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High-Dose Chloroquine for Treatment of Chloroquine-Resistant *Plasmodium falciparum* Malaria

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Background. Due to development of multidrug-resistant *Plasmodium falciparum* new antimalarial therapies are needed. In Guinea-Bissau, routinely used triple standard-dose chloroquine remained effective for decades despite the existence of "chloroquine-resistant" *P. falciparum*. This study aimed to determine the in vivo efficacy of higher chloroquine concentrations against *P. falciparum* with resistance-conferring genotypes.

Methods. Standard or double-dose chloroquine was given to 892 children aged <15 years with uncomplicated malaria during 3 clinical trials (2001–2008) with ≥35 days follow-up. The *P. falciparum* resistance–conferring genotype (*pfcrt* 76T) and day 7 chloroquine concentrations were determined. Data were divided into age groups (<5, 5–9, and 10–14 years) because concentrations increase with age when chloroquine is prescribed according to body weight.

Results. Adequate clinical and parasitological responses were 14%, 38%, and 39% after standard-dose and 66%, 84%, and 91% after double-dose chloroquine in children aged <5, 5–9, and 10–14 years, respectively, and infected with *P. falciparum* genotypes conferring chloroquine resistance (n = 195, $P < .001$). In parallel, median chloroquine concentrations were 471, 688, and 809 nmol/L for standard-dose and 1040, 1494, and 1585 nmol/L for double-dose chloroquine.

Conclusions. Chloroquine resistance is dose dependent and can be overcome by higher, still well-tolerated doses.

Keywords. *Plasmodium falciparum*; *pfcrt*; resistance; chloroquine; efficacy.

Owing to the development and spread of *Plasmodium falciparum* resistant to commonly available monotherapies, the World Health Organization (WHO) recommends artemisinin-based combination therapy for treatment of uncomplicated malaria [1]. However, *P. falciparum* are developing with reduced susceptibility to artemisinin derivatives as well as to partner drugs, highlighting the need for other treatment options [2].

Data from Guinea-Bissau, where triple standard doses of chloroquine were routinely used for decades to treat malaria, are therefore important [3]. In Guinea-Bissau, double-dose chloroquine divided into 2 daily doses for 3 days was as efficacious (≥95%) as artemisinin-based combination therapy for treating uncomplicated malaria in children aged <15 years as late as 2008 [4]. Moreover, doubling the dose doubled the efficacy of chloroquine against *P. falciparum,* with resistance-conferring genotypes [4, 5].

Furthermore, in vivo chloroquine concentrations decrease with decreasing age because young children are relatively underdosed when chloroquine is dosed according to body weight [6]. Thus, concentrations were similar in children aged 2 years given 50 mg/kg and those aged 10–14 years given 25 mg/kg (standard dose) [6]. The current study aimed to determine the correlation between chloroquine concentrations and treatment outcome in children infected with *P. falciparum* with resistance-conferring genotypes.

METHODS

Setting and Participants

Data were obtained from clinical trials conducted at 3 health centers within the Bandim Health and Demographic Surveillance Site in suburban Bissau, Guinea-Bissau. The trials are referred to as study 1 (2001–2004), study 2 (2004–2006), and study 3 (2006–2008) [4, 7, 8]. The study included children aged <15 years, resident within the surveillance site, with fever or a history of fever, monoinfection with *P. falciparum*, a parasite density ≥800 *P. falciparum*/μL, and no signs or symptoms of severe malaria. Patients were included in clinical studies after informed consent from their caretaker. Ethical approval was granted by the ethical review board in Bissau, Guinea-Bissau (Parecet NCP/N19/2006, 019/DHE/2004, and 064/DGSP/2006), the regional ethics committee in Stockholm, Sweden (2005/111-31/1, 2006/1151-31/1, 2011/832-32/2) and the central scientific ethics committee in Denmark (624-01-0042). All studies were registered at ClinicalTrials.gov (study IDs: PSB-2001-chl-amo: NCT00137514; PSB-2004-paracetamol: NCT 00137566; PSB-2006-coartem NCT00426439).

