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Open-Label, Single-Dose, Parallel-Group Study in Healthy Volunteers To Determine the Drug-Drug Interaction Potential between KAE609 (Cipargamin) and Piperaquine

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KAE609 represents a new class of potent, fast-acting, schizonticidal antimalarials. This study investigated the safety and pharmacokinetics of KAE609 in combination with the long-acting antimalarial piperaquine (PPQ) in healthy volunteers. A two-way pharmacokinetic interaction was hypothesized for KAE609 and PPQ, as both drugs are CYP3A4 substrates and inhibitors. The potential for both agents to affect the QT interval was also assessed. This was an open-label, parallel-group, single-dose study with healthy volunteers. Subjects were randomized to four parallel dosing arms with five cohorts (2:2:2:2:1), receiving 75 mg KAE609 plus 320 mg PPQ, 25 mg KAE609 plus 1,280 mg PPQ, 25 mg KAE609 alone, 320 mg PPQ alone, or 1,280 mg PPQ alone. Triplicate electrocardiograms were performed over the first 24 h after dosing, with single electrocardiograms at other time points. Routine safety (up to 89 days) and pharmacokinetic (up to 61 days) assessments were performed. Of the 110 subjects recruited, 99 completed the study. Coadministration of PPQ had no overall effect on exposure to KAE609, although 1,280 mg PPQ decreased the KAE609 maximum concentration (C_{max}) by 17%. The group that received 25 mg KAE609 plus 1,280 mg PPQ showed a 32% increase in the PPQ area under the concentration-time curve from 0 to infinity (AUC_{inf}), while the group that received 75 mg KAE609 plus 320 mg PPQ showed a 14% reduction. Mean changes from baseline in the QT interval corrected by Fridericia's method (QTcF) and the QT interval corrected by Bazett's method (QTcB) with PPQ were consistent with its known effects. PPQ but not KAE609 exposure correlated with corrected QT interval (QTc) increases, and KAE609 did not affect the PPQ exposure-QTc relationship. The QTcF effect for PPQ (least-squares estimate of the difference in mean maximal changes from baseline of 7.47 ms [90% confidence interval, 3.55 to 11.4 ms]) was consistent with the criteria for a positive thorough QT study. No subject had QTcF or QTcB values of >500 ms. Both drugs given alone or in combination were well tolerated, with no deaths, serious adverse events (AEs), or severe AEs reported. Most AEs were mild; upper respiratory tract infections, headache, diarrhea, and oropharyngeal pain were most common. PPQ and KAE609 coadministration had no relevant effect on exposure to either agent, and KAE609 did not affect or potentiate the known effects of PPQ on cardiac conduction.

Malaria-related fatalities have declined substantially in the past 15 years. However, the World Health Organization (WHO) still reported an estimated 207 million malaria cases in 2012, leading to 627,000 deaths (1). Most malaria-related deaths are of children under the age of 5 years, who lack the partial immunity that often protects adults living in areas in which the disease is endemic from severe disease (1). About a decade ago, WHO began recommending artemisinin-based combination therapies as the first-line treatment for uncomplicated *Plasmodium falciparum* infections (2). Alarmingly, resistance to artemisinin has now become geographically widespread across Southeast Asia (3). It is feared that this resistance will become more prevalent and spread globally, as has occurred with all prior classes of antimalarials. Novel, non-artemisinin-based antimalarial drugs are critically important in this changing disease landscape.

KAE609 (cipargamin) is a spiroindolone with potent activity against blood-stage malaria parasites (4, 5). The compound was safe and tolerable and had approximately linear pharmacokinetics (PK) in healthy volunteers at single doses up to 300 mg and multiple doses up to 150 mg once daily for 3 days (6). A phase 2 study of KAE609 in 21 adults with uncomplicated *Plasmodium vivax* ($n = 10$) or *P. falciparum* ($n = 11$) malaria was conducted in Bangkok and on the Thai-Myanmar border (7). Once-daily dosing of KAE609 at 30 mg for 3 days resulted in median parasite clearance (CL) times of approximately 12 h for both *P. vivax* and *P. falciparum*, including in patients with artemisinin resistance. Furthermore, the median parasite clearance half-life ($t_{1/2}$) values were 0.95 h for patients with *P. vivax* and 0.90 h for those with *P. falciparum*. These preliminary data suggest a parasite clearance rate comparable to or faster than that of artemisinin. Treatment with KAE609 was well tolerated by the patients; nausea was the most common adverse event (AE) (7).

Treatment of malaria with monotherapy is strongly discouraged, due to the risk of resistance. Therefore, for clinical use, antimalarial compounds are combined with at least one other drug,

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representing a different mode of action. One potential combination partner for KAE609 is piperaquine (PPQ). PPQ is an approved antimalarial that is currently available in fixed-dose combination with dihydroartemisinin under the label Eurartesim (Sigma-Tau and Medicines for Malaria Venture). Eurartesim is well tolerated (8), although PPQ is known to have effects on cardiac conduction, most notably in terms of exposure-related corrected QT interval (QTc) prolongation, with no plateau of effect described in the range examined and a duration of at least 24 h (9, 10). For KAE609, no significant changes in electrocardiographic (ECG) parameters have been observed in clinical studies conducted to date (7); however, preclinical studies (*in vitro* and in animals) have suggested a potential risk for QTc prolongation.

KAE609 is metabolized by CYP3A but does not appear to induce it, and it has the potential to inhibit CYP3A, based on *in vitro* data (unpublished Novartis data). PPQ is metabolized by CYP3A4 and can also inhibit the enzyme (9); hence, a two-way interaction during drug exposure was hypothesized. The primary objective of this study was to evaluate the safety and drug-drug interaction potential of KAE609 and PPQ. Triplicate ECG evaluations were conducted to evaluate possible QT interval changes in the presence of both drugs.

