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Effect of itraconazole on the pharmacokinetics of faldaprevir in healthy subjects

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Faldaprevir (FDV), a substrate of CYP3A/P-glycoprotein (P-gp), is a selective inhibitor of the hepatitis C virus (HCV) NS3/4 protease. FDV is currently under clinical development for application in interferon-free treatment regimens for patients with chronic HCV infection. Understanding the drug-drug interaction potential of FDV is critical, as certain drug combinations may facilitate the more rapid achievement of steady-state—that is, the ideal drug concentration and balanced metabolic cycle of absorption and elimination that optimize drug efficacy. We thus conducted this study to investigate the effect of itraconazole (ICZ), a strong inhibitor of CYP3A and a moderate inhibitor of P-gp, on the pharmacokinetics (PK) of FDV. Eighteen healthy male and female volunteers participated in this open-label, fixed-sequence study. FDV 120 mg twice daily (BID) was administered on Day 1, followed by 120 mg once daily (QD) from Day 2 until the end of the 10-day study; after 6 days of FDV alone, ICZ 200 mg was added to FDV for an additional 4 days (BID on Day 7 and QD from Day 8 to Day 10). Intensive PK sampling was performed after 6 days of FDV treatment and again after 4 days of combined FDV/ICZ treatment. The adjusted geometric mean (gMean) ratios (%) of area under the concentration curve over dosing interval at steady-state ($AUC_{\tau,ss}$) and maximal concentration at steady-state ($C_{max,ss}$) for combined FDV/ICZ treatment vs. FDV treatment alone were 198.6% and 180.6%, respectively, with 90% confidence intervals (CIs) of 182.4–216.1 and 165.7–196.9. Administration of FDV alone or in combination with ICZ was observed to be safe and well-tolerated. Co-administration with ICZ, however, resulted in an approximately two-fold increase in FDV steady-state exposure. Furthermore, FDV required no dosage adjustment when co-administered with ICZ.

1. Introduction

Faldaprevir (FDV) is a second-generation protease inhibitor used in the treatment of hepatitis C virus infection and is currently being developed for use in an interferon-free regimen for the treatment of HCV infection (Trek Therapeutics 2017a, b). *In vitro*, FDV demonstrates good potency against HCV genotypes (GT) 1, 4, 5, and 6 (White et al. 2010). At a therapeutic dose of 120 mg once daily, in combination with pegylated interferon and ribavirin, FDV has shown high efficacy and good safety profiles in multiple large clinical studies of HCV-infected patients or HCV/HIV co-infected patients (Ferenci et al. 2015; Sulkowski et al. 2013a, b). In addition, proof-of-clinical-concept studies have shown that FDV can be used in interferon-free regimens for the treatment of HCV infection with good efficacy and safety (Zeuzem et al. 2015a,b).

FDV demonstrates nonlinear pharmacokinetics with greater than dose-proportional increases in exposure over the dose range of 4 to 1200 mg in healthy subjects (Sennewald et al. 2014). In HCV-infected patients, when the FDV dose was increased from 120 to 240 mg, exposure increased approximately 5–7 fold (Huang et al. 2016). Food has no clinically relevant effects on FDV absorption (Wu et al. 2016). The elimination half-life of FDV is approximately 20–30 hours (Manns et al. 2011; Sennewald et al. 2014), and therefore FDV is given once daily in the treatment of HCV infection.

FDV is a substrate of CYP 3A (Li et al. 2014). At the therapeutic dose of 120 mg QD, FDV is an inhibitor of CYP2C9 (Cooper et al. 2013; Sabo et al. 2014) and a weak inhibitor of CYP3A4, but has no effect on other CYPs (Sabo et al. 2014). *In vitro* data also suggest that FDV is a substrate of OATP 1B1, a substrate and inhibitor of P-gp, and an inhibitor of UDP-glucuronosyltransferase (UGT1A1) (Cooper et al. 2013; Sabo et al. 2014).

The primary objective of this study was to evaluate the effect of itraconazole, a strong inhibitor of CYP3A and an inhibitor of P-gp, on the pharmacokinetics of FDV.

2. Investigations and results

2.1 Subjects

A total of 18 healthy volunteer subjects (10 males and 8 females) were entered into the ICZ part of this trial and treated. The mean age of the subjects was 39.8 years, ranging from 23 to 50 years, and the mean BMI was 23.8 kg/m^2, ranging from 19.5 to 29.5 kg/m^2. All subjects were white (Table 1). Seventeen of the 18 healthy subjects received FDV alone followed by treatment with FDV + ICZ. One subject withdrew consent after FDV administration on Day 2 due to personal reasons.

Table 1: Demographic and baseline characteristics

Characteristics	Total treated population N=18
Gender, n (%)	
Male	10 (55.6)
Female	8 (44.4)
Race, n (%)	18 (100.0)
White	
Mean age, years (SD)	39.8 (6.8)
Mean weight, kg (SD)	71.8 (14.4)
Mean BMI, kg/m^2 (SD)	23.8 (2.5)

2.2 Pharmacokinetics

The mean plasma concentration-time profiles of FDV at steady state, given alone or with multiple doses of ICZ, indicate that peak concentrations of FDV were reached approximately 3 h after administration when FDV was given alone and at around 4 h after administration when FDV was given in combination with ICZ (Fig. 1).

