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Pharmacokinetics of Quinine and Doxycycline in Patients with Acute Falciparum Malaria: A Study in Africa

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Summary: The pharmacokinetics of quinine was investigated in patients with acute falciparum malaria treated with quinine alone or in the presence of doxycycline. Twenty-six patients divided into two groups of equal number were enrolled in the study. In the absence of doxycycline, the volume of distribution of quinine (mean ± SD) was estimated to be 1.32 ± 0.32 L/kg, and its clearance was 0.125 ± 0.47 L/h/kg, which was only in partial agreement with previously published data. No effect of doxycycline on the pharmacokinetics of quinine was observed. **Key Words:** Acute falciparum malaria—Quinine—Doxycycline—Pharmacokinetics.

Malaria is a major health problem in the world (1). One-third of the population lives in areas where the importance of the disease is growing (2). The situation is further complicated by frequent resistance to chloroquine, common now in Southeast Asia, Central and South America, and Eastern Africa. Quinine has for a long time been considered the treatment of choice for severe chloroquine-resistant falciparum malaria (3). Coadministration of tetracycline antibiotics has been proposed for many years to improve quinine efficacy (4). Encouraging results have not been obtained until recently (5). The potential benefit that may result from such a combination must not be offset by an increased risk of toxicity.

Quinine has a relatively low therapeutic range, with a risk of deafness and cardiac arrhythmias increasing as concentrations rise above 10 μg/ml (6,7). The desirable plasma concentrations range between 5 and 10 μg/ml (8). However, quinine toxicity has probably been overemphasized in severe malaria, and the benefit of high plasma concentrations (up to 20 μg/ml) in the acute phase could outweigh the risks (9). Definitive information on the subject is crucial but still lacking (10), and open to discussion (11,12). Nevertheless, demonstration of the absence of pharmacokinetic interaction between tetracycline antibiotics and quinine seems desirable before the combination of these drugs can be further recommended in acute falciparum malaria.

The pharmacokinetics of quinine has been investigated on multiple occasions and is therefore well known in healthy subjects (13–15), but only relatively few studies have been conducted in patients with acute falciparum malaria (9). It is obvious that a complete pharmacokinetic study of quinine in patients treated for acute falciparum malaria in developing countries raises multiple practical problems. A compromise between the theoretically desirable, ethical, and practical considerations has to be found. The present study was primarily conducted

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in order to demonstrate the absence of a potentially hazardous pharmacokinetic interaction between doxycycline and quinine.

MATERIALS AND METHODS

Patients

Patients were admitted to the Service of Internal Medicine and Infectious Diseases, CHU de Kamengue, in Bujumbura, Burundi, for acute falciparum malaria, diagnosed from clinical examination and laboratory tests including parasitemia. The protocol was accepted by an Ethics Committee. Patients themselves or their relatives gave informed consent to participate in the study.

Patients younger than 15 years or more than 4 months pregnant were excluded from the study. Cardiovascular disease and allergy to any tetracycline were also considered as exclusion criteria, as well as a recent history (7 days or less) of quinine treatment. Thirty-five subjects were included and divided into two groups. Enrollment was stopped after 13 subjects in each group had completed the study. Patients in group A received quinine alone, and those in group B received quinine + doxycycline. All patients were febrile; their characteristics are given in Table 1. Following admission and confirmation of the diagnosis, each patient was weighed and treatment was begun as soon as possible.

TABLE 1. *Demographic characteristics and clinical parameters (mean ± SD) for study groups at time of treatment initiation*

	Group A (quinine alone)	Group B (quinine + doxycycline)
Age (years)	26 ± 10 (16–47)	27 ± 11 (17–61)
Weight (kg)	53 ± 9 (44–74)	53 ± 9 (45–75)
Sex		
Males	9	7
Females	4	6
Parasitemia, range (infected cells/μl)	7–19,589	3–10,440
Hematocrit (%)	42 ± 10 (26–64)	39 ± 9 (22–47)
Albumin (g/L)	37.5 ± 3.8 (32–46)	35.9 ± 3.8 (30–43)
Orosomucoid (g/L)	1.55 ± 0.59 (0.54–2.67)	1.39 ± 0.45 (0.73–2.23)

Ranges are in parentheses.

