Drug-Drug Interaction Analysis of Pyronaridine/Artesunate and Ritonavir in Healthy Volunteers

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Abstract.   A multiple dose, parallel group study was conducted to assess for a drug-drug interaction between the pyronaridine/artesunate (PA) combination antimalarial and ritonavir. Thirty-four healthy adults were randomized (1:1) to receive PA for 3 days or PA with ritonavir (100 mg twice daily for 17 days, PA administered on Days 8–10). Pharmacokinetic parameters for pyronaridine, artesunate, and its active metabolite dihydroartemisinin (DHA) were obtained after the last PA dose and for ritonavir on Days 1 and 10. Ritonavir coadministration did not markedly change pyronaridine pharmacokinetics but resulted in a 27% increase in artesunate area under the curve (AUC) and a 38% decrease in DHA AUC. Ritonavir exposure was increased 3.2-fold in the presence of PA. The only relevant safety observations were increases in liver enzymes, only reaching a clinically significant grade in the PA + ritonavir arm. It was concluded that coadministered ritonavir and PA interact to alter exposure to artesunate, DHA, and ritonavir itself.

INTRODUCTION

Artemisinin-based combination therapies (ACTs) are central to the treatment of acute malaria infection. The ACTs combine artemisinin derivatives, which produce rapid and profound reductions in parasitemia, with longer acting partner drugs capable of eliminating residual parasitemia. The novel ACT Pyramax (PA), a fixed-dose combination of the artemisinin derivative artesunate and the Mannich base derivative pyronaridine tetraphosphate, has been tested in Phase III clinical trials as a 3-day, once daily treatment regimen for adult and pediatric patients with acute uncomplicated falciparum or vivax malaria.

The intent of this study (clinicaltrials.gov ID: NCT01156389) was to assess the pharmacokinetic and safety implications of coadministration of PA and ritonavir, a protease inhibitor commonly used as a pharmacokinetic booster in antiretroviral drug regimens. Ritonavir was selected for study both because of the high prevalence of human immunodeficiency virus (HIV) in malaria-endemic regions, and the substantial overlap between the pharmacokinetic pathways affected by ritonavir administration and the pathways involved in metabolism of pyronaridine and dihydroartemisinin (DHA), the active metabolite of artesunate. Specifically, ritonavir is both an inhibitor and substrate of CYP3A4, CYP2D6, and the drug efflux pump, P-glycoprotein (P-gp).[1–4] Additionally, ritonavir has the capacity for inducing expression of CYP3A4, CYP1A2, and UDP-glucuronosyltransferases (UGTs).[4] Because CYP3A4, CYP2D6, and CYP1A2 are considered likely responsible for pyronaridine metabolism, and UGTs are known to be responsible for DHA metabolism,[5] pharmacokinetic and safety outcomes associated with coadministration of ritonavir, dosed as a pharmacokinetic enhancer,[1] and PA were judged to merit investigation.

MATERIALS AND METHODS

Study design. Medically normal male and female adults, 18 to 55 years of age, able to understand and comply with study procedures were considered for inclusion. Exclusion criteria were as follows: known history or evidence of any clinically significant medical disorder, clinically significant abnormalities in biochemical, hematological, or urine test results, known history of hypersensitivity or adverse reaction to any study drug, HIV seropositivity, presence or recent history of tobacco abuse (≥ 10 cigarettes/day), required chronic use of any prescription medication, or use of over-the-counter medications, including vitamins, analgesics, or antacids, 1 week before the study start. All subjects were required to have normal serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin. Inclusion of non-pregnant female subjects of childbearing potential was contingent on those subjects agreeing to appropriate contraceptive methods. All subjects provided written informed consent for their participation in the trial. This study was approved before starting by the Ethics Committee of both Basel and Swissmedic.

After enrollment, subjects were randomly assigned in a 1:1 ratio to Arm A or Arm B. Figure 1 provides a timeline of study drug administration for each arm. Subjects in Arm A were administered ritonavir soft gelatin capsules (Norvir; Lot no.: 6008303; Abbott Laboratories, Abbott Park, IL) 100 mg once on Day 1, followed by ritonavir 100 mg every 12 hours on Days 2–17; these subjects were additionally administered three or four PA tablets, depending on weight, once daily on Days 8, 9, and 10. Subjects weighing < 65 kg were administered three PA tablets (180 mg pyronaridine tetraphosphate: 60 mg artesunate per tablet) per day; subjects weighing 65 kg or more were administered four tablets per day. Subjects in Arm A were residents in the clinical research unit starting the evening before the first administration of ritonavir on the morning of Day 1 through the last administration of ritonavir on Day 17; these subjects returned for ambulatory clinic visits on Days 22, 29, 36, 43, and 50. Subjects in Arm B were administered three or four PA tablets, depending on weight, on Days 1, 2, and 3. Subjects in Arm B were residents from the evening before the first administration of PA on Day 1 through the morning of Day 4; these subjects returned for ambulatory clinic visits on Days 5, 6, 8, 15, 22, 29, 36, and 43. In both Arm A and Arm B, morning doses of the study drug were administered with 240 mL of mineral water following