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Procedures

Giemsa-stained smears were made from finger-prick blood to identify species and quantify parasite density (per 200 white blood cells), using ×1000 magnification and a sunlit microscope. A slide was considered negative after examination of 100 high-power fields. In all studies, thick and thin films were examined before inclusion and then weekly until the end of the study and whenever medical attention was sought for a child owing to symptoms compatible with malaria.

The following treatments were randomly allocated: 25 or 50 mg/kg of chloroquine or 15 or 30 mg/kg of amodiaquine in study 1 (n = 729), 25 mg/kg of chloroquine with or without paracetamol in study 2 (n = 338), and artemether-lumefantrine or 50 mg/kg of chloroquine in study 3 (n = 378). Only children treated with chloroquine were included in the current study. Chloroquine-phosphate tablets (160 mg; chloroquine base, 100 mg) were donated by Recip, Stockholm, Sweden. Chloroquine base doses were 10 + 10 + 5 mg/kg (25 mg/kg) or 10 + 10, 10 + 10, 5 + 5 mg/kg (50 mg/kg) on days 0, 1 and 2, respectively. After treatment, children were visited weekly until the end of the study, day 35 (studies 1 and 2) or day 70 (study 3). Mothers were interviewed about any medication given since the last visit, and health workers assessed children's health. In addition, parents were asked to bring their child to the health center in case of illness.

We used WHO criteria to define early and late treatment failures. The primary end points for this study were polymerase chain reaction (PCR)–adjusted and unadjusted adequate clinical and parasitological response (ACPR) on day 35.

On day 0, weekly during follow-up, and whenever *P. falciparum* were detected, blood was put on filter papers (Whatman 3MM), dried, and then stored in individual plastic bags until DNA extraction. Blood samples were not collected from the first 200 children in study 1 because filter papers were not available at the beginning of that study. Approximately 25 μL of blood was cut from the filter papers and extracted on an Applied Biosystems Prism 6100 Nucleic Acid Prepstation, according to the manufacturer's protocol for isolation of DNA from whole blood with minor modifications. Extracted DNA was stored at −20°C until use.

Previously described multiplex PCR–restriction fragment length polymorphism methods were used to detect single-nucleotide polymorphisms K76T and N86Y in *P. falciparum* chloroquine resistance transporter (*pfcrt)* and multidrug resistance genes (*pfmdr1*), respectively [9]. Merozoite surface proteins 1 (*pfmsp1*) and 2 (*pfmsp2*) and glutamate-rich protein (*pfglurp*) were amplified using PCR according to WHO recommendations [10]. Recrudescent infections were defined as reappearance of ≥1 band from *pfmsp1, pfmsp2,* and *pfglurp* during follow-up or the reappearance of a band in the successfully amplified gene(s) if other PCR failed.

PCR and restriction products were resolved on 2% agarose gels (Amresco). All gels were stained with a nucleic acid gel stain (ethidium bromide; Biotium) and visualized under UV transillumination (GelDoc; Bio-Rad). Chloroquine and its metabolite desethylchloroquine concentrations were determined with high-performance liquid chromatography, as described elsewhere [11].

Statistical Analysis

Medians and 95% confidence intervals of drug concentrations, parasite densities and age were estimated and compared using quantile regression with bootstrapping (100 repeats). Median parasite densities over time were compared using a quantile regression, with day of follow-up as continuous covariate and the earliest time point as baseline. The proportions of children with parasites on days 0–3 were compared using Fisher exact test. Median ages over time were compared using quantile regression with year as continuous covariate and the earliest time point as baseline. Treatment outcomes were compared using the cumulative odds ratio (Maentel–Haenszel weighted odds ratio) of having ACPR in the groups treated with 25 versus 50 mg/kg. A multiple regression model to distinguish the importance of age (a proxy for immunity) versus drug concentrations was not done because malaria exposure (and therefore immunity) decreased over time. However, the increased ACPR in the age and drug dosage groups presented in Figure 1 was analyzed using logistic regression. Body surface area was estimated from body weight using the Boyd self-adjusting formula [12]. *P. falciparum* with mixed *pfcrt* K76T were considered as having *pfcrt* 76T only. The number of children with both chloroquine resistance–associated *pfcrt* 76T and *pfmdr1* 86Y alleles was too small to justify detailed analyses.

