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TREATMENT OF CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA WITH CHLOROQUINE IN GUINEA-BISSAU

POUL-ERIK KOFOED, FRANCISCO LOPEZ, PETER JOHANSSON, ANITA SANDSTRÖM, KATHRYN HEDEGAARD, PETER AABY, AND LARS ROMBO

Projecto de Saúde de Bandim, Bissau, Guinea-Bissau; Department of Paediatrics, Kolding Hospital, Kolding, Denmark; Laboratório Nacional da Saúde Publica, Bissau, Guinea-Bissau; Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Serum Institute, Copenhagen, Denmark; Division of Infectious Diseases, Thoracic Medicine and Dermatology, Mälarsjukhuset, Karolinska Institute, Stockholm, Sweden

Abstract. Children with symptomatic malaria in Bissau, Guinea-Bissau were randomly assigned to treatment with a 25 mg/kg total dose of chloroquine as recommended by the National Malaria Program or with a higher total dose of 50 mg/kg. Sixty-seven and 62 children, respectively, completed the treatment and were then followed once a week for five weeks. Treatment with a dose of 50 mg/kg was significantly more effective than treatment with 25 mg/kg in preventing recrudescence. The cumulative relative risk (95% confidence interval) of having parasitemia in the low-dose group during follow-up was 0.20 (0.08–0.52) on day 21, 0.38 (0.17–0.86) on day 28, and 0.48 (0.23–0.98) on day 35. Few adverse events were reported, although more children complained of vomiting and diarrhea on day 2 in the high-dose group compared with those in the low-dose group. However, this difference was not statistically significant. We conclude that a dose of 50 mg/kg of chloroquine could be recommended for treatment of *Plasmodium falciparum* malaria in Bissau. To minimize the risk of side effects, this higher dose should be given divided into two daily doses over a three-day period.

INTRODUCTION

Chloroquine is still the most commonly used drug for treatment of *Plasmodium falciparum* in Africa, although resistance has been reported from all endemic areas of the continent. In various health centers in Guinea-Bissau, it has been found that the mean total dose of chloroquine used greatly exceeds the dose of 25 mg/kg recommended by the World Health Organization (WHO).[1] An average dose of 76 mg/kg is used in suburban health centers in the capital of Bissau (Rombo L, unpublished data). Doses greater than the standard one have been shown to be more effective in areas with RI-RII resistance to chloroquine.[2–4] However, the efficacy of such doses and their possible adverse effects in Guinea-Bissau are not known. Therefore, we compared the outcome of therapy in children with symptomatic malaria given a total chloroquine dose of 25 mg base/kg as recommended by the WHO with a total dose of 50 mg/kg, which is closer to what is presently used in Guinea-Bissau. To minimize the risk of adverse effects, the latter dose was divided into a morning and an evening dose; each of these was equal to the single daily dose given the first group.

SUBJECTS AND METHODS

The study was conducted in the semi-urban area of Bandim on the outskirts of Bissau, the capital of Guinea-Bissau. Parents from Bandim who attended the Bandim Health Center before 9:00 AM with children having fever or other symptoms compatible with malaria and stated that the children had not taken any antimalarial drug during the previous week were informed of the study. If they agreed to participate, the children had a thick blood film examined for malaria parasites. Seventy children with a monoinfection (*P. falciparum*) and 20 or more parasites per 200 leukocytes were included in each of the study groups. Children with severe anemia, convulsions, repeated vomiting, or a concurrent infection, and those who for other reasons needed the services of a hospital were not eligible for the study.

Children were enrolled from December 1995 until the end of October 1996. The children were randomized by asking their mothers to draw a closed envelope from a bag containing 10 envelopes for each of the study groups. This block randomization equally distributed the children in the treatment groups during the different seasons of the year.