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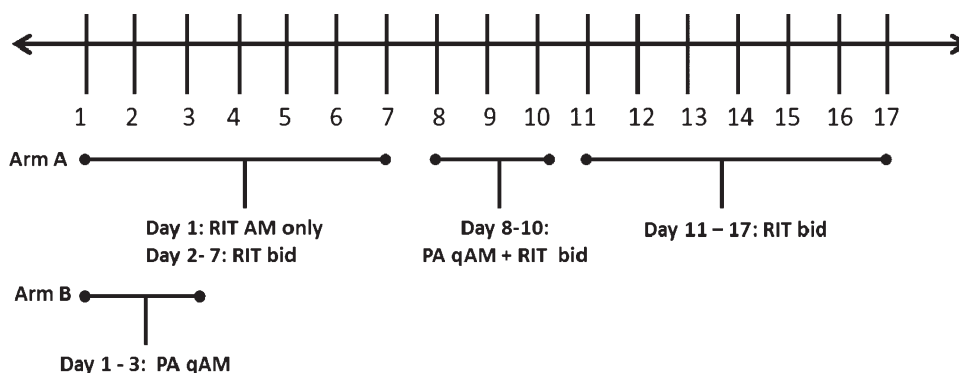
FIGURE 1. Diagram of study drug administration schedule. RIT = ritonavir; PA = pyronaridine/artesunate.

an overnight fast. In Arm A, the evening dose of ritonavir was similarly administered with 240 mL of mineral water.

Intake of grapefruit, grapefruit juice, alcoholic beverages, or caffeine-containing food or beverages within 48 hours before study drug administration was not allowed. Additionally, no medications apart from the study drugs and agents to ameliorate adverse effects were allowed during the course of the study. Acetaminophen and aspirin were to be avoided during the study.

Pharmacokinetic sampling. In Arm A, plasma samples for analysis of ritonavir concentrations were collected on Days 1 and 10 at pre-dose and 1, 2, 3, 4, 6, 8, 9, 12, 18, and 24 hours post-dose. Plasma samples for artesunate/DHA analysis were collected on Day 10 at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours post-dose. Blood samples for pyronaridine analysis were collected on Day 10 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the last dose and at 2, 3, 5, 12, 19, 26, 33, and 40 days after the last dose. In addition, trough concentrations for all analytes were assessed on study Day 8 (pre-dose for pyronaridine and artesunate/DHA) and Day 9 before the morning dosing.

In Arm B, plasma samples for artesunate/DHA analysis were collected on Day 3 at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours post-dose. Blood concentrations for pyronaridine were collected on Day 3 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose, and at 2, 3, 5, 12, 19, 26, 33, and 40 days post-dose. Trough concentrations of pyronaridine, artesunate, and DHA were assessed on study Days 1 and 2 before morning dosing.

For all artesunate/DHA and ritonavir samples, 2 mL of blood was collected in pre-chilled tubes containing fluoride-oxalate and centrifuged for separation of plasma. Plasma was transferred into screw cap cryovials and immediately frozen at or below –80°C until the samples could be shipped to the Clinical Pharmacokinetics Laboratory at the College of Pharmacy, the University of Iowa. For pyronaridine samples, 2 mL of blood was collected in room temperature tubes containing sodium heparin as an anticoagulant. As with plasma samples, whole blood samples were frozen at or below –80°C until the samples could be shipped.

Assay. The liquid chromatography-mass spectrometry analytical method used for determination of artesunate, DHA, and ritonavir was developed from an artesunate/DHA assay described by Naik and others.[6] Briefly, artesunate, α-dihydroartemisinin, ritonavir, and the internal standard artemisinin were extracted from 0.25 mL human plasma using solid phase extraction with Oasis HLB extraction cartridges (Waters, Milford, MA). Analysis was performed on a Shimadzu LCMS-2010A (Shimadzu USA, Columbia, MD) in single ion monitoring positive mode using atmospheric pressure chemical ionization as the interface. Positive ions were measured using selected ion monitoring mode. Chromatography was carried out on a gradient using a Phenomenex Synergi Max-RP, 4 μ, 75 × 2.0 mm column (Phenomenex, Torrance, CA) with an initial mobile phase of 0.04% trifluoroacetic acid, methanol, and acetonitrile (40:45:15) delivered at a flow rate of 0.25 mL/min. Methanol (0.3 mL/min) was added post-column to improve ionization and prevent probe needle clogging. Calibration standards were prepared at concentrations ranging from 1 to 1,400 ng/mL for dihydroartemisinin, 2 to 1,400 ng/mL for artesunate, and 4 to 520 ng/mL for ritonavir. Intraday and interday coefficients of variation were < 15% for dihydroartemisinin, artesunate, and ritonavir.

