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A Study of the Effect of Cyclosporine on Fevipiprant Pharmacokinetics and its Absolute Bioavailability Using an Intravenous Microdose Approach[S]

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ABSTRACT

This drug-drug interaction study determined the effect of cyclosporine, an inhibitor of organic anion transporting polypeptide (OATP) 1B3 and P-gp, on the pharmacokinetics (PK) of fevipiprant, an oral, highly selective, competitive antagonist of the prostaglandin D_2 receptor 2 and a substrate of the two transporters. The concomitant administration of an intravenous microdose of stable isotope-labeled fevipiprant provided the absolute bioavailability of fevipiprant as well as mechanistic insights into its PK and sensitivity to drug interactions. Liquid chromatography–mass spectrometry/mass spectrometry was used to measure plasma and urine concentrations. Geometric mean ratios [90% confidence interval (CI)] for oral fevipiprant with or without cyclosporine were 3.02 (2.38, 3.82) for C_{max}, 2.50 (2.17, 2.88) for AUC_{last}, and 2.35 (1.99, 2.77) for AUC_{inf}. The geometric mean ratios (90% CI) for fevipiprant intravenous microdose with or without cyclosporine were 1.04 (0.86, 1.25) for C_{max}, 2.04 (1.83, 2.28) for AUC_{last}, and 1.95 (1.76, 2.16) for AUC_{inf}. The absolute bioavailability for fevipiprant was approximately 0.3 to 0.4 in the absence and 0.5 in the presence of cyclosporine. The intravenous microdose allowed differentiation between systemic and presystemic effects of cyclosporine on fevipiprant, demonstrating a small (approximately 1.2-fold) presystemic effect of cyclosporine and a larger (approximately twofold) effect on systemic elimination of fevipiprant. Uptake by OATP1B3 appears to be the rate-limiting step in the hepatic elimination of fevipiprant, whereas P-gp does not have a relevant effect on oral absorption.

SIGNIFICANCE STATEMENT

The drug interaction investigated here with cyclosporine, an inhibitor of several drug transporters, provides a refined quantitative understanding of the role of active transport processes in liver and intestine for the absorption and elimination of fevipiprant as well as the basis to assess the need for dose adjustment in the presence of transporter inhibitors. The applied intravenous microdose approach presents a strategy to maximize learnings from a trial, limit the number and duration of clinical trials, and enhance mechanistic drug-drug interaction understanding.

Introduction

Fevipiprant (QAW039) is an oral, competitive antagonist of the prostaglandin D_2 receptor 2 (previously called chemoattractant receptor-homologous molecule expressed on type 2 helper T cells) that dissociates slowly from this receptor (Sykes et al., 2016). In phase II trials, fevipiprant significantly decreased sputum eosinophil counts and reduced airway smooth muscle mass in patients with asthma (Erpenbeck et al., 2016a; Gonem et al., 2016; Bateman et al., 2017; Saunders et al., 2019), but phase III results (ClinicalTrials.gov numbers NCT02555683, NCT02563067, NCT03215758, NCT03226392) did not support submission in this indication.

One aspect of drug development is to build a detailed understanding of the processes underlying the pharmacokinetics (PK) of the drug to determine if, and to what extent, co-medications or other factors can affect its PK. In this context, regulatory authorities encourage obtaining intravenous PK data of drugs in development (European Medicines Agency, 1987). PK parameters requiring intravenous data (such as absolute bioavailability and systemic clearance) can increase the predictability of physiologically based modeling of PK and are generally important for any quantitative estimation of exposure change with, for example, change in formulation, age, partial inhibition of a clearance pathway by DDIs, or organ impairment.

Renal clearance and hepatic elimination via glucuronidation and/or biliary secretion contribute to the elimination of fevipiprant; intravenous PK data can help to estimate the contributions of these clearance pathways more quantitatively. In a human absorption, distribution, metabolism, and excretion (ADME) study of fevipiprant, the fraction

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ABBREVIATIONS: ADME, absorption, distribution, metabolism and excretion; AE, adverse events; AG, acyl-glucuronide; ALT, alanine aminotransferase; AUC_{inf}, area under the plasma concentration–time curve from time zero to infinity; AUC_{last}, area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration; CI, confidence interval; CL, clearance; CLr, renal clearance; ECG, electrocardiogram; F, absolute bioavailability; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LLOQ, lower limits of quantification; m/z, mass to charge ratio; OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein; PK, pharmacokinetics; $t_{1/2}$, terminal half-life; T_{max}, time to reach peak or maximum concentration following drug administration; UGT, uridine 5′-diphospho-glucuronosyltransferase; V, volume of distribution; Vz/F, the apparent volume of distribution during the terminal phase following extravascular administration.

of the oral dose absorbed from the intestine was estimated to be at least 43.5% (42.1% of the total radioactive dose recovered from urine and 1.4% as metabolites in feces) (Pearson et al., 2017). However, the absolute bioavailability, i.e., the fraction of the oral dose reaching the systemic circulation unchanged, could be higher or lower than this minimal estimate of absorption. In vitro data indicate that fevipiprant is taken up via organic anion transporting polypeptide (OATP) 1B3 into the liver, followed by formation of an acyl glucuronide (AG) metabolite by several uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes. Of note, the AG metabolite is the only major circulating metabolite of fevipiprant and is not pharmacologically active (Pearson et al., 2017). Organic anion transporter 3 is responsible for the active renal excretion of fevipiprant (Pearson et al., 2017). Because fevipiprant is a substrate of P-glycoprotein (P-gp, or multidrug resistance protein 1) and UGT enzymes, intestinal efflux and metabolism could have an impact on its absorption and first-pass metabolism, respectively (Pearson et al., 2017). In addition, the hepatic first-pass extraction by OATP1B3-mediated uptake may influence the bioavailability of fevipiprant.

