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Drug–Drug Interaction Assessment of WXFL10203614 with Rifampicin, Itraconazole and Metformin: A Three-Cohort Phase I Study

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Abstract

Aims

To evaluate the effects of rifampicin and itraconazole on the pharmacokinetics (PK) of the Janus kinase 1 (JAK1) inhibitor WXFL10203614 and the effects of steady-state WXFL10203614 on the PK and safety of metformin.

Methods

In this single-centre, open-label, fixed-sequence, self-controlled Phase I study, 54 healthy participants were enrolled into three cohorts. Plasma and urine drug concentrations were determined using validated liquid chromatography–tandem mass spectrometry. Drug–drug interactions were assessed using geometric mean ratios (GMRs) and 90% confidence intervals (CIs) for prespecified PK parameters.

Results

Rifampicin reduced WXFL10203614 systemic exposure by approximately 40% and shortened its elimination half-life by 39%, whereas itraconazole increased exposure by approximately 26% compared with WXFL10203614 alone. Neither treatment produced a clinically meaningful change in maximum plasma concentration (Cmax), suggesting that the interactions primarily affected drug disposition after absorption. At steady state, WXFL10203614 produced no clinically meaningful change in metformin plasma exposure; however, metformin renal clearance and cumulative urinary excretion decreased by approximately 36% and 30%, respectively. These changes were consistent with an effect on renal tubular secretion involving MATE1/MATE2-K. No serious adverse events occurred, and most treatment-emergent adverse events were Grade 1.

Conclusions

WXFL10203614 was sensitive to CYP3A induction, with rifampicin substantially reducing systemic exposure, whereas the effect of itraconazole on exposure was modest. Concomitant use with strong CYP3A inducers should therefore be avoided where possible. Although steady-state WXFL10203614 did not meaningfully increase systemic metformin exposure, the reduction in metformin renal clearance and urinary excretion was consistent with an effect on MATE-mediated renal secretion. These findings support consideration of renal transporter-mediated interactions when WXFL10203614 is co-administered with metformin or other relevant transporter substrates.

Trial registration:

Trial registration CTR20254351, 4 November 2025.

1. Introduction

The Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathway is a central mediator of cytokine signal transduction and plays a pivotal role in the pathogenesis of multiple immune-mediated inflammatory diseases [1, 2]. JAK inhibitors have therefore become an important therapeutic option for rheumatoid arthritis (RA) [3], atopic dermatitis (AD) [4], ulcerative colitis (UC) [5], Crohn's disease (CD) [6], and other immune-mediated inflammatory disorders [7].

WXFL10203614 [8] is a novel, highly selective Janus kinase 1 (JAK1) inhibitor and a Class 1 innovative small-molecule drug that has not yet been approved for marketing in China or elsewhere. It was developed by Wuxi Fuxin Pharmaceutical Research and Development Co., Ltd. for the treatment of the four indications described above. Phase I dose-escalation studies have demonstrated that single oral doses of WXFL10203614 ranging from 1 to 33 mg were safe and well tolerated in healthy participants [8, 9].

Previous pharmacokinetic (PK) studies have characterised the disposition profile of WXFL10203614 and provided the basis for evaluating its drug–drug interaction (DDI) potential [8, 9]. In vitro studies have shown that CYP3A is the primary enzyme responsible for the metabolism of WXFL10203614 and that the compound may also be a substrate of P-glycoprotein (P-gp). In addition, WXFL10203614 inhibits multidrug and toxin extrusion transporters 1 and 2-K (MATE1 and MATE2-K), suggesting a potential risk of transporter-mediated DDIs when co-administered as a perpetrator drug with MATE substrates [10]. However, clinical evidence regarding OCT2/MATE-mediated DDIs involving selective JAK1 inhibitors remains limited, and few clinical studies have specifically investigated this interaction pathway [11].

Concomitant medication use is highly prevalent among patients with immune-mediated inflammatory diseases. Patients with rheumatoid arthritis frequently receive methotrexate, non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and biologic agents in combination [12, 13]. Patients with inflammatory bowel disease are commonly treated with 5-aminosalicylic acid and immunosuppressive agents [14–16], while patients with atopic dermatitis often require multiple topical and systemic therapies. Therefore, a comprehensive evaluation of potential drug–drug interactions (DDIs) is an essential component of the clinical development of new molecular entities and is explicitly recommended by regulatory agencies worldwide. The Technical Guidelines for Drug–Drug Interaction Studies (Trial Version) issued by the Center for Drug Evaluation of the National Medical Products Administration (NMPA) [17], together with the relevant guidance from the US Food and Drug Administration (FDA) and the ICH M12 guideline [18], recommend that clinical DDI studies be conducted using established probe drugs based on in vitro metabolism and transporter data to support dosing recommendations for concomitant medications in product labelling.

Accordingly, three well-established probe drugs were selected to systematically evaluate the CYP-mediated and transporter-mediated DDI potential of WXFL10203614. Rifampicin, a strong CYP3A

inducer, was administered at 600 mg once daily for 5–8 days, a regimen that has been extensively validated to achieve a clinically relevant CYP3A induction effect [19–21]. Itraconazole was selected as a strong CYP3A4 inhibitor and a clinically relevant P-glycoprotein (P-gp) inhibitor [22, 23]. Metformin, a well-established substrate of organic cation transporter 2 (OCT2), multidrug and toxin extrusion transporter 1 (MATE1), and MATE2-K, is eliminated predominantly through active renal tubular secretion [24, 25] and is recommended by the FDA and EMA as a clinical probe substrate for evaluating transporter-mediated DDIs [26]. Although perpetrator drugs commonly used in DDI studies have well-established effects on their intended targets, they are not necessarily mechanism-selective, and their individual effects on different CYP enzymes and transporters may not have been fully characterised in clinical settings. Therefore, interpretation of DDI findings should consider both the enzyme/transporter interaction profile of the perpetrator drug and the magnitude of exposure changes in the victim drug to provide a more reliable understanding of the underlying DDI mechanisms.