The liquid chromatography-mass spectrometry assay used for pyronaridine was previously described by Naik and others.[7] Briefly, liquid-liquid extraction with ether was used for sample preparation, with amodiaquine used as the internal standard. Analysis was performed on a Shimadzu LCMS-2010A (Shimadzu) in single ion monitoring mode using atmospheric pressure chemical ionization as the interface. Chromatography was performed using a Gemini 5 μm C18 3.0 × 150 mm column (Phenomenex) with a 2 mM perfluorooctanoic acid and acetonitrile mixture as a mobile phase delivered at a flow rate of 0.5 mL/min. The mobile phase was delivered in gradient mode. Calibration standards were prepared at concentrations ranging from 5.7 to 855 ng/mL. Pyronaridine intraday and interday coefficients of variation were both < 15%.

Safety analysis. Vital signs and clinical laboratory parameters (hematological, biochemical, and urinalysis) were obtained at screening, Day 1 (pre-dose), and at the study completion visit (Day 50 for Arm A, Day 43 for Arm B) for all subjects. In Arm A, vital signs (blood pressure and heart rate) were additionally assessed on Day 8 and Day 17 and clinical laboratory parameters on Days 8 (pre-dose), 11, 15, and 36. In Arm B, vital signs were additionally assessed on Day 4 and clinical laboratory parameters on Days 4, 8, and 29. Adverse effects were assessed at all visits for subjects in both arms.

Twelve-lead electrocardiogram (ECG) tracings were obtained for subjects in Arm A at screening and at pre-dose, 1.5, 4, and 8 hours post-dose on Day 1 and Day 8. Continuous recording was conducted on Day 10 from pre-dose through 24 hours post-dose. The ECG tracings were also obtained on Day 15 and the study completion visit. In Arm B, ECGs were obtained at screening and at pre-dose, 1.5, 4, and 8 hours post-dose on

Day 1. Continuous recording was conducted on Day 3 from pre-dose through 24 hours post-dose. The ECG tracings were also obtained on Day 8 and at the study completion visit. The ECG tracings and continuous recordings were evaluated in a central ECG laboratory for changes in morphology, central tendency in parameters, and outlier effects. The ECGs were manually read; the QT interval was evaluated after correction by heart rate by means of both the Bazett (QTcB = QT*[HR/60]$^{1/2}$) and the Fridericia (QTcF = QT*[HR/60]$^{1/3}$) correction where QTc stands for heart rate corrected QT interval, the final letter indicates the correction used (thus QTcB stands for QT corrected using the Bazett correction and QTcF stands for QT corrected using the Fridericia correction) and HR stands for heart rate.

Genotyping. Genotyping was used to classify each subject as a CYP2D6 poor, intermediate, or extensive metabolizer.

Pharmacokinetic analysis. The following parameters for pyronaridine, artesunate, DHA, and ritonavir (as applicable) were computed by standard noncompartmental methods for each subject: peak observed concentration (C_{max}), time of peak observed concentration (T_{max}), half-life, area under the concentration-time curve from Hour 0 through the last quantifiable concentration (AUC_{0-t}), area under the concentration-time curve from 0 to infinity (AUC_{0-inf}), area under the concentration-time curve from Hour 0 to the scheduled time of the next dose (AUC_{0-tau}). Half-life was computed as ln(2)/Kel, where Kel is the magnitude of the slope of the log concentration versus time profile during the terminal phase. Kel was only considered estimable if the adjusted R^2 value for the regression line was equal to at least 0.85 and if a concentration decline of at least one-half occurred between the first and last points used in the regression. For ritonavir, T_{max}, C_{max}, C_{12h}, half-life, and AUC_{0-tau} were computed for both Day 1 (0–24 hours post-dose) and Day 10 (0–12 hours post-morning dose) concentration-time profiles. WinNonlin version 5.0 (Pharsight Corporation, St. Louis, MO) was used to perform pharmacokinetic analyses.

Values of C_{max}, AUC_{0-tau}, AUC_{0-inf}, and AUC_{0-t} were normalized to mg/kg doses of 3.25 mg/kg artesunate for artesunate/DHA parameters, 9.72 mg/kg pyronaridine tetraphosphate for pyronaridine parameters, and 1.4 mg/kg ritonavir for ritonavir parameters to reduce variability in pharmacokinetic parameters resulting from the differing mg/kg doses administered to each subject.