Treatment. Chloroquine doses in the 25 mg/kg group were given as 10 mg/kg in the mornings on the first day (day 0) and the second day (day 1) and as 5 mg/kg in the morning on the last day of treatment (day 2). Doses in the 50 mg/kg group were given as 10 mg/kg in the morning and evening on the first and the second days and as 5 mg/kg in the morning and evening on the last day of treatment. Tablets (160 mg of chloroquine phosphate containing 100 mg of chloroquine base; Pharmacia Upjohn, Stockholm, Sweden) were divided in half when necessary. If a child vomited within 30 minutes after receiving the tablets, quinine (10 mg/kg) was given as an intramuscular injection.

Before and during treatment the children or their mothers were asked daily if they had any symptoms related to malaria or to the medication given. At the same time, the axillary temperature was measured using an electronic thermometer.

Ethics. The protocol stated that any study group should be terminated if parasitemia reappeared in 50% or more of at least 40 children. Ethical clearance for this study was obtained from the Ministério da Saúde Pública in Guinea-Bissau.

Exclusion criteria. Children who only received the first dose were excluded. Those admitted to a hospital before the termination of the treatment were excluded from the follow-up because they had received a different treatment regimen. When a child traveled or for other reasons was not seen for two or more consecutive weeks, the follow-up was stopped but the child was included in the analysis until the day of absence.

Blood samples, microscopy, and drug assay. Before inclusion, thick and thin blood films were examined. On day 7, capillary blood samples were drawn for microscopy and drug assay. Thick blood films were also examined on days 14, 21, 28, and 35 after initiation of the treatment.

TABLE 1
Study population and follow-up

Study group	Enrolled	Excluded		Included in the study
		Admitted	Other reasons*	
25 mg/kg	70	2	1	67†
50 mg/kg	70	3	5	62‡

* The children received only the first dose. One mother believed the first dose was sufficient, one mother were ill, one mother was too busy to bring the child, and one child had traveled.
† Of the 67 children in the study, five were lost to follow-up on day 7 (one due to an incorrect address, one moved from the study area, and three had travelled). In additiion, two children were lost to follow-up day 14, one at day 21, and one at day 28 (all due to travel).
‡ Of the 62 children in the study, three were lost to follow-up on day 7 (one due to an incorrect address, one moved from the study area, and one had travelled). In addition, two children were lost to follow-up day 14 and one at day 21 (all due to travel).

Slides were stained with 3% Giemsa and independently examined by two experienced laboratory technicians using binocular microscopes with 100× oil-immersions lenses. Slides were considered negative if no parasites were detected when 2×200 leukocytes had been examined.

Capillary blood samples (100 μL) for the drug assay were dried on filter paper. Samples were kept separate from each other at room temperature until analyses of whole blood concentrations of chloroquine.[5]

Follow-up. Children were visited once a week by a health worker until day 35. If the condition of the child was not satisfactory, the mother was asked to bring the child to the Bandim Health Center. During the study period, the parents were also asked to bring their children to the health center in case of any illness to enable early detection of clinical malaria. All treatments were free of charge during the study period. Mothers of children excluded due to lack of compliance were also visited on day 7 and asked for the reason for not attending. A thick blood film was then obtained and children with parasitemia were treated with Fansidar® (F. Hoffmann LaRoche, Basel, Switzerland) using the dose recommended by the manufacturer, i.e., half a tablet for children two months to four years old, one tablet for children 5–9 years, and two tablets for children more than nine years old. If a child could not be found during follow-up, the reason was elucidated by asking family members or neighbors.

Statistical analysis. The data are presented as grouped failure-times. The outcome of interest was the time until parasites were detected during follow-up. The failure curves were compared using the log rank test for grouped failure-times.[6] The corresponding Mantel-Haenszel relative risk estimates are given,[7] and Miettinens test-based confidence intervals are used.[8] Loss to follow-up between two analysis times has been treated by censoring at the beginning of the time interval. Other differences between and within the two groups have been tested using the chi-square test and the Wilcoxon rank sum test.

RESULTS

The number of children enrolled in each group and reasons for exclusion or loss to follow-up are shown in Table 1. Three children in the 25 mg/kg group were excluded (Table 1). Of the remaining 67 children, 56 received all three doses of chloroquine and eight received two doses. Three children who received two doses of chloroquine were also given one dose of intramuscular quinine, one child due to severe vomiting and two children due to itching caused by the chloroquine. Excluding children who received quinine, the median dose of chloroquine in this group was 25.0 mg/kg (range = 17.5–29.4 mg/kg).