2. Methods

2.1 Study design and treatment

This was a single-centre, open-label, self-controlled, fixed-sequence Phase I drug–drug interaction study (Protocol No. 2025-WXFL10203614-I-013) conducted at the Phase I Clinical Trial Unit of the First Affiliated Hospital of Anhui Medical University. The study protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University and was conducted in accordance with the International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines, the Good Clinical Practice for Drug Clinical Trials issued by the National Medical Products Administration (NMPA), and the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. The study was registered with the Chinese Clinical Trial Registry and Information Disclosure Platform (Registration No. CTR20254351).

The study comprised three cohorts, each enrolling 18 healthy adult participants aged 18–55 years with a body mass index (BMI) of 19.0–26.0 kg/m². All study medications were administered orally 30 ± 1 minutes after the start of breakfast. Cohorts 1 and 2 employed a two-period fixed-sequence design to evaluate the effects of the strong CYP3A inducer rifampicin and the strong CYP3A inhibitor itraconazole, respectively, on the pharmacokinetics of WXFL10203614. Cohort 3 employed a reverse fixed-sequence design to assess the effects of steady-state WXFL10203614 on the pharmacokinetics of metformin.

In Cohort 1, participants received a single oral dose of WXFL10203614 16 mg. After a 7-day washout period, rifampicin 600 mg was administered once daily for multiple doses, and a single 16-mg dose of WXFL10203614 was co-administered after rifampicin had reached steady state.

In Cohort 2, participants received a single oral dose of WXFL10203614 16 mg. Following a washout period, itraconazole was administered at 200 mg twice daily on Day 1 and 200 mg once daily thereafter.

A single 16-mg dose of WXFL10203614 was co-administered after itraconazole had reached steady state.

In Cohort 3, participants first received a single oral dose of metformin hydrochloride 850 mg. After a 3-day washout period, WXFL10203614 16 mg was administered once daily for multiple doses, and a single oral dose of metformin hydrochloride 850 mg was co-administered after WXFL10203614 had reached steady state.

All cohorts completed the prespecified pharmacokinetic blood sampling schedule and safety assessments. In addition, urine samples were collected in Cohort 3 to evaluate the renal excretion of metformin.

The investigational products included WXFL10203614 tablets (4 mg/tablet; batch No. XS250702GL; supplied by Wuxi Fuxin Pharmaceutical Research and Development Co., Ltd.), rifampicin capsules (0.15 g/capsule; Shenyang Hongqi Pharmaceutical Co., Ltd.), itraconazole capsules (0.1 g/capsule; Xian-Janssen Pharmaceutical Ltd.), and metformin hydrochloride tablets (0.85 g/tablet; Merck KGaA). All investigational products met quality specifications and were used within their approved shelf life.

2.2 Participants

Eligible participants were healthy adults aged 18–55 years, of either sex, with a body mass index (BMI) of 19.0–26.0 kg/m², a body weight of at least 50 kg for men and 45 kg for women, and were judged to be in good health based on medical history, physical examination, vital signs, 12-lead electrocardiography (ECG), clinical laboratory tests, posteroanterior chest radiography, and abdominal ultrasonography.

The main exclusion criteria included: use of any prescription medications, over-the-counter medications, traditional Chinese medicines, vitamins, or dietary supplements within 2 weeks before dosing; use of any medications known to inhibit or induce hepatic drug-metabolising enzymes within 30 days before dosing; a history of clinically significant drug hypersensitivity or an allergic constitution; smoking ≥ 5 cigarettes per day within 3 months before screening; consumption of alcohol, xanthine-containing foods or beverages, grapefruit, or other foods known to affect drug metabolism within 48 hours before dosing; clinically significant ECG abnormalities, including a PR interval > 250 ms, QRS duration > 120 ms, or Fridericia-corrected QT interval (QTcF) > 450 ms; and evidence of active infection based on infectious disease screening or T-SPOT. TB testing.

2.3 Pharmacokinetic assessments

In Cohorts 1 and 2, blood samples were collected before dosing and at 16 post-dose time points from 5 minutes to 72.0 hours after dosing on Day 1/Day 14 and Day 1/Day 12, respectively (32 samples per participant), for the determination of plasma WXFL10203614 concentrations. In Cohort 3, blood samples were collected before dosing and at 14 scheduled post-dose time points ranging from 30 minutes to 48.0 hours after dosing on both Day 1 and Day 11 (28 samples per participant) for the determination of plasma metformin concentrations. Urine samples were also collected during the predose interval (− 12

to 0 h) and at prespecified intervals during the 0–24-hour post-dose period to characterise the urinary excretion profile of metformin.

Plasma and urine samples were analysed by Suzhou HaiKe Pharmaceutical Technology Co., Ltd. using validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods. The validated calibration ranges were 0.500–500 ng/mL for WXFL10203614 in plasma, 10.0–1,500 ng/mL for metformin in plasma, and 5.00–2,500 μg/mL for metformin in urine. Bioanalytical method validation and sample analysis were conducted in accordance with the International Council for Harmonisation (ICH) M10 Guideline Bioanalytical Method Validation and Study Sample Analysis and the Guidelines for the Management of Bioanalytical Laboratories for Drug Clinical Trials.

2.4 Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Plasma drug concentrations and pharmacokinetic (PK) parameters were summarised using descriptive statistics. PK parameters are presented as geometric means with geometric coefficients of variation (gCV%). PK parameters were estimated using noncompartmental analysis (NCA) with Phoenix WinNonlin (version 8.3 or later; Certara, Princeton, NJ, USA).

