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A RANDOMIZED CLINICAL TRIAL WITH HIGH DOSE OF CHLOROQUINE FOR TREATMENT OF Plasmodium falciparum MALARIA IN BRAZIL

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SUMMARY

This clinical trial compared parasitological efficacy, levels of in vivo resistance and side effects of oral chloroquine 25 mg/Kg and 50 mg/Kg in 3 days treatment in **Plasmodium falciparum** malaria with an extended followed-up of 30 days. The study enroled 58 patients in the 25 mg/Kg group and 66 in the 50 mg/Kg group. All eligible subjects were over 14 years of age and came from Amàzon Basin and Central Brazil during the period of August 1989 to April 1991. The cure rate in the 50 mg/Kg group was 89.4% on day 7 and 71.2% on day 14 compared to 44.8% and 24.1% in the 25 mg/Kg group. 74.1% of the patients in the 25 mg/Kg group and 48.4% of the patients in the 50 mg/Kg group had detectable parasitaemia at the day 30. However, there was a decrease of the geometric mean parasite density in both groups specially in the 50 mg/Kg group. There was 24.1% of RIII and 13.8% of RII in the 25 mg/Kg group.

Side effects were found to be minimum in both groups. The present data support that there was a high level resistance to chloroquine in both groups, and the high dose regimen only delayed the development of resistance and its administration should not be recommended as first choice in malaria **P. falciparum** therapy in Brazil.

KEYWORDS: Malaria; **Plasmodium falciparum**; Chloroquine; Clinical trial.

INTRODUCTION

Over the last 20 years a drastic increase in the incidence of malaria has been observed in Brazil. Around 90% of the cases have been mostly reported in the Amazon region in areas of new colonizations and mining activities. The intense migration flow to this area, the instability of the population and the difficulties in implementing vector control measures have led to the spread of malaria[8]. Drug treatment has consequently become the main and often the only measure to malaria control in those areas.

Chloroquine has been worldwide used as the main anti-malarial drug in the treatment of both **Plasmodium falciparum** and **Plasmodium vivax** due to its high efficacy against sensitive strains of plasmodium, rapid action, low cost, safety an availability. Although resistance to chloroquine of **P. falciparum** has been reported in the last 30 years[5, 10, 13] chloroquine is still recommended in the official control programme for some tropical regions.

In Brazil, the Ministry of Health has recommended 25 mg/kg of chloroquine as initial treatment for all malaria cases[12]. Higher doses (50-90 mg/kg) have been used parenterally in Central Brazil in an attempt to avoid resistance[2,4] but there is no reliable assessment of a reduced resistance or a possible increased efficacy at this dosage in comparison with the World Health Organization (WHO) standard dose. The main advantage of the use of Chloroquine in **P. falciparum** malaria has been the decrease of mortality rate.

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Considering the difficulties in carrying out field trials in developing countries, with intensive internal migration, high self treatment rates and limited budget for research, it is not surprising that even global public health strategies may be determined by few field trials[9]. This study was designed to assess the safety and cure rate of chloroquine at 25 mg/kg as compared to 50 mg/kg in a 30 day-evaluation of malaria patients from Central Brazil and Amazon basin.

MATERIAL AND METHODS

Study subjects

The study was conducted in the city of Goiânia, Central Brazill at the outpatient clinic of the Malaria Control Program, Fundação Nacional de Saúde (FNS) which treats approximately 90% of all malaria cases in the region. There has not been any report of urban malaria transmission and the origin of most patients has been the Amazon basin and Central Brazil, regions of intense mining activities. The protocol was submitted and approved by the regional official medical ethic council and a written informed consent was collected for all participants.

Patients were selected from August 1989 to April 1991 following the eligible criteria: over 13 years of age, single infection by **P. falciparum**, parasitaemia up to 50,000 trophozoite per mm^3 of blood, no antimalarial therapy in the preceding 7 days, history of at least one malaria episode in the last five years, no severe vomits and diarrhoea or other apparent associated critical illness. Patients were requested and supported to stay in the city for at least 30 days from first day of treatment. Eleven per cent of potential cases did not remain in the city.

