in Brazil[1, 5, 23, 26], while low dose clindamycin (10mg/kg/day) has been effective in patients with **P. falciparum** malaria in Sudan[9]. Moreover, it was observed that multidrug resistant cases of **P. falciparum** malaria in Acre could be cured by clindamycin monotherapy[14].

Clindamycin given as a low dose monotherapy over five days was sufficient to cure patients in both investigated groups, comprised of either semi-immune and non-immune individuals. However, owing to the slow onset of clindamycin's anti-malarial activity, clinical illness usually disappeared by the third day, with all patients becoming parasitologically negative within day 7 after initiation of treatment[19]. This slow clinical and parasitological response is a major limitation of clindamycin treatment and should therefore be restricted to semi-immune patients, since the in vivo drug susceptibility of **P. falciparum** is higher in semi-immune than in non-immune patients, in which a prompt drug response is desirable. Finally, the emergence of clindamycin resistant strains would also be expected using a low dose monotherapy. Therefore a combination therapy such as clindamycin/quinine should be favoured[13].

RESUMO

Susceptibilidade a droga, do *Plasmodium falciparum*, na Amazônia Ocidental, Estado do Acre, Brasil.

Estudos de campo na Amazônia ocidental

(Estado do Acre, Brasil) indicam que as 4-aminoquinolinas, assim como a sua combinação com sulfadoxina-pirimetamina não podem mais ser recomendadas para o tratamento e profilaxia das infecções pelo **P. falciparum** nesta região. A quinina permanece como droga efetiva quando usada corretamente. Entretanto, problemas podem surgir devido aos efeitos colaterais durante sua aplicação por período de 10 dias. Possibilidades de ultrapassar estes problemas combinando curto espaço de administração da quinina com outras drogas estão no momento limitadas devido à falta de um composto associado adequado. Por esta razão, a combinação quinina/clindamicina parece ser a terapêutica mais adequada para a malária pelo **P. falciparum**. Nossos estudos **in vitro** sugerem que a mefloquina é outra alternativa efetiva para o tratamento da malária falciparum nesta região da Amazônia.

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