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ORIGINAL ARTICLE

Multiple administrations of fluconazole increase plasma exposure to ruxolitinib in healthy adult subjects

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Abstract

Purpose Ruxolitinib is metabolized by cytochrome P450 (CYP)3A4 and CYP2C9. Dual inhibitors of these enzymes (like fluconazole) lead to increased ruxolitinib exposure relative to a single pathway inhibition of CYP3A4 or CYP2C9. The magnitude of this interaction, previously assessed via physiologically based pharmacokinetic (PBPK) models, was confirmed in an open-label, phase 1 study in healthy subjects.

Methods The effect of multiple doses (200 mg) of fluconazole on single-dose (10 mg) PK of ruxolitinib was investigated including evaluation of the safety and tolerability. The PK parameters of ruxolitinib alone (reference) were compared to those of ruxolitinib combined with fluconazole (test). The point estimate and corresponding two-sided 90% confidence interval for the difference between means of test and reference parameters were determined.

Results All enrolled subjects ($N=15$) completed the study. When coadministered with fluconazole, geometric means of ruxolitinib PK parameters C_{max}, AUC_{last}, and AUC_{inf} increased by 47%, 234%, and 232%, respectively, vs ruxolitinib alone. The median T_{max} decreased slightly, apparent clearance decreased approximately threefold, and elimination half-life increased approximately 2.5-fold, upon ruxolitinib administration with fluconazole vs ruxolitinib alone. These results were consistent with the prospective predictions from a SimCYP PBPK model. Adverse events (AEs) were reported in six subjects (none were suspected to be related to ruxolitinib); no death or on-treatment serious AE was reported.

Conclusions Coadministration of ruxolitinib with fluconazole significantly increased ruxolitinib systemic exposure; however, no AEs were attributed to ruxolitinib. Concomitant use of ruxolitinib with fluconazole (dose $\leq$ 200 mg) may require dose reduction/modification of ruxolitinib.

Keywords Ruxolitinib · Fluconazole · Drug–drug interaction · CYP3A4 · CYP2C9 · Physiologically based pharmacokinetic model

Introduction

Ruxolitinib is a small molecule tyrosine kinase inhibitor [1, 2] that specifically binds to and inhibits Janus-associated kinases (JAK1 and JAK2). Upon activation by ligand–receptor binding, JAKs activate the signal transducer and activator of transcription (STAT) proteins. The JAK/STAT pathway is one of the critical intracellular signaling cascades regulating the transcription of genes involved in various cell functions like growth, differentiation, proliferation, and survival. Dysregulated JAK–STAT signaling is implicated in tumor cell proliferation and cancer progression [3–6]. Ruxolitinib is approved for the treatment of patients with intermediate or high-risk myelofibrosis [7, 8] and patients with polycythemia vera who are resistant/intolerant to hydroxyurea [9].

Vassilios Aslanis and Kenichi Umehara were employed with Novartis during the study period.

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Ruxolitinib, a Biopharmaceutical Classification System Class I molecule with excellent solubility [10], permeability and dissolution characteristics, exhibits near complete oral absorption and dose proportional systemic exposure (over a dose range of 5–200 mg [11, 12]). Administration with food has little effect on the oral bioavailability of ruxolitinib, with the geometric mean maximum plasma concentration (C_{max}) lowered by 24.3% compared to fasted administration [12]. It is primarily metabolized by cytochrome P450 (CYP)3A4 enzyme system, followed by CYP2C9, with minor contributions of other CYPs. Ruxolitinib exhibits low oral dose clearance (≈ 19 L/h), a small volume of distribution (≈ 80 L), and a short terminal elimination half-life of approximately 3 h [12].

Findings from a drug–drug interaction (DDI) study of ruxolitinib with ketoconazole (a potent CYP3A4 inhibitor) demonstrated that ruxolitinib plasma exposure increased by 91% in combination with ketoconazole compared to ruxolitinib alone [13]. A dose reduction of 50% is recommended when administering ruxolitinib with a potent CYP3A4 inhibitor like ketoconazole, due to reduced systemic clearance of ruxolitinib, which in turn results from inhibition of CYP3A4 metabolism [13, 14]. A physiologically based pharmacokinetic (PBPK) modeling study prospectively predicted that fluconazole (dual CYP3A4/CYP2C9 inhibitor) would increase ruxolitinib plasma concentration–time area under the curve (AUC) by approximately threefold [14]. The respective exposure increase in ruxolitinib when both elimination pathways (CYP3A4 and CYP2C9) are inhibited has not been evaluated so far.

Fluconazole, a moderate CYP3A4/moderate CYP2C9 inhibitor, is poorly metabolized and is mainly eliminated unchanged in the urine, with a long elimination half-life of approximately 30 h [13, 14, 16]. Fluconazole could be coadministered with other azole antifungals like ketoconazole or itraconazole for synergistic activity [17–20].