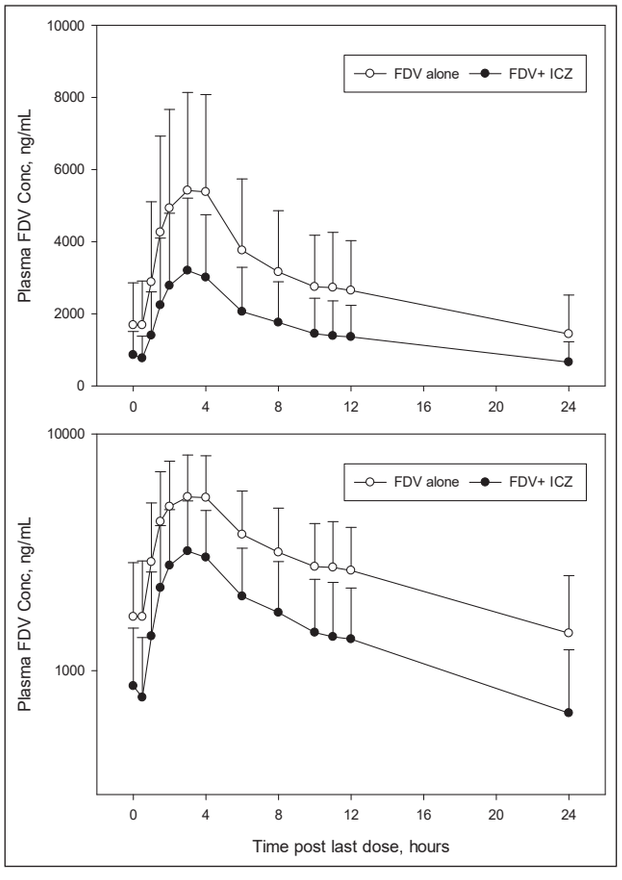

Fig. 1: Plasma FDV concentration–time profile at steady state after administration of FDV alone or in combination with ICZ (up panel linear scale, bottom panel semi-log scale). Note: FDV: faldaprevir; ICZ: itraconazole

Non-compartmental assessment of steady-state pharmacokinetic parameters indicate that exposure to FDV was higher after co-administration with ICZ than after administration of FDV alone. The inter-individual variability of the exposure parameters $AUC_{\tau,ss}$ and $C_{max,ss}$ was moderate, but slightly higher for the reference treatment than for the test treatment (with gCV values of approximately 60% and 50%, respectively) (Table 2, Fig. 2). The geometric mean ratios (%) of FDV+ ICZ vs. FDV alone were 198.6 (90%CI: 182.4 -216.1), 180.6 (90% CI: 165.7- 196.9), and 230.5, for $AUC_{\tau,ss}$ and $C_{max,ss}$, and $C_{24,ss}$ (plasma FDV concentration 24 hours after final dose), respectively. The oral clearance of FDV was reduced by approximately 50% (from 66.8 to 33.6, mL/min) after co-administration with ICZ (Table 2).

2.3 Safety

Adverse effects (AEs) reported during this study are summarized in Table 3. No deaths, other serious adverse events, protocol-specified significant adverse events, or 'other significant' adverse events (according to ICH E3) were reported in this trial. A total of 13 out of the 18 subjects (72.2%) reported at least one adverse event during the treatment periods ('FDV alone' and 'FDV + ICZ'). All adverse events were of mild or moderate intensity. The most frequently reported adverse events at the system organ class (SOC) level were 'nervous system disorders' (11 out of 18 subjects, 61.1%), followed by 'general disorders and administration site conditions' (6 out of 18 subjects, 33.3%). The most frequent adverse event by preferred term was headache (10 out of 18 subjects, 55.6%). Fatigue was reported by 6 out of 18 subjects (33.3%), while all other adverse events were reported by 2 subjects (11.1%) or less. The overall frequency of adverse events was higher during FDV alone than during FDV + ICZ. No clinically relevant finding was reported regarding safety laboratory measurements, ECG recordings, physical examinations, or vital sign measurements. A reversible increase in bilirubin was observed in most subjects: by the end-of-study visit, bilirubin values had returned to baseline levels. Bilirubin increases were predominantly unconjugated and not considered clinically relevant.

Table 3: Summary of adverse events (AEs)

N (%)	FDV alone	FDV + ICZ	Total treated
Number of subjects	18 (100.0)	17 (100.0)	18 (100.0)
Any AE	13 (72.2)	3 (17.6)	13 (72.2)
Drug-related AEs[a]	12 (66.7)	2 (11.8)	12 (66.7)
Serious AEs	0	0	0
Severe AEs	0	0	0
AEs leading to discontinuation	0	0	0
AEs by system organ class[b] Preferred term			
Nervous system disorders	11 (61.1)	0	11 (61.1)
Headache	10 (55.6)	0	10 (55.6)
Dizziness	2 (11.1)	0	2 (11.1)
General disorders and administration site conditions[c]	6 (33.3)	0	6 (33.3)
Gastrointestinal disorders	3 (16.7)	2 (11.8)	3 (16.7)
Nausea	2 (11.1)	1 (5.9)	2 (11.1)
Infections and infestations[d]	2 (11.1)	0	2 (11.1)

[a]Investigator assessed the possible causal relationship between an AE and study medication. [b]AEs reported by ≥2 subjects; more than one AE can occur in a single subject. [c]All fatigue. [d]Both nasopharyngitis.
AE, adverse event; FDV, faldaprevir; ICZ, itraconazole.

