

Quinine Pharmacokinetics and Pharmacodynamics in Children with Malaria Caused by *Plasmodium falciparum*

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The aim of the present study was to assess the pharmacokinetics and the efficacy of a shorter than usual 5-day quinine treatment given orally to children in Cameroon with malaria caused by *Plasmodium falciparum*. Quinine (8.3 mg of base per kg of body weight every 8 h) was administered as a 2% formiate salt syrup for 5 days to 30 children (age range, 0.55 to 6.7 years) with uncomplicated falciparum malaria (initial parasitemia, 1.4×10^3 to $1.8 \times 10^5/\mu\text{l}$). Quinine concentrations in plasma samples (five to nine per patient) were measured by liquid chromatography on days 1 to 3. Parasitemia was counted on days 0, 1, 2, 3, 4, 7, and 14. Pharmacokinetic and pharmacodynamic data were analyzed by population approaches by using NONMEM and WinBugs, respectively. The kinetics of quinine were best described by a one-compartment model with time-varying protein binding. Clearance and the volume of distribution were positively correlated with body weight and increased over time. Parasitemia was undetectable from day 3 to 14 in all children. The time to a 4-log reduction of the initial level of parasitemia (T_{er}) was related to the average quinine concentration from 0 to 72 h (C_{av}) as $T_{\text{er}} = T_{\text{min}} [1 + (C_{50}/C_{\text{av}})^s]$, where sigmoidicity (s) is equal to 2, T_{min} is the time to eradication at infinite C_{av} , and C_{50} is the value of C_{av} for which T_{er} is twice T_{min} . The C_{50} distribution was unimodal, and all C_{50} values were less than 8 mg/liter, while C_{av} ranged from 5.9 to 18.3 mg/liter. The median (10th to 90th percentile) T_{er} was 47 h (range, 39 to 76 h). The efficacy of a 5-day treatment course should be evaluated in a larger clinical trial.

Quinine is still widely used for the treatment of malaria in areas with multidrug-resistant strains. Decreasing sensitivity to quinine has been detected in areas of Southeast Asia (1a), but strains of *Plasmodium falciparum* from Africa are generally highly sensitive to quinine. For children with uncomplicated malaria in areas where parasites remain sensitive, the World Health Organization recommendation is to treat patients with an oral regimen (8 mg/kg of body weight three times per day) for 7 days (9). However, compliance may be poor when children are discharged from hospital. Since compliance is usually better during the first days of treatment, a 5-day treatment course would be more practical, at least for children in Africa. However, the kinetics of quinine and the therapeutic response to quinine may vary with drug formulation, age, race, immunity, the severity of disease, and the sensitivity of the strain (7). Also, the pharmacokinetics (PK) of quinine are known to vary as patients recover from malaria, with an expansion of the volume of distribution and an increase in systemic clearance resulting in a decline of the average concentration of quinine in plasma (8).

In the study described in the present report, the pharmacokinetics of quinine and the relationship of quinine exposure to the therapeutic response were examined in children in Cam-

eroon with uncomplicated malaria caused by *P. falciparum* receiving a 5-day course of oral quinine.

MATERIALS AND METHODS

Patients. The criteria for inclusion in the present study were as follows: age 0.5 to 6 years, a diagnosis of uncomplicated *Plasmodium falciparum* malaria by blood examination, an infrequent occurrence of vomiting, administration of the first dose of quinine within 14 h of the diagnosis, and the provision of written informed consent by the parents. In addition, the expected stay in hospital had to be at least 5 days in order to ensure regular alimentation, close monitoring, and compliance with the study protocol. Patients were not included if they had heart failure, cerebral malaria, a plasma creatinine level higher than the age-adjusted mean + 2 standard deviations (SDs), or transaminase levels greater than twice the normal value or if they had received antimalarial drugs, enzyme inducers, or inhibitors in the preceding month. These drugs were also not allowed during the study (5 days). Patients were excluded if vomiting occurred within 3 h after any quinine administration.

Study design. The study was monocentric, prospective, open, and noncomparative. The protocol was approved by the Ethics Committee of Cochin Hospital, Paris, France. The clinical part of the study was conducted in the Pediatric Unit of Yaoundé Central Hospital, Yaoundé, Cameroon. The number of patients to be enrolled was 30. This sample size was chosen to ensure precise estimates of the pharmacokinetic parameters. After selection for inclusion in the study, the patients received quinine (8.3 mg/kg, expressed as quinine base, every 8 h) as a formiate salt syrup for 5 days (15 administrations). The precisely measured dose was administered in the mouth of the child by using a syringe.