Data on age, sex, occupation in the last 30 days, number of previous malaria episodes, drug treatment, days of symptoms were recorded at enrolment on a standardized form.

Patients assignment

Simple randomization was used in the first four months and as this procedure was unable to provide a balanced distribution of the patients between the groups, randomization in block of 10 patients was used to assign subjects to the two treatment groups (25 mg/kg and 50 mg/kg of chloroquine) in the following 17 months. The study enrolled a total of 124 subjects and this sample size had a 86% power to detect a difference cure rates of 22% or more at day 30, at 5% level of statistical significance.

Treatment Regimens

Chloroquine was administrated orally under supervision as follows:

C25 group - 25 mg/kg being 10 mg/kg on days 1 and 2 and 5mg/kg on the third day.

C50 group - 50mg/kg being 20 mg/kg on days 1 and 2 and 10 mg/kg on the third day.

After medication subjects were observed for 30 minutes. In case of vomiting a new dose of chloroquine was administered. They were asked not to take any other antimalarial drug and return if any medical problem occurred. Side effects were assessed daily, during 7 days after starting treatment, by household visits done by technician from Fundação Nacional de Saúde. The subjects were enquired on vomiting, pruritus and diarrhoea (two or more stools per day).

Chloroquine tablets were prepared by Indústrias Químicas do Estado de Goiás (IQUEGO) and tested independently at the two dosages by Quality Control Reference Centre/Ministry of Health, Brazil.

Parasitological evaluation

A thick smear was prepared for 7 consecutive days since the first day of treatment Day 0 (D0) during the home visits. Since the end point of the study was on D30 appointments slips were given for return visits on D15, D21 and D30, when additional thick blood smears were taken. If patient not returning for clinic appointment were visited at home. Parasite density was determined by counting parasites/100 fields by Giemsa stained thick smear.

The effect of the treatment on the parasitological count was monitored according to the WHO extended fields tests[3]. Chloroquine resistance was defined as RI, if asexual parasites disappear for at least 2 consecutive days but return and are present on day 7 (early RI), or asexual parasites recrudesced within day 30 (delayed RI); resistant

at the RII level, if asexual parasitaemia does not clear, but is reduced to 25% or less of the pre-treatment level during the first 48 hs of treatment, and resistant at the RIII level, if asexual parasitaemia remains above 25% of the pre-treatment level during the first 48 hs of treatment.

Subjects with parasitaemia > 25% of the D0 in D2 or any parasite count on D7, D14 and D30 were considered as chloroquine failure. Retreatment with an alternative drug was given when treatment failure was detected.

Statistical Analysis

The cure rate was calculated as the proportion of patients with no parasitaemia on D7, D14, D21 and D30 in relation to the total number of patients assigned to each group. Patients lost to follow-up were considered as treatment failure in order to avoid distortions in the comparison (intention to treat approach)[15].

Resistance levels were calculated as the number of patients with parasitaemia at different intervals divided by the total number of randomized patients.

The geometric mean parasite density (GMPD) and 95% confidence interval were calculated taking into account the parasitaemia levels of the patients (clearance and no clearance) excluding those considered resistant by the previous time-period evaluation. This GMPD was assessed daily for the first week and on D14, D21 and D30 in order to compare the parasitaemia levels between the two groups.

The data were analysed using the package SPSS/PC+, Computer System, 1987. Chi-Squared tests or Fischer exact test were calculated to measure difference in proportions and Student's test to test differences between means and P values (two sided) smaller than 0.05 were considered statistically significant.

RESULTS

Study Population

During the period of study, 362 subjects with **P. falciparum** malaria were screened. The main reasons for not including patients in the study were: antimalarial treatment in the last 7 days (92 subjects), first episode of malaria (6 subjects), 9 patients were under 14 years of age and 18 patients had parasitaemia more than 50,000 parasites/mm³. One hundred twenty-four patients were enrolled in the study and 102 (82.2%) were followed up for 30 days. Of 66 patients assigned to group C50, 53 (80.3%) completed the 30 days follow-up. The corresponding figure in group C25 was 49/58 (84.5%).