RESULTS

This study includes 521 children given 25 mg/kg and 371 given 50 mg/kg of chloroquine; *pfcrt* K76T alleles were identified in

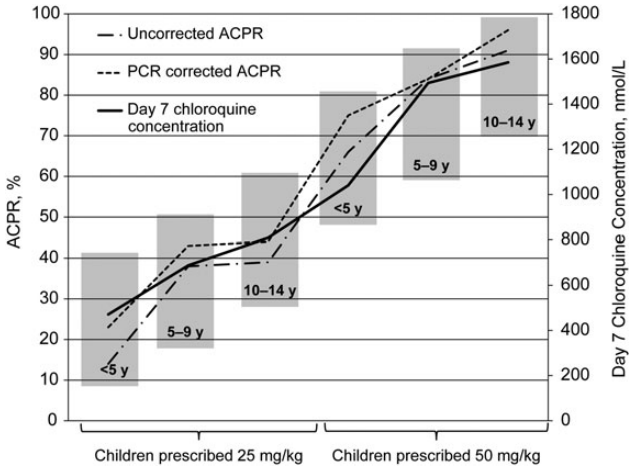

Figure 1. Polymerase chain reaction (PCR)–corrected and uncorrected adequate clinical and parasitological response (ACPR) and chloroquine concentrations among children aged <5, 5–9, or 10–14 years prescribed 25 or 50 mg/kg of chloroquine.

Table 1. Demographic Data, Parasite Density, and Chloroquine Concentrations by Genotype and Chloroquine Dose

	Genotype					
	All			*Pfcrt* 76T		*Pfcrt* K76
Chloroquine dose	25	50	25	50	25	50
No. of children	521	371	112	83	338	224
Age, median (95% CI), y	5.1 (4.7–5.4)	6 (5.5–6.5)	4.9 (4.2–5.6)	6.9 (5.2–8.6)	5.3 (4.9–5.6)	6.2 (5.4–7)
Male-female ratio	266:254	198:173	53:59	41:42	172:166	115:109
Parasite density, median (95% CI), *Plasmodium falciparum*/μL	18 400 (17 029–19 771)	18 400 (16 634–20 166)	19 200 (13 565–24 835)	20 202 (14 865–25 539)	19 200 (17 188–21 212)	18 400 (16 683–20 117)
Chloroquine concentrations						
No. of children	101	250	21	68	59	169
Chloroquine, median (95% CI), nmol/L	545 (466–624)	1331 (1215–1448)	516 (417–615)	1430 (1213–1647)	553 (404–702)	1259 (1136–1382)
Desethylchloroquine, median (95% CI), nmol/L	369 (285–452)	785 (709–861)	335 (247–423)	952 (821–1083)	369 (262–476)	725 (630–820)

Abbreviation: CI, confidence interval.

450 of the children prescribed 25 mg/kg and 307 of those prescribed 50 mg/kg. The discrepancy was due to filter papers not being available at the start of study 1 and to failed PCR (4%). Numbers, age, sex, parasite densities, and chloroquine and desethylchloroquine concentrations are shown in Table 1. The median age (95% confidence interval) was 5.1 (4.9–5.3) years in 2001–2007 and increased to 8.3 (7.1–9.4) years in 2008 ($P < .001$).

Efficacy of 25 Versus 50 mg/kg of Chloroquine for Treatment of Uncomplicated *P. falciparum* Malaria

The day 35 unadjusted and PCR-adjusted cumulative ACPR were 71% and 84% in children aged <15 years treated with 25 mg/kg of chloroquine versus 89% and 94% in those treated with 50 mg/kg (Table 2). No statistical comparison was done because the 2 groups were not fully comparable in terms of age and malaria exposure.

