Cancer Chemother Pharmacol (2012) 69:1247–1253
DOI 10.1007/s00280-011-1819-1

ORIGINAL ARTICLE

The effect of multiple doses of rifampin and ketoconazole on the single-dose pharmacokinetics of ridaforolimus

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Received: 19 November 2011 / Accepted: 28 December 2011 / Published online: 31 January 2012

Abstract
Purpose Ridaforolimus is an inhibitor of the mammalian target of rapamycin protein, with potent activity in vitro and in vivo. Ridaforolimus is primarily cleared by metabolism via cytochrome P450 3A (CYP3A) and is a P-glycoprotein (P-gp) substrate. Since potential exists for ridaforolimus to be co-administered with agents that affect CYP3A and P-gp activity, this healthy volunteer study was conducted to assess the effect of rifampin or ketoconazole on ridaforolimus pharmacokinetics.
Methods Part 1: single-dose ridaforolimus 40 mg followed by rifampin 600 mg daily for 21 days and single-dose ridaforolimus 40 mg on day 14. Part 2: single-dose ridaforolimus 5 mg followed by ketoconazole 400 mg daily for 14 days and single-dose ridaforolimus 2 mg on day 2.
Results Part 1: the geometric mean ratios (GMRs) (90% confidence interval [CI]) for ridaforolimus area under the concentration–time curve to the last time point with a detectable blood concentration ($AUC_{0-\infty}$) and maximum blood concentration (C_{max}) (rifampin + ridaforolimus/ridaforolimus) were 0.57 (0.41, 0.78) and 0.66 (0.49, 0.90), respectively. Both time to C_{max} (T_{max}) and apparent half-life ($t_{1/2}$) were similar. Part 2: the GMRs (90% CI) based on dose-normalized $AUC_{0-\infty}$ and C_{max} (ketoconazole + ridaforolimus/ridaforolimus alone) were 8.51 (6.97, 10.39) and 5.35 (4.40, 6.52), respectively. Ridaforolimus apparent $t_{1/2}$ was ~1.5-fold increased for ketoconazole + ridaforolimus; however, T_{max} values were similar.
Conclusions Rifampin and ketoconazole both have a clinically meaningful effect on the pharmacokinetics of ridaforolimus.

Keywords Pharmacokinetics · Ridaforolimus · Rifampin · Ketoconazole · Drug–drug interaction

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Introduction

Ridaforolimus (also known as MK-8669 and formerly deforolimus) is a unique non-prodrug analog of rapamycin and a potent inhibitor of the mammalian target of rapamycin (mTOR) protein, which in turn acts as a central controller of cell proliferation [1, 2]. Ridaforolimus, in addition to sirolimus, temsirolimus, and everolimus, comprises a family of related rapamycin analogs [3]. The broad antiproliferative activity of rapamycin and the central role of mTOR in control of cell growth support the development of mTOR inhibitors as antitumor agents. The mTOR inhibitors are believed to suppress tumor growth through multiple mechanisms, including antiproliferative, antiangiogenic, and proaptotic effects [2, 4]. Ridaforolimus is being developed for the treatment of a variety of solid tumors, including metastatic sarcoma. The investigated Phase III evaluation (NCT00538239) of ridaforolimus employs a dose of 40 mg once daily for 5 consecutive days per week in repeating weekly cycles.

Previous in vitro studies suggested that the metabolism of ridaforolimus is primarily mediated by cytochrome P450 3A (CYP3A) and that ridaforolimus is a P-glycoprotein (P-gp) substrate [2]. As is the case with ridaforolimus, sirolimus [5], everolimus [6], and temsirolimus [7] are likewise metabolized by CYP3A4 and are P-gp substrates. Metabolism of these compounds is influenced by inducers and inhibitors of CYP3A and P-gp. Taken together, these findings suggest that administration of strong inducers and inhibitors of CYP3A and P-gp may potentially alter ridaforolimus pharmacokinetics.