Sample size determination. The sample size for this trial was calculated for an evaluation of a potential interaction induced by the coadministration of ritonavir on pyronaridine pharmacokinetics. In a previous study using the same PA formulation the highest coefficient of variation for AUC_{0-inf}, AUC_{0-t}, or Cmax was 35.70%, which was observed for AUC_{0-t}. The threshold for a clinically relevant difference was set at 33.33% on the basis of prior knowledge of the dose-effect relationship of pyronaridine. A lack of a relevant interaction in this study was concluded if the 90% confidence interval (CI) for the geometric mean of the test (drug administered with ritonavir)/reference (drug administered without ritonavir) ratio of parameters lay in the 66.67–150.00% range. For a coefficient of variation of 35.70% and an equivalence acceptance range of 66.67–150.00%, a sample size of 15 subjects per treatment arm provides a statistical power of 85%. Two additional subjects per treatment group were recruited to cover for potential withdrawals.

Statistical analysis. To assess the effects of ritonavir on the pharmacokinetics of pyronaridine, artesunate, and DHA, the 90% CI for the geometric mean of the test (drug administered with ritonavir)/reference (drug administered without ritonavir) ratio for the parameters C_{max}, AUC_{0-t}, AUC_{0-inf}, and AUC_{0-tau} were computed using two one-sided t tests. Although the 0.6667–1.5000 interval described previously was used in the formal drug interaction analysis as the no relevant interaction interval for pyronaridine, artesunate, and DHA, this interval was considered to be most clinically relevant to an interaction effect on pyronaridine pharmacokinetics. Given the broad artesunate dosage range recommended by the World Health Organization (WHO) for treatment of acute uncomplicated malaria (2–10 mg/kg/day),[8] and the lack of artesunate dosing above 5 mg/kg/day in the recommended PA dosing scheme, a clinically relevant increase in artesunate/DHA exposure would necessitate a drug interaction effect of substantially greater magnitude than the 33.33% difference delimiting the 0.6667–1.5000 interval. However, a decrease in artesunate/DHA exposure of only slightly > 33.33% could certainly be clinically relevant, particularly for subjects receiving < 3 mg/kg/day of artesunate.

The effects of PA on the pharmacokinetics of ritonavir were assessed by constructing a 90% CI, based on a paired sample t test, for the geometric mean ratio between the AUC_{0-12hr} on Day 10 (during administration of ritonavir with PA) and the AUC_{0-inf} on Day 1. In the absence of any interaction or time-dependent effect on ritonavir pharmacokinetics, this ratio would be expected to equal 1. The software SPSS 17 (SPSS, Inc., Chicago, IL) was used to perform statistical analyses.

RESULTS

Subject demographics. Thirty-four subjects were enrolled in the trial and randomized equally to Arm A and Arm B. In Arm A, 9 of 17 subjects were male; in Arm B, 11 of 17 subjects were male. One subject in Arm B was withdrawn from the study because of vomiting after the first dose of PA; this subject was not included in pharmacokinetic analyses. Four subjects were withdrawn from Arm A because of liver enzyme elevations detected during ritonavir and PA coadministration as detailed under "Safety analysis" below.

In Arm A, three subjects were classified as poor CYP2D6 metabolizers, eight as intermediate CYP2D6 metabolizers, and six as extensive CYP2D6 metabolizers. In Arm B, one subject was classified as a poor CYP2D6 metabolizer, six as intermediate CYP2D6 metabolizers, and nine as extensive CYP2D6 metabolizers.

Safety analysis. No clinically significant changes in the hematologic or urinalysis laboratory parameters were observed during the course of the study. Additionally, no trends were detected in mean or individual subject supine systolic and diastolic blood pressure and pulse rate or in their orthostatic changes upon standing. Although transient changes in blood pressure and pulse rate were noted at isolated time points for some subjects, none of these findings were judged clinically important.

Adverse events occurring in more than two subjects are summarized in Table 1. Diarrhea was the most commonly reported drug-related adverse event after PA administered alone. The incidence of diarrhea was similar in subjects who received PA alone (9 events in 8 of 17 subjects) or in

TABLE 1

Treatment emergent adverse events occurring in more than two subjects for any of the treatments (all causalities)

	Number of subjects with adverse events		
	Arm A (N = 17)		Arm B (N = 17)
	Ritonavir	Ritonavir + PA	PA
Abdominal pain upper	1	3	2
Diarrhea	1	7	8
Nausea		3	3
Fatigue	2	2	1
ALT increased > 3× ULN		5	
Dizziness	1	2	4
Headache	2	4	5
Hot flush		3	

PA = pyronaridine/artesunate; ALT = alanine aminotransferase; ULN = upper limit of normal.

combination with ritonavir (9 events in 7 of 7 subjects). The number of drug-related adverse events reported after ritonavir alone was low, with headache and fatigue being the only two adverse events reported on more than one occasion.