3. Discussion

A drug that is a CYP3A4/5 and P-gp substrate need to be tested with a strong inhibitor of CYP 3A and an inhibitor of P-gp in order to assess the maximal inhibitory effects of CYP 3A and P-gp and to obtain the safety margin under the influence of strong inhibitors for clinical practice. This is particularly important for a drug treating HCV and/or HCV/HIV co-infection, as a large number of agents used to treat these infections are strong inhibitors of CYP 3A and combination treatment is common. Thus, the primary objective of this study was to evaluate effect of steady-state ICZ on the steady-state pharmacokinetics of FDV.

The trial was performed as an open-label study throughout because the treatments were distinguishable from one another. Furthermore, the primary endpoint of the trial was derived from measurements of FDV plasma concentrations, making the potential for bias low.

To study relative bioavailability, the crossover design is considered favourable because each subject serves as his or her own control;

Table 2: Comparison of gMean (gCV%) steady-state pharmacokinetics parameters of FDV after oral administration of FDV alone and in combination with ICZ

PK parameters	FDV Alone (N=17)	FDV + ICZ (N=17)	GMR (%)[a]	90 % CI (%)
AUCτ,ss (h•ng/mL)	29900 (62.8)	59500 (53.8)	198.6	182.4 - 216.1
Cmax,ss (ng/mL)	2780 (61.0)	5030 (49.1)	180.6	165.7 - 196.9
C24,ss (ng/mL)	512 (78.5)	1180 (66.8)	230.5[d]	NE[b]
tmax,ss (h)[c]	3.00 (2.98-4.02)	3.98 (2.98-4.02)	NE	NE
CL/F,ss (mL/min)	66.8 (62.8)	33.6 (53.8)	NE	NE

Values in the table are expressed as geometric mean, gMean (percent geometric coefficient, gCV%), except GMR, 90%CI, and $t_{max,ss}$. GMR and 90% CI were estimated by ANOVA model; [a]: Geometric mean ratio of FDV + ICZ vs. FDV alone; [b]: not estimated; [c]: median value (range); [d]: estimated by gMean of FDV+ICZ/gMean of FDV alone; $AUC_{\tau,ss}$: area under the plasma concentration–time curve over a uniform dosing interval at steady-state; $C_{max,ss}$: steady-state peak concentration; $C_{24,ss}$: steady-state concentration 24 hours post last dose; $t_{max,ss:}$ the time when $C_{max,ss}$ occurred; CL/F_{ss}: oral clearance at steady-state; FDV: faldaprevir; ICZ: itraconazole.

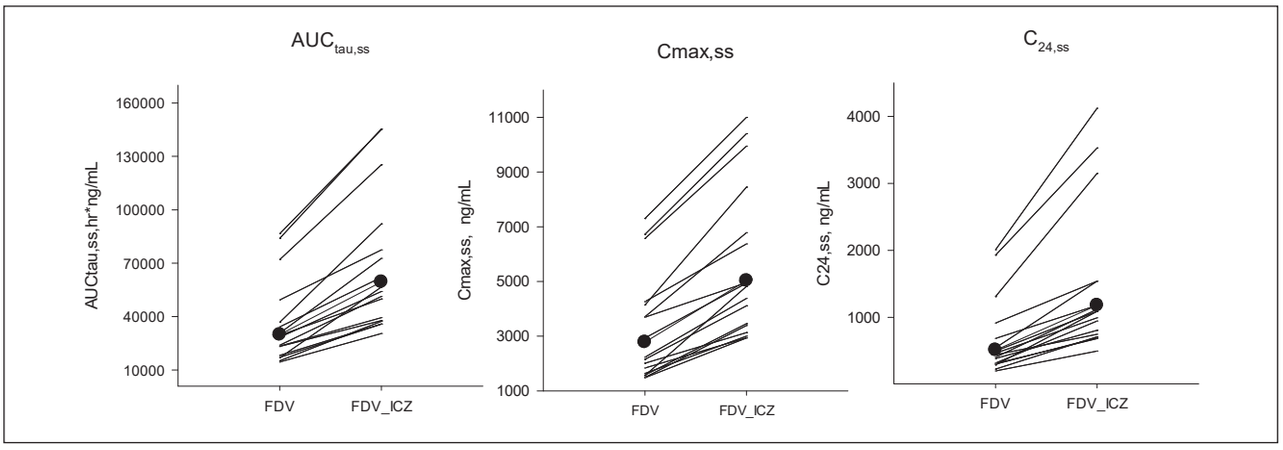

Fig. 2: Individual and gMean values of FDV $AUC_{\tau,ss}$, $C_{max,ss,}$ and $C_{24,ss}$ when FDV was administered alone and in combination with ICZ. Note: line represents individual values, dot represents geometric mean values; FDV: faldaprevir; ICZ: itraconazole.

as a result, inter-subject variability on the effect is removed. This study used a fixed sequence of the two treatments ('FDV' and 'FDV+ICZ') in order to avoid a lengthy washout time (Ke et al. 2014), given that ICZ and its metabolites, as well as FDV, have long half-lives. Per FDA guidance (US Food and Drug Administration 2012), DDI studies need to mimic the clinical situation. For a drug like FDV, which is used for the treatment of chronic HCV infection, an interaction study with a strong CYP3A inhibitor performed at the steady state is a reasonable design. In addition, using this design, the $AUC_{\tau,ss}$ was measured as the primary end point, as opposed to $AUC_{0,\infty}$ (which would have been used if a single dose of substrate (FDV) had been given), thus avoiding the need to use additional doses of ICZ (up to 5 half-life of FDV) during the FDV elimination phase to maintain sufficient CYP 3A inhibition (Liu et al. 2016). This design therefore reduced exposure to ICZ. Although ICZ is a relatively safe drug, as compared to ketoconazole (ICZ is generally considered to be quite safe for exposure ≤ 14 days), it still confers a slight risk of liver toxicity and QTc prolongation in healthy subjects with prolonged exposure, thus, a study design with ICZ exposure time < 7 days is considered preferable (Liu et al. 2016). In this study, a light meal was given before dosing of ICZ and FDV to enhance the bioavailability of the ICZ capsules (Liu et al. 2016). As the study was performed in a cross-over manner, and because food does not have a clinically relevant impact on FDV pharmacokinetics (Wu et al. 2016), food was not expected to exert bias on the evaluation of FDV bioavailability.