Rifampin is a strong CYP3A4 inducer and P-gp inducer, and ketoconazole is a strong CYP3A inhibitor and P-gp inhibitor; co-administration of rifampin and ketoconazole with the sensitive CYP3A probe midazolam resulted in a 43-fold decrease [8] and 16-fold increase [9] in midazolam area under the concentration–time curve (AUC), respectively. Furthermore, rifampin and ketoconazole effects on P-gp substrates have also demonstrated substantive pharmacokinetic changes [10]. The purpose of this open-label, randomized, 2-part, fixed-sequence study was to assess the effects of multiple doses of rifampin or ketoconazole on the single-dose whole-blood pharmacokinetics of ridaforolimus in healthy male subjects.

Methods

Subjects

Healthy men and women 18–45 years of age with a body mass index 20–31 kg/m^2 were eligible for enrollment. Subjects were excluded if they were smokers or had a clinically relevant medical history. Exclusion criteria also included known history of alcohol and/or drug abuse, recent participation in an investigational drug study, and inability to refrain from the use of prescription and non-prescription medications. Subjects agreed to participate in the study by giving written informed consent prior to study commencement. The study was conducted in accordance with principles of Good Clinical Practice and was approved by the Thomas Jefferson University, Office of Human Research Division of Human Subjects Protection, Institutional Review Board and appropriate regulatory agencies.

Study design

This was an open-label, 2-part study. In each part of the study, subjects received two treatments in a fixed-sequence 2-period design, with a 14-day washout period separating treatment periods. The study design included multiple-dose administration of the perpetrator compounds (rifampin and ketoconazole) to achieve or maintain steady-state effect on affected drug-metabolizing enzymes. Based on the past characterization of ridaforolimus pharmacokinetics, single-dose administration of ridaforolimus was sufficiently predictive of the anticipated effect following multiple-dose administration. A fixed-sequence design was incorporated due to the necessity of a significantly long washout interval for a randomized crossover design, which would have affected study conduct.

Part 1 evaluated the effects of multiple-dose rifampin administration on the single-dose pharmacokinetics of ridaforolimus. In Period 1, subjects received a single oral dose of ridaforolimus 40 mg (4 × 10-mg enteric-coated tablet [ECT]) on day 1. In Period 2, subjects received open-label rifampin 600 mg once daily on days 1–21 (standard therapeutic dose, which demonstrates substantive CYP3A4 induction, administered as 2 × 300-mg capsules; Rifadin®, sanofi-aventis, US, LLC, Bridgewater, NJ) and a single co-administered dose of ridaforolimus 40 mg (4 × 10-mg ECT) on day 14 (i.e., the 14th day of rifampin dosing). Ridaforolimus was administered after an 8-h fast. During rifampin-only dosing days, rifampin was administered 1 h before or 2 h after a standard meal, as per dosing recommendations.

Part 2 evaluated the effects of multiple-dose ketoconazole administration on the single-dose pharmacokinetics of ridaforolimus. In Period 1, subjects received a single oral dose of ridaforolimus 5 mg (1 × 5-mg dry-filled capsule [DFC]) on day 1. In Period 2, subjects received 14 days of open-label ketoconazole 400 mg once daily (standard therapeutic dose, which results in clinically meaningful CYP3A inhibition, administered as 1 × 400-mg tablet; Teva Pharmaceuticals, Petach Tikva, Israel) and a single dose of ridaforolimus 2 mg (1 × 2-mg DFC) on day 2 (i.e., the second day of ketoconazole dosing; a reduced dose level of ridaforolimus was given in Part 2 given the potential for elevated concentrations of ridaforolimus when administered with ketoconazole). Ridaforolimus was administered after an 8-h fast. During ketoconazole-only dosing days, a standard light breakfast was served prior to dosing with ketoconazole, as per dosing recommendations. Co-administration of ridaforolimus and ketoconazole in Period 2 occurred on day 2 of ketoconazole dosing, as assessment of an effect after short-term administration predicts the magnitude of change as reliably as following longer-term administration [11].