The majority of patients were males, came from gold mine activities, and more than 50% of the subjects had 1 to 3 episodes of malaria in the last 5 years. The general characteristics of the study participants are shown in Table 1. There were no significant statistical difference between the two treatment groups. Because 10% of the patients in the C25 group had parasitaemia higher

Table 1
Characteristics of malaria **Plasmodium falciparum** patients enrolled in the trial[a]. Central Brazil, 1989-1991.

Baseline Characteristics	Study groups 50 mg	25 mg
Number of patients	66	58
Mean age (yr)	30.1+/-10	30.9+/-9
male proportion	78.8%	81.0%
State of origin		
Mato Grosso	33.3%	44.3%
Pará	27.3%	29.5%
Rondônia	28.8%	16.4%
Rorâima/Amapá/Amazonas	10.6%	9.8%
Occupation in the last 30 days		
mining	74.2%	62.1%
fishing	1.5%	—
agriculture	3.0%	1.7%
trading	6.1%	12.1%
other	15.5%	24.1%
No. of malaria episodes in the last 5 years		
1-3	50.0%	63.8%
4-6	16.7%	15.5%
≥7	33.3%	20.7%
Previous treatment		
chloroquine	15.1%	6.9%
quinine	37.9%	39.6%
more than one	30.3%	37.9%
others	16.7%	15.5%
Days of symptoms at the enrolment		
1-5	83.3%	74.1%
5-10	12.1%	24.1%
≥11	4.5%	1.7%
GMPD[b]	984.6	1563.6
95%CI[c]	681.2-1412.9	1104.3-2183.3

[a] There were no statistical significance difference between study groups.
[b] GMPD = geometric mean parasite density
[c] CI = confidence interval

Table 2

Cumulative cure rate and geometric mean parasite density in **Plasmodium falciparum** malaria patients treated with chloroquine 50 mg/kg and 25 mg/kg.

Cure rate[a](%)	Study groups 50mg	25mg	Total
Total randomized	66	58	124
Day 7			
Clearance	59	26	85
No clearance	2	29	31
Lost of follow-up	5[b]	3	8
Cure rate	89.4	44.8[c]	
Day 14			
Clearance	47	14	61
No clearance	10	40	50
Lost of follow-up	9	4	13
Cure rate	71.2	24.1[c]	
Day 21			
Clearance	27	7	34
No Clearance	27	43	70
Lost of follow-up	12	8	20
Cure rate	40.9	12.1[c]	
Day 30			
Clearance	21	7	28
No clearance	32	43	75
Lost of follow-up	13	8	21
Cure rate	31.8	12.1[c]	

[a] Cure rate=number of clearance patient/total randomized patient x 100
[b] 2 withdrawals and 3 lost to follow-up
[c] P value <0.01

than 10,000 parasites/mm³ compared to 3% in the C50 group, the GMPD was higher in the C25 group, although this difference was not statistically significant.

The withdrawn patients were 1 for adverse effect (vomiting), 1 for deterioration of clinical condition and for the other 20 patients the reasons could not be determined (lost for follow-up). These lost to follow-up were not statistically significant different between the two groups.

Parasitological Findings

Throughout the follow-up period the cure rates were always higher for the C50 group in comparison with the C25 group (Table 2). For C50 group there was almost 90.0% of cure rate at D7 decreasing to 71.2%, 40.9% and 31.8% in D14, D21 and D30, respectively. In contrast, the C25 group started with lower cure rates (44.8)% at D7 following to 24.1%, 12.1% and 12.1% at D14, D21 and D30, respectively, differences statistical significant between the two groups for all periods of follow-up.

There was a striking higher proportion of RIII, RII and RI levels in the C25 group when compared

Table 3

Chloroquine resistance in **Plasmodium falciparum** patients.