Clinically significant findings in biochemical laboratory parameters were observed only for liver function parameters. Elevations in aminotransferases deemed clinically significant by study physicians, with peak values above 3.0× upper limit of normal (ULN), were observed in five subjects, four females and one male, after ritonavir and PA coadministration. Four of these subjects were withdrawn from the study because of these elevations. Among the four subjects withdrawn from the study, the elevations were more than 5× ULN for both ALT and AST; AST was observed for two subjects and for ALT only for one subject. None of the elevations in liver aminotransferases fulfilled Hy's law criteria for drug-induced liver injury as defined in the U.S. Food and Drug Administration (FDA) Guidance on the Premarketing Evaluation of Drug-induced Liver Injury,[9] because all values of serum bilirubin determined during the treatment period remained within the normal range. Additionally, no decreases in serum albumin or increases in international normalized ratio, creatine kinase, or lactate dehydrogenase were observed in these subjects. None of these subjects had increased blood eosinophil counts at any time. The four subjects withdrawn from the study were screened for a panel including the most frequent viruses (cytomegalovirus DNA and immunoglobulin M [IgM], Epstein-Barr, hepatitis A, B, and E IgM, hepatitis C RNA) responsible for viral hepatitis, with negative results in all cases.

The clinically significant ALT increases were first noticed on Day 8 in one subject, Day 11 in three subjects, and Day 15 in a fifth subject. The ALT had returned to the reference range by Days 22, 29, 43, and 50 in one subject each. Values were still high but decreasing by Day 17 (ALT 3.0× ULN down from a peak on Day 13 of 9.8× ULN) in one subject in whom no further follow-up within the study is available; additionally, by the time this subject left the study, AST had returned to the reference range from a peak on Day 11 of 5.4× ULN.

Some of the subjects with clinically significant increases in ALT reported potentially associated symptoms concomitantly with the ALT increase, including two cases of fatigue, two cases of nausea, and one case of decreased appetite. Abdominal pain was also reported by two subjects, but in one of the subjects it was initially reported before the ALT increase and was associated with diarrhea.

Lower grade increases in ALT (values below 3× ULN) were observed in some additional subjects on PA coadministered with ritonavir (up to 6 of 17 total subjects with increased ALT and up to 7 of 17 subjects with increased AST on a given day) and in up to 4 of 16 subjects treated with PA alone. The maximum number of subjects on PA alone with aminotransferase increases was found on Days 4 and 8 for ALT and on Day 8 for AST.

The relative increase in mean ALT (Arm A: ratio of Day 15 value [peak]/Day 1 value was 4.4; Arm B: ratio of Day 8 value [peak]/Day 1 value was 2.2) was higher than the relative increase in mean AST (Arm A: ratio of Day 11 value [peak]/Day 1 value was 2.6; Arm B: ratio of Day 4 value [peak]/Day 1 value was 1.5). No subjects had clinically relevant increases in AST but not in ALT. Increases in ALP were less frequent than those in ALT and AST (up to three subjects receiving ritonavir/PA on Day 15, with these three subjects also having clinically significant increases in ALT, and only one subject receiving PA alone). The influence of CYP2D6 genotype could not be definitively evaluated because of the small number of subjects from each CYP2D6 metabolizer class.

Few changes in ECG morphology were noted during this study. None of the observed findings was considered to be a relevant abnormality of the ventricular rhythm Heart rate corrected QT interval values and did not exhibit any increasing trend after PA administration. Few subjects (QTcB 2 of 17 on ritonavir alone, 1 of 17 on PA + ritonavir and 1 of 17 on PA alone; QTcF 1 of 17 on PA alone) had corrected QT values that exceeded 450 ms and none of the subjects had a value that exceeded 500 ms or a change from baseline in corrected QT exceeding 60 ms.

Pharmacokinetic analysis. The geometric mean half-life of pyronaridine was 14.2 and 13.1 days for Arm A and Arm B, respectively, with peak concentrations occurring on average at 1.8 hours in Arm A and 1.4 hours in Arm B. Further details of the pyronaridine parameters are given in Table 2. The 90% CI for the ratios of geometric means of most pharmacokinetic parameters were contained in the 0.6667–1.500 range, as shown in Table 3. The only exception was C_{max}, for which the upper limit of the 90% CI (point estimate 1.2255 with a 90% CI: 0.9983, 1.5045) extended slightly above the predefined no relevant effect interval. No association was apparent between CYP2D6 genotype and any pyronaridine pharmacokinetic parameter (data not shown).