Pharmacokinetic assessments and analytical methods

Given the relatively low ridaforolimus levels in plasma, the pharmacokinetic findings in this evaluation are based on whole-blood, not plasma, exposures (see Discussion for greater detail). Whole-blood samples (5 mL) were drawn into plastic tubes containing potassium EDTA as the

anticoagulant at pre-dose and specified post-dose time points for pharmacokinetic assessment. To assess the single-dose pharmacokinetics of ridaforolimus in the absence of rifampin (i.e., Period 1 of Part 1) and ketoconazole (i.e., Period 1 of Part 2), whole-blood samples were collected pre-dose on day 1 and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 168 h post-dose. To assess the single-dose pharmacokinetics of ridaforolimus in the presence of rifampin (i.e., Period 2 of Part 1), whole-blood samples were collected pre-dose on day 14 and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 168 h post-dose. Single-dose pharmacokinetics of ridaforolimus in the presence of ketoconazole (i.e., Period 2 of Part 2) were evaluated by collection of whole-blood samples on day 1 at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 288, and 312 h post-dose.

Whole-blood samples were stored frozen at −70°C and shipped on dry ice to Charles River Laboratories, Senneville, Quebec, Canada for analysis. Validated high-performance liquid chromatography—tandem mass spectrometer (HPLC–MS/MS)—assay procedures were used to support the analysis of ridaforolimus in blood samples. Ridaforolimus and its stable isotope labeled internal standard D_6-ridaforolimus were isolated from blood using methyl *tert*-butyl ether liquid–liquid extraction with liquid chromatographic separation on a Waters Atlantis T3 column. A tandem mass spectrometer with a turbo ionspray (TISP) interface in the positive ionization mode was used to detect ridaforolimus and D_6-ridaforolimus ammonium adducts via multiple reaction monitoring in their characteristic precursor → product ion channels at *m/z* 1,007 → 940, 1,013 → 946, respectively.

Two whole-blood assays with different calibration ranges were used to support this study in light of anticipated drug concentration. In Part 1, the lower limit of quantification (LLOQ) for the whole-blood assay was 0.2 ng/mL, and the linear calibration range was 0.2–400 ng/mL. In Part 2, the LLOQ for the whole-blood assay was 0.05 ng/mL, and the linear calibration range was 0.05–100 ng/mL.

Ridaforolimus blood concentrations and actual sampling times were used to determine pharmacokinetic parameters for each treatment in each subject. Values below the LLOQ were replaced with 0. The apparent terminal elimination rate constant (λ) was estimated by regression of the terminal log-linear portion of the blood concentration–time profile (using quantifiable concentrations only); the apparent terminal half-life ($t_{1/2}$) was calculated as the quotient of ln(2) and λ. Onset of the terminal log-linear phase was determined by inspection. AUC to the last time point with a detectable blood concentration (AUC_{0-last}) was calculated using the linear trapezoidal rule for ascending concentrations and the logarithmic trapezoidal rule for descending concentrations. $AUC_{0-\infty}$ was estimated as the sum of AUC_{0-last} and the extrapolated area given by the quotient of the last measured concentration and λ. The maximum blood concentration (C_{max}) and time to C_{max} (T_{max}) were obtained by inspection of the blood concentration data.

Safety measurements

The safety and tolerability of study medication were assessed by clinical evaluation of adverse experiences (AEs) and inspection of other safety parameters including physical examinations, vital sign measurements, 12-lead electrocardiograms (ECGs), and routine laboratory safety tests (hematology, blood chemistry, and urinalysis). AEs were monitored throughout the study and evaluated in terms of intensity (mild, moderate, or severe), duration, severity, outcome, and relationship to study drug.