Treatments

In group A, quinine was administered as Quinimax (Labaz Laboratories, France) at a dose of 20 mg/kg (equivalent to 11.86 mg/kg of quinine base) infused over 4 h (i.e., an initial infusion rate R_1 = 2.97 mg/kg/h of quinine base). This was followed by 15 mg/kg infused over the following 20 h (equivalent to 8.90 mg/kg of quinine base, i.e., an infusion rate R_2 = 0.445 mg/kg/h of quinine base). Finally, 25 mg/kg/day was infused (equivalent to 14.83 mg/kg/day, i.e., a final infusion rate R_3 = 0.618 mg/kg/h of quinine base) for the following 2 days. This dosing regimen was adapted from the usual regimen to optimize pharmacokinetic analysis. In particular, intravenous administration was maintained for 3 days, because early switching to oral dosing would have limited pharmacokinetic interpretation.

In group B, quinine was administered exactly in the same way as previously described (group A). Doxycycline was given intravenously as Vibramycin (Pfizer Laboratories, New York, NY, U.S.A.) at a dose equal to 200 mg every 24 h. The duration of the doxycycline injection was 1 min. The infusion of quinine was started simultaneously with the first injection of doxycycline.

The drugs were dispensed in plastic syringes. Quinine was administered via a Braun perfusor pump to insure a constant rate of infusion. Intravenous fluids were given as required through the same cannula and blood samples were taken from the opposite arm. Acetylsalicylic acid and/or metoclopramide were administered orally if necessary. Oral treatment with quinine was started 72 h after beginning the study.

Blood Collection and Treatment

Blood was taken before treatment and whenever possible at 2, 4, 12, 24, 36, 48, and 72 h after beginning treatment for quinine measurements. Blood was collected into two heparinized Vacutainers, one stored directly at −20°C for measurement of blood concentrations, and the other one immediately centrifuged at approximately 1,500 *g* for 10 min; plasma was separated using the Seraclear devices (Technicon), transferred to tubes resistant to liquid nitrogen (Nunc, Bioblock Scientific), and stored in a freezer at −20°C, for a time period ranging between 1 and 4 months. Samples were then transported by air freight in nitrogen liquid (Cryostorage system) to France for assay.

Assays

Quinine

Quinine was assayed in plasma samples by high-performance liquid chromatography (HPLC) with fluorimetric detection, according to a published method (16), with minor modifications. The limit of quantification of quinine was 0.25 μg/ml. Calibration plots were straight lines over the studied range of concentrations (0.25 to 20 μg/ml). Coefficients of variation for the method were 13.1 and 7.5% at plasma concentrations of 1 and 10 μg/ml, respectively. Results are expressed as quinine base.

Doxycycline

Doxycycline was assayed in plasma samples by HPLC with ultraviolet (UV) detection, according to a published method (17), with minor modifications. The limit of quantification of doxycycline was 0.5 μg/ml. Calibration plots were straight lines over the studied range of concentrations (0.5 to 10 μg/ml). Coefficients of variation for the method were 2.9 and 3.2% at plasma concentrations of 1 and 10 μg/ml, respectively.

Pharmacokinetic and Statistical Analysis

Because of the limited number of data points and for reasons further discussed (cf. the Discussion section), only plasma concentrations measured 4, 48, and 72 h after treatment initiation were used for calculations of pharmacokinetic parameters characteristic of quinine as follows.

Pharmacokinetic parameters characteristic of quinine were calculated assuming instantaneous distribution and first-order elimination (9). The volume of distribution (V_d) was estimated according to

$$V_d = \frac{\text{dose}}{C_T} \cdot \frac{1 - e^{-k_e T}}{k_e \cdot T} \quad (1)$$

where dose is the dose administered during the initial 4-h infusion (11.86 mg/kg), T the duration of the initial infusion (4 h), and C_T the plasma concentration measured at the end of infusion.

