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MEFLOQUINE TREATMENT FOR UNCOMPLICATED FALCIPARUM MALARIA IN YOUNG CHILDREN 6–24 MONTHS OF AGE IN NORTHERN GHANA

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Abstract. Mefloquine (MQ) single dose 20 mg/kg treatment of falciparum malaria was evaluated in 186 children of 6–24 months of age in northern Ghana. There were 15 RII/RIII-type parasitologic failures, all with Day 2 MQ blood levels significantly lower than children whose parasitemias cleared before Day 7 and remained clear through 28 days. Predictors of RII/RIII parasitologic response were vomiting after MQ dosing, Day 2 MQ levels < 500 ng/mL, and undetectable Day 2 levels of the carboxymefloquine metabolite. There were 50 cases of delayed RI parasitologic failure, but 71% of these cases had undetectable Day 28 blood levels of MQ and drug levels in the remaining 29% ranged below the 620 ng/mL level that suppresses MQ sensitive strains of *P. falciparum.* Drug levels among infants that tolerated MQ well were not associated with age, weight, hemoglobin, parasitemia, and pre-existing symptoms of vomiting or diarrhea. An observed recurrent parasitemia of 34,400 trophozoites/μL against a MQ blood concentration of 550 ng/mL was taken as indication of tolerance to suppressive levels of the drug at this location.

INTRODUCTION

Under the conditions of intense malaria transmission commonly seen throughout rural sub-Saharan Africa, *Plasmodium falciparum* rapidly developed resistance to easily affordable drug treatments based on chloroquine or Fansidar.[1,2] Consequently, mefloquine (MQ) became a sensible and popular first-line drug for the prevention of malaria in travelers, and where cost was not a primary limiting factor, this drug was also considered a safe and effective single-dose treatment of uncomplicated *P. falciparum* infection. However, despite limited and carefully supervised use of MQ in combination with sulphadoxine and pyrimethamine (MSP), treatment failure rates in Thailand rose 20-fold in just 4 years and forced an abandonment of the MSP treatment strategy.[3] *In vitro* tests from locations throughout Africa gave early indication that even without prior exposure or drug pressure, strains of malaria circulated that were inherently resistant to MQ.[4,5] The drug was 94% more effective than chloroquine for preventing malaria among Peace Corps volunteers in West Africa during 1992, but cases of MQ prophylaxis failure, confirmed by high drug levels in blood (744–1,275 ng/mL), have now been reported in African states ranging geographically from Tanzania to Sierra Leone.[6,7] While the majority of these have been isolated cases, spanning a dozen years, an unexpectedly high MQ prophylaxis failure rate of 14% was reported during 1998 at a single location in northern Ghana.[8] This high prophylaxis failure rate was even more unusual because it occurred among "malaria-immune" adult Ghanaians who had been randomized to receive standard weekly dosing of MQ in a rigidly supervised double-blind, placebo-controlled prophylaxis trial. This trial had been preceded in 1996 by studies of seasonal malaria incidence in young children and against the likelihood of chloroquine and Fansidar failures, MQ was the drug of choice selected for single dose treatment of their uncomplicated malaria infections.[9] We hypothesized that a retrospective evaluation of the MQ treatment outcomes in these non-immune children, and an analysis of their MQ blood levels would be predictive of the MQ prophylaxis failures that occurred among clinically-immune adults in this same community 2 years later. In other words, we sought details of recurrent parasitemias that occurred in MQ-treated children, confirmation of dosing, and a determination of MQ levels in blood at the time of any early or late treatment failures.

Because of cost and availability, MQ has not been widely used for treatment in Africa, and taken collectively, trials of MQ treatment in African children since the late 1980s have not produced clear indications of either resistant infections or successful treatments. A number of studies have reported that young children tolerate single oral doses of the drug poorly, leading some authorities to advise splitting the dose and to guard against vomiting.[10,11] Several studies have indicated that diarrhea, either a preexisting condition or an adverse side effect of the MQ therapy, is also associated with inadequate MQ drug levels.[12,13] In a study of infant children in French Guyana pre-treated with metoclopramide before gastric tube administration of a single 25 mg/kg dose of MQ, early vomiting occurred in 11.5%, and diarrhea was found to be the most common adverse event of therapy.[14] Host genetic factors, disease condition, and age have also been variably considered responsible for significant pharmacokinetic differences of MQ in whole blood and sera. In one comparative study, healthy adult Swiss men achieved peak plasma MQ levels 3-fold lower than parasitemic adult male Thais given the same dose of MQ.[15] Thai children 5–10 years of age achieved a mean Day 2 blood level of 2,031 ng/mL that was virtually the same as the mean serum level of 2,165 ng/mL measured in Thai adults,[3,16] but pharmacokinetic parameters of C_{max} and area under the curve (AUC) for MQ in Thai infants with malaria were reported to be much higher than in adult Thai patients.[17] African children with malaria seem to attain lower levels of MQ in blood, making treatments and *in vivo* determinations of resistance problematic. Cohorts of Malawian children younger than 5 years of age given single 15- or 25-mg/kg doses of MQ reportedly attained comparable mean Day 2 blood levels of 1,043 and 1,420 ng/mL, respectively, but mean levels in these young children by Day 7 fell to 670 and 718 ng/mL, respectively, with individual measures ranging from 26 to 1,716 ng/mL.[10] Senegalese children > 1