MATERIALS AND METHODS

Subjects. Eligible subjects were healthy male and female subjects, 18 to 45 years of age and weighing at least 50 kg. Vital signs measured at screening and baseline had to be within normal ranges (oral body temperature, 35.0 to 37.5°C; systolic blood pressure, 90 to 140 mm Hg; diastolic blood pressure, 50 to 90 mm Hg; pulse rate, 40 to 90 beats/min). Women of childbearing potential were excluded from this study. Other exclusion criteria included a history or the presence of clinically significant ECG abnormalities or arrhythmias (PR interval of >200 ms, QRS interval of >120 ms, or QT interval corrected by Fridericia's method [QTcF] of >430 ms [male] or >440 ms [female]) and a family history or the presence of long QT syndrome. Subjects were excluded if they had hypersensitivity to any of the study drugs or similar chemical classes, food allergies, or a history of malignancy, autonomic dysfunction, bronchospastic disease, conditions that might alter drug absorption, distribution, metabolism, or excretion, or pancreatic, liver, or renal dysfunction. Subjects with hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, or other immunodeficiency diseases were excluded. Subjects were excluded if they had recently used other investigational drugs, and they were not allowed to ingest grapefruit juice, St. John's wort, or agents that can interact with either KAE609 or PPQ for at least 7 days prior to dosing and during the study. All patients provided written informed consent, and the study was approved by the ethics committee at each participating site.

Study design. This was an open-label, parallel-group, single-dose, randomized study in healthy volunteers. The dose of KAE609 currently being evaluated for use in malaria is 75 mg. The highest approved dose of PPQ used clinically in Eurartesim is 1,280 mg once a day (fasting) for 3 days. Prior to study initiation, SimCYP (Certara) simulations using midazolam as a surrogate for PPQ (substrate for CYP3A4) suggested that KAE609 could potentially increase the exposure to PPQ by up to 2-fold (as a worst-case scenario). Based on the *in vitro* data, an increase in KAE609 exposure by PPQ of up to 4-fold (worst-case scenario) was predicted (DDI Predict version 1.7; Aureus Sciences, France). As PPQ has known exposure-related effects on the QT interval and there was concern regarding potential additional pharmacodynamic (PD) effects on the QT interval, the doses of KAE609 and PPQ were reduced in the arms evaluating each as the subject of the potential drug-drug interaction (see below). The long elimination half-life of PPQ (~22 days) and the potential for further prolongation of the half-life with coadministration with KAE609 made a crossover design nonfeasible, because of the long washout periods that would be required. Therefore, the parallel-group design was selected for this study, to address the pharmacokinetic interactions and safety evaluations for the two study drugs singly and in combination.

The study consisted of four treatment arms, which were conducted in parallel. Arms A through C included one cohort each, whereas arm D included two cohorts. Approximately 108 eligible subjects were to be randomly assigned to the 5 cohorts in the four dosing arms, in a 24:24:24:24:12 ratio. Arm A (cohort 1) received a single morning dose of 75 mg KAE609 plus a single dose of 320 mg PPQ. Arm B (cohort 2) received a single morning dose of 25 mg KAE609 and a single dose of 1,280 mg PPQ. Arm C (cohort 3) received a single morning dose of 25 mg KAE609 only. In cohort 4 in arm D, subjects received a single morning dose of 320 mg PPQ only; in cohort 5 in arm D, subjects received a single morning dose of 1,280 mg PPQ only. Subjects who met the inclusion criteria at screening were admitted to baseline evaluations at the trial sites (day −1). Eligible subjects fasted overnight, were dosed on day 1, and then were domiciled at the site from baseline until 24 h postdosing. Subjects then returned to the site at defined times for an 8-week period (arms A, B, and D) or for an 11-day period (arm C), to undergo safety evaluations and PK sampling. Study completion evaluations were conducted after the last PK sampling on day 61 (end of study). The total study duration for subjects in arms A, B, and D was 89 days. Arm C (KAE609 monotherapy) had a shorter study duration (39 days) than arms A, B, and D due to the shorter half-life of KAE609 (~22 to 25 h) compared to PPQ (~22 days).

Pharmacokinetic assessments. Sampling for pharmacokinetic analysis in arms A, B, and D occurred at times 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, 192, 240, 336, 504, 672, 1,008, and 1,440 h. In arm C (KAE609 alone), pharmacokinetic samples were obtained only to 240 h, using the aforementioned schedule. KAE609 was analyzed in samples to the 240-h time point, and piperaquine was analyzed in samples to 1,440 h. Plasma was isolated within 30 min after blood collection, and tubes were kept frozen at or below −70°C until analysis. KAE609 and PPQ in plasma samples were analyzed separately, using validated high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods.

For the KAE609 assay, a 10-μl aliquot of the reconstituted extract was analyzed by HPLC-MS/MS in multiple reaction monitoring (MRM) mode, using electrospray ionization (ESI) as the ionization technique. KAE609[M6] was used as the internal standard.

For the piperaquine assay, a 50-μl aliquot of internal standard (15.0 ng/ml $^{2}H_{6}$-labeled piperaquine) and 400 μl of methanol were added to a 100-μl plasma sample. The mixture was vortex mixed and centrifuged, the supernatant was dried at 40°C under a stream of nitrogen, and an aliquot of reconstituted sample was injected into a reverse-phase liquid chromatography system (Gemini 5-μm C_{18} 110A column) with a gradient mixture for the mobile phase of 0.1% formic acid and 10 mM ammonium acetate in water (pH 4.5) with 0.1% formic acid and 10 mM ammonium acetate in 95% methanol (pH 6.5). A mass spectrometer in MRM mode was used as the detector, with ESI.