The average half-life of artesunate was 0.410 hours and 0.433 hours in Arm A and Arm B, respectively. The AUC_{0-inf}

TABLE 2

Pharmacokinetic parameters of pyronaridine in subjects administered pyronaridine/artesunate (PA) + ritonavir (Arm A) or administered PA only. C_{max} and area under the curve (AUC) values are normalized to 9.72 mg/kg†

	Arm A (PA + ritonavir) [N = 17]	Arm B (PA only) [N = 16]
Half-life (days)	14.2 (50.3%)‡	13.1 (20.5%)
T_{max} (days)	0.078 (65.5%)	0.059 (25.7%)
C_{max} (ng/mL)	462.0 (28.5%)	377.2 (42.6%)
C_{trough} (ng/mL)	119.2 (47.5%)	114.1 (36.8%)
AUC_{0-t} (day*ng/mL)	955 (38.0%)§	1059 (34.7%)
AUC_{0-inf} (day*ng/mL)	1212 (24.3%)‡	1248 (34.4%)
AUC_{0-tau} (day*ng/mL)	222 (36.5%)	210 (36.8%)

†All values given as geometric mean (geometric %CV).
‡N = 15.
§N = 16.

TABLE 3

Geometric mean ratios (90% confidence intervals [CI]) for the pharmacokinetic parameters of pyronaridine, artesunate, and DHA after administration with ritonavir (Arm A) or without ritonavir (Arm B)

	Ratio: Arm A/Arm B (90% CI)		
	Pyronaridine [$N = 33$]	Artesunate [$N = 33$]	DHA [$N = 33$]
C_{max}	1.2255 (0.9983, 1.5045)	0.9954 (0.6408, 1.5462)	0.7348 (0.5500, 0.9815)
AUC_{0-t}	0.9020 (0.7277, 1.1181)*	1.1470 (0.7833, 1.6797)	0.6432 (0.4875, 0.8486)
AUC_{0-inf}	0.9717 (0.8132, 1.1610)†	1.2707 (0.8481, 1.9038)‡	0.6169 (0.4562, 0.8343)¶
AUC_{0-tau}	1.0590 (0.8589, 1.3058)	1.2691 (0.8476, 1.9001)‡	0.6170 (0.4565, 0.8338)¶

* $N = 32$.
† $N = 31$.
‡ $N = 21$.
¶ $N = 30$.
AUC = area under the curve.

and AUC_{0-tau} estimates were essentially equivalent, as would be expected given the observed rapid elimination of artesunate (Table 4). The 90% CIs for the Arm A/Arm B ratio of geometric means of C_{max}, AUC_{0-t}, AUC_{0-inf}, and AUC_{0-tau} are given in Table 3; these CIs were not wholly contained within the 0.6667–1.500 range, largely because of the high between subject variability associated with the artesunate pharmacokinetic parameters. Considering point estimates for ratios of these parameters, coadministration of ritonavir appeared to result in a negligible effect on C_{max} and mean increases of up to 27% in the AUC values of artesunate.

The DHA pharmacokinetic parameters in the two study arms are presented in Table 4, with the 90% CIs for the ratios of geometric means of C_{max}, AUC_{0-t}, AUC_{0-inf}, and AUC_{0-tau} given in Table 3. The lower limit of the 90% CIs for the Arm A/Arm B ratios of all of these parameters extend below 0.6667, with the upper limit of the CIs falling at 0.9815 for C_{max} and below 0.85 for all AUC parameters. The point estimates for the ratios of geometric means indicate that on average, ritonavir coadministration was associated with decreases in DHA C_{max}, AUC_{0-t}, and AUC_{0-inf} of ~27%, 36%, and 38%, respectively.

Pharmacokinetic parameters for ritonavir are shown in Table 5. The average ritonavir half-life estimates on Day 1 and Day 10 were 6.46 and 3.96 hours, respectively. For all subjects this half-life decrease remained essentially constant from Day 1 to Day 10. The point estimate and 90% CI of the ratio of geometric means for AUC_{0-12hr} on Day10 (steady state) and AUC_{0-inf} on Day 1 was computed to be 3.2001 (90% CI: 2.5678, 3.9882). In the absence of any time-dependent or interaction-dependent changes in ritonavir pharmacokinetics, the accumulation ratio should have resulted in a value of 1.0 rather than 3.2.