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year old treated with a single oral dose of 12.5 mg/kg achieved average Day 2 MQ and CMQ blood levels of 955 and 407 ng/mL, respectively.[18] A more recent evaluation of the MQ 15-mg/kg single dose in young Malawian children reported a mean Day 2 serum level of only 633 ng/mL.[19] Given such variables, uncertainties, and contradictions, we considered that our provision of malaria therapy to Ghanaian infants afforded an opportunity to broaden the knowledge base of MQ treatment in the age and ethnic group at greatest risk of infection, morbidity, and death by malaria.

MATERIALS AND METHODS

Study site. The Kassena Nankana District (KND), situated in the Upper East Region of Ghana at its northern border with Burkina Faso, is an administrative territory of 1,675 km^2 with a population of ~140,000 residents. The flat, open woodland ecology is termed Guinea Savannah, with annual rainfall of ~900 mm occurring from May to October. The ethnic Kassem and Nankan tribes are predominantly subsistence farmers cultivating seasonal millet; however, a dam constructed in 1980 provides irrigation for year-round rice and vegetable cultivation for about one fifth of the district population. Malaria is holoendemic, transmitted by *Anopheles gambiae sensu strictu*, during the wet season and by *An. funestus* at a lower, but continuous, rate across both seasons.[20] The incidence of malaria infection among young children was determined to be 9.1 infections per person-year during the wet season and 4.7 infections per person-year during the dry season.[9] The MQ treatment component of the dry season incidence study constitutes the subject of this report.

Study subjects. Informed consenting parents enrolled their children during November 1996 into a prospective study measuring dry season malaria attack rates. Institutional boards of the US Navy and the Ghanaian Ministry of Health conducted ethical review and granted approval for the conduct of this study. Approximately 28% of children screened were excluded, mainly because of severe anemia (hemoglobin [Hb] < 6.0 g/dL), and were referred for treatment. The enrolled dry season cohort consisted of 259 breast-feeding children 6–24 months of age, all of whom received curative therapy consisting of quinine sulfate (10 mg/kg orally, three times a day, days 1–4), Fansidar (5–10 kg, 1/2 tablet; 11–20 kg, 1 tablet; Day 5), and primaquine (0.25 mg/kg orally, four times a day, days 5–18), except in the case of 17 glucose-6-phosphate dehydrogenase–deficient children. All medications were given as suspensions in a fruit-flavored sugar syrup. Children were visited regularly, with blood films made every 2 weeks or at any occasion of illness consistent with malaria (fever, chills, nausea, vomiting, and malaise). The presence of asexual stages of *P. falciparum* in a Giemsa-stained blood film prompted immediate clinical evaluation and treatment. Uncomplicated clinical malaria and parasitemias < 1% were treated with MQ. Children averaged 6.9 (95% CI, 6.5, 7.3) parasite-free weeks from the end of radical cure to re-infection and the initiation of MQ treatment. Loss of premunition by the radical curative therapy was thought responsible for significantly greater parasitemias in children at the time of reinfection.[9]

Chemotherapy. Mefloquine hydrochloride (Lariam; Hoffman-LaRoche, Basel, Switzerland) uncoated tablets containing 250 mg base were used to prepare a sweet syrup suspension containing 40 mg MQ base/mL. Children were given 0.5 mL of suspension/kg body weight (20 mg base/kg) as a one-time oral dose administered by calibrated plastic syringe. Each child was monitored thereafter for 1 hour, and dosing was repeated if vomiting occurred. The 20-mg/kg body weight dose selected was a compromise between intention to reduce the likelihood of vomiting the dose in the young subjects and intention to affect a full rapid cure. Children unable to tolerate MQ were treated with Fansidar or quinine.

Follow-up. Field workers conducted visits three times weekly to monitor each child. Malaria blood smears were made on days 0, 2, 7, and 28 or at any occasion of illness consistent with malaria. Children who were symptomatic and parasitemic were brought to the hospital for evaluation by a physician. Children with unremitting or recurrent parasitemias during the MQ *in vivo* test were provided Fansidar or quinine treatment. Heparinized capillary tubes were used to collect 100 μL of whole blood on days 2 and 28 or any day of parasitemia that prompted alternative therapy. Capillary blood was blotted onto a Whatman no. 2 filter paper that was air-dried and thereafter kept refrigerated in separate plastic ziplock bags. In the absence of evidence suggesting poor compliance, emesis, or diarrhea at the time of dosing, parasitemias that persisted or recurred within the 7 test days after MQ treatment were classified according to World Health Organization (WHO) criteria as RII- or RIII-type resistance. Clearance followed by recurrence of parasitemia between days 7 and 28 of the test represented either RI-type resistance or re-infection.