After 6 days of 120 mg QD (with a loading dose of 120 BID), steady-state exposure to FDV is expected (Elgadi et al. 2014). After the fourth day of ICZ administration (Day 10 of the study, with the loading dose of ICZ administered on Day 7), the geometric mean plasma ICZ concentration 1.5 h after the final dose was 458 ng/mL (57.6%). This is comparable to or exceeds the exposure reported in the literature following 3 days of pre-treatment with ICZ 100-200 mg/day (Hardin et al. 1988; Jalava et al. 1997a). Multiple DDI studies using 100-200 mg/day of ICZ with a 3-day lead-in time have demonstrated adequately strong CYP3A inhibition with this strategy (Liu et al. 2016).

FDV is a P-gp inhibitor and P-gp substrate at the therapeutic dose of 120 mg QD; drug concentrations in the GI tract (using a standard GI volume of 250 mL (US Food and Drug Administration 2012)) were calculated to be 0.55 mM. If a 10-fold margin is added (US Food and Drug Administration 2012), the estimated GI FDV concentration is 55 µM for the 120 mg dose. The Km value of P-gp was determined to be 11.9 µM using MDCK-MDR1 cell monolayers (Li et al. 2014). As such, FDV may already saturate intestinal P-gp at the therapeutic dose, and therefore, any inhibition of P-gp by other P-gp inhibitors is not likely to further increase FDV absorption. In addition, given ICZ is a not a strong systemic inhibitor of P-gp at the therapeutic dose (Jalava et al. 1997b), the total effect (intestinal + systemic) of ICZ on P-gp of FDV is likely to be limited.

A human ADME study revealed that FDV is metabolized slowly and to a limited extent (Chen et al. 2014). In the study, the most abundant fecal metabolites were two mono hydroxylated metabolites, M2a and M2b, which represented 22% and 20% of fecal radioactivity (22% and 19% of the dose, 41% in total), respectively. There were five additional very minor Phase I fecal metabolites (accounting for approximately 5.5% of the administered dose) (Chen et al. 2014). An *in vitro* study suggested that both M2a and M2b are formed in liver, with a significant contribution from CYP3A4 and a limited contribution from CYP3A5 (Li et al. 2014). If one assumes that the pathway mediated by CYP 3A to form M2a and M2b is completely blocked, the total exposure to FDV ($AUC_{\tau,ss}$) is expected to be increased approximately 1.7-fold based on the formula derived by Rowland and Matin (Rowland et al. 1973). Given that the pathways to form other metabolites of FDV might be also related to the metabolism of CYP3A (Chen et al. 2014), and due to the limited inhibitory effect of ICZ on systemic/intestinal P-gp activities, an additional slight increase in exposure other than inhibition of CYP 3A to generate M2a and

M2b metabolites is expected. Taken together, the approximately 2-fold increase in total exposure ($AUC_{\tau,ss}$) and the 1.80-fold increase in $C_{max,ss}$ seen in the current study approximately matches the estimated 1.7-fold increase expected based on assumption that the CYP3A activities were completely blocked and the limited increase in exposure resulting from P-gp inhibition. Thus, the findings seem to support the notion that CYP 3A activities were probably completely inhibited, or nearly so, by ICZ in the current study design. In the FDV clinical development program, a 1.76-fold increase in $AUC_{0\text{-}24,ss}$ was observed when FDA 120 mg was co-administered with atazanavir/ritonavir (300/100 mg), a moderate CYP3A inhibitor (US Food and Drug Administration 2012), in HCV/HIV co-infected patients (Nelson et al. 2014); in addition, a 1.8-fold increase in trough exposure was observed when darunavir/ritonavir (a moderate CYP3A inhibitor (US Food and Drug Administration 2012)) was co-administered with FDV 100 mg in HCV-HIV co-infected patients (Rockstroh et al. 2014). These data appear to be in a good agreement with the exposure increase observed in the present study.

After co-administration of ICZ, the variability was slightly reduced from 62.8% to 53.8% and from 61.0 to 49.1% for $AUC_{\tau,ss}$ and $C_{max,ss}$, respectively. This appears to be consistent with the fact that FDV's metabolism is mainly mediated by CYP 3A4, with a slight contribution of CYP 3A5 (Li et al. 2014). It has been reported that CYP 3A5 shows a polymorphism (Roy et al. 2005), leading to variability in exposure; after inhibition with a strong CYP 3A5 inhibitor, the variability of FDV's PK parameters related to exposure is expected to be reduced.