Drug–drug interaction analyses were performed using the drug interaction analysis set (DIS). The primary PK parameters, including the maximum plasma concentration (Cmax), area under the plasma concentration–time curve from time zero to the last quantifiable concentration (AUC_{0-t}), and area under the plasma concentration–time curve from time zero extrapolated to infinity ($AUC_{0-\infty}$), were natural log-transformed before analysis. For Cohort 3, renal clearance over the 0–24-hour interval (CLr,0–24 h) and the cumulative fraction of dose excreted unchanged in urine over the same interval (Fe0–24 h) were additionally analysed. Analysis of variance (ANOVA) and the two one-sided tests (TOST) procedure were performed on the log-transformed data to estimate geometric mean ratios (GMRs; combination treatment/reference treatment) and corresponding 90% confidence intervals (CIs). A clinically meaningful interaction was considered absent if the entire 90% CI for the GMR was contained within the predefined no-effect boundaries of 80.00%–125.00%. Time to maximum plasma concentration (Tmax) was compared using the paired Wilcoxon signed-rank test. All statistical tests were two-sided, with a significance level of $\alpha = 0.05$.

3. Results

3.1 Participant disposition

A total of 134 individuals were screened, of whom 80 failed screening. Fifty-four healthy participants were enrolled across the three cohorts (18 participants per cohort), and all received at least one dose of the study drug. Overall, 52 participants (96.30%) completed the study. In Cohort 1, two participants discontinued prematurely: one withdrew consent for personal reasons, and one discontinued because of an adverse event that, in the investigator’s judgment, precluded further participation. Both participants

discontinued before receiving WXFL10203614 in Period 2, and their data from Period 1 were included in the relevant analysis sets. All 18 participants in Cohorts 2 and 3 completed the study (Figure 1). Baseline demographic characteristics of participants in the three cohorts are summarised in Table 1.

Table 1. Demographic characteristics of the full analysis set (FAS)

Characteristic	Cohort 1 (N = 18)	Cohort 2 (N = 18)	Cohort 3 (N = 18)
Age (years), mean ± SD	37.3±9.61	32.8±8.12	31.2±10.15
Sex, n (%)			
male	12(66.67)	13(72.22)	13(72.22)
female	6(33.33)	5(27.78)	5(27.78)
Ethnicity, n (%)			
han	18(100.00)	17(94.44)	18(100.00)
other	0(0.00)	1(5.56)	0(0.00)
Body weight (kg), mean ± SD	64.57±8.504	65.91±10.160	62.57±7.475
Height (cm), mean ± SD	165.50±8.848	167.69±7.589	165.92±7.871
Body mass index (kg/m²),mean ± SD	23.50±1.760	23.31±2.005	22.70±1.875

Note: Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m²). Age and BMI were automatically calculated by the electronic data capture (EDC) system.

3.2 Effect of rifampicin on the pharmacokinetics of WXFL10203614

A total of 18 participants in Cohort 1 were included in the pharmacokinetic analysis set, and 16 participants who completed both treatment periods were included in the drug interaction analysis set (Table 2 and Figure 2). Following single-dose administration of WXFL10203614 alone (N = 18), the geometric mean Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were 268.30 ng/mL, 4781.97 h·ng/mL, and 4926.08 h·ng/mL, respectively. The median Tmax was 3.00 h, the terminal elimination half-life ($t_{1/2}$) was 12.82 h, and the apparent oral clearance (CL/F) was 3.25 L/h. Following co-administration of WXFL10203614 with rifampicin (N = 16), the geometric mean Cmax was 238.20 ng/mL, whereas the geometric mean AUC_{0-t} and $AUC_{0-\infty}$ decreased to 2826.13 and 2843.41 h·ng/mL, respectively. The median Tmax was 1.75 h, the $t_{1/2}$ was 7.77 h, and the CL/F increased to 5.63 L/h. Paired analysis of the 16 participants who completed both treatment periods showed that the GMR for Cmax was 88.72% (90% CI, 76.57%–

102.80%). Compared with WXFL10203614 administration alone, co-administration with rifampicin decreased AUC_{0-t} and $AUC_{0-\infty}$ by 39.83% and 40.75%, respectively, with GMRs of 60.17% (90% CI, 53.25%–67.97%) and 59.25% (90% CI, 52.40%–66.98%). The 90% CIs for both AUC parameters were entirely outside the predefined no-effect boundaries of 80.00%–125.00%, with the upper bounds of the 90% CIs below 80.00%. The median Tmax was reduced from 3.00 h to 1.75 h (P = 0.0231).

Table 2. Summary of the pharmacokinetic parameters of WXFL10203614 co-administered with rifampicin (PKPS)

Parameter (unit)	GeoMean(GeoCV%)	
	WXFL10203614 alone (N = 18)	WXFL10203614 + rifampicin (N = 16)
Tmax*(h)	3.0030(1.003□4.026)	1.7545(0.503□3.010)
Cmax (ng/mL)	268.3(22.21)	238.2(27.05)
$AUC_{0-t(}$h*ng/mL)	4781.971(19.4163)	2826.131(21.7211)
$AUC_{0-\infty}$(h*ng/mL)	4926.077(20.1423)	2843.405(21.5917)
AUC%Extrap(%)	2.122(82.1267)	0.538(55.2228)
$t_{1/2}$(h)	12.822(16.0454)	7.769(17.4534)
λz(1/h)	0.0541(16.0454)	0.0892(17.4534)
Vz/F(L)	60.081(21.3823)	63.073(21.1014)
CL/F(L/h)	3.248(20.1423)	5.627(21.5917)
MRT_{0-t}(h)	17.673(9.5044)	10.932(16.0378)
$MRT_{0-\infty}$(h)	19.511(14.1931)	11.274(15.8232)

Note: Data for *T*_{max} are presented as **median (min, max)**.