A moderate dual CYP3A4 and CYP2C9 inhibitor such as fluconazole has a larger effect on the PK of a dual substrate of CYP2C9 and CYP3A4, such as ruxolitinib, compared to a single pathway inhibitor like ketoconazole on CYP3A4.

The present study investigated the ruxolitinib–fluconazole drug interaction by determining the effect of multiple doses of fluconazole (400 mg followed by 200 mg doses) on the PK of ruxolitinib administered as a single dose (10 mg) in an open-label, study in healthy subjects. The results of this study informed the dosing recommendations for ruxolitinib administered with dual CYP3A4/CYP2C9 inhibitors like fluconazole.

Materials and methods

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Establishment of a ruxolitinib physiologically based pharmacokinetic model

Before initiating the phase 1 study, a PBPK model of ruxolitinib was established to estimate the victim DDI risks on CYP3A4 and CYP2C9. The effect of the dual CYP3A4 and CYP2C9 inhibitor fluconazole [doses 100 mg, 200 mg, and 400 mg orally (po), twice daily (bid)] on the PK of ruxolitinib (10 mg po, bid) was prospectively evaluated [14]. This PBPK model of ruxolitinib established by Umehara et al. [14] was used without any refinement for prediction of the effect of fluconazole on the PK of ruxolitinib. The previously established PBPK model by Shi et al. [15] was not used in this analysis.

Effect of fluconazole on the pharmacokinetics of ruxolitinib

Study design

This was a single-center, open-label, 2-period fixed-sequence study to evaluate the effect of fluconazole (dual CYP3A4 and CYP2C9 inhibitor) on the PK of ruxolitinib in healthy study participants. The enrollment of 15 eligible healthy male or female subjects was planned to obtain 12 evaluable subjects, considering a nonevaluability rate of 20% was reached (including dropout).

The study consisted of a screening phase and a treatment phase (Supplementary Fig. 1). The subjects were screened for eligibility within a 28-day screening period (day − 28 to day − 2). This was followed by a treatment phase starting with baseline assessment on day − 1, treatment period (day 1–day 9), and end of treatment [day 10 and safety follow-up (day 39)]. The treatment period consisted of drug administration and predose and postdose PK sampling according to the evaluation schedule. To assess any potential drug interaction with fluconazole, the ruxolitinib dose of 10 mg was selected, as it represented a clinically relevant dose of ruxolitinib with a sufficient safety margin. Ruxolitinib was administered alone on day 1 (period 1), and it was coadministered with fluconazole (period 2), separated by a washout period of at least 1 day between the ruxolitinib administrations to avoid drug accumulation or carryover effect after the first administration. Fluconazole administration schedule consisted of a loading oral dose of 400 mg on day 2 to attain plasma concentrations close to steady state, followed by daily oral doses of 200 mg for 7 days [day 3–day 9 (including coadministration with ruxolitinib on day 7)]. The end of treatment evaluation was performed on day 10, followed by a safety follow-up on day 39. The study was completed when the last subject completed the treatment phase.

Study population

Study population comprised healthy adult male and female subjects of non-childbearing potential (18–55 years). Subjects were required to be free of clinically significant findings from medical history, physical examination, vital signs, electrocardiogram, and laboratory tests to fulfill the study eligibility criteria. The key exclusion criteria were sexually active males (unless they used contraception during treatment and for 7 days after stopping treatment), history of malignancy, bronchospastic disease, cardiac disease, immunodeficiency diseases, chronic infection (hepatitis B or C virus), and any surgical or medical condition that might significantly impact the absorption, distribution, metabolism, or excretion of drugs (per investigator discretion).

Pharmacokinetic evaluation

Blood samples were collected by venipuncture or via an indwelling catheter on the forearm vein. The sampling times for the PK of ruxolitinib were as follows: day 1 to predose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdose; day 7 to predose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h postdose. The corresponding sampling times for the PK of fluconazole were as follows: day 2 to predose; day 4 to predose; day 6 to predose; day 7 to predose; day 8 to predose; day 9 to predose; and day 10–24 h postdose. Blood samples were then processed [within 30 min of collection (centrifuged at 2000×g rpm at a temperature of 4 °C for 10 min)] to obtain plasma for the bioanalytical evaluations. Plasma samples of ruxolitinib were analyzed by liquid chromatography–mass spectrometry (LC/MS) with a lower limit of quantification of 0.5 ng/mL for ruxolitinib and of 10.0 ng/mL for fluconazole. Pharmacokinetic parameters of ruxolitinib were determined from blood concentration–time profiles of administered alone (period 1) and administered with fluconazole (period 2) using noncompartmental methods with Phoenix version 6.4 software from Certara Inc, Princeton, New Jersey, USA. The PK analysis set included all subjects who provided at least one evaluable PK profile from either the treatment period 1 or 2. The criteria for evaluability of period 1 profile were the following: subject received planned ruxolitinib dose [10 mg on day 1 (under fasting)], did not vomit within 2 h of receiving the dose, and provided at least one primary PK parameter (C_{max}, AUC_{last}, or AUC_{inf}). AUC values were calculated based on linear trapezoidal linear interpolation method in Phoenix version 8. The secondary PK parameters to be evaluated included time to reach maximum plasma concentration (T_{max}), $T_{1/2}$, apparent total body clearance of drug from the plasma (CL/F), and apparent volume of distribution during terminal phase (Vz/F). Fluconazole concentrations were used to check if the subjects had been adequately exposed to the inhibiting drug.