Analysis of MQ levels in blood. Coded filter paper blood blots were sent to the Department of Clinical Chemistry, Falun Central Hospital, Falun, Sweden, where high-performance liquid chromatography (HPLC) was used to determine concentrations of MQ and its main metabolite, carboxymefloquine (CMQ).[21] Sensitivity of this assay was 95 ng/mL (0.25 μmol/L) for MQ and 75 ng/mL (0.20 μmol/L) for CMQ. It was reported that 5–10% degradation of MQ could be expected from our handling of the samples before extraction and HPLC. Based on confirmed parasitemia, patient's illness and dosing history, concentrations of MQ in blood at the time of parasitemia, and the threshold of 620 ng/mL (1.67 μmol/L) blood concentration of MQ considered to be an effective barrier against the appearance of sensitive strains of *P. falciparum* in the bloodstream,[7,22] a determination of sensitivity, resistance, or poor drug absorption was made.

Statistical analysis. Descriptive statistics were performed for baseline characteristics of the enrolled children and their parasitemias. Student *t* test was used to compare levels of MQ and CMQ in cured cases and those considered early or late treatment failures. χ^2 and Fisher exact tests were used to compare proportions. Simple linear regression analysis was used to explore the relationship between drug levels and vomiting or diarrhea. For graphing and statistical purposes, MQ and CMQ values below the limits of detection were arbitrarily assigned a value midway between that threshold value and zero.

RESULTS

A total of 193 children with uncomplicated, slide-confirmed falciparum malaria were evaluated and enrolled into the MQ *in vivo* test. No symptoms were reported or observed for 29

infants with parasitemias ranging from 40 to 26,000/μL. Among 164 infants with illness, fever was their dominant symptom, reported by 81% of mothers. Additional symptoms or conditions reported were chills/rigor (46%), diarrhea (39%), vomiting (38%), apathy/listless (26%), respiratory illness (6.7%), and altered mental state (2%). Two cases were excluded because of concomitant anti-malarial use, and five individuals were lost to follow-up. Enrollment characteristics of 186 children (Table 1) that were followed to an endpoint show that anemia (Hb < 8.0 g/dL), measured fever (> 37.5°C), and high-density parasitemia (> 20,000 asexual forms/μL) were conditions present in about one third of the children. Therapy was supervised and directly observed in 98.7% of the cases. Vomiting within the post-dosing observation period and re-dosing occurred in 18 (9.3%) children.

Parasitemias cleared by Day 2 in 51% (93/181) and by Day 7 in 92% (170/185) of treated cases. Among 15 cases classified as RII (9) and RIII (6) failures (Table 2) with persistent (13) or recurrent, (2) parasitemias observed on or before Day 7, records revealed histories of vomiting (3), diarrhea (2), or both (4), emesis of the dose (2), and unmonitored dosing (1). Geometric mean (GM) parasitemia for this group was 3,083/μL at the start of therapy and 349/μL on Day 7. Figure 1A shows that blood concentrations of MQ in these 15 cases ranged on Day 2 from below detectable level to 350 ng/mL, with a mean significantly below that of a random selection of "sensitive" cases (Table 3) that cleared and remained clear through the 28 day test period (RII/RIII: 172 ng/mL versus S: 561 ng/mL; $P < 0.0001$). Day 2 MQ and/or CMQ levels were below the limits of detection in eight cases. There was no apparent cause for early treatment failure among three children with observed compliance and no record of vomiting or diarrhea. Day 2 levels of MQ (210, 255, and 305 ng/mL) in these exceptions, while being among the highest in this group, were far below the lower 95% confidence limit of the drug in whole blood (467 ng/mL) from children who were cured by the treatment.

Late recurrent parasitemias ranging from 40 to 64,000/μL (GM, 10,560/μL) developed in 50 children (50/186 = 27%). Twelve of these cases were symptomatic and appeared between days 15 and 26. An additional 38 recurrent, but largely asymptomatic, RI-type parasitemias were detected in the scheduled Day 28 screen. MQ blood level analysis selected

TABLE 1
Characteristics of the study population at enrollment

Characteristic	Values
No. enrolled	186
Males:females	90:96
Mean age (mo)	15.51 (95% CI: 14.7–16.3)
Age range	6–25
Mean body weight (kg)	8.29 (95% CI: 8.1–8.5)
Mean hemoglobin (g/dL)	8.6 (95% CI: 8.3–8.8)
No. anemic (Hb < 8.0)	69 (37%)
Geometric mean (GM) parasite density/μL	3,363 (95% CI: 2,430–4,653)
No. febrile (%)	69 (37%)
GM parasite density of febrile cases	8,670 (95% CI: 5,433–13,836)
GM parasite density of non-febrile cases	2,138 (1,268–3,606)
No. parasitemias > 20,000/μL	52 (28%)