3.3 Effect of itraconazole on the pharmacokinetics of WXFL10203614

All 18 participants in Cohort 2 completed both treatment periods and were included in the drug interaction analysis set (Table 3 and Figure 3). Following single-dose administration of WXFL10203614 alone, the geometric mean Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were 273.00 ng/mL, 4436.98 h·ng/mL, and 4528.84 h·ng/mL, respectively. The median Tmax was 2.01 h, the terminal elimination half-life ($t_{1/2}$) was 12.23 h, and the apparent oral clearance (CL/F) was 3.53 L/h. Following co-administration of WXFL10203614 with itraconazole, the geometric mean Cmax was 278.30 ng/mL, with a GMR of

101.93% (90% CI, 94.22%–110.26%), which was entirely within the predefined no-effect boundaries of 80.00%–125.00%. The geometric mean AUC_{0-t} and $AUC_{0-\infty}$ increased to 5572.12 and 5735.26 h·ng/mL, representing increases of 25.58% and 26.64%, respectively, compared with WXFL10203614 administration alone. The corresponding GMRs were 125.58% (90% CI, 114.07%–138.25%) and 126.64% (90% CI, 114.48%–140.08%), respectively, and neither of the 90% CIs was fully contained within the predefined no-effect boundaries. Median Tmax was not significantly different between treatments (2.00 h; P = 0.4616). The $t_{1/2}$ increased to 13.29 h, whereas CL/F decreased to 2.79 L/h.

Table 3. Summary of the pharmacokinetic parameters of WXFL10203614 co-administered with itraconazole (PKPS)

Parameter (unit)	GeoMean (GeoCV%)	
	WXFL10203614 alone (N = 18)	WXFL10203614 + itraconazole (N = 18)
Tmax*(h)	2.0075(1.005□4.005)	2.0035(0.503□4.024)
Cmax (ng/mL)	273.0(15.28)	278.3(12.63)
AUC_{0-t}(h*ng/mL)	4436.984(17.9072)	5572.118(16.4163)
$AUC_{0-\infty}$(h*ng/mL)	4528.838(18.6489)	5735.260(17.4367)
AUC%Extrap(%)	1.546(87.7683)	2.250(73.6358)
$t_{1/2}$(h)	12.228(19.9054)	13.294(20.0102)
λz(1/h)	0.0567(19.9054)	0.0521(20.0102)
Vz/F(L)	62.325(19.6429)	53.505(20.9167)
CL/F(L/h)	3.533(18.6489)	2.790(17.4367)
MRT_{0-t}(h)	16.708(12.3559)	18.525(10.8042)
$MRT_{0-\infty}$(h)	18.074(17.0681)	20.443(16.9300)

Note: Data for *T*_{max} are presented as **median (min, max)**. **N** denotes the number of participants in the pharmacokinetic parameter set (PKPS) who received the corresponding treatment.

3.4 Effect of WXFL10203614 on the pharmacokinetics of metformin

All 18 participants in Cohort 3 completed both treatment periods and were included in the drug interaction analysis set (Table 4 and Figure 4). Following single-dose administration of metformin alone,

the geometric mean Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were 1709.30 ng/mL, 11,938.19 h·ng/mL, and 12,165.61 h·ng/mL, respectively. The median Tmax was 2.50 h, the terminal elimination half-life ($t_{1/2}$) was 5.08 h, and the apparent oral clearance (CL/F) was 69.87 L/h. Following administration of metformin during steady-state WXFL10203614 treatment, the geometric mean Cmax was 1783.40 ng/mL, with a GMR of 104.33% (90% CI, 93.57%–116.32%). The geometric mean AUC_{0-t} and $AUC_{0-\infty}$ were 13,357.99 and 13,549.11 h·ng/mL, respectively, with corresponding GMRs of 111.89% (90% CI, 100.60%–124.44%) and 111.37% (90% CI, 100.28%–123.68%). The 90% CIs for Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were all entirely contained within the predefined no-effect boundaries of 80.00%–125.00%. Median Tmax was delayed from 2.50 h to 3.50 h (P = 0.0118), the $t_{1/2}$ was slightly prolonged to 5.44 h, and no appreciable change in CL/F was observed. For urinary pharmacokinetic parameters, co-administration with WXFL10203614 reduced the cumulative amount of unchanged metformin excreted in urine over 24 hours (Ae0–24 h) from 298.87 mg to 209.25 mg. Renal clearance over 0–24 hours (CLr,0–24 h) and the cumulative fraction of dose excreted unchanged in urine over the same interval (Fe0–24 h) decreased by 36.49% and 29.98%, respectively, with GMRs of 63.51% (90% CI, 54.33%–74.22%) and 70.02% (90% CI, 58.40%–83.93%). Neither of the corresponding 90% CIs for these two parameters was contained entirely within the predefined no-effect boundaries of 80.00%–125.00%.

Table 4. Summary of the pharmacokinetic parameters of metformin administered alone and co-administered with steady-state WXFL10203614 (PKPS)

Parameter (unit)	GeoMean (GeoCV%)	
	Metformin alone (N = 18)	Metformin + steady-state WXFL10203614 (N = 18)
Tmax*(h)	2.5040(1.003–4.021)	3.5035 (2.003–4.025)
Cmax (ng/mL)	1709.3 (19.89)	1783.4(19.08)
AUC_{0-t}(h*ng/mL)	11938.191(16.5807)	13357.992(21.2216)
$AUC_{0-\infty}$(h*ng/mL)	12165.610(16.4173)	13549.114(20.8804)
AUC%Extrap(%)	1.767(35.6182)	1.288(46.2688)
$t_{1/2}$(h)	5.075(29.2291)	5.435(34.1573)
λz(1/h)	0.1366(29.2291)	0.1275(34.1573)
Vz/F(L)	511.518(29.8571)	491.943(28.9968)
CL/F(L/h)	69.869(16.4173)	62.735(20.8804)
MRT_{0-t}(h)	6.181(9.3983)	6.713(16.8279)
$MRT_{0-\infty}$(h)	6.734(10.1510)	7.201(17.9851)

Note: Data for *T*_{max} are presented as **median (min, max)**. **N** denotes the number of participants included in the pharmacokinetic parameter set (PKPS) who received the corresponding treatment.