Pharmacokinetic/safety correlation. Analysis of artesunate, DHA, ritonavir, and pyronaridine pharmacokinetic parameters (unadjusted for mg/kg dosing to reflect individual patient exposure) in subjects with clinically significant hepatic enzyme elevations yielded no clear pattern for artesunate, DHA, or ritonavir parameters. However, within Arm A these subjects displayed four of the top five pyronaridine C_{max} values, with these four values corresponding to the first, second, fourth, and seventh highest C_{max} values in the combined Arm A and Arm B population. The three highest AUC_{0-tau} values in Arm A were observed in the three Arm A subjects displaying ALT elevations more than 5× ULN. These three values corresponded to the first, third, and fourth highest AUC_{0-tau} values in the combined population. Overall, despite the trend toward higher pyronaridine C_{max} and AUC_{0-tau} values among subjects experiencing clinically significant aminotransferase elevations, there was notable overlap in the magnitude of parameters in subjects displaying and those not displaying significant liver enzyme elevations.

DISCUSSION

The objective of this study was to determine whether there was a drug interaction with pharmacokinetic or safety implications between ritonavir, administered at doses used for pharmacokinetic boosting, and fixed dose PA in healthy subjects. On the basis of the 90% CI for the ratio of geometric means of C_{max}, there was an effect, on average, of ritonavir on pyronaridine pharmacokinetics. However, these findings appear to lack clinical relevance as the 90% CI was very close to the acceptance range. For artesunate, the high degree of variability among the subjects in C_{max} and AUC values resulted in wide CIs for the ratios of those values between the two study groups. On average, although C_{max} values were similar in the presence and absence of ritonavir, artesunate

TABLE 4

Pharmacokinetic parameters of artesunate and dihydroartemisinin (DHA) in subjects administered pyronaridine/artesunate (PA) + ritonavir (Arm A) or administered PA only*

	Artesunate		DHA	
	Arm A (PA + ritonavir) [$N = 17$]	Arm B (PA only) [$N = 16$]	Arm A (PA + ritonavir) [$N = 17$]	Arm B (PA only) [$N = 16$]
Half-life (hours)	0.410 (28.0%)†	0.433 (39.5%)‡	2.04 (52.6%)¶	2.18 (42.4%)§
T_{max} (hours)	1.05 (61.4%)	0.75 (54.2%)	1.77 (40.5%)	1.39 (29.1%)
C_{max} (ng/mL)	95.1 (108%)	95.5 (61.9%)	539.5 (63.9%)	734.3 (37.6%)
AUC_{0-t} (hours*ng/mL)	120 (86%)	105 (55.5%)	1151 (61.2%)	1790 (35.1%)
AUC_{0-inf} (hours*ng/mL)	157 (70.5%)†	124 (49.3%)‡	1164 (63.9%)¶	1887 (34.4%)§
AUC_{0-tau} (hours*ng/mL)	157 (70.5%)†	124 (49.1%)‡	1162 (63.8%)¶	1884 (34.2%)§

* C_{max} and area under the curve (AUC) values are normalized to an artesunate dose of 3.25 mg/kg. All values given as geometric mean (geometric %CV).
† $N = 9$.
‡ $N = 13$.
¶ $N = 16$.
§ $N = 14$.

	Day 1 [$N = 17$]	Day 10 [$N = 17$]
Half-life (hours)	6.46 (27.3%)	3.96 (25.1%)
T_{max} (hours)	3.46 (51.6%)	2.27 (39.5%)
C_{max} (ng/mL)	492 (81.1%)	2751 (52.4%)
C_{12hr} (ng/mL)	147.7 (59.3%)	416 (57.7%)
AUC_{0-12hr} (hours*ng/mL)	3072 (73.8%)	14825 (51.8%)
AUC_{0-inf} (hours*ng/mL)	4633 (62.4%)	
AUC_{0-24hr} (hours*ng/mL)	4127 (65.2%)	

*C_{max} and area under the curve (AUC) values are adjusted for 1.4 mg/kg q12hr dose. All values given as geometric mean (geometric %CV).

AUC values appeared to increase. However, given that artesunate is hydrolyzed through esterase activity,[10] no clear mechanism for an interaction between artesunate and ritonavir is apparent. Any interaction effect ritonavir may be exerting on artesunate pharmacokinetics is likely minor in relation to the extreme underlying between subject variability in artesunate pharmacokinetics.

More definitive interaction effects were apparent for DHA, with both peak concentrations and exposure to DHA being lower in the setting of ritonavir coadministration. The DHA is eliminated through conjugation with glucuronic acid by UGTs.[5] Induction of the metabolizing UGTs by ritonavir could certainly account for the lower DHA AUC values detected in subjects receiving ritonavir. Additionally, the observed reduction in DHA AUC is consistent with reductions in DHA exposure observed when artemether/lumefantrine was coadministered with lopinavir/ritonavir (400 mg/100 mg bid).[11] Artemether/lumefantrine coadministration was carried out at the end of the second week of lopinavir/ritonavir administration. Coadministration with lopinavir/ritonavir resulted in DHA C_{max} and AUC_{0-t} reductions of 36% and 45%, respectively. There was a trend toward decreases in these measures for artemether, the parent drug, as well; however, because these decreases were smaller than those found for DHA, ritonavir induction of DHA metabolism likely contributed to the observed reductions in DHA exposure.

