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LACK OF A PHARMACOKINETIC INTERACTION BETWEEN AZITHROMYCIN AND CHLOROQUINE

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Abstract. A study was conducted to investigate a possible pharmacokinetic interaction between azithromycin and chloroquine. Twenty-four subjects received azithromycin, 1,000 mg a day for three days, followed by a washout period, then azithromycin, 1,000 mg plus chloroquine 600 mg base on days 1 and 2, and azithromycin, 1,000 mg plus chloroquine 300 mg base on day 3 of the final period. A second group of 16 subjects received chloroquine, 600 mg base on days 1 and 2, then 300 mg base on day 3. Blood samples were obtained serially up to 624 hours after the day 3 dose in each period. Log transformed maximum concentration and area under the curve values of azithromycin and chloroquine were compared using 90% confidence intervals calculated from appropriate analysis of variance models. Ninety percent confidence intervals for all pharmacokinetic parameters were contained within the interval 80–125%, which indicates the absence of a clinically relevant pharmacokinetic interaction.

INTRODUCTION

A combination azithromycin/chloroquine regimen is currently being developed for the treatment of chloroquine-resistant malaria. Synergy of antimalarial effect has been reported with chloroquine in combination with azithromycin *in vitro*.[1] A pilot clinical trial showed promising results in the treatment of *Plasmodium falciparum* malaria in India: 28 days after taking chloroquine or azithromycin, clinical success was observed in only 27% and 33% of patients, respectively, but when the two drugs were taken in combination, clinical success was achieved in 97% of patients.[2] The mechanism of synergy between these two agents is not understood. Chloroquine resistance has been reversed by co-administration of agents, such as verapamil, with the ability to inhibit p-glycoprotein-mediated efflux.[3] It is not known whether azithromycin is such an inhibitor; however, azithromycin has been shown to be a p-glycoprotein substrate.[4] Thus, we investigated whether alterations in human pharmacokinetics of either compound could be responsible for at least part of the increased effectiveness of these compounds when given together.

MATERIALS AND METHODS

Study design. This was a single-center, phase 1, open-label, randomized, multiple-dose (three days) study conducted in two groups of healthy volunteers. Forty subjects who fulfilled the entry criteria were randomly assigned to one of two groups (group 1, 24 subjects; group 2, 16 subjects). The three-day regimens of azithromycin and chloroquine were identical to regimens studied in the pilot clinical trial and the chloroquine regimen was consistent with the standard World Health Organization three-day chloroquine treatment regimen.[5] Group 1 received azithromycin (Zithromax®; Pfizer Inc., Ann Arbor, MI), 1,000 mg every day for three days during the first period. After a 3–5-week washout, group 1 participants received azithromycin, 1,000 mg plus chloroquine (Aralen®; Bayer, Myerstown, PA), 1,000 mg (600 mg base) on days 1 and 2, and azithromycin, 1,000 mg plus chloroquine 500 mg (300 mg base) on day 3. Group 2 subjects received chloroquine, 1,000 mg (600 mg base) on days 1 and 2 and 500 mg (300 mg base) on day 3. The effect of chloroquine on azithromycin pharmacokinetics was to be examined by within-subject comparisons of azithromycin data obtained during the two regimens received by group 1. However, because of the long half-life of chloroquine, between-group comparisons were used to investigate the effect of azithromycin on chloroquine pharmacokinetics.

Subjects of any race that met the following criteria were eligible to participate in the study: good health as determined by medical history, physical examination, clinical laboratory measurements, and electrocardiogram (ECG); age 18–60 years inclusive; either male or female of non–child-bearing potential; and with a body weight $\geq$ 50 kg. Subjects could not participate in the study if any of the following conditions existed: history of retinopathy, liver disease, or alcoholism; retinal or visual changes attributable to either 4-aminoquinoline compounds or any other etiology; psoriasis or porphyria; hypersensitivity to azithromycin, any other macrolide antibiotics, or 4-aminoquinoline compounds; use of any medication not considered acceptable by the clinical investigators during the 14-day period before the start of the study; donation of a unit of blood or participation in a study of investigational or marketed drugs during the 30-day period before initiation of treatment with study drug; or significant urine concentration of any drug that could interfere with the study. All doses were taken between 7:00 AM and 11:00 AM, after an overnight fast, with 240 mL of tap water. Food was restricted for at least one hour after each dose on days 1 and 2, and for at least four hours after dosing on day 3. Safety assessments consisted of clinical observation, physical examination, vital signs measurement, clinical laboratory measurements, and adverse events. Twelve-lead ECGs were recorded at screening and closeout for all subjects. In addition, for subjects in the chloroquine alone and chloroquine plus azithromycin treatment groups, ECGs were recorded predose on day 1 and 3–5 hours postdose on days 1 and 3.