3.5 Safety

A total of 54 healthy participants (18 participants per cohort) were included in the safety analysis set (Table 5). No serious adverse events (SAEs), serious adverse reactions (SARs), or deaths occurred in any cohort during the study. The majority of adverse events (AEs) were Grade 1 in severity, did not require concomitant medications or other medical interventions, and resolved or recovered.

Table 5. Summary of treatment-emergent adverse events (Safety Set [SS])

Category	Cohort1, n(%) (N=18)	Cohort2, n(%) (N=18)	Cohort3, n(%) (N=18)
Treatment-related adverse events	8（44.44）	11（61.11）	12（66.67）
Adverse events leading to study discontinuation	1（5.56）	0（0.00）	0（0.00）
Adverse events	9（50.00）	11（61.11）	12(66.67)
Grade 1	8(44.44)	11（61.11）	12(66.67)
Grade 2	1(5.56)	0（0.00）	0(0.00)
Grade ≥3	0(0.00)	0（0.00）	0（0.00）

Note: Data are presented as **n (%)**. Percentages were calculated based on the total number of participants in each cohort. Severity was counted once per participant according to the highest CTCAE grade reported.

In Cohort 1, one participant experienced a Grade 2 elevation in liver enzymes during the WXFL10203614 monotherapy period, which was considered possibly related to the study drug by the investigator and resulted in study discontinuation. No other AEs leading to study discontinuation occurred during the combination treatment period. In Cohort 1, the incidence rates of AEs following administration of WXFL10203614 alone and in combination with rifampicin were 38.89% (7/18 participants; 13 events) and 37.50% (6/16 participants; 19 events), respectively. The incidence rates of adverse drug reactions (ADRs) were 33.33% and 31.25%, respectively. In the combination treatment period, the most frequently reported events were increased blood pressure (18.75%; 3 participants, 12 events) and increased heart rate (12.50%; 2 participants, 3 events). The incidence and severity of AEs did not increase compared with WXFL10203614 monotherapy. However, rifampicin, a strong CYP3A inducer, markedly reduced WXFL10203614 exposure (by approximately 40%). This interaction was considered clinically relevant and may potentially reduce the therapeutic efficacy of WXFL10203614.

In Cohort 2, the incidence rates of AEs following administration of WXFL10203614 alone and in combination with itraconazole were 38.89% (7 participants) and 44.44% (8 participants), respectively. AEs were mainly classified within the cardiac disorders system organ class. The incidence of sinus bradycardia increased from 11.11% during WXFL10203614 monotherapy to 27.78% during combination treatment. One participant experienced first-degree atrioventricular block. All cardiac-related events were Grade 1 in severity, required no concomitant medications or other medical interventions, and primarily resolved or recovered. Although itraconazole increased WXFL10203614 exposure by approximately 26%, no clinically relevant safety impact was observed.

In Cohort 3, following co-administration with WXFL10203614, the incidence rates of AEs and ADRs associated with metformin both increased from 38.89% to 50.00% (9/18 participants; 16 events). New-onset increases in serum creatinine (16.67%) and decreases in estimated glomerular filtration rate (eGFR) (11.11%) were observed during combination treatment. These changes were consistent with the reduction in metformin renal clearance (CLr, 0–24 h decreased by approximately 36.49%), suggesting that WXFL10203614 inhibited MATE1/MATE2-K-mediated renal excretion of metformin.

In conclusion, WXFL10203614 was generally well tolerated when administered alone or in combination with rifampicin, itraconazole, or metformin, and no new safety signals were identified. The reduction in WXFL10203614 exposure caused by rifampicin was considered clinically relevant, and concomitant administration should be avoided where possible. The increase in WXFL10203614 exposure associated with itraconazole was not considered to have clinically relevant safety implications. Concomitant use of WXFL10203614 with metformin should also be avoided where possible because of the potential risk of metformin accumulation resulting from inhibition of renal excretion.

4. Discussion

This study represents a systematic clinical drug–drug interaction (DDI) assessment of WXFL10203614, a selective JAK1 inhibitor. Based on in vitro metabolic enzyme phenotyping and transporter evaluation results, three well-established probe drugs were selected to characterise the DDI potential of WXFL10203614 in three cohorts of 54 healthy Chinese participants (52 participants completed the study, including 16 participants in Cohort 1). WXFL10203614 was generally well tolerated when administered alone or in combination with rifampicin, itraconazole, or metformin, with no serious adverse events (SAEs) reported, and the majority of adverse events (AEs) being Grade 1 in severity.

Regarding pharmacokinetics, rifampicin decreased WXFL10203614 systemic exposure by approximately 40%, whereas itraconazole increased exposure by only approximately 26%. WXFL10203614 reduced the renal clearance of metformin by approximately 36%, while resulting in only a modest increase in plasma metformin exposure. Collectively, the three cohorts characterised the DDI profile of WXFL10203614 as both a victim drug (Cohorts 1 and 2) and a perpetrator drug (Cohort 3).The integrated interpretation of Cohorts 1 and 2 provides clear mechanistic insights into the DDI profile of WXFL10203614. In both cohorts, no meaningful changes in Cmax were observed, whereas alterations were primarily reflected in