The lower limits of quantitation (LLOQs) for KAE609 and PPQ are 1 ng/ml and 0.5 ng/ml, respectively. The linearity ranges for KAE609 and PPQ are 1 to 5,000 ng/ml and 0.5 to 250 ng/ml, respectively. Accuracy and precision for both KAE609 and PPQ were within acceptable limits for study validation. For KAE609, at different concentrations of quality control samples, the coefficient of variation (CV) (precision) and bias (accuracy) were as follows: 2 ng/ml, CV, 10.3%; bias, −4.5%; 5 ng/ml, CV, 4.0%; bias, −5.0%; 400 ng/ml, CV, 5.6%; bias, −2.3%; 2,000 ng/ml, CV, 4.2%; bias, −4.0%; 4,000 ng/ml, CV, 4.6%; bias, −5.8%. Similarly, for PPQ, the values were as follows: 1.5 ng/ml, CV, 7%; bias, −0.7%; 15 ng/ml, CV, 4.8%; bias, −1.3%; 75 ng/ml, CV, 3.7%; bias, −1.9%; 200 ng/ml, CV, 4.2%; bias, −4.0%. Concentrations below the limit of quantification were treated as zero in summary statistics and for the calculation of PK parameters. No formal imputation for missing data was performed. PK parameters of KAE609 and PPQ were determined from the plasma concentration-time data using the recorded sampling times and noncom-

partmental methods with WinNonlin Phoenix (version 6.2). The following PK parameters were determined: maximum concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve (AUC) from 0 to t (AUC_{0-t}) ($t = 24$ h for KAE609 and $t = 24$, 168, and 672 h for PPQ), AUC from 0 to the last measurable point (AUC_{last}), AUC from 0 to infinity (AUC_{inf}), half-life ($t_{1/2}$), CL/bioavailability (F), and apparent volume of distribution during the terminal phase (V_z)/F.

Safety assessments. Safety assessments consisted of recording all adverse events (AEs), with their severity and relationship to study drugs, using the Common Terminology Criteria for Adverse Events (CTCAE) version 4 grading scale. Regular monitoring of hematological, blood chemistry, and urinary parameters (plus testing for HIV, HBV, and HCV at screening) was performed at local laboratories for each center. In addition, regular assessments of vital signs, full physical examination findings, and liver safety parameters and monitoring for pregnancies were undertaken. Standard 12-lead ECGs were performed predosing and 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h, and 96 h postdosing. These time points were chosen to capture the period of potential maximal exposure risk. ECG evaluations were performed either as single assessments or in triplicate (baseline through 24 h), approximately 1 min apart starting approximately 1 min before the scheduled time. Interpretation of the tracings was performed by a qualified physician.

Statistical analyses. Pharmacokinetic parameters were summarized descriptively by treatment group as well as analyzed inferentially. The logarithmically transformed primary PK parameters (C_{max}, AUC_{last}, and AUC_{inf}) for KAE609 and PPQ were analyzed using a linear fixed-effects model with treatment as a fixed effect. The analysis was done on the natural logarithmic scale for PK parameters (C_{max}, AUC_{last}, and AUC_{inf}), and the differences in adjusted means, with 90% confidence intervals (CIs), were calculated for the comparisons of 75 mg KAE609 plus 320 mg PPQ versus 320 mg PPQ, 1,280 mg PPQ plus 25 mg KAE609 versus 25 mg KAE609, and 1,280 mg PPQ plus 25 mg KAE609 versus 1,280 mg PPQ. All results for the defined comparisons were back-transformed to the original scale to present adjusted geometric mean ratios (GMRs) and corresponding 90% confidence intervals. Data for all subjects with at least one PK parameter were considered in the statistical analysis.

For ECG values, the means of triplicate readings were calculated before the statistical analysis was performed. These values were obtained by subtracting the mean of triplicate predose QTcF (or QT interval corrected by Bazett's method [QTcB]) values from the mean of triplicate or single-point postdose QTcF (or QTcB) values for each subject and time point. The change from baseline for QTcF (or QTcB) for each time point was analyzed using a linear fixed-effects model with treatment as a fixed effect and the baseline value (taken as 0 h on day 1) as a covariate. The differences in adjusted means, with 90% confidence intervals, were calculated for the comparisons of 75 mg KAE609 plus 320 mg PPQ versus 320 mg PPQ, 75 mg KAE609 plus 320 mg PPQ versus 1,280 mg PPQ, and 75 mg KAE609 plus 320 mg PPQ versus 25 mg KAE609; 1,280 mg PPQ plus 25 mg KAE609 was contrasted with 1,280 mg PPQ, with 320 mg PPQ, and with 25 mg KAE609. Similar analyses were performed separately for the maximal changes from baseline for QTcF for KAE609 concentrations alone and in the presence of PPQ and for PPQ concentrations alone and in the presence of KAE609, using a linear fixed-effects model with treatment as a fixed effect and the concentrations of KAE609 and PPQ at maximal change as covariates. The differences in adjusted means, with 90% confidence intervals, were calculated for the comparisons of 75 mg KAE609 plus 320 mg PPQ versus 320 mg PPQ, 1,280 mg PPQ plus 25 mg KAE609 versus 1,280 mg PPQ, and 1,280 mg PPQ plus 25 mg KAE609 versus 25 mg KAE609. Similar analyses were also performed for the QTcB, PR interval, and QRS interval.

The study was designed to have 80% power to detect at least a 30% difference in AUC or C_{max}, assuming CVs of 32% for KAE609 and 24% for PPQ, based on a conservative review of prior clinical data, if there were at least 20 subjects per KAE609 or PPQ arm in the comparisons. To account for potential dropouts, the target enrollment was 25 per arm except for the arm with 1,280 mg PPQ alone ($n = 12$). The arm with 1,280 mg PPQ was not for direct PK comparisons but was for additional exposure-response data and safety data on PPQ.