Safety evaluation

The secondary objective of the study was to characterize the safety of a single oral dose of ruxolitinib when administered with and without fluconazole in healthy volunteers. The safety set included all subjects who received at least one dose of study medication (ruxolitinib or fluconazole). The safety assessments included all adverse events (AEs) occurring during on-treatment period. Adverse events were summarized by number and percentage of subjects having at least one AE, having at least one AE in each primary system organ class and for each preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) coding. A subject with multiple occurrences of an AE was counted only once in the respective AE category. The safety summary included all assessments available for the laboratory parameter collected no later than 30 days after the last date of study treatment administration.

Statistical analysis

A formal statistical analysis was performed for PK parameters (C_{max}, AUC_{last}, and AUC_{inf}). The comparison of interest was ruxolitinib + fluconazole (test) vs ruxolitinib alone (reference). A linear mixed model (Eq. 1) was fitted to the log-transformed PK parameters to assess the effect of fluconazole on the PK of a single oral dose of 10 mg ruxolitinib. The model included treatment as a fixed factor and subject as a random factor for the analysis. The point estimates and 90% confidence intervals (CIs) for the geometric mean ratio of test vs reference treatment were determined. The model-based intra-subject and inter-subject variations and coefficient of variance (CV%) were presented for the primary PK parameters.

$$\log(PK_{ij}) = T_i + u_j + \varepsilon_{ij} \quad (1)$$

with T_i representing the treatment i ($i=1$ for ruxolitinib and $i=2$ for ruxolitinib + fluconazole), u_j representing the random effect for patient j (for j = 1, …, n subjects) and ε_{ij} representing the residual error.

The median and the range of the differences of T_{max} values of ruxolitinib were calculated for test vs reference. The individual subject ratios of test vs reference along with geometric mean ratio and 90% CI for primary PK parameters were displayed. The descriptive statistics (n, mean, CV%, standard deviation (SD), median and geometric mean, and geometric CV%, minimum and maximum) were presented by treatment for all PK parameters except T_{max} (only n, median, minimum, and maximum were presented). For the comparisons of interest for C_{max}, AUC_{inf}, and AUC_{last}, the individual subject ratios (test/reference) and the geometric mean ratio and 90% CI obtained from linear mixed model were plotted.

Results

These results presented here are simulations of the PBPK model of ruxolitinib established by Umehara et al. [14].

Physiologically based pharmacokinetic analysis

An AUC ratio of ruxolitinib (10 mg po, single dose at day 1 and day 7) with coadministration of fluconazole (400 mg po at day 2, followed by 200 mg po, once daily (qd) at day 3–9) was predicted to be 2.76 (Table 1). The ratio was within a twofold error compared to the clinical observation of 3.32 (Table 1), although underestimation was shown. The time–plasma concentration profiles of ruxolitinib in the absence and presence of fluconazole as observed and predicted are shown in Fig. 1a, b, respectively. As shown in Fig. 1c, the simulated plasma concentrations over time of fluconazole reached steady state with the 1- to 2-day treatment, resulting in maximal DDI effects of fluconazole on the ruxolitinib PK at day 6 during the entire simulation period (Fig. 1).

Effect of fluconazole on the pharmacokinetics of ruxolitinib in the clinical phase 1 study

Overall, 15 healthy subjects (median age, 44.0 years; males, 86.7%; median body mass index (BMI), 25.28 kg/m^2) were enrolled in the study. The baseline characteristics are presented in Supplementary Table 1. All 15 subjects received treatment and completed the study per the protocol.