Statistical analysis

Individual $AUC_{0-\infty}$ and C_{max} values for ridaforolimus were natural log-transformed and analyzed with linear mixed-effects models appropriate for a 2-period fixed-sequence study design to evaluate the blood pharmacokinetics of ridaforolimus in the presence and absence of rifampin (Part 1) or ketoconazole (Part 2). The models included a fixed effect for treatment and a random effect for subject. Prior to analysis, individual $AUC_{0-\infty}$ and C_{max} values obtained in Part 2 of the study following the administration of 5 mg ridaforolimus alone (i.e., Period 1) were dose normalized to a 2-mg dose to allow for a direct comparison with the $AUC_{0-\infty}$ and C_{max} results obtained following 2 mg ridaforolimus plus 400 mg ketoconazole (i.e., Period 2 [see "Discussion" section for PK considerations regarding dose normalization]). Two-sided 90% confidence intervals (CIs) were constructed from the mixed-effects models for the differences in least squares (LS) means of log-transformed $AUC_{0-\infty}$ and C_{max} values between treatments. The mean differences and these limits were back transformed to the original scale to obtain the geometric mean ratios (GMRs) and 90% CIs of $AUC_{0-\infty}$ and C_{max} (ridaforolimus + rifampin/ridaforolimus alone in Part 1; ridaforolimus + ketoconazole/ridaforolimus alone in Part 2).

Results

Demographics and baseline characteristics

Twenty healthy male subjects were enrolled in the study (10 subjects each in Parts 1 and 2). For Part 1, body mass index range was between 21.9 and 30.3 kg/m^2, and age range was 23–45 years. For Part 2, body mass index range

was between 22.0 and 30.9 kg/m^2, and age range was 29–45 years. Of the 10 subjects enrolled in Part 1, five were black, and five were white; none were Hispanic or Latino in ethnicity. In Part 2, eight were black, and two were white; none were Hispanic or Latino in ethnicity.

Two subjects each in Parts 1 and 2 did not complete the study, all for reasons not considered related to study medication. Eight subjects in both Parts 1 and 2 contributed complete pharmacokinetic data; the remaining two subjects who discontinued in both Parts 1 and 2 contributed partial pharmacokinetic results. In Part 1, one subject discontinued after the 6-h time point in the rifampin + ridaforolimus treatment period (i.e., Period 2). Thus, $AUC_{0-\infty}$ and apparent $t_{1/2}$ could not be calculated for this subject in this period. The Period 1 (ridaforolimus alone) pharmacokinetic data and C_{max} and T_{max} data from Period 2 for this subject were included. An additional subject discontinued after the 96-h time point in the rifampin + ridaforolimus treatment period (Period 2). The $AUC_{0-\infty}$ and apparent $t_{1/2}$ results from this subject for this period were excluded from the analysis. However, C_{max} and T_{max} values for this subject from Period 2, as well as all Period 1 (ridaforolimus alone) data, were included in the analysis. In Part 2, one subject discontinued following the first dose of ketoconazole alone in the ketoconazole + ridaforolimus treatment period (i.e., Period 2). All Period 1 (ridaforolimus alone) pharmacokinetic data from this subject were included in the analysis. Another subject discontinued after the 96-h time point in the ketoconazole + ridaforolimus treatment period. The $AUC_{0-\infty}$ and apparent $t_{1/2}$ results from this subject in this period were excluded from the analysis. However, C_{max} and T_{max} values from this period, as well as all Period 1 (ridaforolimus alone) data from this subject, were included in the analysis. All 20 subjects enrolled in this study were included in the evaluation of safety and tolerability.

Effect of rifampin on ridaforolimus pharmacokinetics

The mean whole-blood concentration–time profiles of ridaforolimus when administered alone or following concomitant administration of rifampin are illustrated in Fig. 1. The mean whole-blood concentration–time profile of ridaforolimus was reduced following the administration of multiple doses of rifampin. The summary statistics for $AUC_{0-\infty}$, C_{max}, T_{max}, and apparent $t_{1/2}$ as well as the GMRs (co-administration [ridaforolimus + rifampin]/ridaforolimus alone; 90% CI) for whole-blood ridaforolimus are provided in Table 1. The GMRs and corresponding 90% CI for the $AUC_{0-\infty}$ and C_{max} of ridaforolimus were 0.57 (0.41, 0.78) and 0.66 (0.49, 0.90), respectively. The median T_{max} and apparent $t_{1/2}$ values for ridaforolimus were similar in the absence and presence of rifampin.