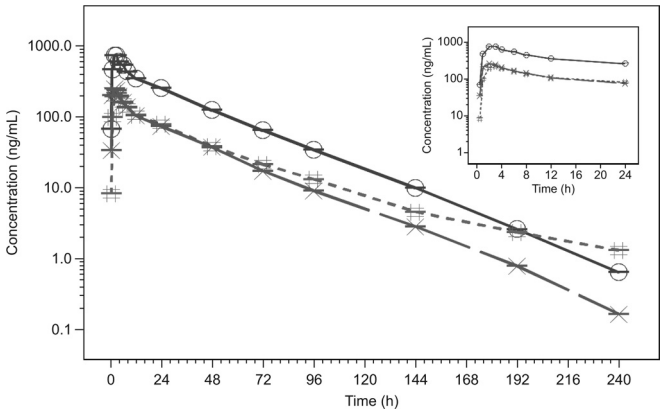

FIG 1 Semilogarithmic plots of arithmetic mean KAE609 plasma concentrations according to time and treatment group. Inset, first 24 h after dosing.

RESULTS

Subjects. A total of 110 subjects were enrolled and randomized, with 25 in arm A (cohort 1; 75 mg KAE609 plus 320 mg PPQ), 24 in arm B (cohort 2; 25 mg KAE609 plus 1,280 mg PPQ), 25 in arm C (cohort 3; 25 mg KAE609), 24 in cohort 4 in arm D (320 mg PPQ), and 12 in cohort 5 in arm D (1,280 mg PPQ). Overall, 90% of subjects completed the study. Discontinuation rates were highest in the groups that received 1,280 mg PPQ (3/12 subjects [25%]) and 75 mg KAE609 plus 320 mg PPQ (5/25 subjects [20%]). For all except one of the subjects who discontinued, the reason was withdrawal of consent. The remaining subject (in the group that received 25 mg KAE609) discontinued due to a protocol violation; this subject had a pulse rate outside the permissible range at baseline and discontinued on day 7. All subjects in each treatment group had evaluable PK and PD data up to the time of discontinuation and so were included in the PK and PD analysis sets. Baseline demographic characteristics were similar between treatment arms. All subjects were male, with a mean age of 25.8 years and a mean weight of 75.7 kg. Most subjects (81.8%) were Caucasian.

PK parameters for KAE609 in plasma in the presence and absence of PPQ. KAE609 concentration-time profiles according to treatment group are shown in Fig. 1 (arithmetic means) and Fig. 2 (medians). The apparent flattening of the semilogarithmic curve in Fig. 1 for the group that received 25 mg KAE609 plus 1,280 mg piperaquine was due to a single outlier (subject 1002 00051). This subject had a KAE609 concentration at 192 h of 45.1 ng/ml, with corresponding concentrations for the other subjects in this treatment group ranging from 0 to 3.14 ng/ml. Semilogarithmic plots of median KAE609 concentrations (which minimized the effects of outliers) did not show flattening of the curve for the group that received 25 mg KAE609 plus 1,280 mg piperaquine (Fig. 2).

All KAE609 PK parameters measured for the group that received 25 mg KAE609 plus 1,280 mg PPQ were similar to those observed for the group that received 25 mg KAE609 (Table 1). Calculation of the geometric mean ratios (GMRs) for the primary KAE609 PK parameters for the two treatment groups indicated

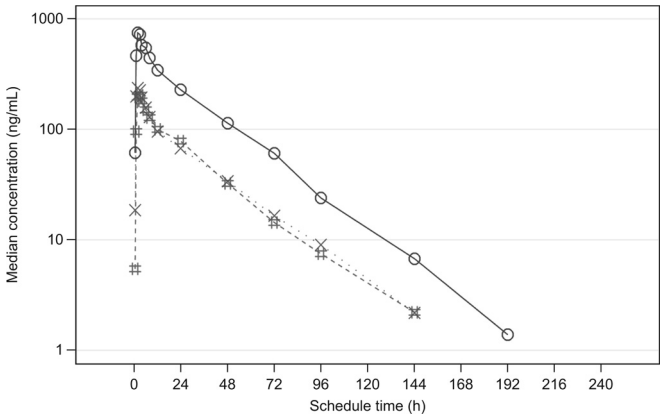

FIG 2 Semilogarithmic plots of median KAE609 concentrations according to time and treatment group (full profile). ○, 75 mg KAE609 plus 320 mg PPQ; #, 25 mg KAE609 plus 1,280 mg PPQ; ×, 25 mg KAE609.

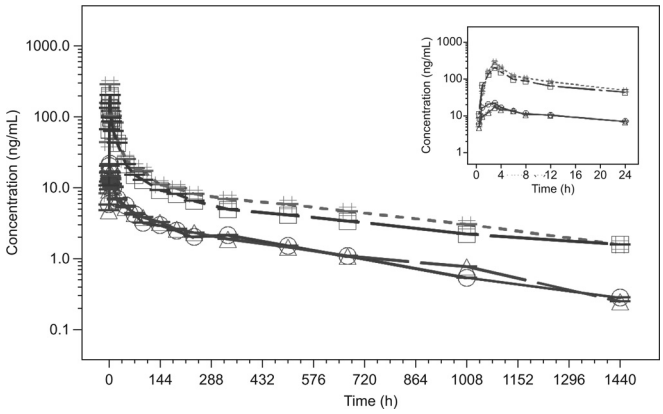

FIG 3 Semilogarithmic plots of arithmetic mean piperaquine plasma concentrations according to time and treatment group. ○, 75 mg KAE609 plus 320 mg PPQ; #, 25 mg KAE609 plus 1,280 mg PPQ; △, 320 mg PPQ; □, 1,280 mg PPQ. Inset, first 24 h after dosing.

that exposure to KAE609 was not affected by coadministration of PPQ, i.e., for AUC_{inf}, the GMR was 1.03 (90% CI, 0.87 to 1.22), and for AUC_{last}, the GMR was 1.02 (90% CI, 0.86 to 1.20). There was, however, a 17% decrease in the KAE609 C_{max} in the presence of 1,280 mg PPQ (GMR, 0.83 [90% CI, 0.73 to 0.94]).