Clinical assessment. Fever (temperature, $>38^\circ\text{C}$), cough, vomiting, and inappetence were recorded every day for 5 days and then on days 7 and 14. Side effects were recorded at the same times.

Concentration measurements. Nine blood samples per patient were obtained by venous puncture at time zero and 1, 2, 3, 4, 8, 24, 48, and 56 h after the onset of treatment. The blood samples were collected in heparinized tubes. Following centrifugation, all plasma samples were stored at -20°C until analysis. The plasma quinine concentrations in all samples were determined by liquid chro-

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TABLE 1. Characteristics of the prior distributions for the Bayesian analysis of the pharmacodynamic model

Parameter	Characteristic ^a			
Stage 2	$T_{\min} \sim \text{LN}(\mu_{\log}, \sigma_{\log}^2)$		$C_{50} \sim \text{LN}(\mu_{\log}, \sigma_{\log}^2)$	
Stage 3: prior distribution	$\mu_{\log} \sim \text{LN}()$	$1/\sigma_{\log}^2 \sim G()$	$\mu_{\log} \sim \text{LN}()$	$1/\sigma_{\log}^2 \sim G()$
Mean of prior distribution	36 h	1	6 mg/liter	1
CV of prior (%)	50	100	50	100

^a \sim , distributed as; LN, lognormal distribution; G, gamma distribution; μ_{\log} and σ_{\log}^2 , the mean and the variance of the log-transformed parameters, respectively.

matography with fluorescence detection after liquid-liquid extraction. Three quality controls (2, 6, and 8 mg/liter) were used with each series. The interday coefficients of variation (CVs) for the controls were less than 10%, and their bias was less than 5%. The limit of quantification was 1 mg/liter.

Pharmacodynamic (PD) measurements. Blood samples were collected in EDTA-containing tubes at time zero; 12, 24, 48, 72, and 96 h; and days 7 and 14. Thin smears were prepared from each blood sample (three slides per sample), the slides were stained with Giemsa stain, and the parasitized erythrocytes were counted. Counting was done in microscopic fields containing approximately 200 erythrocytes, and the level of parasitemia was expressed as the number of parasites per 100 erythrocytes. A negative smear was defined as one in which no asexual form was seen in 100 microscopic fields.

Pharmacokinetic analysis. The data were analyzed by a population approach. The basic model was a one-compartment open model with first-order absorption and elimination rates. The pharmacokinetic parameters were the absorption constant (K_a), the apparent volume of distribution (V/F), and the apparent elimination clearance (CL/F). The last two parameters are known to increase with time during the first days of treatment, owing to the decrease of the concentration of α 1-glycoprotein acid, the binding protein of quinine in plasma, which results in an increasing unbound fraction (f_u) (5). To account for time-varying protein binding, f_u was assumed to increase linearly with time (t) from 0 to 72 h, according to the equation $f_u = b \cdot (t - 36) + 0.15$, where b is a slope parameter, and the intercept (0.15) is the typical value of f_u at 36 h (4). The median values of V/F and CL/F were assumed to be related to the child's body weight (BW). Hence, the model for the median parameters CL/F and V/F was $CL/F = f_u(\theta_1 + \theta_5 \cdot \text{BW})$ and $V/F = f_u(\theta_2 + \theta_6 \cdot \text{BW})$, where the θ 's are fixed effects. The individual parameters (CL/F , V/F , K_a , and b) were assumed to arise from a multivariate lognormal distribution, with the median and variance-covariance to be estimated. The residual error model (i.e., the model for the discrepancies between the observed and the predicted concentrations [C_{obs} and C_{pred} , respectively]) was $C_{\text{obs}} = C_{\text{pred}} \cdot \exp(\epsilon)$, where ϵ is a random variable with a normal distribution, zero mean, and variance to be estimated. With this model, the CV of the residual error is approximately constant and is equal to the SD of ϵ .

Since the pharmacokinetic model was nonlinear, it was implemented as a set of differential equations. The area under the predicted concentration-versus-

time curve (AUC) was calculated by numerical integration from 0 to 72 h. The average concentration (C_{av}) was estimated as $\text{AUC}/72$.

Parameters were estimated by using NONMEM with the first-order conditional estimation method (1). Hypothesis testing (e.g., comparison of alternative models) was based on the likelihood ratio test. The level of significance was 0.01. Goodness of fit was assessed by visual examination of various residual scatterplots.