Efficacy of Chloroquine for Treatment of *P. falciparum* With the Chloroquine Resistance-Conferring *pfcrt* 76T Genotype

Figure 1 and Table 3 show PCR-corrected and PCR-uncorrected treatment outcomes, day 7 chloroquine concentrations, and the amount of chloroquine prescribed per square meter of body surface area. ACPR increased with increasing chloroquine concentrations and increasing amounts of chloroquine prescribed per square meter of body surface area, peaking at 91%–96% ACPR in children aged 9–14 years receiving 50 mg/kg. It also increased with increasing age when stratified by age groups (<5, 5–9, and 10–14 years) and chloroquine dose (25 or 50 mg/kg) ($P < 0.001$).

Parasite Clearance

Median parasite densities and the proportion of children with parasites on days 0–3 did not differ significantly by genotype or age group (Figure 2).

Factors Potentially Contributing to Treatment Failure When *pfcrt* 76T–Carrying Parasites Were Treated With 50 mg/kg of Chloroquine

The following were compared between children with ACPR and those with late treatment failure, reinfection, both after receipt

Table 2. PCR-Corrected and Uncorrected Treatment Outcomes With 25- or 50-mg/kg Chloroquine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Children Aged <15 Years (2001–2008)

	Chloroquine Dose Given	
Outcome	25 mg/kg	50 mg/kg
Children at day 0, No.	521	371
Day 35 ACPR, %		
Uncorrected	71	89
PCR corrected	84	94
Early treatment failure, No.	16	9
Late treatment failure, No.	56	10
Reinfections, No.	67	17
Lost to follow-up or withdrawn from study, No.	77	65

Abbreviations: ACPR, adequate clinical and parasitological response; PCR, polymerase chain reaction.

Table 3. PCR-Corrected and Uncorrected Treatment Outcomes With 25- or 50-mg/kg Chloroquine for the Treatment of Uncomplicated *Plasmodium falciparum* With the Chloroquine Resistance–Causing *pfcrt* 76T Genotype in Children Aged <15 Years Between (2001–2008)

	Chloroquine Dose					
Outcome	25 mg/kg			50 mg/kg		
Age group, y	<5	5–9	10–14	<5	5–9	10–14
No. at day 0	58	37	17	24	33	27
Day 35 ACPR, %						
Uncorrected	14%	38%	39%	66%	84%	91%
PCR corrected	23%	43%	44%	75%	84%	96%
Early treatment failure, No.[a]	5	0	0	1	0	0
Late treatment failure, No.[a]	29	18	8	4	5	1
Reinfections, No.	13	4	2	2	0	1
Lost to follow-up or withdrawn from study, No.	4	6	2	4	4	5
Median chloroquine concentration, median (95% CI), nmol/L	471 (404–537)	688 (491–885)	809 (570–1048)	1040 (928–1152)	1494 (1285–1703)	1585 (1309–1860)
Chloroquine dose prescribed, median (95% CI), mg/m^2	551 (533–568)	645 (630–660)	742 (723–760)	1047 (1033–1061)	1217 (1202–1233)	1408 (1376–1436)

Abbreviations: ACPR, adequate clinical and parasitological response; CI, confidence interval; PCR, polymerase chain reaction.

[a] This table includes only children with *pfcrt* 76T, accounting for the apparent discrepancy in numbers of treatment failures, especially early treatment failures, between this table and Table 2.

of 50 mg/kg of chloroquine: concentrations of chloroquine, desethylchloroquine, and chloroquine plus desethylchloroquine; the amount of chloroquine prescribed per square meter of body surface; age; and parasite densities (Table 4). No associations with treatment failure were seen.