AUC and terminal elimination half-life, suggesting that the interactions mainly occurred during the elimination phase rather than during absorption. Rifampicin accelerated the systemic clearance of WXFL10203614 through induction of intestinal and hepatic CYP3A activity, potentially with concomitant induction of P-glycoprotein (P-gp). In contrast, the contribution of CYP3A-mediated metabolism to the overall clearance of WXFL10203614 appears to be limited, which may explain the ceiling effect observed with CYP3A inhibition by itraconazole.The finding that the induction effect was substantially greater than the inhibition effect indicates an interaction phenotype characterised by sensitivity to CYP3A induction but relative tolerance to CYP3A inhibition. This pattern is consistent with previously reported DDI characteristics of CYP3A-metabolised JAK inhibitors, such as upadacitinib [27, 28], suggesting that exposure reduction caused by strong CYP3A inducers represents a potential class-wide risk for this therapeutic category. These findings directly support the clinical strategy of avoiding concomitant administration with strong CYP3A inducers while not requiring dose adjustment when co-administered with strong CYP3A inhibitors. For the metformin interaction, WXFL10203614 produced minimal changes in plasma metformin exposure but substantially reduced urinary excretion parameters. The reduction in metformin renal clearance, together with the decreased cumulative fraction excreted unchanged in urine, was consistent with inhibition of MATE1/MATE2-K-mediated renal tubular secretion. The in vitro inhibitory activities of WXFL10203614 against MATE1 and MATE2-K (IC_{50} values of 0.835 and 0.736 μmol/L, respectively) further support these clinical findings and identify renal tubular secretion as the primary site of interaction. Similar mechanisms have been reported for interactions between metformin and other MATE/OCT2 inhibitors, including cimetidine and dolutegravir [29, 30].

These findings also indicate that evaluation of transporter-mediated DDIs based solely on plasma AUC may underestimate the potential interaction risk, and urinary PK parameters provide a necessary complementary assessment for renal transporter-mediated DDIs. Notably, the direction of changes in serum creatinine elevation and reduced glomerular filtration rate in the combination treatment group was consistent with the decrease in renal clearance, accompanied by an increased incidence of adverse events. Although metformin has a relatively wide therapeutic window and the short-term increase in exposure was limited, prolonged co-administration or use in patients with impaired renal function may still present a potential risk of metformin accumulation and, consequently, lactic acidosis.

The strengths of this study include the prospective selection of probe drugs guided by prior in vitro data, the self-controlled design that effectively minimised inter-individual variability, and the integration of plasma and urinary pharmacokinetic assessments to provide mechanistic confirmation. The in vitro CYP3A metabolic phenotype and MATE inhibitory activity of WXFL10203614 were both confirmed in the clinical setting.

In conclusion, this study demonstrates that WXFL10203614 was generally well tolerated when administered alone or in combination with rifampicin, itraconazole, or metformin. The DDI characteristics of WXFL10203614 have been established, including avoidance of concomitant use with strong CYP3A inducers, no requirement for dose adjustment when co-administered with strong CYP3A4/P-gp inhibitors, and avoidance of concomitant use with MATE substrates. These findings provide a scientific

basis for combination medication recommendations in the prescribing information and for the management of concomitant medications in future clinical studies.

Declarations

Author Contributions

Jinmei Zhou and Mengshuang Gao contributed to data collection, pharmacokinetic analysis and manuscript drafting. Daolei Zhou was responsible for participant management during the clinical study. Zhiwei Zhang and Huan Zhang contributed to study design and supervision. Yu Sun and Huan Zhou conceived the study, supervised the research and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Consent for Publication

Not applicable. No individual participant data or identifiable information are presented in this manuscript.

Ethics Approval The study protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University. The study was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice (ICH-GCP), and applicable national regulations.

Consent to publish All authors have reviewed and approved of publishing this manuscript.

Competing Interests Zhiwei Zhang and Huan Zhang are employees of Zhuohe Pharmaceutical Group Co., Ltd. The remaining authors declare that they have no competing interests.

Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

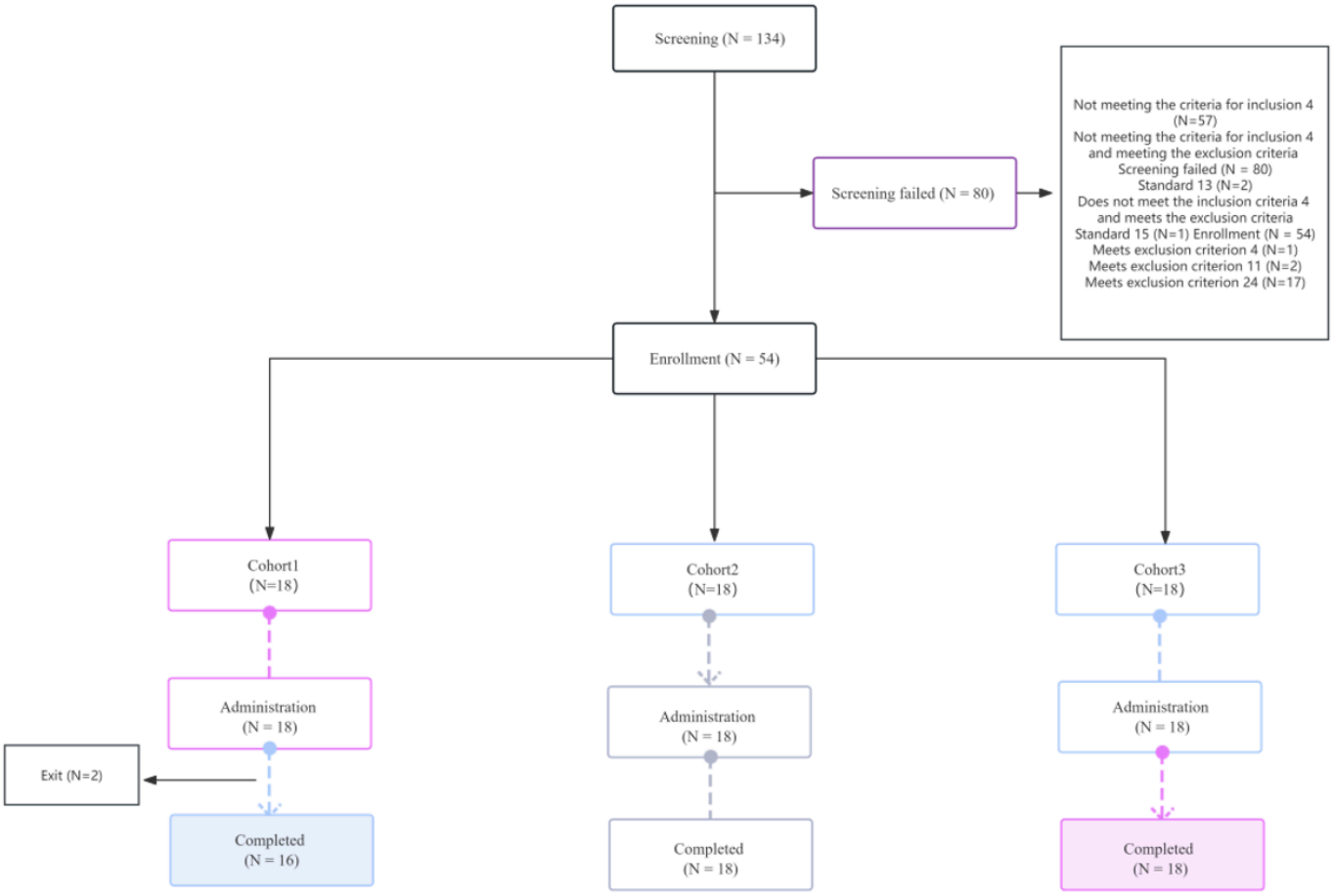


Figure 1

Flowchart of participant distribution

Note: Cohort 1 received WXFL10203614 plus rifampicin; Cohort 2 received WXFL10203614 plus itraconazole (participant B009 was of Tibetan ethnicity); and Cohort 3 received WXFL10203614 plus metformin.

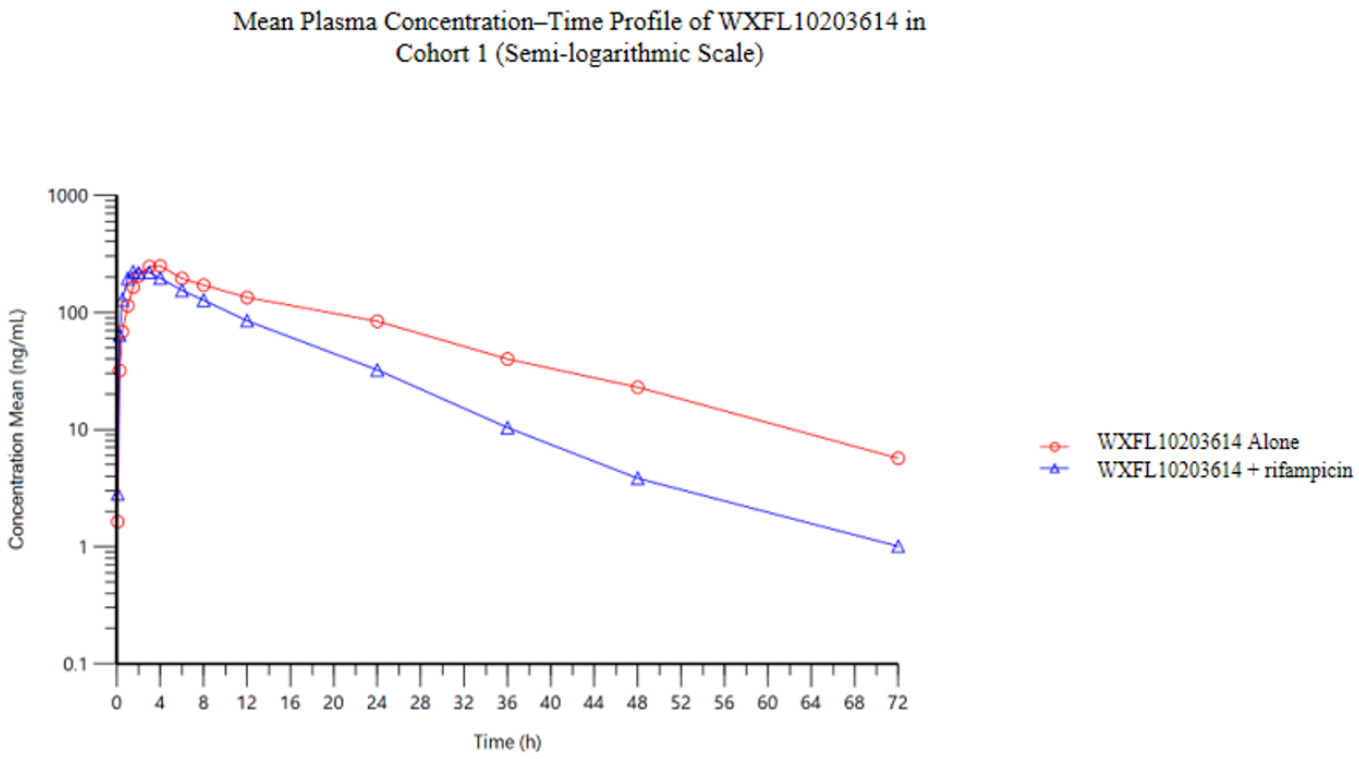


Figure 2

Mean plasma concentration–time profiles of WXFL10203614 after administration of WXFL10203614 alone or in combination with rifampicin (semi-logarithmic scale).