Eight children in the 50 mg/kg group were excluded (Table 1). Of the remaining 62 children, 46 received all six doses of chloroquine, nine received five doses, and two received four doses. Three children were given one dose of quinine and two children were given two doses of quinine due to severe vomiting on the day of enrollment. The median dose of chloroquine for children not receiving quinine in this group was 50.0 mg/kg (range = 33.0–59.0 mg/kg).

There was no difference in the distribution of sex, age, and body weight between the children in the two treatment groups (Table 2). Children excluded or not completing the follow-up did not differ from the children enrolled.

Signs and symptoms. There were no differences in the reported symptoms between the two groups (Table 3). At inclusion, the most common symptoms reported were fever (97%), vomiting (49%), diarrhea (23%), and headache (85%). On day two, 40% reported fever, 10% still vomited, 9% had diarrhea, and 15% complained of a headache. Two children in the 25 mg/kg group refused to take the last chloroquine tablets due to itching. Temperatures measured at inclusion were the same in the two groups with a mean of 38.4°C (range = 35.9–40.5°C). After two days of treatment only 2% had temperatures > 38°C.

Parasitemia. On inclusion, the median parasitemias in the 25mg/kg and 50 mg/kg groups were 8,125 and 11,400 parasites per microliter of blood, respectively (assuming a leukocyte count of 5,000 per microliter of blood). During follow-up, 93% and 95% of the planned blood films, respectively, were obtained. On day 7, 10 (16%) children in the low-dose group had parasitemia, whereas parasites were not found until day 21 in the 50 mg/kg group. The cumulative relative risk (95% confidence interval) of having parasitemia was 0.38 (0.17–0.86) on or before day 28 and 0.48 (0.23–0.98) on or before day 35 (Table 4). Except for one child in the 25 mg/kg group and one in the 50 mg/kg group, all children with parasitemia were at some time during follow-up treated for clinical malaria on or before day 35.

Drug analyses. Median whole blood concentrations of chlo-

TABLE 2
Description of the children in the study

Treatment group		No. of children		Age (years)* range	Weight (kg)* range
		Male	Female		
25 mg/kg	All children enrolled	32	38	0.4–13 (3.9)	7.6–39.5 (14.3)
	Children excluded or not completing follow-up	4	8	0.7–8 (3.9)	7.0–25.0 (14.0)
50 mg/kg	All children enrolled	30	40	0.8–13 (3.2)	6.3–64.0 (14.1)
	Children excluded or not completing follow-up	6	8	1.0–10 (4.0)	8.0–25.1 (14.9)

* Values in parentheses are medians.

TABLE 3
Symptoms reported by the mothers on inclusion of their children into the study and on day 2

	Treatment group			
	Day 0		Day 2	
Symptoms	25 mg/kg	50 mg/day	25 mg/kg	50 mg/day
Vomiting	33/67 (49%)	31/62 (50%)	5/61 (8%)	8/59 (14%)
Diarrhea	16/67 (24%)	14/61 (23%)*	3/61 (5%)	8/59 (14%)
Headache	58/67 (87%)	49/61 (80%)*	9/61 (15%)	9/59 (15%)
Nausea	11/67 (16%)	11/61 (18%)*	0/61 (0%)	0/59 (0%)
Itching	7/66 (11%)*	3/61 (5%)*	3/61 (5%)	0/59 (0%)
Fever	64/66 (97%)*	59/61 (97%)*	22/61 (36%)	24/58 (41%)*

* Information was missing for one child.

roquine at day 7 are shown in Table 5. In the low-dose group, no statistical difference was found between the concentrations on day 7 in the children who became parasitemic and the children who did not have parasitemia during follow-up. In the 50 mg/kg group, the concentration of chloroquine was significantly higher in the group with parasitemia during follow-up than in the group without parasitemia. No correlation was found between age and drug concentrations on day 7 in any of the groups.