Considering that the rate constant for elimination, k_e, was equal to 0.04 h^{-1}, which corresponds to the mean value of the elimination half-life ($t_{1/2}$ = 18 h) previously reported in comparable situations (9), Eq. (1) becomes

$$V_d = 0.92 \frac{\text{dose}}{C_T} \quad (2)$$

The dose was equal to 11.86 mg/kg, C_T was expressed in μg/ml, and V_d was expressed in L/kg.

Clearance (CL) was calculated from Eq. (3), considering that plasma concentrations of quinine measured at 48 and 72 h represented steady-state concentrations:

$$CL = \frac{R_3}{C_{ss}} \quad (3)$$

R_3 is the final infusion rate previously defined (R_3 = 0.618 mg/kg/h) and C_{ss} the steady-state plasma concentration obtained from the arithmetic mean of concentrations measured at 48 and 72 h, expressed in μg/ml. Clearance was therefore expressed in L/h/kg.

The elimination half-life ($t_{1/2}$) was obtained from Eq. (4), and expressed in h:

$$t_{1/2} = 0.693 \cdot \frac{V_d}{CL} \quad (4)$$

A Student's t test was used to compare mean values of quinine pharmacokinetic parameters, obtained in patients treated by the drug alone (group A) and in the presence of doxycycline (group B). An alpha level of 0.05 was defined as a significant difference.

RESULTS

Mean ± SD plasma concentrations of quinine calculated in each group of patients are shown in Fig. 1. Quinine plasma concentrations measured at any time in the two groups were virtually identical (8.76 ± 2.02 and 7.99 ± 2.98 μg/ml, respectively). Pharmacokinetic parameters calculated for quinine, in the control phase and doxycycline phase of the study, are presented in Table 2.

Mean ± SD doxycycline plasma concentrations are presented in Fig. 2, together with the curve simulated from parameters previously determined by one of us in young healthy volunteers (18). This figure illustrates the good agreement between measured and simulated data, especially at trough levels.

DISCUSSION

Cotreatment with doxycycline had no effect on quinine disposition as assessed from measurements

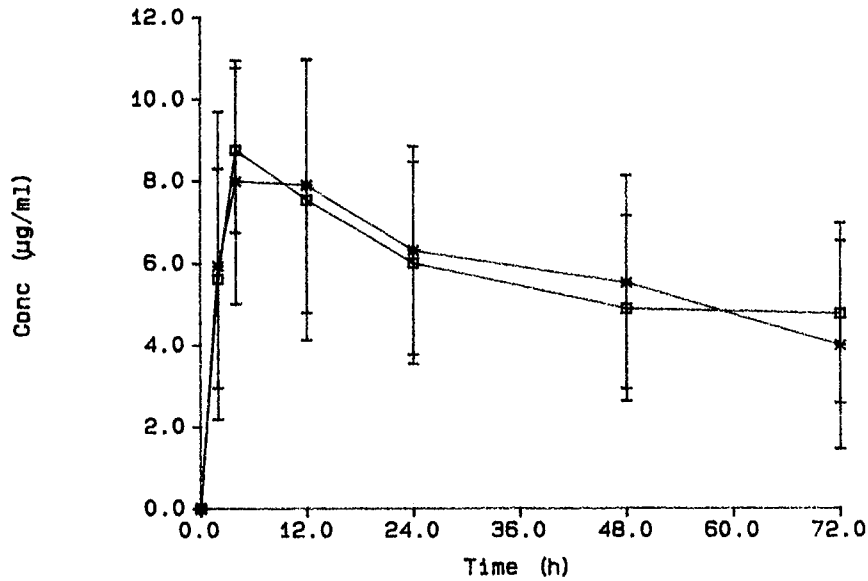

FIG. 1. Mean (SD) plasma concentrations of quinine in patients treated by quinine alone (□) and by quinine plus doxycycline (*).

of plasma concentrations. The two drugs may therefore be coadministered without increasing the risk of toxicity related to high plasma levels of quinine.