In each study period, blood samples were drawn before dosing on day 3 and at 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 168, 240, and 624 hours, except that 240- and 624-hour samples were not obtained when azithromycin was dosed alone. Samples of venous blood (5 mL for azithromycin and/or 7 mL

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for chloroquine) were withdrawn into vacuum blood collection tubes containing either no anticoagulant (for azithromycin) or sodium heparin (for chloroquine). After each collection, blood samples were centrifuged and plasma was separated. Plasma samples were stored frozen in polyethylene tubes at –20°C until analyzed for azithromycin or chloroquine and desethylchloroquine (its major and active metabolite) concentrations.

Ethical approval. The protocol and consent documents were reviewed and approved by the Institutional Review Board of the Pfizer Research Clinic. The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice, the Declaration of Helsinki, and in compliance with United States Food and Drug Administration regulations for informed consent and protection of subject rights as described in 21 Code of Federal Regulations 50, 56, and 312. Written informed consent was obtained from all participants. Subjects were told that they were free to withdraw from the study at any time.

Bioanalytical methods. Serum concentrations for azithromycin were measured by a validated liquid chromatography/electron capture method at BAS Analytics (West Lafayette, IN). The lower limit of quantitation was 10.4 ng/mL. The precision of the assay was within 5.9% and the accuracy ranged from –1.7% to 4.3%.

Plasma concentrations for chloroquine and its metabolite desethylchloroquine were measured by a liquid chromatography/mass spectrometry/mass spectrometry method at Bioassay Laboratory, Inc. (Houston, TX). The lower limit of quantitation was 1 ng/mL for chloroquine and 0.5 ng/mL for desethylchloroquine. The precision of the assay was within 9.5% for chloroquine and 8.5% for desethylchloroquine and the accuracy ranged from –0.1% to 4.6% for chloroquine and –3.2% to –1.0% for desethylchloroquine.

Pharmacokinetic methods. Pharmacokinetic azithromycin, chloroquine, and desethylchloroquine parameters were calculated from individual subject's concentration-time profiles by non-compartmental methods using WinNonlin Pro version 2.1 (Pharsight Corp., Mountain View, CA). Maximum observed concentration (C_{max}) and time of occurrence (t_{max}) were determined by inspection. The terminal phase rate (k) constant was estimated as the absolute value of the slope of a linear regression during the apparent terminal phase of the natural-logarithm (ln) transformed concentration-time profile. The terminal half-life ($t_{½}$) was calculated as $t_{½} = \ln(2)/k$. The area under the concentration time curve (AUC) to the last quantifiable concentration ($AUC_{0–tlqc}$) was calculated using the linear trapezoidal method and extrapolated to infinite time ($AUC_{0–\infty}$) by the equation $AUC_{0–\infty} = AUC_{0–tlqc} + (\text{last quantifiable concentration})/k$.

Statistical methods. Log-transformed Cmax and AUC values for azithromycin and chloroquine were the primary parameters used in the assessment of interaction. The effect of chloroquine on azithromycin pharmacokinetics was evaluated within group 1. Parameter values were evaluated by an analysis of variance (ANOVA) model containing a fixed treatment and a random subject effect. The estimated treatment differences and the 90% confidence intervals for the differences were anti-log transformed back to the ratio of original scale. Absence of an interaction would be concluded if the anti-log-transformed confidence intervals for the true ratios of the test (azithromycin given together with chloroquine) to reference (azithromycin given alone) were to lie within the 80–125% range for both Cmax and AUC.

The effect of azithromycin on chloroquine pharmacokinetics was evaluated by comparison of groups 1 and 2. Examination of subject demographics showed that the mean weight of the chloroquine alone group was approximately 10 kg less than that of the azithromycin plus chloroquine group (Table 1). Since plots of weight versus chloroquine Cmax and AUC values (Figure 1) showed a correlation, Cmax and AUC values were corrected for subject weight (parameter value × subject weight) and weight-corrected parameter values were evaluated by an ANOVA model containing a fixed treatment effect. The estimated treatment differences and the 90% confidence intervals for the differences were anti-log transformed back to the ratio and confidence intervals of original scale. Absence of an interaction would be concluded if the anti-log-transformed confidence intervals for the true ratios of the test (azithromycin given in combination with chloroquine) to reference (chloroquine given alone) lie within the 80–125% range for both Cmax and AUC.