Coadministration of PA resulted in substantially increased exposure to ritonavir, suggesting that either total body clearance decreased and/or the extent of ritonavir absorption increased. From *in vitro* evidence, pyronaridine is an inhibitor of CYP2D6, which is involved in the metabolism of ritonavir, and of P-gp, which is involved in the efflux transport of ritonavir.[1,3,12,13] Either CYP2D6 or P-gp inhibition could produce the observed increase in exposure. If the increase in exposure stemmed primarily from CYP2D6 inhibition, some increase in ritonavir half-life on Day 10, as compared with Day 1, would be anticipated. However, half-life decreased or remained essentially constant, suggesting that the increased ritonavir exposure may have stemmed primarily from pyronaridine inhibition of P-gp mediated ritonavir efflux. It should be noted that the design of this study does not allow for effects of PA on ritonavir pharmacokinetics to be differentiated from any intrinsic time-dependency of ritonavir pharmacokinetics. Hsu and others[14] evaluated time-dependent ritonavir pharmacokinetics and determined that pre-dose concentrations trended downward when examined from Day 5 to Day 16 of ritonavir administration; the authors propose ritonavir auto-induction as the source of the decline in pre-dose concentrations. In this study, ritonavir auto-induction would be expected to decrease Day 10 AUC_{0-tau} relative to Day 1 AUC_{0-inf}. Because a marked increase was observed instead, it is highly likely that drug interaction effects, rather than time-dependent changes in ritonavir pharmacokinetics, are responsible.

The PA administered alone for 3 days was well tolerated, with gastrointestinal adverse effects being most commonly reported. Following PA coadministration with ritonavir, a similar pattern of adverse events was observed with the exception of clinically significant, but reversible, increases in ALT, with or without increases in AST occurring in five subjects. These increases returned to baseline during Days 22 to 50 in four of the subjects, whereas in the fifth it was decreasing by Day 17 (ALT 3.0× ULN on Day 17 down from a peak of 9.8× ULN on Day 13 and with three values in between decreasing as compared with the previous one). Although there was a trend toward higher pyronaridine C_{max} and AUC_{0-tau} levels in these five subjects, there was overlap between these parameters in subjects displaying and not displaying liver enzyme elevations. This was particularly true when considering the combined Arm A and Arm B population.

The PA therapy administered alone to healthy subjects has previously resulted in liver enzyme elevations, but the incidence and severity of the elevations observed in Arm A of the present trial would not be anticipated based upon previous PA trials.[15,16] Ritonavir itself has been associated with hepatic enzyme elevations in clinical trials.[1] However, ritonavir exposure alone cannot account for elevations observed in this trial. In particular, despite the elevated Day 10 ritonavir concentrations, ritonavir exposure in this trial would still remain below exposure resulting from the 600 mg twice daily ritonavir regimen used for antiretroviral therapy in many ritonavir clinical trials.[1] Additionally, such trials typically involved HIV-positive patients who, unlike the healthy volunteers in our study, could present with comorbidities and receive comedications predisposing them to ALT and AST elevations. Therefore, PA and ritonavir exposure considered separately cannot account for the observed liver enzyme elevations in this trial. Instead, coadministration of ritonavir and PA appear to have increased the likelihood of liver enzyme elevations in a manner that cannot be accounted for by the observed pharmacokinetic parameters from any one of the examined moieties.

Although this study did not allow for the precise mechanism of the hepatic enzyme elevations associated with PA and ritonavir coadministration to be elucidated, two definitive pharmacokinetic conclusions can be drawn from this trial. First, PA coadministration appears to substantially increase ritonavir exposure, likely caused by pyronaridine P-gp inhibition of ritonavir efflux. Second, ritonavir coadministration substantially decreases exposure to DHA. This finding is of particular clinical relevance because currently available ACTs contain artesunate or artemether, which are metabolized to DHA, or DHA itself. The reduced exposure to DHA associated with ritonavir coadministration poses an increased risk of suboptimal antimalarial efficacy; the clinical effects of reduced exposure may be augmented by the immunocompromised state of the patient population receiving ritonavir therapy. Given this risk, the pharmacokinetic and clinical effects of ritonavir coadministration on DHA pharmacokinetics are deserving of further investigation, optimally in an HIV-positive patient population, to determine if dose adjustments of

artemisinin derivatives should be considered when treating malaria patients receiving chronic ritonavir therapy.

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