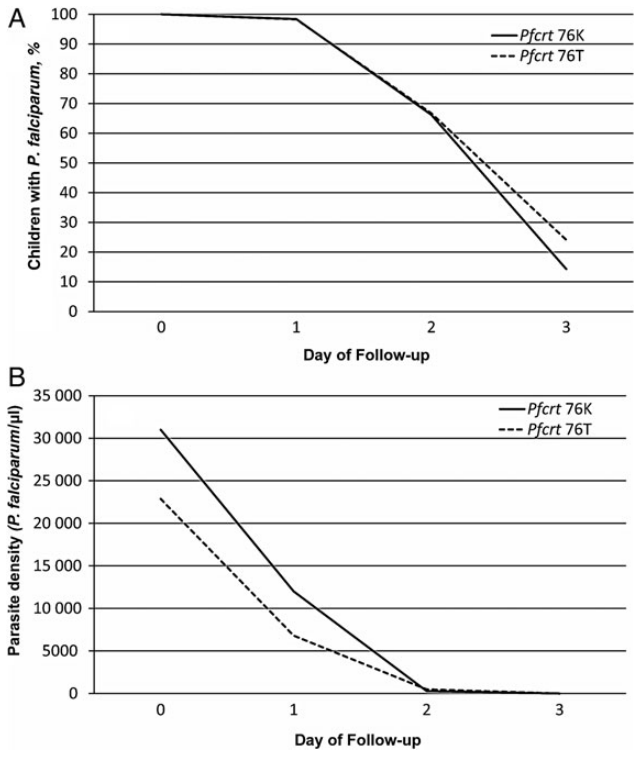

Figure 2. Parasite clearance on days 0–3 by *pfcrt* K76T allele after treatment with 50 mg/kg of chloroquine. *A*, Proportion of children with *Plasmodium falciparum* in the blood according to microscopy on days 0–3 after treatment with 50 mg/kg of chloroquine by *pfcrt* K76T allele. Data are pooled from studies conducted 2001–2004 and 2006–2008. *B*, Median parasite density on days 0–3 in children treated with 50 mg/kg of chloroquine by *pfcrt* K76T allele (2006–2008).

Among children treated with 50 mg/kg who had treatment failure, 3 were aged <2 years and 1 aged <3 years. The *P. falciparum* density was 133 320/µL in 1 child who was 5 years old and 97 561/µL in another who was 12 years old. The remaining 4 children were 5–8 years old and had *P. falciparum* densities <45 000/µL. All children were infected with *P. falciparum* that had the chloroquine resistance–causing *pfcrt* 76T. The chloroquine concentration was not determined in 1 child, which explains why there are 10 treatment failures listed here but only 9 in Table 4, where drug concentrations are presented.

Chloroquine Dose Needed to Eradicate *Plasmodium falciparum* with *pfcrt* 76T in Guinea-Bissau

Figure 3 shows the dose of chloroquine given per square meter of body surface area, the age of children, and the outcome after treatment with 25 or 50 mg/kg of chloroquine. Parasites were not seen after treatment with a total dose of ≥1500 mg/m^2. The highest prescribed amount in children with recrudescence and reinfection were 1364 and 1409 mg/m^2, respectively. These 2 children had day 7 chloroquine concentrations of 1815 and of 1814 nmol/L, respectively.

DISCUSSION

The linear correlation between ACPR and chloroquine concentrations that culminated in 91%–96% ACPR when *P. falciparum* with resistance-conferring *pfcrt* 76T genotypes were treated is remarkable. It indicates that chloroquine resistance is a relative phenomenon that can be overcome by higher but well-tolerated doses of chloroquine. It also indicates that higher doses of chloroquine can approximate the WHO-recommended 95% efficacy for new antimalarials when used to treat *P. falciparum* considered to be chloroquine resistant. Although ours is the

Table 4. Day 7 Chloroquine and Desethylchloroquine Concentrations, Day 0 Parasite Densities, and Age of Children With ACPR, Late Treatment Failure, or Reinfection After Treatment With 50 mg/kg

Finding	ACPR		Late Treatment Failure		Reinfection		Late Treatment Failure Plus Reinfection	
	No. of Children	Median Value (95% CI)	No. of Children	Median Value (95% CI)	No. of Children	Median Value (95% CI)	No. of Children	Median Value (95% CI)
Chloroquine concentration, nmol/L	197	1380 (1235–1525)	9	1273 (745–1801)	10	1090 (595–1585)	19	1170 (786–1554)
Desethylchloroquine concentration, nmol/L	195	801 (705–897)	8	809 (624–994)	10	647 (430–864)	18	760 (616–904)
Chloroquine plus desethylchloroquine concentration, nmol/L	195	2249 (2057–2442)	8	2300 (1761–2840)	10	1737 (1048–2426)	18	1954 (1309–2600)
Chloroquine dose prescribed, mg/m^2	270	1200 (1175–1225)	10	1185 (1076–1293)	17	1117 (997–1238)	27	1141 (1062–1221)
Age, y	270	6.5 (5.7–7.3)	10	5.1 (2–8.3)	17	3 (0.3–5.7)[a]	27	4.1 (1.9–6.3)
Parasite density, *Plasmodium falciparum*/μL	269	16 800 (13 644–19 956)	10	14 800 (0–49 421)	17	17 200 (14 368–20 032)	27	17 200 (11 491–22 909)