Pharmacokinetics

The concentration–time profiles of reference (ruxolitinib) and test (ruxolitinib + fluconazole) groups are presented in Fig. 2. Ruxolitinib showed rapid absorption in both the reference and test groups, with a median T_{max} of 1.0 h and 0.5 h, respectively (Table 2). However, the slight decrease in T_{max} for the test group compared to the reference group indicated a slightly delayed absorption of ruxolitinib in the presence of fluconazole. C_{max} of ruxolitinib increased when coadministered with fluconazole as compared to ruxolitinib alone [geometric mean ratio, test/reference = 1.47 (Table 2)]. The elimination of ruxolitinib was slower when administered with fluconazole than when administered alone, as suggested by the respective slopes of the terminal phase of the concentration–time profile of test and reference groups in Fig. 2. The individual plasma concentration–time profile for ruxolitinib (with or without fluconazole) is presented in Supplementary Fig. 2.

Table 1 PBPK predicted systemic exposure changes of ruxolitinib in the absence and presence of coadministration of fluconazole

Ruxolitinib	Fluconazole	N	C_{max} (ng/mL)		AUC (ng h/mL)		AUC ratio	
			Predicted	Observed	Predicted	Observed	Predicted	Observed
10 mg po, single dose at day 1 and day 7[a]	Control	N[b]	169 (86.1–330)	97.5 (72.6–150)	809 (297–3235)	340 (204–746)	–	–
	400 mg po qd at day 2, followed by 200 mg po qd from day 3–9		229 (128–447)	143 (96.3–188)	2014 (679–5840)	1130 (615–2300)	2.76 (2.01–4.45)	3.32 (90% confidence interval: 3.02–3.65)
10 mg po, bid at the last dose[c]	Control	200	180 (90.40–340)	NA	729 (270–2228)	NA	–	–
	100 mg po, qd		247 (125–517)	NA	1326 (451–4033)	NA	1.82 (1.41–2.70)	NA
	200 mg po, qd		302 (150–679)	NA	1895 (612–5945)	NA	2.60 (1.80–4.34)	NA
	400 mg po, qd		399 (188–975)	NA	2989 (930–9491)	NA	4.10 (2.58–7.40)	NA

Results of simulations with the PBPK model of ruxolitinib established by Umehara et al. [14]

AUC area under the plasma concentration–time curve, *bid* twice daily, C_{max} maximum plasma concentration, *NA* not applicable, *po* orally, *qd* once daily. Pharmacokinetic data based on plasma concentration are represented as geometric mean with ranges as given in parentheses, otherwise noted

[a] AUC = AUC_{inf}

[b] $N = 15$ for observations; $N = 100$ for predictions

[c] Ruxolitinib and fluconazole were administered for 10 days. AUC = $AUC_{(0–12h)}$