TABLE 2
Patient descriptions, symptoms at enrollment, follow-up parasitemia, parasitologic classification, and whole blood levels of MQ and CMQ metabolite in MQ-treated cases of falciparum malaria with RII/RIII parasitologic outcomes

Patient characteristics at enrollment					Symptoms before treatment				Parasite densities/μL			Drug and metabolite levels (ng/mL)				
ID#	Sex/age	Hb	Weight	Temp.	Fever	Vomiting	Diarrhea	Score*	D0	D2–6	D7	Class	D2-MQ	D2-CMQ	D28-MQ	D28-CMQ	Comments
ETF-1	M/19	9.1	6	36.1	N	N	Y	1	120	Negative	160	RII	47.5	125	47.5	37.5	Suspect incomplete dosing
ETF-2	M/7	14.9	6	36.1	N	Y	Y	3	1,200	1,640	320	RIII	47.5	37.5	47.5	37.5	Vomiting reported
ETF-3	F/22	10.5	12	37.1	Y	N	Y	3	30,960	22,120	120	RII	47.5	37.5	47.5	37.5	Vomiting reported
ETF-4	M/19	9.1	7	36.6	N	N	N	0	480	200	840	RII	95	37.5	47.5	37.5	Dose vomited
ETF-5	F/19	8.9	9	38.5	Y	Y	N	3	36,000	20,440	560	RII	130	37.5	47.5	37.5	Vomiting reported
ETF-6	F/11	9.3	8	38.4	Y	Y	Y	5	17,360	800	160	RII	140	37.5	47.5	37.5	Dose confirmed vomited
ETF-7	M/13	11.2	10	36.6	N	N	N	0	80	Negative	2,000	RIII	145	115	165	37.5	Dose vomited
ETF-8	F/10	6.6	9	35.1	Y	Y	N	3	18,000	1,600	160	RII	155	37.5	375	37.5	Vomiting reported
ETF-9	F/22	7.1	10	36.3	N	N	N	0	120	600	5,880	RIII	165	230	47.5	37.5	Unsupervised dose of MQ
ETF-10	F/21	6	8	36.3	Y	Y	N	3	13,200	2,200	240	RII	190	80	47.5	37.5	Vomiting reported
ETF-11	F/16	8.4	8	38.0	Y	N	N	2	12,400	2,160	120	RII	210	125	47.5	37.5	No apparent cause for failure
ETF-12	F/22	6.8	10	36.8	N	N	N	0	120	520	1,000	RIII	255	300	47.5	85	No apparent cause for failure
ETF-13	M/20	9.3	7	36.8	N	N	Y	1	16,680	400	400	RII	295	37.5	47.5	37.5	Mother reported diarrhea
ETF-14	M/19	6.6	8	38.0	Y	N	N	3	36,480	34,000	40	RIII	305	185	47.5	37.5	No apparent cause for failure
ETF-15	M/18	9.4	7	36.2	Y	Y	Y	5	7,840	76,000	D4 S/P	RIII	350	140	47.5	37.5	Vomiting reported
Mean		17.2	8.9	8.3	36.8	53%	40%	40%	2.1	3,083	2,632	349		171.8	104.2	77.2	40.7	
95% CI		2.4	1.1	0.8	0.5				0.9					48.7	41.6	44.4	6.21	

* Illness Score based on number of indivudal symptoms reported by the child's mother. Weight is given in kilograms, hemoglobin as grams per decaliter (g/dL), and age in months.