Confidence intervals for weight-corrected desethylchloroquine pharmacokinetic parameter values were used as an aid in data interpretation. Statistical analysis was performed using WinNonlin Pro version 2.1.

RESULTS

Subject disposition and safety. Forty subjects entered the study and 39 completed the study. One subject was discontinued on the third day of the chloroquine alone treatment because of an adverse event of abnormal vision (difficulty focusing); the abnormal vision resolved two days after discontinuation. A full concentration-time profile was not obtained for that subject; therefore; data from that subject were not used in pharmacokinetic evaluations.

A total of 209 adverse events were reported, 151 of which were considered associated with treatment. Adverse events occurred with the greatest frequency in the body as a whole and the digestive system. The most frequent adverse events considered associated with treatment with azithromycin alone (n = 24 subjects) were abdominal pain (10 subjects), headache (7 subjects), diarrhea (6 subjects), and eructation (4 subjects). The most frequent adverse events considered associated with treatment with chloroquine alone (n = 16 subjects)

TABLE 1
Demographic data for subjects whose data was used in pharmacokinetic parameter analyses

	Group 1 (n = 24)	Group 2 (n = 15)
Sex		
Males	19	10
Females	5	5
Age, years		
Mean	39.3	40.7
Minimum–maximum	26–57	21–60
Height, cm		
Mean	179	174
Minimum–maximum	160–193	154–188
Weight, kg		
Mean	89.5	78.1
Minimum–maximum	56.8–112	58.7–91.9