Although pharmacokinetic parameters characteristic of quinine were virtually similar in the presence and absence of doxycycline, they were only in partial agreement with values previously estimated in patients treated for acute falciparum malaria (9). Estimates of the volume of distribution were only slightly different, since White et al. calculated a V_d of 1.18 ± 0.37 L/kg (mean ± SD) in patients with cerebral malaria (9), and we obtained a value of 1.32 ± 0.32 L/kg in patients receiving quinine alone (Table 2). Conversely, estimates of clearance presented major discrepancies, although interindividual variation in this parameter was greater than that in V_d. We report a clearance value (CL = 0.125 ± 0.047 L/h/kg corresponding to 2.08 ± 0.78 ml/min/kg) that is about twice that previously estimated (9) in patients (0.92 ± 0.42 ml/min/kg). As a consequence, different estimates of half-life were obtained. Our estimate of clearance was close to that obtained by White et al. in healthy volunteers (14), but further interpretation could be risky. Since a control group with healthy volunteers was lacking, the effect of acute falciparum malaria on quinine disposition was beyond the scope of this study.

Different approaches were used by White et al. (9) and by ourselves in order to calculate the pharmacokinetic parameters characteristic of quinine in patients. This may account, at least in part, for the observed discrepancies.

Inaccurate estimation of $t_{1/2}$ may have had consequences for our estimation of V_d. However, the error was minimal. For example, even if $t_{1/2}$ were only half of that expected (9 h instead of 18 h), Eq. (2) would overestimate V_d by less than 10%, which can be neglected for practical purposes.

We estimated CL from plasma concentrations of quinine measured 48 and 72 h after starting treat-

TABLE 2. *Pharmacokinetic parameters characteristic of quinine administered alone and in the presence of doxycycline in patients with acute falciparum malaria*

	V_d[a] (L/kg)	CL[b] (L/h/kg)	$t_{1/2}$ (h)
Group A: quinine alone			
Mean ± SD	1.32 ± 0.32	0.125 ± 0.047	7.99 ± 3.08
Range	(0.976–2.01)	(0.073–0.213)	(3.16–12.4)
Group B: quinine + doxycycline			
Mean ± SD	1.44 ± 0.48	0.145 ± 0.085	7.79 ± 4.20
Range	(0.864–2.30)	(0.070–0.333)	(1.98–16.1)

[a] Estimated from data collected 4 h after treatment initiation.
[b] Estimated from data collected 48 and 72 h after treatment initiation.

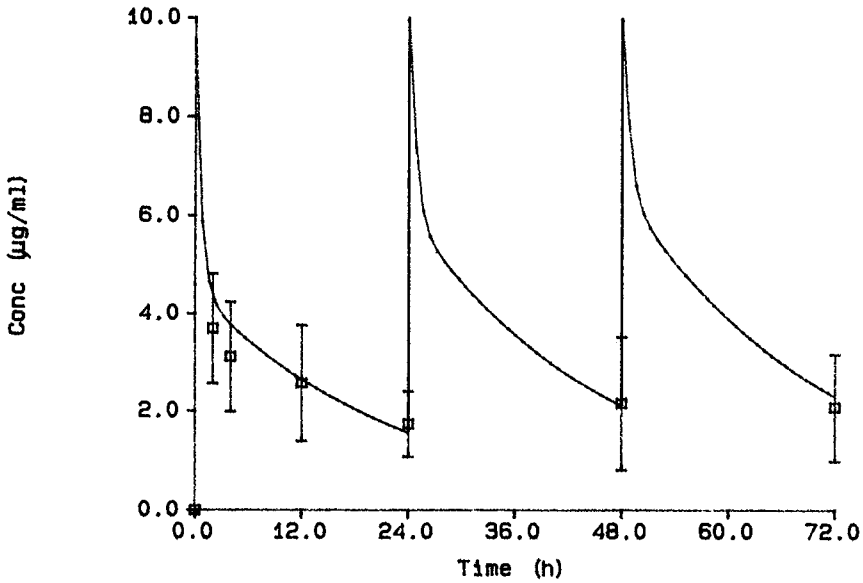

FIG. 2. Mean (SD) plasma concentrations of doxycycline (□) in patients treated by quinine plus doxycycline. The curve (solid line) results from simulations with parameters previously obtained in young healthy volunteers: $C = 5.07\, e^{-1.47t} + 4.48\, e^{-0.044t}$ (from ref. 18).

ment, i.e., only 24 and 48 h after the final administration was initiated. At that time, the steady-state was not completely achieved and therefore our estimates of *CL* are probably slightly erroneous.