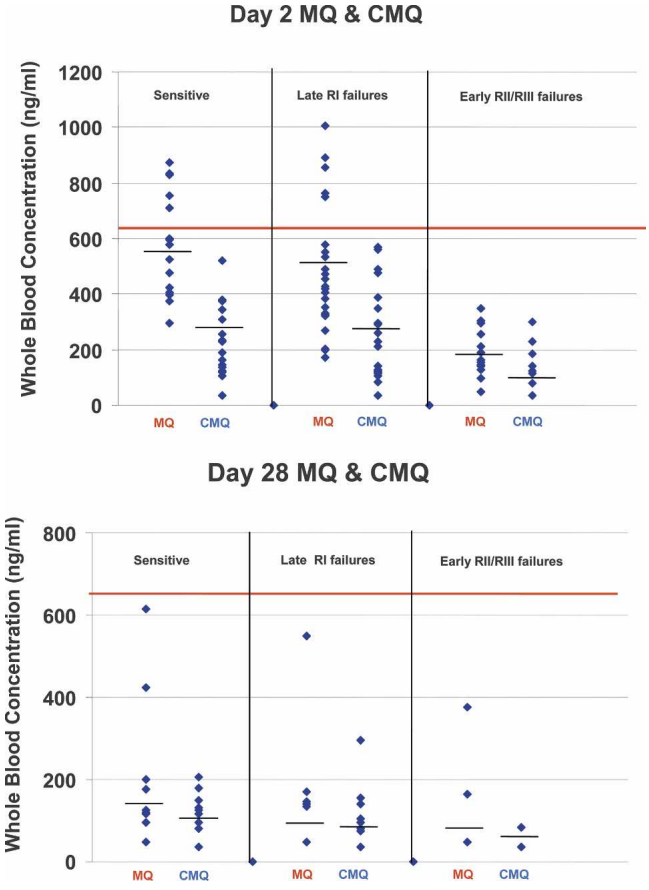

FIGURE 1. Scatterplot of whole blood concentrations determined for MQ and its carboxylic acid metabolite (CMQ) on days 2 (**A**) and 28 (**B**) after oral treatment with 20 mg/kg MQ base in young Ghanaian children whose responses to treatment were classified as sensitive (cure), delayed RI-type parasitologic failures, or RII/RIII early parasitologic failure. The solid line at 620 ng/mL designates the MQ blood concentration considered to be the threshold level required for suppression of sensitive *P. falciparum* infections. This figure appears in color at www.ajtmh.org.

the 12 symptomatic cases and 12 others with parasitemias > 6,680/μL (Table 4). Day 2 levels of MQ > 500 ng/mL characterized 33% of children with RI-type late recurrences compared with 44% of children who remained clear through 28 days. Mean Day 2 drug and metabolite levels in these late parasitemias were also lower, but not significantly different, from that of sensitive cases (MQ:, 467_{RI} versus 561_{S} ng/mL; $P = 0.28$; CMQ: 220_{RI} versus 229_{S} ng/mL; $P = 0.90$). Day 2 levels of MQ typically exceeded those of CMQ, averaging 2.6, 3.1, and 3.4 times higher in the RII/RIII, RI, and S groups, respectively.

Day 2 levels of MQ < 500 ng/mL and Day 2 levels of CMQ below the level of detection were both strongly associated with vomiting after dosing ($P < 0.0007$) and RII/RIII ($P < 0.001$) parasitologic responses. There was a stronger negative association on Day 2 between levels of CMQ and pretreatment vomiting ($r = -0.41$) than between MQ and vomiting ($r = -0.21$). We found no association between mother's report at baseline of diarrhea in her child and Day 2 levels of either MQ or CMQ. No association was seen between levels of MQ or CMQ and parasitemia, age, weight, or hemoglobin.

Across all outcome groups, Day 28 MQ levels (Figure 1B; Tables 2–4) were well below the putative boundary level of

TABLE 3

Patient descriptions, symptoms at enrollment, follow-up parasitemia, parasitologic classification, and whole blood levels of MQ and CMQ metabolite in MQ-treated cases of falciparum malaria that were cured

Patient characteristics at enrollment					Symptoms before treatment				Parasite densities/μL					Drug and metabolite levels (ng/mL)			
ID#	Sex/age	Hb	Weight	Temp.	Fever	Vomiting	Diarrhea	Score*	D0	D2–6	D7	D8–25	D26–28	Class	D2-MQ	D2-CMQ	D28-MQ	D28-CMQ
S-1	F/7	10.6	8	36.1	Y	N	N	1	320	Negative	Negative	Negative	Negative	S	295	135	47.5	37.5
S-2	F/18	9.1	9	35.5	N	N	N	1	10,040	80	Negative	ND	Negative	S	295	380	125	150
S-3	M/23	6.8	8	36.7	Y	N	Y	3	23,200	200	Negative	ND	Negative	S	375	120	47.5	125
S-4	M/6	8.3	6	37.2	N	N	Y	2	24,640	400	Negative	ND	Negative	S	395	125	95	115
S-5	M/24	10.4	11	36.1	Y	Y	Y	5	88,000	400	Negative	ND	Negative	S	405	105	47.5	80
S-6	F/15	10.4	8	37.9	Y	Y	N	3	8,840	80	Negative	ND	Negative	S	425	255	115	105
S-7	F/13	9.9	8	35.2	Y	Y	N	3	1,200	Negative	Negative	Negative	Negative	S	475	230	47.5	37.5
S-8	M/7	6.9	8	37.8	Y	Y	N	4	28,000	Negative	Negative	Negative	Negative	S	525	37.5	120	37.5
S-9	F/10	9.9	9	37.1	Y	N	N	2	21,840	40	Negative	ND	Negative	S	580	375	175	180
S-10	M/29	10.1	11	37.4	Y	N	N	1	3,880	Negative	Negative	ND	Negative	S	595	310	47.5	130
S-11	M/7	11.5	7	35.9	Y	Y	Y	3	880	Negative	Negative	ND	Negative	S	600	190	615	125
S-12	M/18	9.3	10	39.9	Y	N	Y	4	3,600	120	Negative	ND	Negative	S	710	145	425	115
S-13	F/16	8.8	7	36.3	Y	N	Y	3	4,960	Negative	Negative	ND	Negative	S	755	165	47.5	37.5
S-14	F/21	9.9	10	35.6	N	N	N	0	1,200	120	Negative	ND	Negative	S	830	345	200	95
S-15	M/18	11.4	12	38.9	Y	N	N	3	1,800	Negative	Negative	Negative	Negative	S	835	520	47.5	150
S-16	F/12	9.3	7	36.8	Y	Y	Y	3	840	Negative	Negative	Negative	Negative	S	875	235	175	37.5
Mean	15.25	9.5	8.7	36.9	81%	38%	44%	2.6	4915	136					560.6	229.5	148.6	103.6
95% CI	3.3829	0.7	0.8	0.6				0.6							95.0	62.7	77.5	26.8

* Illness Score based on number of individual symptoms reported by child's mother. Weight is given in kilograms, hemoglobin as grams per decaliter (g/dL), and age in months.