PK parameters for PPQ in plasma in the presence and absence of KAE609. Exposure to PPQ was influenced by coadministration of KAE609, but the effects with increasing KAE609 doses were inconsistent. Figure 3 presents a semilogarithmic plot of mean PPQ concentrations over time according to treatment group.

Coadministration of 25 mg KAE609 with 1,280 mg PPQ modestly increased PPQ exposure, whereas coadministration of 75 mg KAE609 with 320 mg PPQ appeared to yield similar PPQ exposure (Fig. 3 and Table 2). Geometric means for C_{max}, AUC_{last}, and AUC_{inf} were compared for the different cohorts (25 mg KAE609 plus 1,280 mg PPQ versus 1,280 mg PPQ, and 75 mg KAE609 plus 320 mg PPQ versus 320 mg PPQ). This analysis indicated that coadministration of 75 mg KAE609 with 320 mg PPQ led to a 10% decrease in AUC_{last} (GMR, 0.90 [90% CI, 0.72 to 1.12]) and a 14% decrease in AUC_{inf} (GMR, 0.86 [90% CI, 0.70 to 1.07]) for PPQ, together with a 12% increase in C_{max} (GMR, 1.12 [90% CI, 0.85 to 1.49]). In contrast, coadministration of 25 mg KAE609 with 1,280 mg PPQ was associated with a 28% increase in AUC_{last} (GMR, 1.28 [90% CI, 0.96 to 1.72]) and a 32% increase in AUC_{inf} (GMR, 1.32 [90% CI, 0.98 to 1.78]). A 28% increase in PPQ C_{max} was also observed in the presence of 25 mg KAE609 (GMR, 1.28 [90% CI, 0.90 to 1.81]). The reasons for the inconsistent and nonsignificant dose effects of KAE609 on PPQ exposure are unclear.

Safety and tolerability. In this study, 54.5% of subjects experienced at least one adverse event (AE). None of the AEs was serious, none was graded 3 or 4, and all resolved by the end of the study; no patient discontinued due to an AE. The highest overall rate of AEs was observed in the group that received 1,280 mg PPQ. The most common AEs in all treatment groups were classified as infections and infestations, nervous system disorders, and gastrointestinal disorders; no other system or organ class showed AEs affecting more than 10% of the total safety set. There were no AEs affecting the cardiovascular system. The most common AE overall was upper respiratory tract infection, followed by headache in all groups except the group that received 75 mg KAE609 plus 320 mg PPQ (for which oropharyngeal pain was the second most common AE) (Table 3). In addition to upper respiratory tract infections, headache, and oropharyngeal pain, other AEs that occurred in more than one subject were diarrhea, gastritis, abdominal pain, and dry skin. The treatment groups showed some differences in the rates of AEs, but there were no obvious patterns and the differences are difficult to interpret, given the small numbers of AEs.

KAE609 does not affect QTc. PPQ is known to increase the

TABLE 1 Plasma PK parameters for KAE609 in the presence and absence of piperaquine

Parameter	Value for treatment group receiving:		
	75 mg KAE609 + 320 mg PPQ ($n = 25$)[a]	25 mg KAE609 + 1,280 mg PPQ ($n = 24$)	25 mg KAE609 ($n = 25$)[b]
C_{max} (mean ± SD [%CV]) (ng/ml)	760 ± 176 (23.2)	226 ± 60.0 (26.6)	269 ± 57.5 (21.3)
T_{max} (median [range]) (h)	3.00 (1.00–46.5)	3.00 (2.00–6.00)	3.00 (1.00–12.0)
AUC_{0-24} (mean ± SD [%CV]) (μg · h/ml)	9.35 ± 1.90 (20.4)	2.77 ± 0.80 (28.7)	2.93 ± 0.70 (23.9)
AUC_{last} (mean ± SD [%CV]) (μg · h/ml)	18.6 ± 5.53 (29.8)	5.87 ± 3.97 (67.6)	5.35 ± 1.44 (26.8)
AUC_{inf} (mean ± SD [%CV]) (μg · h/ml)	18.6 ± 5.55 (29.8)	6.10 ± 4.54 (74.5)	5.42 ± 1.45 (26.7)
$t_{1/2}$ (mean ± SD [%CV]) (h)	24.4 ± 6.82 (28.0)	26.4 ± 13.0 (49.1)	25.2 ± 9.12 (36.2)
V_z/F (mean ± SD [%CV]) (liters)	149 ± 46.4 (31.2)	172 ± 56.9 (33.1)	170 ± 49.3 (28.9)
CL/F (mean ± SD [%CV]) (liters/h)	4.34 ± 1.16 (26.6)	5.01 ± 1.76 (35.1)	4.98 ± 1.53 (30.7)

[a] $n = 23$ for AUC_{last}, AUC_{inf}, $t_{1/2}$, and V_z/F.
[b] $n = 24$ for AUC_{last}, AUC_{inf}, $t_{1/2}$, and V_z/F.