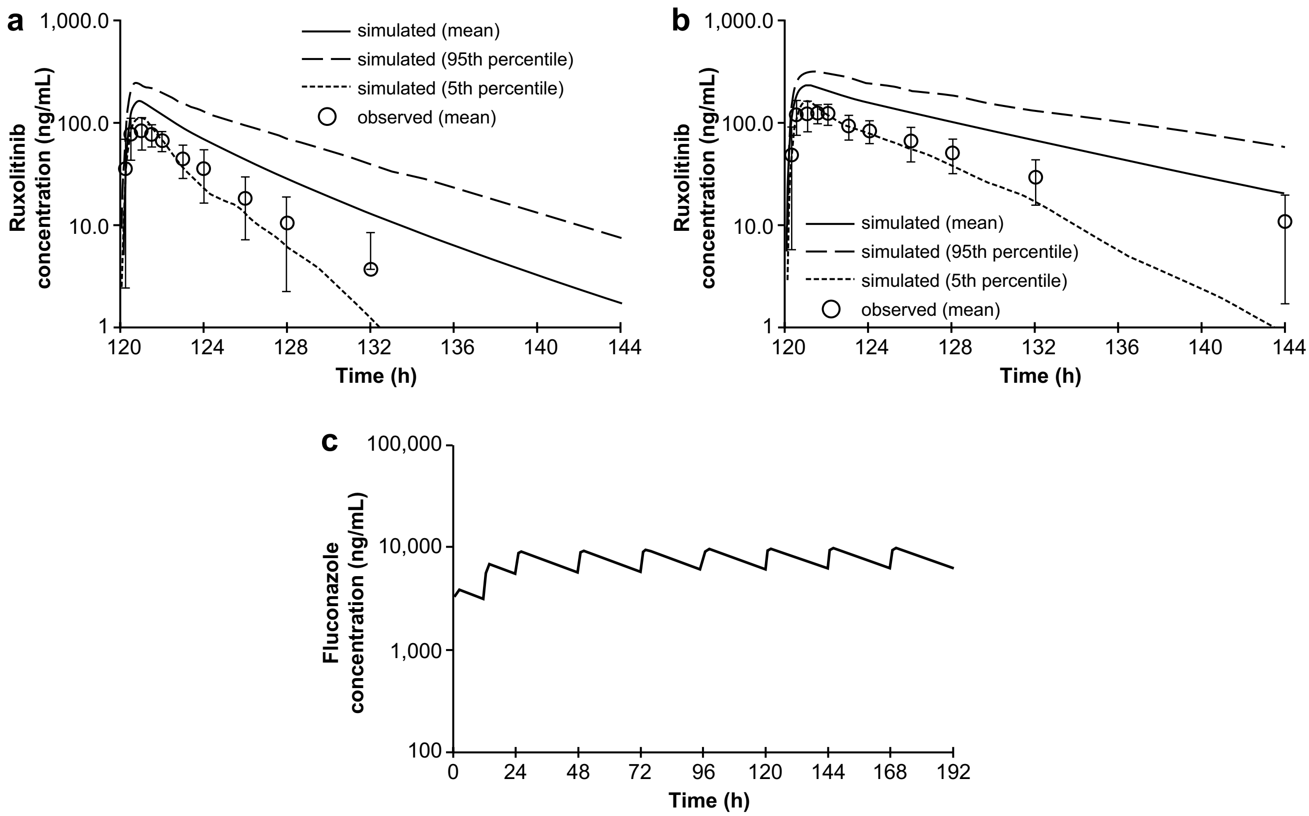

Fig. 1 Simulated and observed **c**hanges in ruxolitinib plasma concentrations in the presence and absence of fluconazole over time [results of simulations with the PBPK model of ruxolitinib established by Umehara et al. [14]. Plasma concentration profiles of ruxolitinib (10 mg, single dose at day 1 and day 7 in the absence (**a**) and presence (**b**) of fluconazole (400 mg orally, once daily at day 2, followed by 200 mg orally, once daily from day 3–9) were simulated ($N=100$). **c** Mean simulated plasma concentration–time profiles of fluconazole in healthy volunteers ($N=100$). The solid and broken lines represent simulated mean time–plasma concentration profiles and the 5th/95th percentile of the total virtual populations, respectively. Open circles are the measured values ($N=15$)]

Upon coadministration with fluconazole, the primary PK parameters C_{max}, AUC_{last}, and AUC_{inf} (geometric mean) of ruxolitinib increased by 47%, 234%, and 232%, respectively, when compared to ruxolitinib alone (Table 3).

Overall, the PK parameters displayed low to moderate variability. From the linear mixed model, the intra-subject variability for the primary PK parameters was low (15–17%) and close to the reference value (20.0%) used in the sample size calculation (Table 4). The inter-subject variability ranged from low to moderate (12–35%, Table 4).

The individual subject ratios of the test to reference groups and geometric mean ratios (90% CI) for plasma ruxolitinib PK parameters are presented in Supplementary Fig. 3. These ratios for C_{max}, AUC_{last}, and AUC_{inf} were greater than 1 (except for 1 data point), indicating that there was a significant increase in the exposure of ruxolitinib when coadministered with fluconazole vs ruxolitinib alone. The spread of the individual subject ratios and geometric mean ratio was significantly conserved for these parameters, highlighting low inter-subject variability.

The secondary PK parameter summaries for ruxolitinib are presented in Supplementary Table 2. The apparent CL/F was decreased approximately threefold in the presence of fluconazole (geometric mean = 29.4 L/h for ruxolitinib alone vs 8.85 L/h for the combination) consistent with the increased exposure. The geometric mean $T_{1/2}$ or elimination half-life for ruxolitinib was approximately 2.5 times longer when combined with fluconazole (geometric mean = 2.22 h for ruxolitinib alone vs 5.91 h for the combination), indicating a slower elimination of ruxolitinib in the presence of fluconazole. The Vz/*F* also decreased in the presence of fluconazole (geometric mean = 94.0 L for ruxolitinib alone vs 75.5 L for the combination).

Safety

Overall, six subjects (40.0%) reported at least one AE. The most common AEs were back pain and dizziness (two subjects (13.3%) each). The other AEs included hot flushes, paresthesia, pollakiuria, pruritus, and taste alteration (one