DISCUSSION

Chloroquine is likely to be used in the future in Guinea-Bissau since most of the resistance to it is RI/RII, which implies that the drug still has a temporary effect. The continued use of chloroquine is understandable since the drug is cheap, always available, and only associated with rather minor adverse side effects.

One way to prolong the usefulness of this drug would be to increase the dose. This has previously been shown to be effective by resulting in higher cure rates than the standard dose of 25 mg/kg.[2–4,9] It is reasonable to believe that the effect of a higher chloroquine dose would not only prolong the time to recrudescence, but also to cure additional individuals with malaria.

The elimination half-life of chloroquine increases with time after the last dose. When the dose of chloroquine for treatment is increased, the elimination time for any concentration interval is therefore longer, implying that parasites are also exposed to subtherapeutic concentrations of chloroquine for a longer time than following treatment with a standard dose. This might promote resistance to the drug. Conversely, higher concentrations of chloroquine will be parasiticidal in more children, resulting a higher cure rate, diminishing the parasite load, and reducing transmission.

TABLE 4
Children with parasitemia during follow-up

	No. (%) of children with parasitemia per no. of children observed*		Relative risk (test-based confidence interval)†
Group	25 mg/kg	50 mg/kg	
Day 7	10/62 (16%)	0/59 (0%)	Not defined
Day 14	3/50 (21%)	0/57 (0%)	Not defined
Day 21	5/46 (30%)	4/56 (7%)	0.20 (0.08–0.52)
Day 28	0/40 (30%)	4/52 (14%)	0.38 (0.17–0.86)
Day 35	2/40 (34%)	3/48 (20%)	0.48 (0.23–0.98)‡

* Values in parentheses are cumulative percentages of children with parasitemias. Two children were lost to follow-up in each group on day 14, one on day 21, and one child in the 25 mg/kg group on day 28.
† Miettinen's test-based confidence interval.
‡ $P = 0.045$, by log rank test.

At the present time, the magnitude of *in vitro* chloroquine resistance in Bissau has been the same over a period of at least five years despite the use of higher than recommended doses (Rombo L, unpublished data), indicating that the use of these higher doses has not resulted in the development of resistance.

In the present study, no parasites were detected during the first two weeks after treatment with the higher dose of 50 mg/kg. The percentages of children with parasites at day 28 and at day 35 were similar to what we found after seven days of treatment with Quinimax/quinine.[10,11] This indicates that chloroquine is still effective when used at higher doses than at the dose recommended by the WHO.

The children in the two study groups were eventually at similar risk of acquiring new malaria infections. With longer follow-up, the occurrence of new infections would therefore reduce the relative difference between the groups (Table 4).

Chloroquine is considered to be a toxic drug with a narrow therapeutic range. It is probably the most commonly used drug for suicide and suicidal attempts in Africa and is also the cause of a substantial number of suicides in industrialized countries.[12] The acute toxicity of chloroquine is limited to transient but very high concentrations in the blood during an incomplete distribution phase, especially after parenteral administration. However, when 15 mg of chloroquine diphosphate per kilogram was infused over a four-hour period, no serious side effects were observed.[13]

Peak concentrations of chloroquine after oral administration are obtained within 1–3 hours. The drug is then distributed into several compartments with varying affinities to the drug. Only 0.1% of the absorbed drug remains in the plasma.[14] The major distribution of chloroquine occurs within hours and results in substantially lower trough concentrations at the time of the next dose. Furthermore, orally administered chloroquine gives a peak plasma concentration that is only 20% of the concentration obtained after parenteral administration.[14]

When the last evening dose of approximately 7.5 mg/kg was given to the group receiving a total dose of 50 mg/kg in our study, the attained peak concentrations were low enough to be considered safe. In accordance with this, only minimal side effects were found in previous studies using higher than recommended doses of chloroquine.[2,4,9] In the present study, no serious side effects were observed, although three children in the low-dose group complained of itching, two of them so severe that they refused to take the last tablets. On the day of enrollment, five children in the high-dose group received quinine intramuscularly due to vomiting compared with only one child in the low-dose group. However, at the time of vomiting,