TABLE 2 PK parameters for piperaquine in the presence and absence of KAE609

Parameter	Value for treatment group receiving:			
	75 mg KAE609 + 320 mg PPQ ($n = 25$)[a]	1,280 mg PPQ + 25 mg KAE609 ($n = 24$)[b]	320 mg PPQ ($n = 24$)[c]	1,280 mg PPQ ($n = 12$)[d]
C_{max} (mean ± SD [%CV]) (ng/ml)	24.2 ± 16.8 (69.4)	299 ± 191 (63.7)	20.4 ± 8.40 (41.2)	219 ± 124 (56.6)
T_{max} (median [range]) (h)	3.00 (1.00–46.5)	3.00 (2.00–6.00)	3.00 (1.00–12.0)	3.00 (1.00–4.00)
$AUC_{0–24}$ (mean ± SD [%CV]) (μg · h/ml)	0.27 ± 0.14 (53.0)	2.30 ± 1.00 (43.2)	0.243 ± 0.09 (38.9)	1.84 ± 0.84 (45.8)
$AUC_{0–168}$ (mean ± SD [%CV]) (μg · h/ml)	0.84 ± 0.40 (47.4)	5.15 ± 1.89 (36.8)	0.850 ± 0.33 (38.2)	4.15 ± 1.47 (35.4)
$AUC_{0–672}$ (mean ± SD [%CV]) (μg · h/ml)	1.72 ± 0.92 (53.4)	8.49 ± 2.84 (33.5)	1.74 ± 0.68 (39.4)	6.70 ± 2.13 (31.8)
AUC_{last} (mean ± SD [%CV]) (μg · h/ml)	2.07 ± 1.28 (61.6)	10.8 ± 3.53 (32.7)	2.16 ± 0.98 (45.4)	8.36 ± 2.64 (31.5)
AUC_{inf} (mean ± SD [%CV]) (μg · h/ml)	2.60 ± 1.49 (57.2)	12.0 ± 3.70 (30.9)	2.82 ± 1.13 (40.1)	8.99 ± 2.86 (31.8)
$t_{1/2}$ (mean ± SD [%CV]) (h)	510 ± 339 (66.6)	485 ± 113 (23.3)	551 ± 284 (51.5)	536 ± 172 (32.1)
V_z/F (mean ± SD [%CV]) (liters)	99,300 ± 49,500 (49.9)	83,400 ± 35,000 (41.9)	96,700 ± 44,500 (46.0)	125,000 ± 71,700 (57.5)
CL/F (mean ± SD [%CV]) (liters/h)	155 ± 80.9 (52.4)	120 ± 50.0 (41.7)	131 ± 59.9 (45.6)	155 ± 46.1 (29.8)

[a] $n = 22$ for AUC_{last}, $n = 21$ for $t_{1/2}$ and V_z/F, and $n = 19$ for AUC_{inf}.
[b] $n = 21$ for AUC_{last}, AUC_{inf}, $t_{1/2}$, and V_z/F.
[c] $n = 22$ for AUC_{inf}, $t_{1/2}$, and V_z/F.
[d] $n = 9$ for AUC_{last} and $n = 7$ for AUC_{inf}, $t_{1/2}$, and V_z/F.

QTc (9, 10). Therefore, ECG evaluations were performed for all cohorts using QTcF values in the primary analysis. No mean increase in QTcF was seen except for the two treatment groups that received 1,280 mg PPQ (Fig. 4). There appeared to be little difference in ECG results between the group that received 1,280 mg PPQ alone and the group that received 25 mg KAE609 plus 1,280 mg PPQ or between the group that received 320 mg PPQ alone and the group that received 320 mg PPQ plus 75 mg KAE609. The group that received 25 mg KAE609 did not show an increase in QTcF at any time point.

A statistical analysis of treatment comparisons for maximal changes in QTcF from baseline for the first 24 h postdose is presented in Table 4; it uses a linear fixed-effects model with treatment as a fixed effect and the PPQ concentration at maximal change as a covariate. There were no statistically significant differences in the comparisons of 25 mg KAE609 plus 1,280 mg PPQ versus 1,280 mg PPQ and 75 mg KAE609 plus 320 mg PPQ versus 320 mg PPQ. This was also the case when the analysis was performed with treatment as a fixed effect and PPQ C_{max} as a covariate (data not shown). This further indicates that KAE609 does not affect the QTcF increases observed with PPQ. A statistical analysis of the comparison of 25 mg KAE609 plus 1,280 mg PPQ versus 25 mg KAE609 with treatment as a fixed effect and KAE609 concentration as a covariate (Table 4) showed a significantly greater maximal change from baseline for 25 mg KAE609 plus 1,280 mg PPQ than for 25 mg KAE609 (least-squares means of 11.2 and 3.70 ms, respectively, with a least-squares estimate of the difference of 7.47 ms [90% CI, 3.55 to 11.4 ms]; $P = 0.0025$) (Table 4). Similar results were obtained when treatment was used as a fixed effect and KAE609 C_{max} as a covariate (data not shown). None of the subjects in any of the other treatment groups demonstrated an increase in QTcF of >30 ms or a newly occurring QTcF value of ≥450 ms. A similar analysis of QTcB values was in agreement with the QTcF results. These data confirmed that PPQ is significantly associated with QTc increases, but they showed that KAE609 alone does not affect QTc and the drug does not potentiate the cardiac effects of PPQ when given in combination.

Data were pooled across treatment groups to investigate the exposure effects of KAE609 or PPQ alone or in combination on the change in QTcF from baseline. There was no apparent relationship between KAE609 concentrations and changes in QTcF from baseline; this was the case for KAE609 alone and in the pres-

TABLE 3 Incidences of AEs according to preferred terms

Preferred term[a]	No. (%) of subjects					
	75 mg KAE609 + 320 mg PPQ ($n = 25$)	25 mg KAE609 + 1,280 mg PPQ ($n = 24$)	25 mg KAE609 ($n = 25$)	320 mg PPQ ($n = 24$)	1,280 mg PPQ ($n = 12$)	Total ($n = 110$)
At least one AE	12 (48.0)	15 (62.5)	10 (40.0)	14 (58.3)	10 (83.3)	61 (55.5)
Upper respiratory tract infection	4 (16.0)	6 (25.0)	4 (16.0)	8 (33.3)	8 (66.7)	30 (27.3)
Headache	0 (0.0)	2 (8.3)	4 (16.0)	4 (16.7)	2 (16.7)	12 (10.9)
Diarrhea	0 (0.0)	2 (8.3)	2 (8.0)	1 (4.2)	0 (0.0)	5 (4.5)
Oropharyngeal pain	3 (12.0)	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.6)
Photosensitivity reaction/sunburn[b]	1 (4.0)	1 (4.2)	1 (4.0)	0 (0.0)	0 (0.0)	3 (2.7)
Gastritis	0 (0.0)	2 (8.3)	0 (0.0)	1 (4.2)	0 (0.0)	3 (2.7)
Contusion	0 (0.0)	1 (4.2)	1 (4.0)	1 (4.2)	0 (0.0)	3 (2.7)
Abdominal pain	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.3)	0 (0.0)	2 (1.8)
Myalgia	1 (4.0)	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)	2 (1.8)
Lethargy	0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)	1 (8.3)	2 (1.8)
Dry skin	0 (0.0)	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.8)