A rapid change in quinine clearance may not be excluded as a confounding factor. Such a phenomenon may be observed during the initial days of the acute episode, when patients are critically ill and have unstable physiology. As an example, plasma concentrations of α-glycoproteins rise during the acute phase of malaria infection (19), with presumably complex consequences on quinine disposition because of extensive (19) and nonlinear binding over the range of concentrations encountered during therapy (20). Constancy of drug disposition characteristics seems unlikely when conditions of the patients deteriorate quickly, requiring fluid replacement and sometimes other treatments such as intermittent dialysis (21). Although it is admitted that quinine clearance is depressed during acute falciparum malaria, and increases in convalescent patients restudied 1 month after the acute episode (9), there is virtually no information on the constancy or variation of quinine pharmacokinetics in a shorter period of time, when the most important changes in physiopathologic status are observed. It was only reported that the quinine elimination half-life was lengthened in two patients during fever, and returned to normal after fever (13).

In order to optimize the dosing regimen on a rational basis, the effects of V_d and *CL* on plasma levels of quinine at different stages of treatment have to be clearly distinguished, as was not always the case. For example, the fall in quinine plasma levels observed in convalescent patients has been erroneously attributed to an increase in both *CL* and V_d (9), and is still perpetuated in the most recent literature (22). In convalescent patients, quinine plasma concentrations are independent of V_d [Eq. (3)]. An increase in that parameter would only be responsible for a lengthening of $t_{1/2}$ and consequently for lesser fluctuations of quinine plasma concentrations during intermittent dosing, without an effect on the mean concentration.

The time that elapses between the beginning of the acute episode of falciparum malaria and the attainment of effective plasma concentrations of quinine is supposed to be of major prognostic importance (23). Therefore, correct estimate of V_d of quinine at the initiation of treatment is crucial because this parameter determines the optimal loading dose. The interindividual variation in the value of V_d normalized to body weight was relatively small (Table 2). Consequently, using a mean value of V_d equal to 1.3 L/kg for the loading dose calculation would allow achievement of plasma concentrations of quinine at the end of the initial infusion close to the desirable value in most patients, even with highly variable and possibly unstable physiopathologic status. This was observed experimentally since plasma concentrations of quinine at the end of infusion ranged between 6.5 and 11.9 µg/ml with a mean ± SD equal to 8.8 ± 2.0 µg/ml in the control group.

Precise knowledge of clearance seems to be less important in clinical practice because clearance has

virtually no influence on plasma concentrations of quinine during the initial hours of treatment. However, it has recently been proposed to increase the dose of quinine if parasitemia does not fall by 75% within 48 h (21). Precise knowledge of clearance should then be required since at this stage of treatment this parameter should be solely responsible for quinine plasma levels [Eq. (3)].

In conclusion, this study provided complementary information on the pharmacokinetics of quinine in acute falciparum malaria, which are only in partial agreement with previously published data (9). Discrepancies are not totally explained, and because of experimental constraints, poor estimates of clearance may have been obtained both by White et al. (9) and by ourselves. Further experiments would be necessary in order to elucidate that question. Although these conflicting results would not affect dosing recommendations for treatment initiation, they would have practical consequences in severe cases when an increase in the dose of quinine may be required after several days of treatment. Finally, it was demonstrated that doxycycline has no effect on quinine disposition and that consequently these two drugs may be used together to treat cerebral malaria without increasing the risk of toxicity.