Resistance level[a]	50 mg n=66		25 mg n=58		X^2	P value
RIII	1	(1.5)	14	(24.1)	12.4	<0.01
RII	0	(- -)	8	(13.8)	9.6	<0.01[b]
RI early	1	(1.5)	7	(10.3)	6.7	0.02[b]
RI delayed	30	(45.4)	14	(24.1)	6.1	0.01

[a] number of resistant patients/number of randomized patients.
[b] Fisher Exact test

Table 4

Geometric mean parasite density in **Plasmodium falciparum** malaria patients treated with chloroquine 50 mg/kg and 25 mg/kg.

GMPD[a]	Study groups 50 mg	25 mg
Days		
D1	412.4(410.7-415.1)[b]	470.9 (466.9-474.9)
D2	20.8(16.2-25.4)	95.4(88.3-102.4)
D3	2.2(0.5-3.7)	24.2(19.9-30.5)
D4	-	8.9(3.8-14.0)
D5	-	12.9(6.6-19.2)
D6	-	9.6(3.6-15.6)
D7	1.1(0.5-1.7)	21.1(12.0-30.2)
D14	2.4(0.1-4.7)	28.2(10.9-45.5)
D21	13.5(3.6-23.4)	9.3(undef-28.1)
D30	4.2(undef-9.8)	-(undef)

[a] GMPD = geometric mean parasite density
[b] ( ) = number in parenthesis are CI 95%
undef = undefined

to C50 group. Most patients showed recrudescence of asexual parasites after the initial clearance particularly in the C25 group (Table 3).

In all two groups, there was approximately 80% reduction of parasitaemia on D2 when compared to GPMD in D0. The lowest values of GMPD were observed on D4 in the C25 group with no detectable parasitaemia in C50 group from D4 to D6. However, there was an increase parasitaemia levels in both groups after D6 (Table 4).

Side Effects

Occurrence of vomiting, diarrhoea and pruritus were not of major importance. Only one patient was withdrawn from the study on D1 in the C25 group treatment for excessive vomiting.

Of patients without vomiting and/or diarrhoea at the enrolment 8 (15.7%) of 51 patients in the C50 group treatment and 4 (9.1%) of 44 patients in the C25 group vomited at least once at home ($p>0.05$). In both groups occurrence of vomiting disappeared on D3, except one patient in C50 group treatment. Eight (15.7%) of 51 patients in the C50 group and 3 (7.0%) of 43 patients in the C25 group reported diarrhoea only during treatment days ($p>0.05$). Thirteen (19.7%) and 9 (15.5%) patients reported itching in the C50 group and in the C25 group, respectively, during treatment and follow-up ($p>0.05$). In only one patient (C25 group) the pruritus occurred after the treatment and remained until D7.

DISCUSSION

Since **P. falciparum** resistance to chloroquine has been detected in Brazil[1,2,21] the official Malaria Control Programme has recommended several alternative drugs. However due to the appearance of resistance for these new treatment [14,17], the use of chloroquine has still been considered as a public health option[12].

Intravenous use chloroquine in higher dosage has shown some increased efficacy in the treatment hospitalized patients with **P. falciparum** malaria in Brazil[4] but nor proper assessment of oral chloroquine in higher dosage has been done regionally.

In the present study the cure rate was significantly higher in the group treated with 50 mg/kg as compared with 25 mg/kg in 7, 14, 21 and 30 day evaluation. For the C50 group there was a rapid clearance reaching null values on D4 up to D6. The results of decreased parasitaemia with higher dosages of chloroquine was previously reported for children in Africa. In those studies, increasing the doses of chloroquine delayed the appearance of recrudescence beyond 7 days. In Burundi's study despite of the small number of the enroled patients the recrudescence occurred for a period directly related to the dose of chloroquine used (to 35, 40 and 50 mg/kg)[7]. The Rwandan surveillance study to follow the efficacy of chloroquine also suggested that 50 mg/kg dosage had no role to play in the control programme since the failure in treatment would occur in the following week[20].