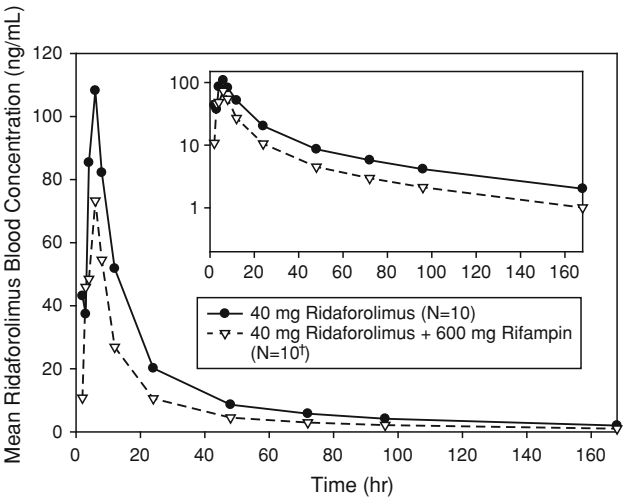

Fig. 1 Mean blood concentration profiles for ridaforolimus following the administration of a 40-mg dose of ridaforolimus alone or following concomitant administration of multiple dose of 600 mg rifampin with 40 mg ridaforolimus in healthy fasted male subjects in Study Part 1 (*linear scale*) (*inset*; *semi-log scale*). †One subject discontinued after the 6-h time point. Another subject discontinued after the 96-h time point

Effect of ketoconazole on ridaforolimus pharmacokinetics

The mean whole-blood concentration–time profiles of ridaforolimus when administered alone or following concomitant administration of multiple doses of ketoconazole are illustrated in Fig. 2. The mean whole-blood concentration–time profile of ridaforolimus was increased following ketoconazole administration. The summary statistics for dose-normalized $AUC_{0-\infty}$, C_{max}, T_{max}, and apparent $t_{1/2}$ as well as the GMRs (co-administration [ridaforolimus + ketoconazole]/ridaforolimus alone; 90% CI) for whole-blood ridaforolimus are provided in Table 2. The GMRs and corresponding 90% CI for the dose-normalized $AUC_{0-\infty}$ and C_{max} of ridaforolimus were 8.51 (6.97, 10.39) and 5.35 (4.40, 6.52), respectively. Ridaforolimus apparent $t_{1/2}$ was ~1.5-fold increased for ridaforolimus when administered with ketoconazole; however, T_{max} values were similar.

Safety and tolerability

Single doses of ridaforolimus dosed in combination with rifampin or ketoconazole were generally well tolerated. There were no clinically important changes in laboratory, vital signs, or ECG safety parameters. No serious clinical or laboratory AEs were reported in either part of the study, and no volunteers discontinued due to AEs considered related to study therapy. In Part 1, a total of 30 clinical AEs were reported. Of these AEs, three were judged by the

Table 1 Summary of whole-blood pharmacokinetic parameters of ridaforolimus following the administration of a 40-mg dose of ridaforolimus alone or concomitant administration of multiple-dose 60 mg rifampin with 40 mg ridaforolimus in healthy fasted male subjects in Part 1

Pharmacokinetic parameter	Ridaforolimus alone			Rifampin + ridaforolimus			(Rifampin + ridaforolimus/ridaforolimus alone)		rMSE[b]
	N	GM	95% CI	*N*[a]	GM	95% CI	GMR	90% CI	
$AUC_{0-\infty}$ (ng/mL·h)[c]	10	2,011.57	1,510.01, 2,679.73	8	1,138.32	829.33, 1,562.43	0.57	0.41, 0.78	0.3563
C_{max} (ng/mL)[c]	10	111.69	80.08, 155.77	10	74.06	53.10, 103.29	0.66	0.49, 0.90	0.3789
T_{max} (h)[d]	10	6.00	[4.00, 8.03]	10	6.0	[3.00, 6.02]			
Apparent $t_{1/2}$ (h)[e]	10	62.3	7.8	8	60.8	8.8			