Abbreviations: ACPR, adequate clinical and parasitological response; CI, confidence interval.

[a] The median age of children with reinfection was lower than that of children with ACPR ($P = .005$).

Figure 3. Chloroquine dose needed to eradicate *Plasmodium falciparum* with *pfcrt* 76T in Guinea-Bissau.

first study to show this, the results are supported by a similar increase of ACPR with age and dose of chloroquine taken among Afghan refugees receiving 25 mg/kg over 3 days or 40 mg/kg over 5 days for the treatment of *pfcrt* 76T–carrying *P. falciparum* [13].

Not assessing malaria-specific immunity is a limitation of this study, because improved ACPR with age could be an effect of immunity. However, if immunity was of significant importance, decreased efficacy would be expected in the <5-year age group receiving 50 mg/kg compared with the 10–15-year age group receiving 25 mg/kg. Instead, ACPR increased from 39%–44% to 66%–75%, indicating that drug concentrations are of paramount importance. The study is also limited because it is a post hoc analysis of a subset of children included into randomized trials. Theoretically, there could be genetic changes that reverse chloroquine resistance while maintaining the *pfcrt* 76T genotype, as seen in French Guiana [14]. However, if this was prevalent, no dose response would be seen, and the 25-mg/kg dose should have been efficacious.

Chloroquine resistance is primarily mediated by mutations in *pfcrt* that enable transport of chloroquine out of the parasite digestive vacuole [15–17]. This transport can be saturated, and *pfcrt* already operates near its maximum capacity at the drug concentrations obtained with 25 mg/kg [18]. Increasing the dose thus enables toxic concentrations of chloroquine to accumulate inside the digestive vacuole, resulting in parasite death by disrupting heme metabolism and possibly by inducing programmed cell death [19].

It follows that chloroquine concentrations must be maintained if the *pfcrt*-mediated transport is to be continuously interrupted. In line with this, in vitro data indicate that *P. falciparum* clearance is dependent on time over minimal inhibitory concentration (MIC) rather than peak concentrations [20]. However, chloroquine concentrations rapidly decrease

primarily due to redistribution soon after oral intake, and it was estimated that concentrations in adults were subtherapeutic for approximately 12 hours during the first 24 hours after a single 10-mg/kg dose [21]. A loading dose followed by smaller 12 hourly doses has been suggested to overcome this [21]. The multiple daily dosing employed in Guinea-Bissau was thus probably optimal. The correlation between day 7 concentrations and the considerable improvement of ACPR in children receiving 2 daily doses instead of a single dose are also in line with this.

It is not known how long concentrations above the MIC must be maintained. However, it is probable that a total dose of 1500 mg/m^2 body surface area given as split daily doses will result in concentrations that are sufficient to ensure ACPR. This equates to an increase from 25 to approximately 40 mg/kg for a 70-kg adult. However, for a 10-kg child (approximately 2 years old) this equates to an increase from 25 to approximately 75 mg/kg. At first sight, this is alarming given the potential toxicity of chloroquine, but children are underdosed and higher doses are necessary to attain similar drug concentrations to those found in adults [6] Interestingly, approximately 75 mg/kg was the total dose routinely taken as divided doses over 5 days in Guinea-Bissau [3, 22]. These are only approximations that need to be assessed in pharmacokinetic and safety studies. It is also probable that lower total doses can achieve the same concentrations if a slow-release formulation of chloroquine is used.