The 2-fold increase in FDV exposure over dosing with 120 mg is not considered clinically relevant, as in the FDV clinical development program, both 240 mg and 120 mg were tested in the Phase II/III program and were shown to have comparable safety profiles (Ferenci et al. 2015) (120 mg is the dose being selected as therapeutic dose for further development). Also the FDV exposure after 240 mg was 5-fold (based on trough concentration) (Huang et al. 2016) to 7-fold (based on $AUC_{0\text{-}24,ss}$, Boehringer Ingelheim data on file) higher than after 120 mg in HCV infected patients. The merely 2-fold increase in exposure observed in this study would be well within the safety margin observed in the large FDV Phase II/III trials, and, therefore dose adjustment is not required when FDV is co-administered with ICZ. However, we should recognize that FDV is currently being developed into an all-oral combination for the treatment of HCV infection, the effects of ICZ on other components of the combination may also need to be evaluated.

As an important component in the treatment of HIV/HCV co-infection, NS3/4A protease inhibitors are expected to be co-administered with antivirals such as protease inhibitors. Due to potential drug-drug interaction resulting from inhibition of CYP 3A pathways, many of the licensed NS3/4A protease inhibitors are not recommended (AbbVie 2014; Janssen 2017) or are contraindicated (Merck 2016) for co-administration with ritonavir-boosted protease inhibitors. Even for the most recently licensed all-oral agents, the issue remains. For example, co-administration of atazanavir, darunavir, and lopinavir with MAVYRET is not recommended due to a marked increase in exposure to glecaprevir (AbbVie 2017); and co-administration of VOSEVI with atazanavir or lopinavir is not recommended due to a significantly increased exposure to voxilaprevir (Gilead 2017). Compared to these aforementioned licensed agents, and given that no clinically relevant interactions with protease inhibitors or other antivirals with strong CYP 3A inhibition potential were observed or are expected, FDV demonstrates a clear advantage in the treatment of patients with HIV/HCV co-infection.

All AEs observed in this study were of mild or moderate intensity. Even though the exposure to FDV was approximately 2-fold higher after co-administration with ICZ, the overall frequency of adverse events was higher during treatment with FDV alone in 13 out of 18 subjects (72.2%) than during FDV+ICZ (3 out of 17 subjects; 7.6%). This suggests that the higher incidence of adverse events during treatment with FDV alone may be not exposure driven. However, as no placebo group was included in this trial, no firm conclusions can be made. The observed reversible increases in unconjugated bilirubin were not considered clinically relevant, as this is a known effect of FDV treatment. Overall, administration of FDV alone or in combination with ICZ was generally safe and well tolerated by the healthy male and female subjects in this trial. In summary, administration of FDV alone or in combination with ICZ was generally safe and well-tolerated. Co-administration with ICZ resulted in an approximately 2-fold increase in FDV steady-state exposure. This interaction is considered clinically irrelevant and no dose adjustment is required when FDV is co-administered with ICZ.

4. Experimental

4.1 Subjects

Healthy male and female subjects aged 18 to 50 years old with a body mass index of 18.5 to 29.9 kg/m^2 were eligible for enrollment in the study. All subjects provided written informed consent prior to participation. Exclusion criteria included any finding in the medical examination of gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological or hormonal disorders; past surgery of the gastrointestinal tract that could have interfered with the kinetics of the study drug; relevant chronic or acute infection; currently active diseases requiring medical treatment; or current drug or alcohol abuse. Use of any drugs that could influence the results, such as nutraceuticals and herbal remedies, was restricted for 7 days prior to the administration of study drug, as were prior use of long half–life (>24 hours) drugs (within 1 month), use of any investigational drug (within 60 days), excessive physical activities (within 7 days), and blood donation (within 1 month). Concomitant administration of oral contraceptives or any food product known to alter CYP P450 enzymes or P-gp activity was prohibited.

4.2. Study design

This was a Phase I, open-label, fixed-sequence study of 18 healthy male and female volunteers to evaluate the effect of ICZ 200 mg once-daily (QD) on the PK of FDV (Fig. 3).

Faldaprevir 120 mg QD, with 120 mg BID loading dose on Day 1, was administered orally for the entire study duration. ICZ 200 mg QD capsules (Sempera, Janssen-Cilag GmbH, Neuss, Germany, 100 mg, unit strength), after a 200 mg BID loading dose on Day 7, was added to FDV from Day 8 onwards. ICZ and FDV were given 30 minutes after breakfast or dinner. Intensive PK sampling was performed on Day 6 (for FDV alone) on Day 10 (for FDV+ICZ) (Fig. 3). This trial was conducted at the Human Pharmacology Centre (HPC) of Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany, from February 21 to May 27, 2013, in accordance with the International Conference on Harmonization guideline for Good Clinical Practice and the principles of the Declaration of Helsinki. Before study initiation, the clinical trial protocol, the subject information, and the informed consent form were reviewed by the responsible local Independent Ethics Committee (Landesärztekammer Rheinland-Pfalz, Mainz, Germany). The clinical trial application was also reviewed by the German Competent Authority (BfArM, Bonn, Germany).

4.3. Blood sampling

Intensive PK samples were taken at predose (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 hours after FDV administration. Additional trough plasma samples (pre-dose) for FDV/ICZ or post dose samples (ICZ) were taken as indicated in Fig. 3. At each time point, 2.7 mL blood were taken from a forearm vein in a tripotassium ethylenediaminetetraacetic acid (K3-EDTA) blood drawing tube. Centrifugation was carried out within 60 minutes after blood sampling at 2,000 to 4,000 x g for 10 min at 4 – 8°C. Two aliquots of plasma samples were stored in individually labeled polypropylene tubes at -20°C for analysis.