TABLE 4

Patient descriptions, symptoms at enrollment, follow-up parasitemia, parasitologic classification, and whole blood levels of MQ and CMQ metabolite in MQ-treated cases of falciparum malaria with delayed RI-type parasitologic outcomes

Patient characteristics at enrollment					Symptoms before treatment				Parasite densities/μL						Drug and metabolite levels (ng/mL)				
ID#	Sex/age	Hb	Weight	Temp.	Fever	Vomiting	Diarrhea	Score*	D0	D2–6	D7	D8–25	D26–28	Class	D2-MQ	D2-CMQ	D28-MQ	D28-CMQ	Comments
LTF-1	F/9	9.7	6	37.1	Y	N	N	1	400	Negative	Negative	200	13,600	RI	47.5	37.5	47.5	37.5	Vomiting reported
LTF-2	F/14	8.8	9	38.1	Y	Y	N	3	32,200	7,560	Negative	9,200	Negative	RI	170	37.5	47.5	37.5	
LTF-3	F/8	8.4	7	36.2	Y	Y	Y	4	200	Negative	Negative	560	Negative	RI	200	37.5	47.5	37.5	Vomiting reported
LTF-4	F/24	11.2	9	36.3	N	N	N	0	80	Negative	Negative	ND	34,400	RI	205	295	550	75	
LTF-5	M/16	8.1	10	35.9	Y	N	N	1	32,000	12,760	Negative	D15-28160		RI	270	130	47.5	105	
LTF-6	M/24	8.8	9	36.1	Y	Y	Y	3	23,600	80	Negative	5,200	120	RI	320	85	47.5	37.5	
LTF-7	M/18	7.3	7	36.6	Y	Y	Y	4	3,080	Negative	Negative	D16-7800		RI	325	105	47.5	37.5	
LTF-8	F/16	8.2	9	38.8	Y	Y	Y	4	128,000	6,400	Negative	960	80	RI	330	37.5	47.5	37.5	
LTF-9	F/18	7.9	8	37.5	Y	Y	N	3	10,800	Negative	Negative	ND	6,880	RI	355	115	145	37.5	
LTF-10	M/16	8.9	9	37.2	Y	N	Y	2	23,200	Negative	Negative	ND	8,400	RI	385	140	47.5	37.5	
LTF-11	F/12	8	6	37.9	Y	N	N	3	160	120	Negative	156,000	520	RI	405	120	47.5	75	Vomiting reported
LTF-12	M/22	9.7	7	34.9	N	N	N	0	22,000	160	Negative	ND	26,000	RI	420	260	47.5	140	Vomiting reported
LTF-13	M/24	9.5	9	36.5	Y	Y	N	2	14,000	640	Negative	5,920	3,440	RI	430	475	47.5	37.5	Vomiting reported
LTF-14	F/10	7.7	8	36.8	Y	N	N	2	76,000	240	Negative	ND	32,400	RI	455	115	170	37.5	
LTF-15	F/20	8.7	10	38.1	Y	Y	N	4	76,800	400	Negative	ND	6,680	RI	470	125	47.5	140	
LTF-16	F/13	5.6	7	37.4	Y	N	N	2	9,600	Negative	Negative	ND	20,280	RI	490	390	135	37.5	
LTF-17	F/22	10.3	9	38.5	Y	N	N	3	21,920	800	Negative	D23-24800		RI	535	210	47.5	37.5	
LTF-18	F/15	7.6	8	35.7	Y	N	N	2	24,400	Negative	Negative	ND	16,400	RI	550	560	47.5	80	
LTF-19	M/7	9.5	7	36.2	Y	Y	N	3	31,440	Negative	Negative	8,040	Negative	RI	580	85	47.5	37.5	
LTF-20	F/21	7.6	9	38.7	Y	N	N	2	3,400	Negative	Negative	ND	64,000	RI	750	490	47.5	155	
LTD-21	F/24	7.8	11	36.0	Y	N	Y	2	4,320	Negative	Negative	ND	6,720	RI	765	570	47.5	37.5	
LTF-22	M/23	9	7	36.2	Y	N	N	1	480	160	Negative	ND	36,800	RI	855	290	140	295	
LTF-23	M/6	8.7	7	38.9	Y	N	N	3	34,680	22,800	Negative	ND	15,280	RI	890	230	47.5	95	Vomiting reported
LTF-24	F/23	10.5	9	36.1	Y	N	Y	3	16,480	Negative	Negative	ND	9,000	RI	1,005	350	47.5	37.5	
Mean	16.9	8.6	8.2	37.0	92%	38%	29%	2.4	7,836	863		3,739	7,058		467.0	220.4	85.1	71.8	
95% CI	2.4	0.5	0.5	0.4				0.5							96.7	68.4	42.5	24.4	

* Illness Score based on number of individual symptoms reported by the child's mother. Weight is given in kilograms, hemoglobin as grams per decaliter (g/dL), and age in months.