Pharmacodynamic model. In *P. falciparum* malaria, parasitemia is known to exhibit cyclic fluctuations due to the sequestration of mature parasites (6). However, owing to the sparse sampling schedule for parasitemia measurements, it was not possible to describe precisely the effect of quinine on the kinetics of parasitemia. The effect of quinine was measured by determination of the time required for a 10^4 -fold reduction in the initial level of parasitemia (T_{er}). The factor of 10^4 was chosen because it was of the order of the initial parasitemia. T_{er} was estimated for each patient as $4/k$, where k is the slope of the regression line of \log_{10} parasitemia versus time during the first 3 days of treatment. For antibacterial agents, k is usually related to the concentration of the drug by a Hill model. Hence, T_{er} was expected to be related to quinine exposure (e.g., C_{av}) by an inverse Hill model, such as $T_{\text{er}} = T_{\min} \cdot [1 + (C_{50}/C_{\text{av}})^s]$, where T_{\min} is the time to eradication at infinite C_{av} , C_{50} is the value of C_{av} for which T_{er} is twice T_{\min} , and s is a sigmoidicity coefficient. The parameters of this model cannot be estimated with reasonable precision because three parameters must be determined but only one T_{er} value is measured per individual. Therefore, a Bayesian approach (2) was applied to the following three-stage hierarchical model. The independent variable was C_{av} . Individual values of C_{av} were the post hoc estimates obtained from the population model for pharmacokinetics. At the first stage, the T_{er} observations are assumed to arise from a normal distribution with the mean equal to the predicted value of T_{er} . At the second stage, the distribution of T_{\min} and C_{50} are assumed to be lognormal, with the moments (mean and variance) to be estimated. At the third stage, the moments of these lognormal distributions are given a prior distribution: a lognormal prior distribution for the mean and a gamma prior distribution for the precision (i.e., the inverse of variance). These prior distributions were moderately informative, as shown in Table 1. Since parasitemia was undetectable at 72 h in all children, the prior distribution of the mean T_{\min} was centered on 72/2, which is equal to 36 h. The prior distribution of the mean C_{50} was centered on 6 mg/liter, which is the lower

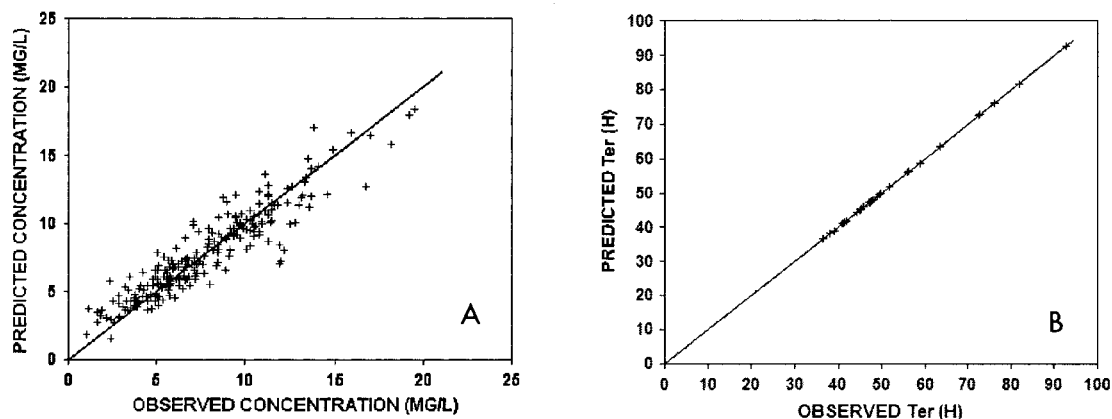


FIG. 1. (A) Predicted versus observed quinine concentrations in plasma; (B) predicted versus observed time to 4-log reduction of *Plasmodium falciparum* initial parasitemia. The lines of identity are shown indicated.

TABLE 2. Values of the parameters of the population model for quinine pharmacokinetics

Parameter	Point estimate (SE)	Interindividual CV (%)
Clearance parameters, ^a θ_1 (liter/h), θ_5 (liters/h/kg)	0 (fixed), 0.53 (0.05)	CL/F, 33 ^b
Volume parameters, ^a θ_2 (liters), θ_6 (liters/kg)	57 (28), 3.8 (2.0)	V/F, 32 ^b
K_a (1/h)	0.934 (0.244)	113
b (1/h)	0.001 (fixed)	37
Variance (ϵ)	0.048 (0.011)	

^a See text for definitions. θ_1 and θ_5 are related to unbound drug clearance; θ_2 and θ_6 are related to unbound volume of distribution. A free fraction of 0.15 is assumed.