[a] AEs are presented in descending order of total frequency.
[b] These two preferred terms were combined due to their clinical similarity on the basis of reported terms.

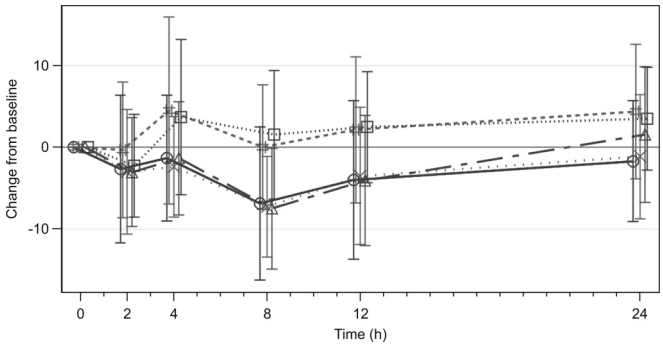

FIG 4 Arithmetic means (and standard deviations) of changes in QTcF from baseline according to time and treatment group. At predosing and 2, 4, 8, 12, and 24 h postdosing, triplicate ECG assessments were made. The average of the triplicate assessments was calculated, and the change from baseline is shown. Baseline was taken at 0 h on day 1. ○, 75 mg KAE609 plus 320 mg PPQ; #, 25 mg KAE609 plus 1,280 mg PPQ; △, 320 mg PPQ; □, 1,280 mg PPQ; ×, 25 mg KAE609.

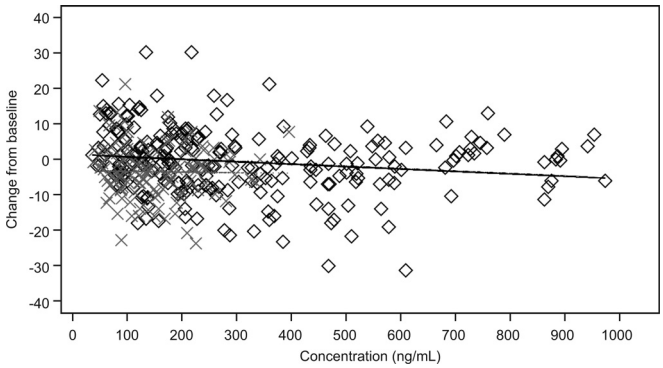

FIG 5 KAE609 concentration effects on changes in QTcF from baseline for KAE609 alone and in the presence of piperaquine. ◇, KAE609 plus PPQ; ×, 25 mg KAE609. Solid line, regression for KAE609 plus PPQ; dotted line, regression for 25 mg KAE609. $R^2 = 0.0273$ for KAE609 plus PPQ; $R^2 = 0.0003$ for 25 mg KAE609.

ence of PPQ (Fig. 5). The R^2 for KAE609 plus PPQ was 0.0273 ($P = 0.01$), and that for KAE609 alone was 0.0003 ($P = 0.8365$). For PPQ, there was an indication of a concentration-dependent effect on changes in QTcF from baseline, but this response was not affected by the presence of KAE609 (Fig. 6). The R^2 for KAE609 plus PPQ was 0.0709 ($P < 0.0001$), and that for PPQ alone was 0.0693 ($P = 0.0004$).

DISCUSSION

The current study assessed the safety, tolerability, and drug-drug interaction potential of KAE609 and PPQ administered alone or in combination to healthy subjects. Both drugs, when administered alone or coadministered, appeared to be well tolerated by the study volunteers. There were no serious adverse events, severe adverse events, or adverse events that led to discontinuation from the study. The most commonly reported AEs were upper respiratory tract infections, headache, diarrhea, and oropharyngeal pain. There were no consistent differences between the treatment groups, and there were no obvious increases in the rates of specific AEs when PPQ and KAE609 were coadministered, compared with administration of PPQ alone.

The ability of KAE609 and PPQ to affect cardiac conduction when given alone or in combination was also tested. PPQ is known to be associated with QTc prolongation in human studies (9, 10) and, although no significant changes in ECG parameters were observed in clinical studies of KAE609 (7), preclinical evaluations indicated this as a potential risk (unpublished Novartis data). The data from this study indicated that the mean maximal changes in QTcF and QTcB from baseline following a single PPQ dose were consistent with the known effects of the drug. A difference in QTcF of 7.47 ms (90% CI, 3.55 to 11.4 ms) was found for PPQ plus KAE609 versus KAE609 alone. The labeling for Eurartesim indicates a much greater QTcF increase after 3 days of dosing, which achieves approximately 3 times the PPQ exposure of a single dose. The magnitude of the change observed in this study and the Eurartesim labeling would be consistent with a positive thorough QTc study according to ICH E14 guidelines. In contrast, a single dose of KAE609 administered alone did not show an effect on the QTc, and QTcF and QTcB changes showed no correlation with KAE609 concentrations. Furthermore, coadministration of KAE609 did not potentiate the effects of PPQ on QTc. No cardiovascular AEs were reported for any subject.