4.4. Bioanalytical methods

A validated high-pressure liquid chromatography–tandem mass spectrometry method was used to quantify concentrations of FDV in EDTA plasma (Huang et al. 2015). The calibration range was from 10.0 to 10,000 ng/mL for FDV, the assay accuracy (deviation %) was in the range of -1.0 to 2.3% and the assay precision (CV %) was in the range of 0.6 -12.5%.

A validated high–pressure liquid chromatography–tandem mass spectrometry method was used to quantify concentrations of ICZ and 2-hydroxy-itroconazole in EDTA plasma. The calibration range was from 0.5 to 500 ng/mL for ICZ, the assay accuracy (deviation %) was < 4% and the assay precision (CV %) was < 2%. The calibration range was from 0.5 to 500 ng/mL for 2-hydroxy-itraconazole, the assay accuracy (deviation %) was < 7% and the assay precision (CV %) was ≤ 3%.

4.5. Safety assessments

The investigator evaluated the general health of the subjects for participation in the trial by carrying out medical evaluations throughout the trial. In addition to obtaining a medical history and performing a physical examination, the evaluation included a review of the informed consent, demographics, inclusion/exclusion criteria, vital signs (blood pressure and pulse rate), 12-lead ECG and safety laboratory tests. Adverse

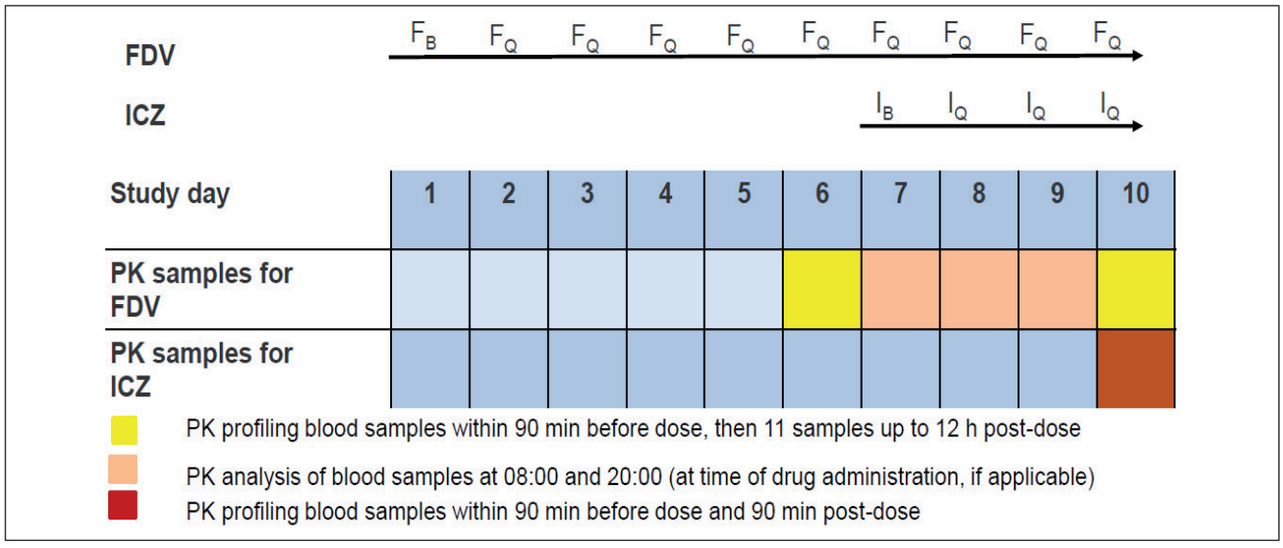

Fig. 3: Study design. Note: FDV, faldaprevir; FB, FDV 120 mg BID (loading dose); FQ, FDV 120 mg QD; ICZ, itraconazole; IB, ICZ 200 mg BID (loading dose); IQ, ICZ 200 mg QD; PK, pharmacokinetic

events (AEs) were assessed throughout the study. All AEs and serious adverse events (SAEs) persisting at the end of the study were followed until clinical resolution, return to the subject's baseline or until follow-up was deemed sufficient by the investigator.

4.6 Pharmacokinetic analysis

PK analyses of FDV in plasma were conducted by noncompartmental techniques using WinNonlin software version 5.2 (Pharsight, Mountain View, CA, USA). The details of these methods have been described previously (Joseph et al. 2015). Briefly, actual sampling times were used for all calculations except for pre-dose times, which were set to zero. The area under the plasma concentration–time curve over a uniform dosing interval at steady-state ($AUC_{\tau,ss}$) was calculated using the trapezoidal rule, with the linear up/log down algorithm. Steady-state peak concentrations ($C_{max,ss}$) and plasma concentration 24 hours post last dose at steady-steady ($C_{24,ss}$) were determined directly from the reported data. $T_{max,ss}$ is the time when $C_{max,ss}$ occurred. Oral clearance at steady-state (CL/F_{ss}) was calculated as dose/ $AUC_{\tau,ss}$. Summary statistics were reported for all parameters.

4.7. Statistical analysis

The effect of ICZ on the relative bioavailability of FDV was determined on the basis of the PK parameters $AUC_{\tau,ss}$, $C_{max,ss}$, of FDV. The statistical model used for the analysis of relative bioavailability was an analysis of variance (ANOVA) model on the logarithmic scale. Point estimated of bioavailability, the ratios of the geometric means (gMeans) and 2-sided 90% confidence intervals (CIs) were calculated.