620 ng/mL needed to suppress susceptible strains of *P. falciparum*. Drug and/or metabolite levels were below the limits of detection in 7 of the 16 "sensitive" cases and in 22 of the 24 late recurrent cases. Parent drug was more frequently detected in sensitive cases than in RI cases (S: 56% versus RI: 22%; $P = 0.02$), but mean MQ levels were not significantly different in the two groups. The CMQ metabolite was more often detected than MQ (40% versus 31%) and at concentrations exceeding MQ in 17 of 29 cases (mean ratio of CMQ to MQ: 1.4) where either parent drug or metabolite was measurable.

One case of drug tolerance was documented in which a parasitemia of 34,400/μL on Day 28 was observed against an MQ concentration of 550 ng/mL (1.46 μmol/L). With a conceivable 10% loss of MQ from this sample as a result of time and storage conditions, the whole blood concentration at the time of parasitemia may have been as high as 605 ng/mL, with plasma levels even higher.

DISCUSSION

Apart from their critical need for malaria care during this most vulnerable period in their lives, young children of 6–24 months of age are ideal for showing the effect of a drug, alone, against malaria parasites and yield a valid *in vivo* test outcome for drug resistance that is free from the confounding effects of maternally and naturally acquired immunity. Lower MQ failure rates in adults compared with young children have been attributed to immunity, owing to comparable Day 2 drug levels in the two groups.[3] Our findings in this select population of African infants yield no firm evidence of innate RII-RIII resistance in the parasites and indicate that low drug levels resulting from emesis, vomiting, poor absorption, and/or incomplete dosing account for all of the 15 RII/RIII parasitologic responses that occurred. Based on the low levels of MQ measured 48 hours after dosing in children who achieved better treatment outcomes, we believe that re-infection, and not late recrudescence, accounted for the majority of late recurrent parasitemias seen. In the absence of molecular evidence showing genotype differences between baseline and recurrent parasitemias, additional support for this supposition derives from the malaria incidence measured among these same children immediately preceding their MQ treatment. Assuming full compliance, normal absorption, and a normal decline by Day 10 after receiving treatment to MQ blood levels below the minimum inhibitory threshold of 620 ng/mL, there would have been 3,078 child-days (~8.4 child-years) of re-infection risk leading up to the Day 28 endpoint of our MQ *in vivo* test. From the calculated 4.7 infections per child-year determined during that dry season,[9] an expected 40 infections would have occurred in our children. The difference between the 50 late recurrent parasitemias observed and the 40 that were expected is presumed to relate to less than ideal absorption of the drug and reduced sensitivity to MQ in a fraction of the circulating *P. falciparum* strains.

Well before any use of MQ in Africa, *P. falciparum* strains from Ghana and Ivory Coast showed tolerance to the highest levels of MQ in the standard WHO *in vitro* micro-test.[23] *In vitro* test results from Senegal, Mali, Cameroon, and Nigeria also gave indication of reduced sensitivity to MQ in the absence of direct pressure resulting from the use of that drug in their populations.[6] However, among the *in vivo* trials that have followed, reasonable validation based on demonstration of parasitemia and high levels of MQ in blood has only been documented from Northern Cameroon.[5] Two separate trials of the 25-mg/kg dose in Nigerian children ranging from 6 months to 10 years of age reported 28-day cure rates of 93% and 95%, respectively, both of which were supported by *in vitro* measures of reduced MQ susceptibility, but neither were validated by MQ blood levels at the time of recurrence.[24,25] In Northern Cameroon, among children 1–10 years of age, 13% of cases showed persistent or rising parasitemias during the 7 days after MQ 25-mg/kg single dose treatment.[5] Based on Day 3 MQ levels > 500 nmol/L in these six children, RII-RIII resistance to MQ was considered proven. In the absence of prior MQ use in that area and supported by *in vitro* evidence, investigators hypothesized MQ resistance in Northern Cameroon to be a by-product of quinine resistance.[5]