^b The correlation coefficient between CL/F and V/F was 0.64.

bound of the therapeutic range for quinine when it is administered by continuous infusion (3). The coefficient of sigmoidicity, s , was fixed at either 1, 2, 3, or 4. The posterior distributions of the mean and the CV of T_{min} and C_{50} were obtained by Monte Carlo Markov chain simulation by using WinBugs 1.4 (6a). Convergence was assessed by checking the stability of the posterior distributions. Goodness of fit was assessed by visual examination of the residual scatterplots and the posterior distributions. In particular, a multimodal posterior distribution was considered indicative of a conflict between the prior distribution and the data. Sensitivity to assumptions about the prior distribution was determined by fitting the model with different assumptions.

A graph of the relationship between T_{er} and C_{av} was obtained from a population simulation of the PD model. Individual profiles of the T_{er} versus C_{av} relationship were generated for 200 fictive individuals by drawing samples of T_{min} and C_{50} in their posterior distribution. These 200 profiles were summarized by drawing the curve for the mean value of T_{min} and C_{50} and plotting the SDs of the 200 values of T_{er} for C_{av} equal to 6, 10, and 13 mg/liter.

RESULTS AND DISCUSSION

Patient characteristics. A total of 30 children (17 boys, 13 girls) were enrolled in the study. Their characteristics were as follows (means \pm SDs): age, 2.8 ± 1.7 years (age range; 0.55 to 6.7 years); body weight, 13.6 ± 3.8 kg; erythrocyte count, $(3.44$

TABLE 3. Characteristics of the posterior distributions for the Bayesian analysis of the pharmacodynamic model

Parameter	Posterior distribution		
	Mean	5th percentile	95th percentile
Mean T_{min} (h)	35	32	38
Interindividual CV T_{min} (%)	45	33	59
Mean C_{50} (mg/liter)	6.6	4.3	9.2
Interindividual CV C_{50} (%)	67	42	107

$\pm 0.88) \times 10^6/\mu\text{l}$; white blood cell count, $10,000 \pm 5,200/\mu\text{l}$; and creatinemia 5.8 ± 1.9 mg/liter. The median initial parasitemia was $16,500/\mu\text{l}$ (range, 1,404 to $176,000/\mu\text{l}$).

Clinical assessment. The follow-up rate was 100%. The numbers of patients exhibiting a clinical sign at day 5 and the numbers of patients exhibiting this sign at inclusion were 1 and 28, respectively, for fever; 2 and 11, respectively, for cough; 0 and 9, respectively, for vomiting; and 1 and 25, respectively, for inappetence. Vomiting had ceased in all patients by day 3. Fever, cough, and inappetence had disappeared in all patients by day 7. No side effect related to quinine treatment was observed.

Pharmacokinetics of quinine. The final model for quinine pharmacokinetics is the model described in Materials and Methods. The fit of this model was significantly better than that of reduced models with no covariate (i.e., models that do not account for variation of CL or V with body weight: $\theta_5 = 0$ or $\theta_6 = 0$) or reduced models with time-independent protein binding ($b = 0$). In particular, the latter reduced model was unable to fit the final minimum plasma concentrations. The interindividual variability of CL/F and V/F was better correlated with body weight than with age. Hence, it is more appropriate to adjust the quinine dose with respect to body weight than with respect to age, at least among children in this age range. The intercept of the clearance model (θ_1) was not significantly