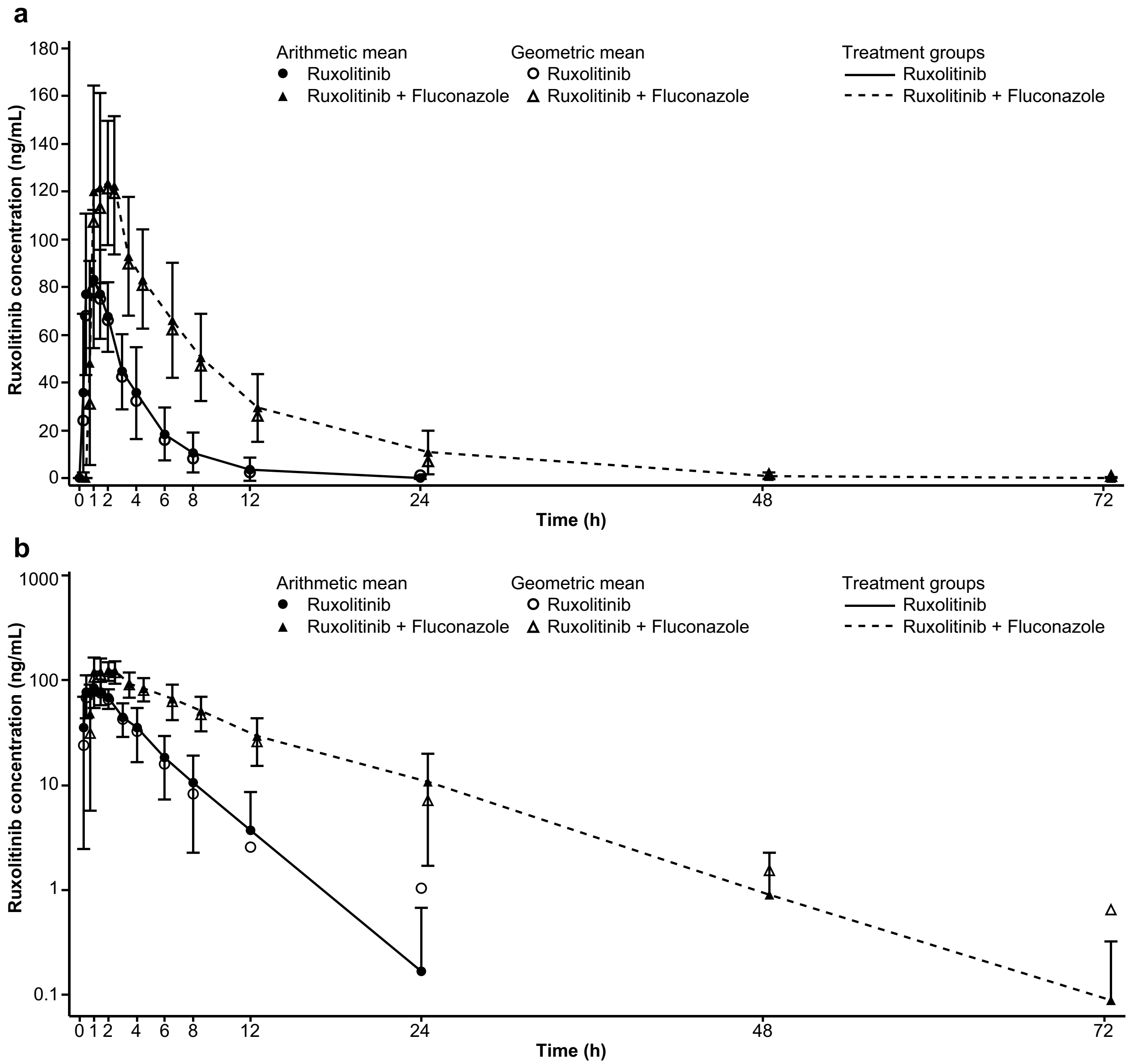

Fig. 2 Linear view (**a**) semi-logarithmic view (**b**) of geometric mean and arithmetic mean (standard deviation) plasma concentration–time profiles for ruxolitinib (with or without fluconazole). Zero concentrations at individual time points are excluded from geometric mean computation

subject (6.7%) each, Supplementary Table 3). All the AEs were grade 1, except back pain (grade 2). No grade 3 or 4 or serious AEs were reported. Four subjects experienced AEs that were suspected to be related to fluconazole, whereas none of the AEs were suspected to be related to ruxolitinib (investigator discretion). All the AEs resolved within 2 days of occurring and did not require study treatment modification/interruption. There were no deaths during the treatment and safety follow-up periods.

Discussion

Based on the findings from the in-house PBPK model of ruxolitinib that was established for estimating the victim DDI risks on CYP3A4 and CYP2C9 [14], it was recommended to avoid the use of fluconazole dose of greater than 200 mg daily with ruxolitinib. The PBPK model (with optimized $f_{m,CYP}$ values) was established using the systemic exposure increase of ruxolitinib (10 mg) after

Table 2 Statistical analysis of primary pharmacokinetic parameters and T_{max} for ruxolitinib

PK parameter (unit)	Treatment	N[a]	Adjusted geometric mean	Comparison	Treatment comparison		
					Geometric mean ratio	90% CI	
						Lower	Upper
C_{max} (ng/mL)	Reference	15	97.5				
	Test	15	143	Test/reference	1.47	1.33	1.63
AUC_{last} (ng h/mL)	Reference	15	331				
	Test	15	1100	Test/reference	3.34	3.01	3.69
AUC_{inf} (ng h/mL)	Reference	15	340				
	Test	15	1130	Test/reference	3.32	3.02	3.65
T_{max} (h)	Reference	15	1.00	Test − reference	−0.0167	−3.50	1.52
	Test	15	0.500				