GM geometric least squares mean; *GMR* geometric least squares mean ratio ([rifampin + ridaforolimus]/ridaforolimus alone); *rMSE* square root of conditional mean squared error (residual error) from the linear mixed effect model; $AUC_{0-\infty}$ the sum of AUC_{0-last} and the extrapolated area given by the quotient of the last measured concentration and λ

[a] One subject discontinued after the 6-h time point in the Rifampin + Ridaforolimus treatment period (i.e., Period 2). Thus, $AUC_{0-\infty}$ and apparent half-life ($t_{1/2}$) could not be calculated. An additional subject discontinued after the 96-h time point in the Rifampin + Ridaforolimus treatment period. $AUC_{0-\infty}$ and apparent $t_{1/2}$ results from this subject from this period were excluded. However, maximum blood concentration (C_{max}) and time to C_{max} (T_{max}) values from these subjects were included

[b] rMSE*100% approximates the within-subject % coefficient of variation on the raw scale

[c] Back-transformed least squares mean and confidence intervals (CIs) from linear mixed-effects model performed on natural log-transformed values

[d] Median [min, max] reported for T_{max}

[e] Harmonic mean, jack-knife standard deviation reported for apparent $t_{1/2}$

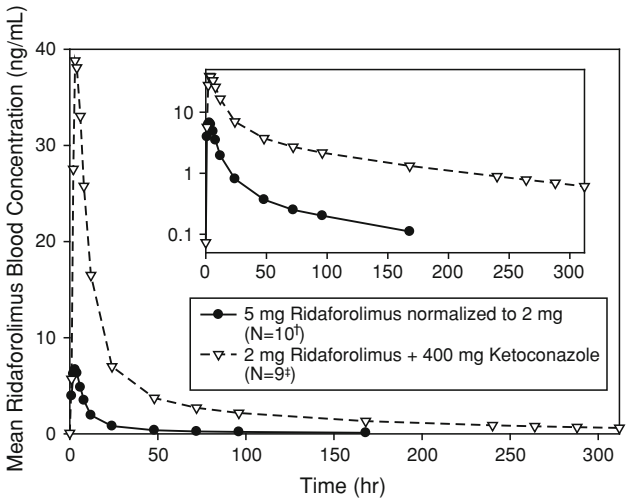

Fig. 2 Mean blood concentration profiles for ridaforolimus following the administration of a 5-mg dose of ridaforolimus alone or concomitant administration of multiple-dose 400 mg ketoconazole with 2 mg ridaforolimus in healthy fasted male subjects in Study Part 2 (*linear scale*) (*inset*; *semi-log scale*). †The 5 mg ridaforolimus blood concentration profile was normalized to 2 mg to allow for comparison with the Ketoconazole + Ridaforolimus group. ‡One subject discontinued prior to the Ketoconazole + Ridaforolimus treatment period. Another subject discontinued after the 96-h time point

investigator to be related to study drug. Frequent bowel movements and nausea were reported once with rifampin administration alone. Somnolence was reported in one subject after administration of rifampin alone and also in combination with ridaforolimus. In Part 2, a total of 19 clinical AEs were reported, with two judged to be related to study drugs. Headache (ketoconazole administration alone) and abdominal distension (ketoconazole and ridaforolimus administration) were reported. All drug related AEs were mild in intensity and of limited duration.

Discussion

Both in vitro data obtained with ridaforolimus and clinical data obtained with other rapamycin analogs suggested that ridaforolimus would potentially be sensitive to CYP3A inhibition and CYP3A4 induction. Preclinical data suggest that ridaforolimus is metabolized almost exclusively by CYP3A, and that ridaforolimus is a P-gp substrate. In a previous clinical evaluation of the structurally and metabolically similar compound everolimus, the AUC of everolimus was reduced by 63% in the presence of rifampin [12]. Similarly, previous evaluations with ketoconazole demonstrated a 15-fold increase in exposure for everolimus [13] and a 10.9-fold increase in exposure for sirolimus [5]. Accordingly, a clinical pharmacokinetic evaluation of ridaforolimus with rifampin and ketoconazole was conducted.