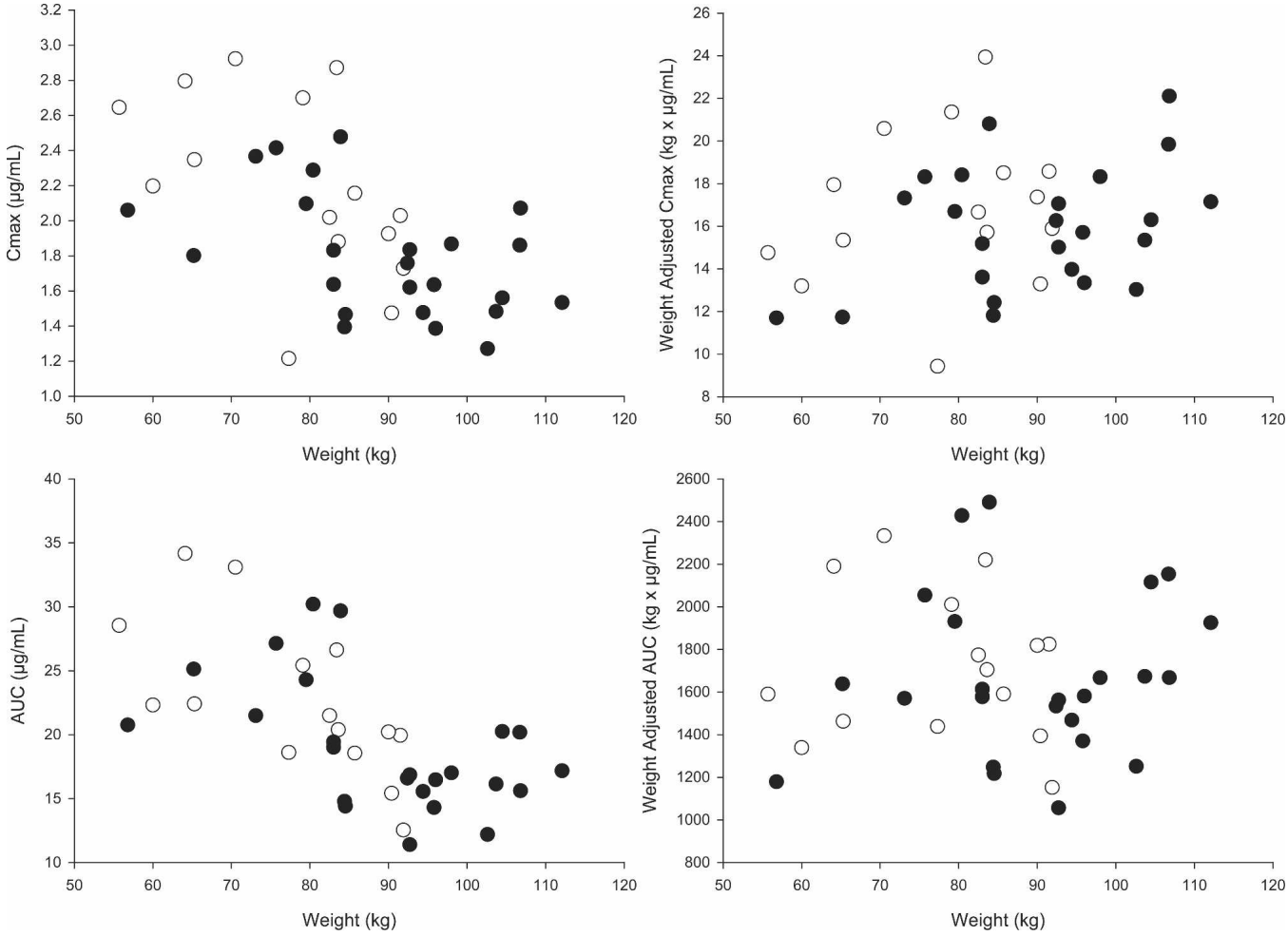

FIGURE 1. Individual subject's raw (left panels) and weight-adjusted (right panels) chloroquine Cmax (top panels) and AUC(0–∞) (bottom panels) values as a function of body weight after administration of chloroquine alone (○) or with azithromycin (●). AUC = area under curve.

were headache (6 subjects), abdominal pain (3 subjects), and nausea (3 subjects). The most frequent adverse events considered associated with treatment with azithromycin plus chloroquine (24 subjects) were diarrhea (16 subjects), abdominal pain (12 subjects), headache (10 subjects), dyspepsia (5 subjects), flatulence (4 subjects), dizziness (2 subjects), somnolence (2 subjects), and eructation (2 subjects). All adverse events were mild or moderate in intensity with the exception of one severe incidence of diarrhea during treatment with azithromycin plus chloroquine and one severe incidence of headache during treatment with chloroquine alone. There were no serious adverse events or deaths during this study. Adverse events were consistent with the product labels. No clinically significant abnormalities were noted in physical examinations, vital signs, or individual ECGs. However, increases in QTc values using Fridericia's correction for the chloroquine alone and the azithromycin plus chloroquine treatment groups were evident on day 1 (mean ± SD difference baseline to day: chloroquine, 5.2 ± 9.3 msec; azithromycin plus chloroquine, 7.6 ± 11.2 msec) and were maximal on day 3 (chloroquine, 13.7 ± 7.4 msec; azithromycin plus chloroquine, 19.9 ± 16.3 msec); mean values returned to the range of initial values at closeout for both treatment groups. Although mean increases were numerically greater for the azithromycin plus chloroquine group when compared with the chloroquine alone group, a post hoc inferential analysis indicated no statistically significant difference between the chloroquine alone and azithromycin plus chloroquine treatment groups post-dose on day 1 or day 3. Administered alone and together, chloroquine and azithromycin were overall well tolerated in this study.

Effect of chloroquine on azithromycin. Mean serum azithromycin concentration-time profiles for azithromycin administered alone and azithromycin administered with chloroquine are shown in Figure 2. Mean pharmacokinetic parameter values are shown in Table 2 along with ratios and confidence intervals.

Based on similar tmax and Cmax values, the rate of azithromycin absorption after administration with chloroquine was similar to that of azithromycin administered alone. The mean tmax value with chloroquine was approximately 20 minutes shorter and the mean Cmax value was 15% greater than corresponding values without chloroquine. Based on AUC(0–∞) values, extent of azithromycin absorption after administration with chloroquine was similar to that of azithromycin administered alone. The mean AUC(0–∞) value with chloroquine was within 3% of that without chloroquine. The 90% confidence intervals for the ratio of treatment mean Cmax and