A slow-release formulation would be the most obvious way to maintain concentrations above MIC for *pfcrt* 76T–carrying *P. falciparum*. The in vivo MIC that must be maintained by a slow-release preparation to eliminate *P. falciparum* is beyond this study. However, average peak whole-blood concentrations when adults took a single approximately 10-mg/kg dose were approximately 2000 nmol/L [21, 23]. Furthermore, parasite densities decreased equally rapidly irrespective of treatment and *pfcrt* K76T allele, even in the youngest children. Importantly, parasite densities decreased considerably between day 0 and day 1. Thus, the peak concentrations attained on day 0, even in young children, are sufficient to result in concentrations of chloroquine that are lethal to *pfcrt* 76T–carrying *P. falciparum*. It should therefore at least not be necessary to exceed 2000 nmol/L in a slow-release preparation to achieve concentrations that will eliminate *P. falciparum* with *pfcrt* 76T in Guinea-Bissau.

Acute toxicity seems to be due to the vasodilatory effects and negative inotropism of chloroquine linked to peak plasma concentrations that are avoided by dividing large total doses into multiple smaller daily doses [21]. In line with this, approximately 77 mg/kg divided into 2–3 doses per day for 5 days and 50 mg/kg divided into 2 daily doses for 3 days were well tolerated [3, 4, 7]. Furthermore, 100-mg/kg given as 10 plus 10 mg/kg for 5 days has been used to treat 5–15-year-old children with Giardia [24]. In the past, 15-mg/kg single daily doses for 3 weeks were given to children with amoebic liver abscess [25]. Thus, even substantially higher total doses than those used in Guinea-Bissau can be well tolerated.

Despite the linear correlation between ACPR and chloroquine concentrations, we did not find significantly lower drug concentrations in children with treatment failure compared with ACPR after receiving 50 mg/kg. The number of children with treatment failure was low (n = 10), and their ages, parasite densities, and varied drug concentrations probably account for the lack of difference.

Chloroquine resistance was imported to Africa from Southeast Asia during the late 1970s and then spread throughout the continent [26]. In Guinea-Bissau and the rest of Africa, the *pfcrt* 72–76 haplotype CVIET is predominant and seems to be the primary determinant of resistance [26]. CVIET has been shown to be among the most efficient chloroquine transporters [18]. It therefore seems highly probable that higher concentrations can overcome this mechanism of resistance, irrespective of the origin of *P. falciparum*. In line with this, a total 40-mg/kg dose over 5 days increased the efficacy of chloroquine from 16% to 49% in Afghan refugees despite suboptimal daily dosing [13]. However, regional variations in other resistance-associated genes may further decrease *P. falciparum* chloroquine susceptibility [27]. Additional high-dose chloroquine studies would therefore be of great value.

The prevalence of chloroquine-resistant parasites has decreased in multiple African settings after the withdrawal of chloroquine. The possibility of reintroducing chloroquine has therefore been raised. However, *pfcrt* 76T prevalence can increase from approximately 25% to approximately 100% over a transmission season [28]. Reintroducing 25 mg/kg is therefore likely to rapidly select *pfcrt* 76T resulting in increased treatment failures. However, the prevalence of *pfcrt* 76T was stable at <25% when approximately triple standard-dose chloroquine was routinely used in Guinea-Bissau, indicating that *pfcrt* 76T–carrying parasites were disadvantaged compared with chloroquine-sensitive ones [29]. Because 76T is essential for resistance, this suggests that high-dose chloroquine represents an exceptional hurdle for the development of resistance, probably due to a loss of parasite fitness [28, 29]. Thus, it is essential to reconsider how chloroquine is dosed before considering reintroduction. This study also has implication for potential drug combinations that include chloroquine, such as chloroquine plus azithromycin, which is currently being evaluated [30].

To conclude, we found a linear correlation between increasing chloroquine concentrations and increasing ACPR, culminating in 91%–96% ACPR when chloroquine was used to treat *P. falciparum* with resistance-conferring mutations. The 91%–96% efficacy indicates that high-dose chloroquine can fulfill WHO efficacy criteria. For pharmacodynamic, pharmacokinetic, and safety reasons higher doses should optimally be given as slow-release preparations, and further studies are needed.

Notes

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