In fact our data revealed that considering the extending the follow up to D30 even for the C50 group, more than 45% of patients had RI delayed. Similar results were found in other report which suggested that the extended follow-up was more important than the determination of parasite densities for the evaluation of drug efficacy and public health intervention[19]. Although a simplified "in vivo" test with 7 days follow-up has been described recently[16] it may not be adequate for surveillance purpose. Considering that most of our patients were from mining activities and rural settlements, 30 days follow-up is a difficult task. Beside this problem our study showed that almost 40% of the total malaria patients had to be excluded due to previous recent drug intake because self-treatment is very common in the region.

Nevertheless in the short term follow-up (48 hours) for the standard dose of chloroquine (35 mg/Kg) there was more than 37% (22/58) of unaltered or increased parasitaemia (RIII or RII) while this type of resistance level occurred in only 1 patient (1.5%) in the higher dosage group.

The lowest parasitaemia count were found on D4 for both groups indicating that the clinical evaluation to modify therapy should not rely on parasitaemia count on D4 specially in areas where resistant strains of **P. falciparum** have already been detected.

In the present study the "in vivo" testing of chloroquine in a sample of malaria patients coming from Amazon region and Central Brazil showed chloroquine resistant up to 72% (43/58) at the dose of 25 mg/Kg. These results may represent a reli-

able sample of the resistance strains of **P. falciparum** in Brazil since the Amazon region concentrate 90% of all malaria cases in the country. 93% of chloroquine resistance had already been reported in previous study among patients from a neighbouring State[18]. Considered that chloroquine resistance appers to be caused from selections of mutant resistant strains due to drug pressure[6, 11], even the administration of higher dosage of chloroquine could favour the length of time of carrier state and consequently the widespread of resistant strains due to the great number of RI delayed observed in our study.

In conclusion, the present data support that there was a high level resistance to chloroquine in both groups, and the high dose regimen only delayed the development of resistance. Thus, the chloroquine should not be recommended in malaria **P. falciparum** therapy in Brazil.

RESUMO

Ensaio clínico aleatório duplo cego com cloroquina em dose alta para tratamento da malária por *Plasmodium falciparum* no Brasil.

Comparou-se a eficácia parasitológica, níveis de resistência "in vivo" e efeitos colaterais da cloroquina oral nas dosagens de 25 mg/kg e 50 mg/kg no tratamento da malária por **Plasmodium falciparum** com seguimento de 30 dias. O estudo foi conduzido de agosto de 1989 a abril de 1991 e incluiu 124 pacientes, selecionados aleatoriamente em blocos de 10 pacientes, do ambulatório da Fundação Nacional de Saúde-Goiânia, Brasil. Todos os pacientes eram procedentes da Bacia Amazônica e Brasil - Central, sendo 58 alocados no grupo de 25 mg/kg (C25) e 66 no grupo de 50 mg/kg (C50). Os efeitos colaterais foram mínimos em ambos os grupos. A taxa de cura no C50 foi 89,4% no dia 7 e 71,2% no dia 14 enquanto para o C25 as taxas foram de 44,8% e 24,1%, respectivamente. Setenta e quatro por cento dos pacientes do C25 e 48,4% no C50 apresentaram parasitemia detectável no dia 30. Entretanto, houve uma queda da média geométrica da densidade parasitária (MGDP) em ambos os grupos, especialmente no C50. Resistência tipo III e II foi detectada respectivamente em 24,1% e 13,8% dos pacientes no grupo C25. No grupo de 50 mg/kg não foi detectado nenhum caso de RII registrando-se apenas um caso de RIII. Um grande número de RI tardio foi detectado em ambos os grupos, o que poderia retardar o tempo de portador e contribuir para disseminação de cepas resistentes. Desta forma, o presente estudo conclui que cloroquina, em qualquer das doses testadas, não deve ser utilizada no tratamento da malária por **P. falciparum**, em nosso meio.

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