TABLE 5
Median whole blood chloroquine concentrations on day 7*

Treatment group	Children with parasitemia on or before day 35	Children without parasitemia on or before day 35	All children finishing follow-up
25 mg/kg	506 nmol/L (365, 656)	574 nmol/L (430, 874)	541 nmol/L (421, 767)
50 mg/kg	1,820 nmol/L (1419, 2,272)	1,187 nmol/L† (971, 1,740)	1,420 nmol/L (999, 1,810)

* Numbers in parentheses are the 25% percentile and 75% percentile values given in nmol/L.
† $P < 0.05$.

three of the children in the high-dose group had received only a single dose of chloroquine, which was the same dose given to the low-dose group. On day 2, gastrointestinal symptoms (vomiting and diarrhea) were reported more frequently in the 50 mg/kg group, although the difference was not statistically significant.

Parasites that are show RI–RII resistance to chloroquine are inhibited by higher concentrations of the drug, but multiply once again when concentrations decrease.[15] We therefore expected that parasitemic children would have lower drug concentrations on day 7. This was found in the group treated with 25 mg/kg, although the difference was small. The similarity of the drug concentrations in children with detected parasites and those without parasites indicates that the difference in outcome was probably not due to varying kinetics of the drug but to differences in drug resistance of the parasites and/or differences in host immunity. Drug concentrations after a total dose of 25 mg/kg were very similar to what we observed in Tanzania.[16] We were surprised to observe that chloroquine concentrations were significantly higher in children with parasites in the high-dose group than in children without detected parasites. The reason for this difference is not known.

The higher dose of chloroquine increases the efficacy of treating uncomplicated malaria in children in Guinea-Bissau without a significant increase in side effects. Therefore, chloroquine still has an important role. However, due to the kinetics of this drug, it is important to give the higher dose twice a day to minimize possible severe side effects. Even if we did not find 50 mg/kg to be a toxic dose, a further increase of the dosage would probably be associated with more frequent adverse effects. Policy makers should therefore be aware of the potential implications before increasing dosages even more.

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Authors' addresses: Poul-Erik Kofoed, Department of Paediatrics, Kolding Hospital, DK 6000, Kolding, Denmark, Telephone: 45-7-636-2230, Fax: 45-7-553-9144, E-mail: PKO@KS.VejleAmt.dk. Francisco Lopez, Projecto de Saúde de Bandim, Apartado 861, 1004 Bissau Codex, Guinea-Bissau, Telephone: 245-20-14-89, Fax: 245-20-16-72. Peter Johansson, Division of Infectious Diseases, Karolinska Sjukhuset, Stockholm, Sweden, E-mail: peter.joh@telia.com. Anita Sandström, SAREC, Sida, SE-105 25 Stockholm, Sweden, Telephone: 46-8-698-5345, Fax: 46-8-698-5656, E-mail: anita.sandstrom@sida.se. Kathryn Hedegaard, Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Seruminstitut, Artillerivej 5, 2300 Copenhagen, Denmark, Telephone: 45-3-268-3965, Fax: 45-3-268-3965, E-mail:Kah@ssi.dk. Peter Aaby, Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Seruminstitut, Artilerivej 5, 2300 Copenhagen, Denmark, Telephone: 45-3-268-3268, Fax: 45-3-268-3165, E-mail: psb@sol.gtelecom.gw. Lars Rombo, Mälarsjukhuset, S-63188 Eskilstuna, Sweden, Telephone: 46-1-610-3551, Fax: 46-1-610-3519, E-mail: Lars.Rombo@mse.dll.se

Reprint requests: Poul-Erik Kofoed, Department of Paediatrics, Kolding Hospital, DK 6000, Kolding, Denmark, Telephone: 45-7-553-3222 extension 2282, Fax: 45-7-553-9144, E-mail: PKO@KS.VejleAmt.dk

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