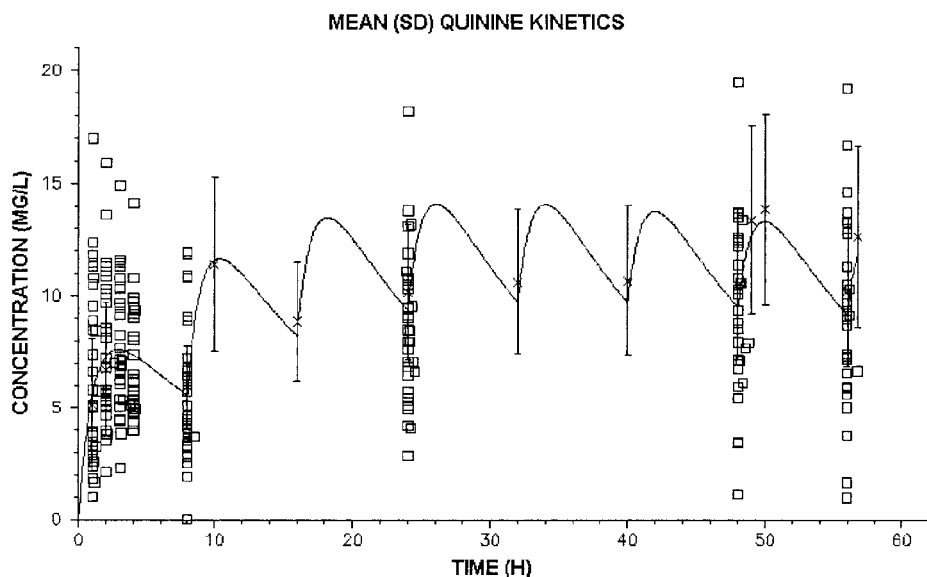


FIG. 2. Kinetics of mean plasma quinine concentration. The dose is 8.3 mg/kg every 8 h. The line is the curve yielded by the mean parameters. The crosses are the mean predictions. The vertical lines represent ± 1 SD. The squares are the observed concentrations for the 30 children.

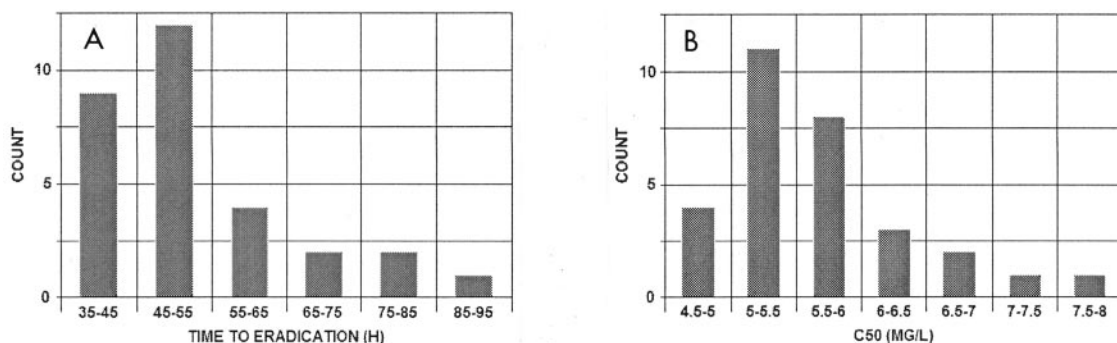


FIG. 3. (A) Histogram of the observed time to a 4-log reduction of *Plasmodium falciparum* initial parasitemia; (B) histogram of the average concentration of quinine in plasma yielding half the maximal antimicrobial effect.

different from 0; therefore, it was fixed to 0. Since the unbound fraction of quinine had not been measured, the typical value of b , the slope parameter for f_u , could not be estimated and was fixed to 0.001/h to be consistent with the available data (but interindividual variability of b was allowed). With this value, the typical value of f_u increased from 0.114 at the onset of treatment to 0.186 at 72 h. For a 15-kg child, the typical values of quinine CL/F and V/F increased during the same period from 0.91 to 1.48 liters/h and from 13 to 21 liters, respectively. These values are similar to those obtained by White et al. (8) in adults with uncomplicated malaria caused by *P. falciparum* (CL is 1.21 liters/h and V is 25 liters for a 15-kg child). By contrast, the typical elimination half-life is independent of time; for a 15-kg child it is 9.1 h. The plot of predicted concentrations (based on the post hoc estimates of the parameters) versus observed concentrations shows random scatter (Fig. 1A). The estimates of the parameters of the final model

are shown in Table 2. The CV of the residual error was 22%. Figure 2 shows the mean curve of quinine kinetics and the interindividual variability superimposed on the observed data.