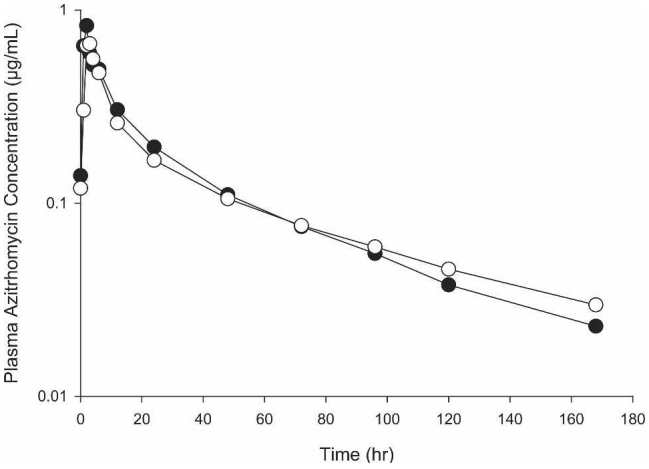

FIGURE 2. Mean serum azithromycin concentration-time profiles after three days of administration of azithromcyin alone (○) or with chloroquine (●).

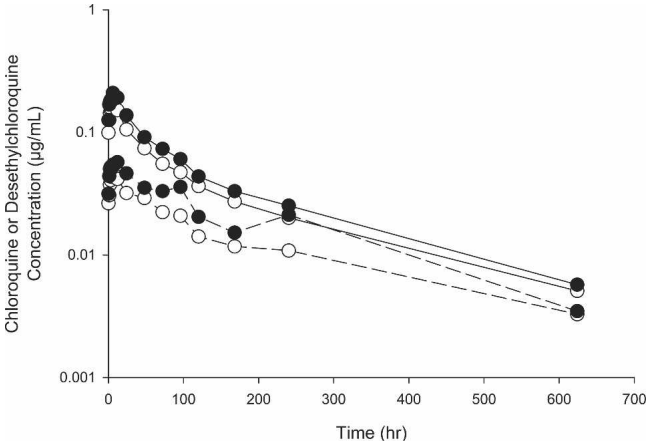

FIGURE 3. Mean plasma chloroquine (solid lines) and desethylchloroquine (dashed lines) concentration-time profiles after three days of administration of chloroquine alone (○) or with azithromcyin (●).

AUC(0–∞) values, based on log-transformation, were within the 80–125% range. Azithromycin elimination t½ values were similar for each treatment, averaging approximately 74 hours. Thus, chloroquine has no clinically relevant effect on azithromycin pharmacokinetics.

Effect of azithromycin on chloroquine. Mean plasma chloroquine concentration-time profiles for chloroquine administered alone and chloroquine administered with azithromycin are shown in Figure 3. Mean pharmacokinetic parameter values are shown in Table 3 along with ratios and confidence intervals.

Based on similar tmax and weight-corrected Cmax values, the rate of chloroquine absorption after administration with azithromycin was similar to that of chloroquine administered alone. The mean tmax value with azithromycin was approximately 30 minutes shorter and the mean Cmax value was 5% lower than corresponding values without chloroquine. Based on AUC(0–∞) values, extent of chloroquine absorption after administration with azithromycin was similar to that of chloroquine administered alone. The mean AUC(0–∞) value with azithromycin was within 5% of that of chloroquine administered alone. The 90% confidence intervals for the ratio of treatment mean Cmax and AUC(0–∞) values, based on log-transformation, were within the 80–125% range. Chloroquine terminal t½ values were similar for each treatment, averaging approximately 195 hours. Evaluation of desethylchloroquine pharmacokinetic data supported the similarity between treatments. Thus, azithromycin has no clinically relevant effect on chloroquine pharmacokinetics.

DISCUSSION

Azithromycin is incompletely absorbed after oral administration with an absolute bioavailability of 38% after oral administration. It has an apparent-steady state volume of distribution of approximately 31 L/kg. The plasma protein binding of azithromycin is concentration dependent, ranging from 51% at 0.2 µg/mL to 7% at 2 µg/mL. Approximately 6% of the dose is eliminated unchanged in the urine. Biliary excretion, predominately as unchanged drug, is a primary route of elimination. The metabolism of azithromycin has not been fully elucidated. Azithromycin has a terminal elimination half-life of approximately 68 hours.[6]