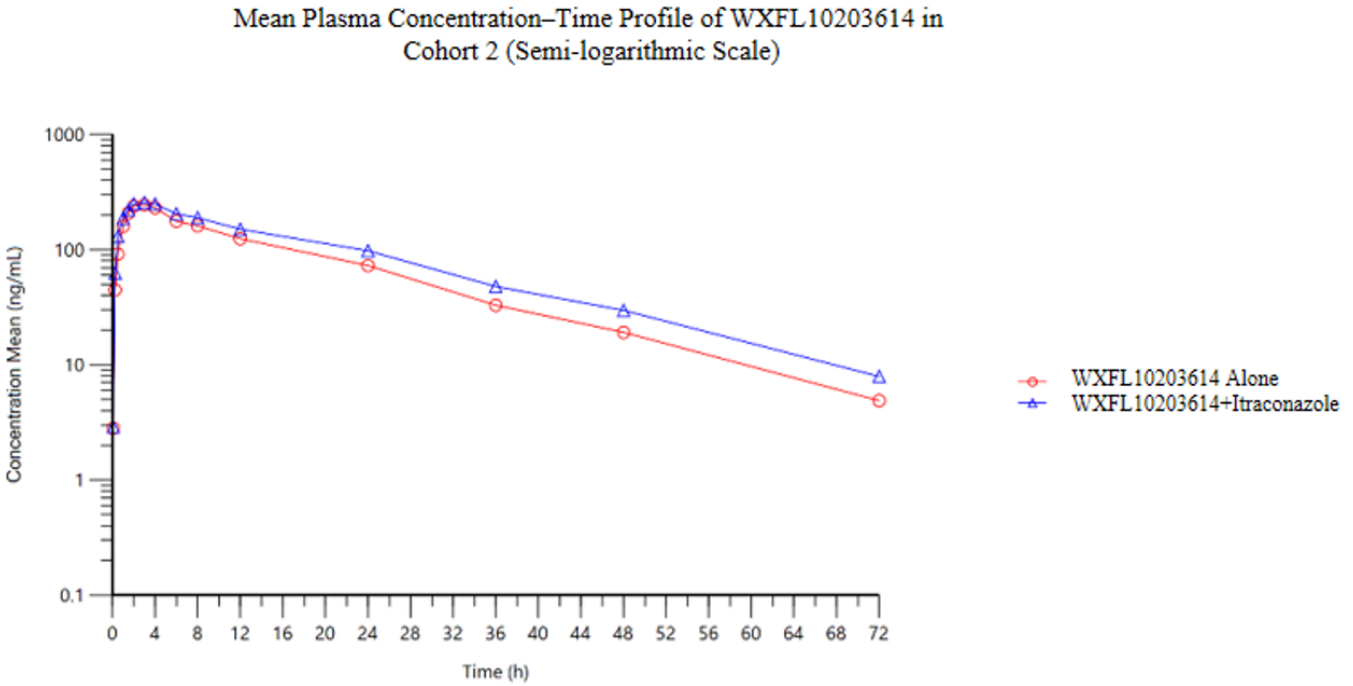


Figure 3

Mean plasma concentration–time profiles of WXFL10203614 following administration of WXFL10203614 alone and co-administration with itraconazole (semi-logarithmic scale).

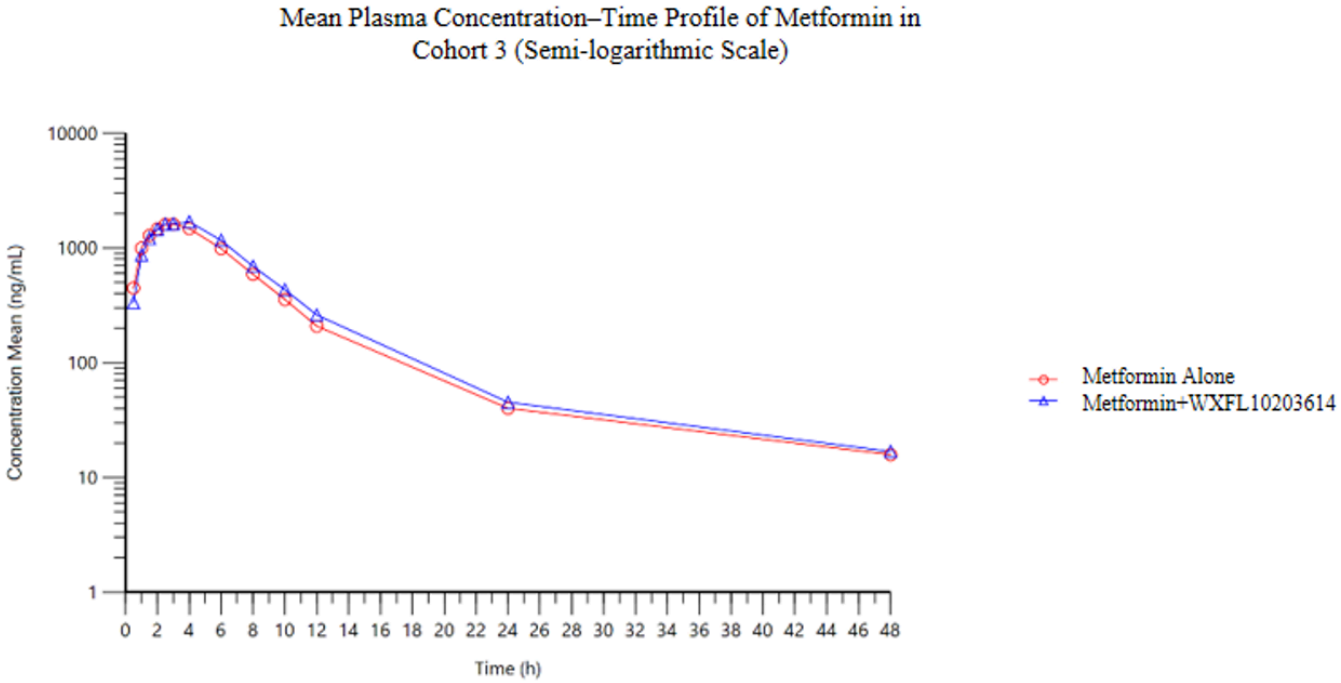


Figure 4

Mean plasma concentration–time profiles of metformin following administration of metformin alone and co-administration with steady-state WXFL10203614 (semi-logarithmic scale).