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References

AbbVie (2014) VIEKIRA PAK (ombitasvir, paritaprevir, and ritonavir tablets; dasabuvir tablets) [package insert], AbbVie Inc., North Chicago, IL. .
AbbVie (2017) MAVYRET (glecaprevir and pibrentasvir) tablets [package insert], AbbVie Inc., North Chicago, IL
Chen LZ, Rose P, Mao Y, Yong CL, St George R, Huang F, Latli B, Mandarino D, Li Y (2014) Mass balance and metabolite profiling of steady-state faldaprevir, a hepatitis C virus NS3/4 protease inhibitor, in healthy male subjects. Antimicrob Agents Chemother 58: 2369–2376.
Cooper C, Conway B, Ghesquiere W, R L, A R, Chandoik N, E T, Sane R, Ting N, Mensa F, Elgadi M, Sabo JP (2013) Pharmacokinetic interactions of faldaprevir and deleobuvir (BI 207127) and their individual and combined effects on selected cytochrome p450 (CYP) probe substrates in genotype 1 hepatitis C infected patients. Hepatology 58: 730A-760A (Poster 1083).
Elgadi M, Yong CL, Wruck J, Cooper C, Huang F, Stern JO (2014) Pharmacokinetics of faldaprevir following mutiple oral risisng doses in healthy volunteers and subjescts with gilbert's syndrom. 2014 American Society for Clinical Pharmacology and Therapeutics (ASCPT) Annual Meeting, March 18-22, 2014, Atlanta, GA. Poster # PII-022.
Ferenci P, Asselah T, Foster GR, Zeuzem S, Sarrazin C, Moreno C, Ouzan D, Maevskaya M, Calinas F, Morano LE, Crespo J, Dufour JF, Bourliere M, Agarwal K, Forton D, Schuchmann M, Zehnter E, Nishiguchi S, Omata M, Kukolj G, Datsenko Y, Garcia M, Scherer J, Quinson AM, Stern JO, Group STS (2015) STARTVerso1: A randomized trial of faldaprevir plus pegylated interferon/ribavirin for chronic HCV genotype-1 infection. Journal of hepatology 62: 1246–1255.
Gilead (2017) VOSEVI (sofosbuvir, velpatasvir, and voxilaprevir) tablets, [package insert], Gilead Sciences, Inc. Foster City, CA
Hardin TC, Graybill JR, Fetchick R, Woestenborghs R, Rinaldi MG, Kuhn JG. (1988). Pharmacokinetics of itraconazole following oral administration to normal volunteers. Antimicrob Agents Chemother 32: 1310–1313.
Huang F, Haertter S, Quinson AM (2016) Effect of intrinsic factors on the exposure of faldaprevir in HCV-infected patients: pooled analysis of data from three faldaprevir phase III studies Paper presented at the 2016 American College of Clinical Pharmacology (ACCP) Annual Meeting, Washington DC.
Huang F, Moschetti V, Lang B, Halabi A, Petersen-Sylla M, Yong CL, Elgadi M (2015) Pharmacokinetics, safety, and tolerability of faldaprevir in patients with renal impairment. Antimicrob Agents Chemother 59: 251–257.
Jalava KM, Olkkola KT, Neuvonen PJ. (1997a) Itraconazole greatly increases plasma concentrations and effects of felodipine. Clin Pharmacol Ther 61: 410–415.
Jalava KM, Partanen J, Neuvonen PJ (1997b) Itraconazole decreases renal clearance of digoxin. Ther Drug Monit 19: 609–613.
Janssen (2017) OLYSIO [package insert], Titusville NJ: Janssen Therapeutics, Division of Janssen Products, LP.
Joseph D, Schobelock MJ, Riesenberg RR, Vince BD, Webster LR, Adeniji A, Elgadi M, Huang F (2015) Effect of steady-state faldaprevir on the pharmacokinetics of steady-state methadone and buprenorphine-naloxone in subjects receiving stable addiction management therapy. Antimicrob Agents Chemother 59: 498–504.
Ke AB, Zamek-Gliszczynski MJ, Higgins JW, Hall SD (2014) Itraconazole and clarithromycin as ketoconazole alternatives for clinical CYP3A inhibition studies. Clin Pharmacol Ther 95: 473–476.
Li Y, Zhou J, Ramsden D, Taub ME, O'Brien D, Xu J, Busacca CA, Gonnella N, Tweedie DJ. (2014) Enzyme-transporter interplay in the formation and clearance of abundant metabolites of faldaprevir found in excreta but not in circulation. Drug Metabol Dispos 42: 384–393.
Liu L, Bello A, Dresser MJ, Heald D, Komjathy SF, O'Mara E, Rogge M, Stoch SA, Robertson SM (2016) Best practices for the use of itraconazole as a replacement for ketoconazole in drug-drug interaction studies. J Clin Pharmacol 56: 143–151.
Manns MP, Bourliere M, Benhamou Y, Pol S, Bonacini M, Trepo C, Wright D, Berg T, Calleja JL, White PW, Stern JO, Steinmann G, Yong CL, Kukolj G, Scherer J, Boecher WO. (2011) Potency, safety, and pharmacokinetics of the NS3/4A protease inhibitor BI201335 in patients with chronic HCV genotype-1 infection. J Hepatol 54: 1114–1122.
Merck (2016) ZEPATIER (elbasvir and grazoprevir) [package insert], Whitehouse Station NJ: Merck & Co. Inc.
Nelson M, Arasteh K, Jain MK, Soriano V, Madruga J, Furtado J, Battegay M, Huang F, Manero M, Dieterich D (2014) Effect of faldaprevir on atazanavir pharmacokinetics in patients with HIV/HCV Co-infection. 2014 Conference On Retroviruses And Opportunistic Infections (CROI), Boston MA. 3-6 March 2014.
Rockstroh JK, Valantin MA, Mallolas J, Puoti M, Pineda JA, Ingiliz P, Nunez M, Huang F, Vinisko R, Dieterich DT (2014) Pharmacokinetics of faldaprevir and antiretrovirals in patients with HIV/HCV Co-infection. Paper presented at the 2014 Conference On Retroviruses And Opportunistic Infections (CROI), Boston, MA.
Rowland M, Martin SB (1973) Kinetics of drug-drug interactions. J Pharmacokinet Biopharm 1: 553-567.
Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ, Roger M (2005) CYP3A5 genetic polymorphisms in different ethnic populations. Drug Matabol Disposi 33: 884–887.
Sabo JP, Kort J, Ballow C, Haschke M, Battegay M, Fuhr R, Girlich B, Schobelock M, Feifel U, Lang B, Li Y, Elgadi M (2014) Clinical assessment of potential drug