Ridaforolimus is administered at the 40-mg dose level in the Phase III evaluation (unpublished data NCT00538239). Given the potential for elevated concentrations of ridaforolimus following administration with ketoconazole, a reduced 2-mg dose of ridaforolimus was used when administered with ketoconazole. To ensure blood concentrations were sufficiently above the LLOQ when ridaforolimus was administered alone, a 5-mg dosage strength formulation was

Table 2 Summary of dose-normalized whole-blood pharmacokinetic parameters of ridaforolimus following the administration of a 5-mg dose of ridaforolimus alone or concomitant administration of multiple-dose 400 mg ketoconazole with 2 mg ridaforolimus in healthy fasted male subjects in Part 2

Pharmacokinetic parameter	Ridaforolimus alone[a]			Ketoconazole + ridaforolimus			(Ketoconazole + ridaforolimus/ridaforolimus alone)		rMSE[c]
	N	GM	95% CI	*N*[b]	GM	95% CI	GMR	90% CI	
$AUC_{0-\infty}$ (ng/mL·h)[d]	10	113.04	92.47, 138.18	8	961.52	772.53, 1,196.74	8.51	6.97, 10.39	0.2234
C_{max} (ng/mL)[d]	10	7.5	6.31, 9.04	9	40.45	33.51, 48.82	5.35	4.40, 6.52	0.2308
T_{max} (h)[e]	10	3.02	[2.00, 6.00]	9	3.00	[3.00, 6.02]	–	–	–
Apparent $t_{1/2}$ (h)[f]	10	80.5	11.6	8	119.0	15.7	–	–	–

GM geometric least squares mean, *GMR* geometric least squares mean ratio ([rifampin + ridaforolimus]/ridaforolimus alone), *rMSE* square root of conditional mean squared error (residual error) from the linear mixed effect model, $AUC_{0-\infty}$ the sum of AUC_{0-last} and the extrapolated area given by the quotient of the last measured concentration and λ

[a] Ridaforolimus administered alone as 5 mg; as described in the text $AUC_{0-\infty}$ and maximum blood concentration (C_{max}) parameters dose normalized to 2 mg to allow for comparison with Ketoconazole + Ridaforolimus group. The non-dose-normalized $AUC_{0-\infty}$ and C_{max} GM (95% confidence internal [CI]) following a single 5-mg oral dose of ridaforolimus alone were 282.60 (240.25, 332.42) ng/mL·h and 18.88 (15.42, 23.13) ng/mL, respectively

[b] One subject discontinued prior to the Ketoconazole + Ridaforolimus treatment period (i.e., Period 2). Another subject discontinued after the 96-h time point in the Ketoconazole + Ridaforolimus treatment period. $AUC_{0-\infty}$ and apparent half-life ($t_{1/2}$) results from this subject from this period were excluded. However, C_{max} and time to C_{max} (T_{max}) values from this subject were included

[c] rMSE*100% approximates the within-subject % coefficient of variation on the raw scale

[d] Back-transformed least squares mean and CIs from linear mixed-effects model performed on natural log-transformed values