Preclinical data also suggested a potential risk for phototoxicity from KAE609 exposure. Three light-related AEs were reported. All were of mild intensity and they did not exhibit a pattern suggesting a dose-response relationship for either KAE609 or PPQ. Two of these AEs were suspected by the investigators to be related to a study drug (one on day 2 in the group that received 75 mg KAE609 plus 320 mg PPQ, and one on day 3 in the group that

TABLE 4 Statistical analysis of comparisons for mean maximal changes in QTcF from baseline

Treatment comparison	LS mean maximal change from baseline (ms)[a]						LS estimate of difference (90% CI) (ms)	*P*
	75 mg KAE609 + 320 mg PPQ	25 mg KAE609 + 1,280 mg PPQ	320 mg PPQ	1,280 mg PPQ	1,280 mg PPQ + 25 mg KAE609	25 mg KAE609		
75 mg KAE609 + 320 mg PPQ vs 320 mg PPQ	5.04		5.90				−0.86 (−4.41 to 2.68)	0.6864
25 mg KAE609 + 1,280 mg PPQ vs 1,280 mg PPQ		9.50		7.12			2.37 (−2.08 to 6.82)	0.3775
25 mg KAE609 + 1,280 mg PPQ vs 25 mg KAE609					11.20	3.70	7.47 (3.55–11.4)	0.0025

[a] At predosing and 2, 4, 8, 12, and 24 h postdosing, triplicate ECG assessments were available. Averages of triplicate assessments were calculated, and the values for baseline and changes from baseline were derived accordingly. The repeated measurements were not taken into account for the calculation of statistics. The maximal changes from baseline for ECG intervals of less than 24 h were analyzed using a linear fixed-effects model with treatment as a fixed effect and KAE609 and PPQ concentrations at maximal changes as covariates. LS, least-squares.

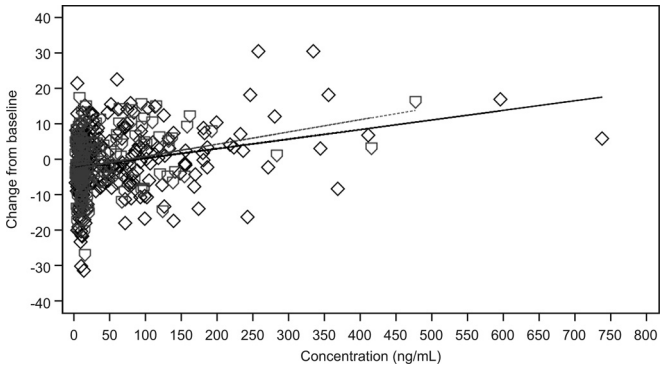

FIG 6 Piperaquine concentration effects on changes in QTcF from baseline for piperaquine alone and in the presence of KAE609. ◇, KAE609 plus PPQ; shields, PPQ. Solid line, regression for KAE609 plus PPQ; dotted line, regression for PPQ. $R^2 = 0.0709$ for KAE609 plus PPQ; $R^2 = 0.0693$ for PPQ.

received 25 mg KAE609). The investigators' interpretation was that these subjects had increased vision sensitivity to light. The subject in the group that received 25 mg KAE609 reported a long history of intermittent similar symptoms. The third case involved sunburn (on day 27) in a subject in the group that received 25 mg KAE609 plus 1,280 mg PPQ. This was not suspected by the investigators to be related to a study drug, as the subject had experienced sunburn after falling asleep on a beach.

This study evaluated the effects of combination treatment on exposure to each drug. Both KAE609 and PPQ are substrates for CYP3A4. PPQ is also a CYP3A inhibitor, based on the approved label for Eurartesim (9), while *in vitro* studies (unpublished Novartis data) have shown that KAE609 does not induce CYP3A but has a low potential to inhibit the enzyme. Prior to study initiation, SimCYP simulations using midazolam (a well-characterized high-clearance CYP3A4 substrate) as a surrogate for PPQ (CYP3A4 substrate) suggested that KAE609 could potentially increase the exposure to PPQ by up to 2-fold (as a worst-case scenario). Based on the *in vitro* interaction data and limited clinical data, an increase in KAE609 exposure of up to 4-fold (worst-case scenario) with PPQ was predicted (DDI Predict). The results from this study indicated a low degree of interaction, as coadministration of PPQ was found to have no overall effect on exposure to KAE609 in healthy subjects, although the KAE609 C_{max} was decreased by 17% in the group that received 75 mg KAE609 plus 320 mg PPQ, compared with the group that received 320 mg PPQ. Coadministration of KAE609 had inconsistent and nonsignificant effects on exposure to PPQ. The reasons for this observation are unknown, but both comparisons had high variability, as shown by the wide confidence intervals. The study was designed to have at least 80% power to detect differences in AUC or C_{max} in geometric mean ratio comparisons for KAE609 and PPQ if there were at least 20 subjects per arm. To account for potential dropouts, the study actually enrolled more than 20 subjects per arm; therefore, the power to detect differences was greater. It is unlikely that these small observed effects following coadministration would be clinically relevant, given the long exposure time for PPQ. Other KAE609 and PPQ pharmacokinetic parameters were not affected by coadministration of the partner drug.

On the basis of this study, KAE609 and PPQ could be administered in combination without the need for dose adjustment for either compound. PPQ coadministration had no effect on exposure to KAE609, and the effects of KAE609 on PPQ exposure were small and inconsistent. As the effects of inhibition would be found with a single dose and no time-dependent effects for either drug have been observed, it is probable that these results can be extrapolated to multiple-dose use. KAE609 does not appear to have a significant effect on cardiac conduction and does not potentiate the known effects of PPQ.

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The study was designed, data reviewed and analyzed, and the manuscript drafted by D.S.S., J.P.J., M.K., G.L., and S.M.; P.G. and J.L. conducted the study and reviewed the data. D.S.S. was responsible for the overall study. All authors reviewed and approved the manuscript.

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