Reference treatment: ruxolitinib; test treatment: ruxolitinib + fluconazole. Model is a linear mixed effects model of the log-transformed PK parameters. Treatment as a fixed factor and subject as a random factor were included in the model. Results were back transformed to get adjusted geometric mean, geometric mean ratio, and 90% CI. For T_{max}, median is presented under "adjusted geometric mean," median difference under "geometric mean ratio," and minimum and maximum differences under "geometric mean ratio" 90% CI

AUC area under the plasma concentration–time curve, AUC_{inf} AUC from time zero to infinity, AUC_{last} AUC from time zero to the last measurable concentration sampling time, *CI* confidence interval, C_{max} maximum plasma concentration, *PK* pharmacokinetics, T_{max} time to reach maximum plasma concentration

[a] N = number of observations used for analysis

Table 3 Primary pharmacokinetic parameters for ruxolitinib

Parameter	Statistics	Ruxolitinib (reference)	Ruxolitinib + fluconazole (test)
C_{max} (ng/mL)	*N*	15	15
	Geometric mean	97.5	143
	Geometric CV%	21.0	19.2
	Median	94.1	137
	Min–max	72.6–150	96.3–188
AUC_{last} (ng h/mL)	*N*	15	15
	Geometric mean	331	1100
	Geometric CV%	35.0	40.6
	Median	315	997
	Min–Max	202–736	612–2290
AUC_{inf} (ng h/mL)	N	15	15
	Geometric mean	340	1130
	Geometric CV%	35.8	39.7
	Median	319	1040
	Min–Max	204–746	615–2300

AUC area under the plasma concentration–time curve, AUC_{last} AUC from time zero to the last measurable concentration sampling time, AUC_{inf} AUC from time zero to infinity, C_{max} maximum plasma concentration, *CV* coefficient of variance, *PK* pharmacokinetics, *N* number of subjects with corresponding evaluable PK parameters

Table 4 Pharmacokinetic parameters and intra- and inter-subject variability for ruxolitinib

Parameter (unit)	*N*	Intra-subject variance	Intra-subject CV%	Inter-subject variance	Inter-subject CV%
C_{max} (ng/mL)	15	0.03	16.1	0.01	11.9
AUC_{last} (ng h/mL)	15	0.03	15.9	0.11	33.9
AUC_{inf} (ng h/mL)	15	0.02	15.0	0.11	34.3

Model is a linear mixed effects model of the log-transformed PK parameters. Included in the model were treatment as a fixed factor and subject as a random factor

AUC area under the plasma concentration–time curve, AUC_{last} AUC from time zero to the last measurable concentration sampling time, AUC_{inf} AUC from time zero to infinity, C_{max} maximum plasma concentration, *CV* coefficient of variance, *PK* pharmacokinetics. *N* number of subjects used in the model analysis

coadministration of a CYP3A4 inhibitor ketoconazole (200 mg po, bid) [14]. The model successfully predicted the magnitude of the inhibition potential of fluconazole on the PK of ruxolitinib (Table 1). Umehara et al. prospectively predicted the effect of coadministered fluconazole (200 mg) on the PK of ruxolitinib (10 mg) at steady state, estimating an AUC ratio of 2.76 (Table 1). While the previous and current DDI simulations were conducted by multiple and single oral administration of the victim drug ruxolitinib, respectively, assessments of the victim DDI risks will not be affected by different dose frequencies. Due to the relatively rapid elimination profiles of ruxolitinib as illustrated in Fig. 1a, b and historical PK data [12], no accumulation of the systemic exposure of ruxolitinib after multiple oral dosing was expected.

In model building for ruxolitinib, the reduced fractions metabolized of ruxolitinib by coadministration of ketoconazole were mainly distributed to CYP2C9-mediated metabolism, assuming that this pathway was not affected by the ketoconazole inhibition effect [14]. Accordingly, an $f_{m,CYP2C9}$ value in the model was calculated to be ~0.56. There has been no strong CYP2C9 inhibitor available to verify the $f_{m,CYP2C9}$ value by conducting a respective clinical DDI study [21]. By comparing the predicted AUC changes of ruxolitinib in the presence and absence of fluconazole to the corresponding clinical observations (Table 1), the estimated $f_{m,CYP2C9}$ of 0.56 was verified. For DDI simulations, no further optimization of input parameters including the $f_{m,CYP2C9}$ value is required.