Previous studies in healthy volunteers have demonstrated that chloroquine is almost completely absorbed after oral administration with an absolute bioavailability of 78–89%.[7] It has a large volume of distribution with estimates ranging from 116 to 800 L/kg when estimated from plasma chloroquine concentrations.[7,8] Chloroquine binding to plasma proteins is concentration independent; there is a modest steroselective difference because 66.6% of the (+)-chloroquine and 45.9% of (–)-chloroquine is bound to plasma proteins.[9] Approxi-

TABLE 2

Summary of mean azithromycin pharmacokinetic parameter values following three days of oral adminsitration of azithromycin alone or with chloroquine*

Parameter	Least-squares mean values		Ratio (test:reference)	90% confidence interval
	Azithromycin alone (reference)	Azithromycin with chloroquine (test)		
Number	24	24		
Cmax, µg/mL	0.805	0.922	115	105–124.8
tmax, hours	2.38	2.00	84.1	NA
AUC(0–tlqc), µg · hour/mL	16.9	18.7	111	104–117
AUC(0–∞), µg · hour/mL	19.9	20.5	103	96.2–110
t½, hours	74.0	73.3	99.0	80.5–118

* NA = not applicable; AUC = area under curve.

TABLE 3

Summary mean of chloroquine and desethylchloroquine pharmacokinetic parameter values following three days of oral administration of chloroquine alone or with azithromycin*

Parameter	Least-squares mean values: Chloroquine alone (reference) n = 15	Least-squares mean values: Chloroquine with azithromycin (test) n = 24	Ratio (test:reference)	90% confidence interval
		Chloroquine		
Weight-adjusted parameters				
Cmax, kg · μg/mL	16.5	15.6	95.1	85.1–106
AUC(0–tlqc), kg · μg · hour/mL	1,561	1,510	96.7	86.4–108
AUC(0–∞), kg · μg · hour/mL	1,690	1,626	96.2	85.3–109
Nonweight-adjusted parameters				
tmax, hours	6.60	6.08	92.2	NA
t½, hours	206	185	89.6	80.0–99.3
		Desethylchloroquine		
Weight-adjusted parameters				
Cmax, kg · μg/mL	4.99	4.57	91.7	76.1–110
AUC(0–tlqc), kg · μg · hour/mL	658	625	95.0	80.2–113
AUC(0–∞), kg · μg · hour/mL	761	726	95.4	80.2–113
Nonweight-adjusted parameters				
tmax, hours	13.2	6.79	51.5	NA
t½, hours	247	239	96.5	76.6–116

* AUC = area under curve; NA = not applicable.

mately 50% of a given dose is recovered unchanged in the urine. Chloroquine is metabolized predominately through dealklyation to desethylchloroquine, bis desethylchloroquine, and 7-chlor-4-aminoquinoline. Plasma desethylchoroquine concentrations reach 20–30% of parent concentrations after single doses and 36–48% of parent at steady state.[7,10] Half-lives of chloroquine and deschloroquine are long, both ranging from 20 to 60 days. Results from the present study are consistent with previous observed chloroquine data with the exception of half-life. The sampling scheme used in the present study was insufficient to capture the true terminal phase.

Azithromycin demonstrates antimalarial activity *in vitro* against both chloroquine-sensitive and -resistant *P. falciparum* and has been successfully used as chemoprophylaxis.[11,12] It has a proven safety record in children and lack of teratogenicity in nonclinical studies. In addition, early neonatal mortality rate and rate of low birth weight were reduced compared with no intervention in a large study of women treated with azithromycin during pregnancy as part of a preventive therapy regimen for sexually transmitted diseases.[13] Furthermore, azithromycin has shown synergy with chloroquine *in vitro*. The combination of azithromycin with chloroquine may provide important advantages over existing treatment options, not only with respect to the potential for use in children and pregnant women, but also with respect to factors such as safety, cost, and tolerability.

The present study was undertaken to rule out a systemic drug-drug interaction as the cause of the apparent synergy of azithromycin and chloroquine given in combination to treat chloroquine-resistant malaria. Since both drugs may be modulators of similar transporter systems, it was not inconceivable that the synergy was due to an increased exposure to one or both of the agents because of improved bioavailability or a decrease in clearance. The pharmacokinetic parameters observed in this study were consistent with those reported previously for azithromycin and chloroquine when administered to healthy subjects. Furthermore, the similarity of pharmacokinetic parameters between treatments in this study clearly demonstrate that the apparent synergy is not the result of a systemic drug-drug interaction.

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