Pharmacodynamics of quinine. Parasitemia was undetectable at 72 h in all children, and no rebound was observed on day 7 or 14. The distribution of T_{er} is shown in Fig. 3A. T_{er} was longer than 72 h for four children, for which the decline of parasitemia was not log-linear. The median (10th to 90th percentile) T_{er} was 47 h (range, 39 to 76 h). Hence, the median parasite reduction rate, i.e., the ratio of the initial parasitemia to the parasitemia at 48 h (7), was 10^4 . The median T_{er} was shorter than the typical parasite clearance time observed in adults from Thailand: 71 h (3) and 73 h (4). Part of this discrepancy may be due to the higher sensitivities of the strains (1a) or to the effect of acquired immunity (7) or race. The characteristics of the posterior distribution of the parameters of the pharmacodynamic model are described in Table 3. The

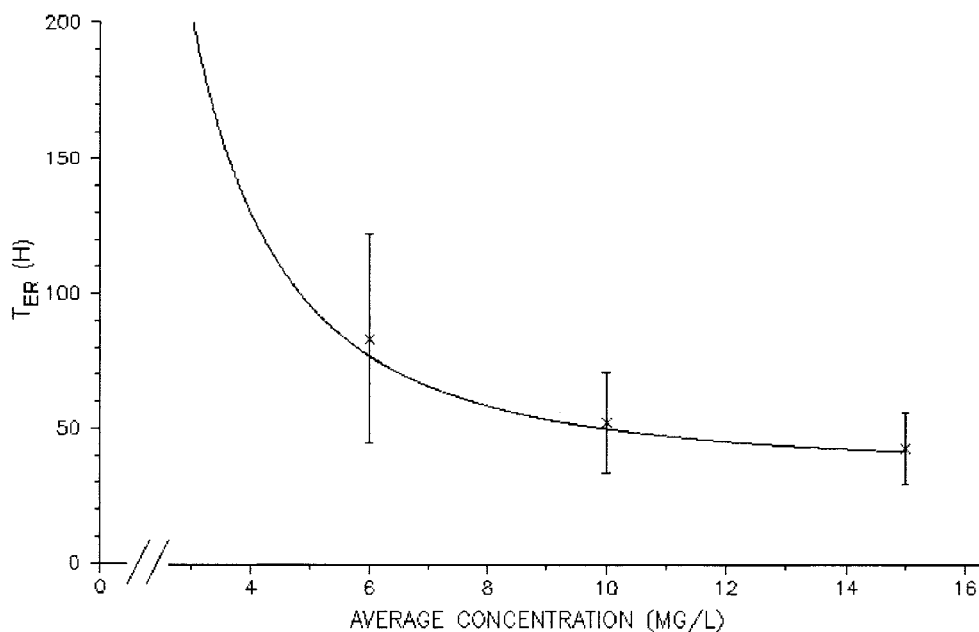


FIG. 4. Time to to 4-log reduction of *Plasmodium falciparum* initial parasitemia as a function of average concentration of quinine in plasma. The line is the curve yielded by the mean parameters. The crosses are the mean predictions. The vertical lines represent ± 1 SD.

best fit was obtained by setting s equal to 2. The posterior distributions were reasonably narrow, with a single mode. The plot of the predicted T_{er} versus the observed T_{er} showed a very close agreement, as demonstrated in Fig. 1B. The histogram of individual C_{50} point estimates (the mean of the posterior distribution for each individual) is shown in Fig. 3B. The distribution was unimodal, and all C_{50} values were less than 8 mg/liter. The interindividual variation of the pharmacodynamic parameters was not correlated with age. Figure 4 shows the curve of the T_{er} -versus-quinine average concentration relationship with its interindividual variability. This curve shows that T_{er} does not shorten significantly when the average concentration is higher than 10 mg/liter; however, the interindividual variability of T_{er} is reduced at higher concentrations. This value (10 mg/liter) has to be compared with the average concentration of quinine in plasma, which ranged from 5.9 to 18.3 mg/liter and with that in children receiving the standard dose (R) of 25 mg/kg/day, based on the mean clearance at 36 h, which is R/CL equal to 12.5 mg/liter. Moreover, the time to a 6-log reduction in the initial level of parasitemia is $6/k$, i.e., 1.5 T_{er} . Since the 90th percentile of T_{er} is 76 h, the corresponding value for a 6-log reduction is 114 h. Hence, the 5-day treatment (120 h) should be able to eradicate the parasite from 90% of children with an initial parasitemia of $10^6/\mu\text{l}$, provided that the slope of the parasitemia decay is independent of the initial parasitemia.

Overall, these data suggest that, at least in Cameroon, parasitemia of children with uncomplicated malaria may be cleared by the quinine formiate syrup following a 5-day course of treatment with 25 mg/kg/day. However, the sample size was

small and the follow-up (14 days) was too short to draw a conclusion about efficacy. A larger clinical trial is needed to test this hypothesis.

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