[e] Median [min, max] reported for T_{max}

[f] Harmonic mean, jack-knife standard deviation reported for apparent $t_{1/2}$

administered as the reference treatment. The use of dissimilar dosage strengths for ridaforolimus when administered alone and with ketoconazole mirrors the approach taken in an evaluation of everolimus when dosed with ketoconazole [14]. The formulation of ridaforolimus is a 10-mg ECT formulation. To accommodate the reduced 2- and 5-mg dosage strengths used in this investigation, a DFC formulation was used instead of the ECT. Although the DFC formulation differs from the formulation proposed for marketing (i.e., ECT), the DFC formulation was administered both when ridaforolimus was given alone and in combination with ketoconazole to avoid a potential confounding effect of formulation. As with other rapamycin analogs, ridaforolimus exhibits saturable binding to erythrocytes that results in a relatively high blood to plasma ratio [3, 14, 15]. Given the relatively low ridaforolimus levels in plasma, the pharmacokinetic results in this evaluation are based on whole-blood, not plasma, exposures. Erythrocyte binding may be an important contributor to the nonlinear whole-blood pharmacokinetics for ridaforolimus. Following intravenous administration of ridaforolimus, increases of whole-blood exposure were less than proportional with dose at the higher dose groups investigated; however, relatively low doses were characterized with approximately dose-proportional behavior [16]. Similarly, following oral dosing, ridaforolimus exhibits less than proportional increases of whole-blood exposure with dose [17], particularly following administration of oral doses of 40 mg and greater. The observed systemic exposures associated with the 2- and 5-mg dose levels were within the range of the dose-exposure relationship for ridaforolimus that was sufficiently linear to permit an evaluation of the data based on dose-normalized pharmacokinetic parameters.

Following concomitant administration of ridaforolimus with ketoconazole, the $AUC_{0-\infty}$ and C_{max} of ridaforolimus were increased on average by 8.51- and 5.35-fold, respectively. The mean 8.51-fold increase in ridaforolimus $AUC_{0-\infty}$ following administration with ketoconazole was consistent with expectations given the 15-fold increase in exposure for everolimus [13] and a 10.9-fold increase in exposure for sirolimus following co-administration with ketoconazole [5]. As with the other rapamycin analogs, given that the magnitude of the interaction with a CYP3A inhibitor exceeds fivefold, ridaforolimus may likewise be classified as a sensitive CYP3A substrate [18]. Since the proposed market ECT formulation is available only in a 10-mg dose potency, it would not be possible to nullify the anticipated increase in ridaforolimus exposure following administration of a strong CYP3A and P-gp inhibitor with a dose adjustment; rather, strong CYP3A and P-gp inhibitors should be avoided.

With regard to rifampin induction effects, the mean 43% reduction of ridaforolimus $AUC_{0-\infty}$ following administration of ridaforolimus with rifampin approximated the 63%

reduction in everolimus AUC when administered with rifampin [12]. Patients should avoid the use of concomitant strong CYP3A4 inducers with ridaforolimus; however, if a patient requires co-administration of a strong CYP3A4 inducer, a dose increase (e.g., by 50% to 60 mg daily) can be accommodated and may be considered, if warranted. It is of note that there are no clinical pharmacokinetic data with this dose adjustment in patients receiving strong CYP3A4 inducers.

Conclusions

The study results demonstrated that co-administration of single-dose ridaforolimus and multiple doses of rifampin or ketoconazole is generally well tolerated in healthy male subjects. Ridaforolimus pharmacokinetics is affected to a clinically meaningful extent by rifampin and ketoconazole, a strong CYP3A/P-gp inducer and inhibitor, respectively. Patients should avoid the use of concomitant strong CYP3A4 inducers with ridaforolimus; however, if a patient requires co-administration of a strong CYP3A4 inducer, a dose increase (e.g., by 50% to 60 mg daily) may be considered, if warranted; similarly, strong CYP3A and P-gp inhibitors should be avoided.

Acknowledgments Funding for this study was provided by Merck, Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ. The authors thank Kathleen Newcomb and Kristen Lewis (Merck Sharp & Dohme, Corp.) for assistance with the editing and formatting of this manuscript.

Conflicts of interest M. Stroh, J. Palcza, J. McCrea, S. Marsilio, S. Breidinger, D. Panebianco, A. Johnson-Levonas, K. Orford, G. Murphy, N. Agrawal, M. Trucksis, J. Wagner, and M. Iwamoto are current or former employees of Merck Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ, and may own stock or hold stock options in the company. W. K. Kraft is a full-time employee of Thomas Jefferson University, which received funding from Schering-Plough Corporation (now Merck Sharp & Dohme Corp.) for the conduct of this study.

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