Surprisingly, among Ghanaian children with good treatment outcomes, the 20-mg/kg MQ dose did not seem to be well absorbed, because Day 2 concentrations of drug > 500 ng/mL, a level associated with treatment success,[5,10,12,18,19] were measured in only 33% of the children. Mefloquine is rapidly absorbed, attains maximum blood levels within 24 hours of oral dosing, and concentrations of ~1,000 ng/mL are typically attained in older children and adults by the 25-mg/kg treatment dose.[26–28] Early treatment failures of MQ in Malawian children were strongly associated with MQ blood levels < 500 ng/mL on Day 2. These low Day 2 drug levels were in turn associated with vomiting, which occurred in 40% of children given a 15-mg/kg single dose and in 29% of those who received a 25-mg/kg single dose. Surprisingly, among children who did not vomit within 30 minutes of treatment, Day 2 blood levels of MQ were comparable in both groups, but 18% still had Day 2 MQ levels < 500 ng/mL. Because unremitting or recurrent parasitemias developed within the 28-day test period in comparable proportions of low (62%) and high (55%) dose groups, the authors refrained from any interpretation of MQ resistance and called attention to the problems of erratic drug absorption and vomiting in very young children.[19] In this regard, it seemed unusual that we saw so few RII/RIII-type MQ failures in Ghanaian children, and we suspect that parasitemias at that time and location were relatively susceptible to the drug.

To date, the MQ prophylaxis failures that were reported among adult Ghanaians have not been verified by drug levels and may not have occurred in all cases by MQ-resistant parasites. Based on the low blood levels of MQ seen in their children, wide inter-individual variation in MQ pharmacokinetics, low blood levels associated with lighter parasitemia, older age, and ethnic/genetic factors, it seems possible that the high failure rate of MQ prophylaxis in 6 of 46 Ghanaian adults also resulted from poor drug absorption and inadequate blood levels.

Mefloquine performed poorly in Ghanaian infants, an age group already prone to vomiting and diarrhea, and widespread use of this drug, alone, for treatment of malaria may exacerbate any existing low level resistance in the circulating malaria. The mean Day 2 blood level of MQ we measured in Ghanaian infants who tolerated MQ dosing well (mean: 501 ng/mL; 95% CI: 431–571; range: 170–1,005 ng/mL) was lower than that reported for Senegalese (955 ± 74 ng/mL) or Malawian (633 ± 343 ng/mL) children, and was grossly below

the Cmax reported for Thai children (2,031 ± 831 ng/mL).[3,16,18,19,28] In retrospect of the globally low drug levels achieved, we are also led to consider the possibility that our formulating MQ as a suspension in a high-fructose, pineapple-flavored syrup may somehow have altered the drug's chemistry and/or subsequent bioavailability.

The carboxylic acid metabolite of MQ is inactive against malaria but is deemed to have value in monitoring compliance during prophylaxis.[29] Our purpose in presenting CMQ data for MQ-treated Ghanaian infants is that of verifying drug consumption, providing an indication of the metabolism of parent MQ in this relatively under-studied group and showing its potential for predicting early treatment failures. Great individual variability is typically seen in blood levels of MQ and CMQ under both therapeutic and prophylactic regimens, but pregnancy, parasitemia, and age are known determinants of MQ absorption, distribution, and elimination.[26–28,30] Relevant to our findings in Ghanaian children are those from Thailand which found that children 6–24 months of age achieve peak levels, tissue distribution, and elimination of MQ more rapidly than older children and adults.[17] From this, assuming a 12-hour peak in MQ blood levels, such as that seen in young Thai children, our Day 2 measurement, 36 hours later, might be expected to show the heightened accumulation of CMQ over MQ in Ghanaian children.[31] This was not apparent, however, and even at Day 28, we did not see ratios of CMQ:MQ that approached those of 2–6 that have been reported for adults on weekly prophylaxis.[29,32] The stronger negative correlation we observed on Day 2 between CMQ levels and vomiting was noteworthy, and although our study was not intended or powered to examine the relationship between drug/metabolite levels and clinical condition of these children, no correlation was seen between diarrhea at baseline and blood levels of either MQ or CMQ measured on Day 2. Multiple studies have reported an association between diarrhea and low MQ drug levels in blood.[11–14,16,] Diarrhea, anemia, and vomiting as conditions before MQ dosing were not predictive of low Day 2 levels of MQ or treatment failure in our Ghanaian children, but vomiting after the MQ dosing observation period was clearly related to low drug levels and early treatment failure.

In summary, a single oral dose treatment of a 20-mg/kg MQ suspension to breast-feeding Ghanaian infants was well tolerated, but resulted in whole blood levels of the drug on Day 2 that were surprisingly low, even among those children that had tolerated the suspension without vomiting. Early RII/RIII-type parasitologic failures that occurred in 8% of the infants were associated with vomiting after dosing, Day 2 blood levels of MQ < 500 ng/mL, and undetectable Day 2 levels of CMQ. Vomiting and diarrhea before dosing were not associated with treatment failure. Despite no prior use of MQ for treatment at this location, one instance was found of reduced sensitivity by the parasite to this drug. Such early evidence does provide some rationale and credence for multiple cases of MQ prophylaxis failure that occurred at the same location 3 years later. Ghana thus joins a number of other African nations that pose a risk to travelers of MQ prophylaxis failure. We consider the collective results of this study and others to be a warning against further use and/or promotion of treatment by MQ alone for falciparum malaria in this high transmission area of northern Ghana.

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