REFERENCES

1. Gentilini D. Le paludisme: une situation critique. *Med Sci* 1989;9:716–7.
2. OMS. Situation du paludisme dans le monde 1986–1987. Parties I et II. *Rev Epidemiol Hebd* 1989;64:241–54.
3. Hall A. The treatment of severe falciparum malaria. *Trans R Soc Trop Med Hyg* 1977;71:367–9.
4. Reacher M, Campbell CC, Freeman J, Doberstyn EB, Branding-Benett AD. Drug therapy for Plasmodium falciparum malaria resistant to pyrimethamine-sulfadoxine (Fansidar). *Lancet* 1981;2:1066–9.
5. Giboda M, Denis MB. Response of Kampuchean strains of Plasmodium falciparum to anti-malarials: in-vivo assessment of quinine and quinine plus tetracycline; multiple drug resistance in-vitro. *J Trop Med Hyg* 1988;91:205–11.
6. Dyson EH, Proudfoot AT, Prescott LF, Heyworth R. Death and blindness due to overdoses of quinine. *Br Med J* 1985; 291:31–3.
7. Bateman DN, Blain PG, Woodhouse KW, et al. Pharmacokinetics and clinical toxicity of quinine overdosage: lack of efficacy of techniques intended to enhance elimination. *Q J Med* 1985;54:125–31.
8. Franke U, Proksch B, Müller M, Risler T, Ehninger G. Drug monitoring of quinine by HPLC in cerebral malaria with acute renal failure treated by haemofiltration. *Eur J Clin Pharmacol* 1987;33:293–6.
9. White NJ, Looareesuwan S, Warrel DA, Warrel MJ, Bunnag D, Harinasuta T. Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. *Am J Med* 1982;73:564–72.
10. Hofheinz W, Merkli B. Quinine and quinine analogues. In: Peters W, Richards WHG, eds. *Antimalarial drugs II*. Berlin, Heidelberg, New York: Springer, 1984.
11. Hall A. Dangers of high-dose quinine and over-hydratation in severe malaria. *Lancet* 1985:1453.
12. Warrel DA, White NJ, Looareesuwan S, et al. Dangers of high-dose quinine and over-hydratation in severe malaria—a reply. *Lancet* 1985:1453–4.
13. Trenholme GM, Williams RL, Rieckmann KH, Frischer H, Carson PE. Quinine disposition during malaria and during induced fever. *Clin Pharmacol Ther* 1976;19:459–67.
14. White NJ, Chanravanich P, Krishna S, Bunch C, Silamur K. Quinine disposition kinetics. *Br J Clin Pharmacol* 1983;16: 399–403.
15. Lockey D, Bateman DN. Effect of oral activated charcoal on quinine elimination. *Br J Clin Pharmacol* 1989;27:92–4.
16. Jamaludin A, Mohamad M, Navaratnam V, et al. Relative bioavailability of the hydrochloride, sulphate and ethyl carbonate salts of quinine. *Br J Clin Pharmacol* 1988;25:261–3.
17. Eksborg S, Ehrsson H, Lönnroth U. Liquid chromatographic determination of tetracycline in plasma and urine. *J Chromatogr* 1979;185:583–91.
18. Fourtillan JB, Lefebvre MA, Saux MC, Becq-Giraudon B. Étude pharmacocinétique de la doxycycline chez l'homme par chromatographie liquide haute performance: administrations de doses uniques de 200 mg par voie intraveineuse et par voie orale. *Nouv Presse Med* 1980;9:77–81.
19. Silamut K, White NJ, Looareesuwan S, Warrell DJ. Binding of quinine to plasma proteins in falciparum malaria. *Am J Med* 1985;34:681–6.
20. Mihaly GW, Ching MS, Klejn MB, Paull J, Smallwood A. Differences in the binding of quinine and quinidine to plasma proteins. *Br J Clin Pharmacol* 1987;24:769–74.
21. Looareesuwan S, Charoenpan P, Ho M, et al. Fatal plasmodium falciparum malaria after an inadequate response to quinine treatment. *J Infect Dis* 1990;161:577–80.
22. Edstein MD, Prasitthypayong A, Sabchareon A, Chongsuphajaiziddhi T, Webster KH. Simultaneous measurement of quinine and quinidine in human plasma, whole blood, and erythrocytes by high-performance liquid chromatography with fluorescence detection. *Ther Drug Monit* 1990;12:493–500.
23. Warrel DA, Looareesuwan S, Warrel MJ, et al. Dexamethasone proves deleterious in cerebral malaria: double blind trial in 100 patients. *N Engl J Med* 1982;306:313–9.