interactions of faldaprevir, a hepatitis C virus protease inhibitor, with darunavir/ritonavir, efavirenz, and tenofovir. Clin Infect Dis 59: 1420–1428.

Sabo JP, Kort J, Ballow C, Kashuba AD, Haschke M, Battegay M, Girlich B, Ting N, Lang B, Zhang W, Cooper C, O'Brien D, Seibert E, Chan TS, Tweedie D, Li Y (2015) Interactions of the hepatitis C virus protease inhibitor faldaprevir with cytochrome P450 enzymes: in vitro and in vivo correlation. J Clin Pharmacol 55: 467–477.

Sennewald R, Narjes N, Yong CL, Nehmiz N, Huang F, Stern JO (2014) Safety, tolerbility, and pharmacokinetics of faldaprevir after single rising doses in healthy subjects. 2014 American Society for Clinical Pharmacology and Therapeutics (ASCPT) Annual Meeting, March 18-22, 2014, Atlanta, GA. Poster # PII-025.

Sulkowski MS, Asselah T, Lalezari J, Ferenci P, Fainboim H, Leggett B, Bessone F, Mauss S, Heo J, Datsenko Y, Stern JO, Kukolj G, Scherer J, Nehmiz G, Steinmann GG, Bocher WO (2013a) Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naive patients with chronic genotype 1 HCV: SILEN-C1 trial. Hepatology 57: 2143–2154.

Sulkowski MS, Bourliere M, Bronowicki JP, Asselah T, Pawlotsky JM, Shafran SD, Pol S, Mauss S, Larrey D, Datsenko Y, Stern JO, Kukolj G, Scherer J, Nehmiz G, Steinmann GG, Bocher WO (2013b) Faldaprevir combined with peginterferon alfa-2a and ribavirin in chronic hepatitis C virus genotype-1 patients with prior nonresponse: SILEN-C2 trial. Hepatology 57: 2155–2163.

Trek Therapeutics (2017a) A Study of Faldaprevir, Ribavirin and TD-6450 in Participants With Genotype 4 Hepatitis C Virus Infection. Retrieved from http://clinicaltrials.gov/show/NCT02593162

Trek Therapeutics (2017b) A Study of Faldaprevir, TD-6450 and Other Antivirals in Participants With Genotype 1b Hepatitis C Virus Infection. Retrieved from http://clinicaltrials.gov/show/NCT02716428

US Food and Drug Administration (2012) Guidance for industry: drug interaction studies—study design, data, analysis, implications for dosing, and labeling recommendations. .

White PW, Llinas-Brunet M, Amad M, Bethell RC, Bolger G, Cordingley MG, Duan J, Garneau M, Lagace L, Thibeault D, Kukolj G (2010) Preclinical characterization of BI 201335, a C-terminal carboxylic acid inhibitor of the hepatitis C virus NS3-NS4A protease. Antimicrob Agents Chemother 54: 4611–4618.

Wu J, Giessmann T, Lang B, Elgadi M, Huang F (2016) Investigation of the effect of food and omeprazole on the relative bioavailability of a single oral dose of 240 mg faldaprevir, a selective inhibitor of HCV NS3/4 protease, in an open-label, randomized, three-way cross-over trial in healthy participants. J Pharm Pharmacol 68: 459–466.

Zeuzem S, Dufour JF, Buti M, Soriano V, Buynak RJ, Mantry P, Taunk J, Stern JO, Vinisko R, Gallivan JP, Bocher W, Mensa FJ (2015a) Interferon-free treatment of chronic hepatitis C with faldaprevir, deleobuvir and ribavirin: SOUND-C3, a Phase 2b study. Liver Int 35: 417–421.

Zeuzem S, Soriano V, Asselah T, Gane EJ, Bronowicki JP, Angus P, Lohse AW, Stickel F, Mullhaupt B, Roberts S, Schuchmann M, Manns M, Bourliere M, Buti M, Stern JO, Gallivan JP, Voss F, Sabo JP, Bocher W, Mensa FJ (2015b). Efficacy and safety of faldaprevir, deleobuvir, and ribavirin in treatment-naive patients with chronic hepatitis C virus infection and advanced liver fibrosis or cirrhosis. Antimicrob Agents Chemother 59: 1282–1291.