Of note, the exposure changes of ruxolitinib when exposed to a CYP2C9 inducer could not be adequately estimated because of the lack of a verified PBPK inducer model. For CYP3A4, a verified induction model existed (rifampicin) and the respective simulation could be performed [14]. In addition, for PK profiles of ruxolitinib as a single agent, the current PBPK model showed a certain overestimation of the systemic exposures compared to the clinical observations (Table 1; Fig. 1) and as reported by Umehara et al. [14].

Nevertheless, it was demonstrated that the ruxolitinib model could successfully be used for the evaluation of victim DDI risks due to single and multiple pathway inhibitions on CYP2C9 and/or CYP3A4. The current model will be applicable in estimating the inhibition potential on ruxolitinib in untested case scenarios (e.g., pediatric subjects).

The DDI study conducted by Shi et al. in healthy adult volunteers confirmed that coadministration of ketoconazole with ruxolitinib resulted in a clinically important drug interaction effect on ruxolitinib PK/pharmacodynamics (PD) [13]. Study results showed an approximate twofold increase in the ruxolitinib systemic exposure. The combined PK/PD data suggested a 50% of reduction of starting doses of ruxolitinib, when administered concomitantly with a potent CYP3A4 inhibitor.

Fluconazole is a moderate inhibitor of CYP2C9/CYP3A4 [16, 17, 22]. Ruxolitinib is metabolized by CYP3A4 with equivalent contributions from CYP2C9 [12, 23]. Coadministration of ruxolitinib with fluconazole involves a potential risk of increase in ruxolitinib exposure [14, 23, 24]. A ruxolitinib PBPK model developed by Shi et al. predicted that 100–200 mg daily dose of fluconazole would increase ruxolitinib plasma concentration area under the curve approximately twofold [15]. The findings from the PBPK modeling study by Umehara et al. matched the predictions from the study by Shi et al. [14, 15].

The DDI study of ruxolitinib with fluconazole was conducted to evaluate the effect of multiple doses of fluconazole (200 mg and 400 mg) on single-dose PK of ruxolitinib (10 mg) in healthy volunteers. The comparison of concentration–time profiles of reference (ruxolitinib alone) and test groups (ruxolitinib + fluconazole) showed that C_{max} increased by almost 50%, AUC_{last} and AUC_{inf} increased by at least three times in the test group when compared to the reference group (Tables 2, 3; Fig. 2). The individual subject ratios of the test to reference groups for plasma ruxolitinib PK parameters (C_{max}, AUC_{last}, and AUC_{inf}) also indicated a significant increase in the exposure of ruxolitinib when coadministered with fluconazole vs ruxolitinib alone (Supplementary Fig. 3).

Coadministration of ruxolitinib with fluconazole considerably increased the systemic exposure to ruxolitinib in healthy subjects. However, there were no AEs attributed to ruxolitinib during this study, and the limited number of AEs that were attributed to fluconazole was mainly grade 1. This resultant increase in systemic exposure may require the ruxolitinib dose to be reduced/modified (by approximately 50%) when it is being administered with fluconazole doses of less than or equal to 200 mg (ruxolitinib prescribing information [24]). It is also recommended to avoid the use of fluconazole doses of greater than 200 mg daily with ruxolitinib.

Concomitant administration of a dual CYP3A4/CYP2C9 inhibitor with ruxolitinib may warrant close monitoring of drug plasma concentration and frequent monitoring of safety and efficacy [24], especially in the low platelet population. However, no safety concerns with ruxolitinib alone or when coadministered with fluconazole were identified in this study, and both treatments were well tolerated.

Ruxolitinib has proven to be a valuable therapeutic option for patients with intermediate or high-risk myelofibrosis and patients with polycythemia vera who are resistant/intolerant to hydroxyurea. The therapeutic potential of ruxolitinib is also being evaluated for patients with leukemia, such as JAK2 + acute myeloid leukemia. Concomitant administration of antifungal agents (prophylaxis or therapy) is often required in these settings. Our study provides critical insights into the effect of fluconazole on the ruxolitinib–PK profile and the translation of these effects on the pharmacodynamic aspects. However, these findings warrant careful interpretation as they are implied for healthy subjects as opposed to ruxolitinib-treated patients in a clinical trial or real-world setting.

The estimation of changes in the ruxolitinib systemic exposures with coadministration of CYP3A4 and CYP2C9 perpetrators using the PBPK model by Umehara et al. [14] provided critical insights for the evaluation of ruxolitinib–fluconazole drug interaction in healthy subjects and the interpretation of the clinical data. The systemic exposure increase observed in the clinical DDI study was comparable to the prospective prediction results using the PBPK model.

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Compliance with ethical standards

Conflict of interest FH, TO, SB, AB, WZ, and BG are all employees of Novartis. VA and KU were employed with Novartis during the study period. BG, FH and TO own stocks of Novartis.

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