The aim of this study was to determine the effect of oral cyclosporine, an inhibitor of OATP1B3 (Shitara et al., 2012) and P-gp (Kovarik and Koelle, 1999), on the PK of fevipiprant and also to increase the overall understanding of fevipiprant PK. Cyclosporine has no inhibitory effects on the other elimination pathways of fevipiprant, that is, UGTs [no inhibition was reported at time of survey in the Metabolism and Transport Drug Interaction Database (https://sop.washington.edu/department-of-pharmaceutics/research/drug-interaction-database/)] and organic anion transporter 3 (El-Sheikh et al., 2013). An intravenous microdose of stable (i.e., nonradioactive) isotope-labeled fevipiprant ([$^{13}C_2^{15}N_2$]fevipiprant) (Fig. 1) was coadministered with unlabeled oral fevipiprant to determine its absolute bioavailability and systemic clearance, both in the absence and presence of cyclosporine. Labeled and unlabeled fevipiprant are identical biologically, i.e., their interaction with, for example, the target or drug transporters is not expected to differ, but they can be distinguished analytically by mass spectrometry. This allows intravenous and oral data to be obtained on the same drug, from the same study participant, at the same time without the risk of a bias of the intravenous PK data because of the microdose in case of PK nonlinearity (Lappin et al., 2006). In the context of the DDI assessment, this allowed determination of systemic (from fevipiprant intravenous administration) as well as "presystemic plus systemic" (from fevipiprant oral administration) effects of cyclosporine, adding mechanistic granularity.

Materials and Methods

Study Objectives. The primary objective was to determine the effect of cyclosporine on the following three key PK measures of orally administered fevipiprant in healthy volunteers: 1) the area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration (AUC_{last}); 2) the area under the plasma concentration–time curve from time zero to infinity (AUC_{inf}); and 3) the maximum plasma concentration (C_{max}).

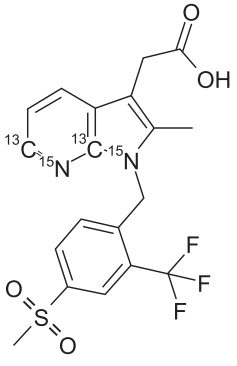

Fig. 1. Structure of fevipiprant showing the positions of the stable isotope-labeling ($^{13}C_2^{15}N_2$) used for the intravenous microdosing.

The secondary objectives were to determine 1) the absolute bioavailability (F) and the absolute disposition parameters of fevipiprant, i.e., clearance (CL) and volume (V) of distribution, by administering an intravenous microdose of stable isotope-labeled fevipiprant concomitantly with the oral dose; 2) the effect of cyclosporine on the PK of the intravenous microdose of fevipiprant (V, CL, AUC_{last}, and AUC_{inf}); 3) the safety and tolerability of fevipiprant administered both orally and intravenously, with and without coadministration of cyclosporine; and 4) the effect of cyclosporine on the PK of the major AG metabolite of oral fevipiprant. The study protocol was approved by the Institutional Review Board for the study center, and the study was conducted according to the ethical principles of the Declaration of Helsinki.

Study Design. This was an open-label, single-sequence, two-period, crossover study with two treatment periods separated by a washout period of 7–14 days (Fig. 2). The study was carried out at IQVIA (formerly QuintilesIMS) (Overland Park, KS). The investigational drugs, fevipiprant 150 mg film-coated tablets and fevipiprant 100 μg labeled intravenous microdose ([$^{13}C_2^{15}N_2$]fevipiprant) (Fig. 1), were prepared and released by Novartis Technical Research and Development. Cyclosporine 100 and 25 mg soft gelatin capsules (Neoral Novartis Pharma AG, Basel, Switzerland) were commercially available and sourced locally by the site.

In Treatment Period 1, study participants were admitted to the clinic on day −1 for baseline evaluations at least 12 hours before dosing. On day 1, they received a single oral dose of fevipiprant 150 mg, followed by an intravenous microdose of labeled fevipiprant (100 μg) 1 hour (±5 minutes) later. Participants fasted for 8 hours before the oral dose administration and continued to fast for 2 hours afterward, and they were confined to the clinic for approximately 28 hours following the oral study drug administration, during which time blood and urine samples for PK analysis and safety assessments were taken. On days 3–5, participants returned to the study site for outpatient safety and PK visits. They returned to the clinic for Treatment Period 2 following a washout period; overall, 7–14 days separated day 1 in Treatment Period 1 from nominal day 16 in Treatment Period 2. Anyone that prematurely discontinued from Treatment Period 1 was required to complete an early termination visit (day 15 visit).

In Treatment Period 2, participants were admitted to the clinic on day 15 for baseline evaluations at least 12 hours before dosing. On days 16–19, they received oral doses of cyclosporine 175 mg twice daily, and on day 20, they received a single oral dose of cyclosporine 175 mg in the morning. On day 17, participants received a single oral dose of fevipiprant 150 mg in parallel with the morning cyclosporine dose (± approximately 3 minutes), followed by administration of an intravenous microdose of labeled fevipiprant (100 μg) 1 hour (±5 minutes) later. On all dosing days, participants fasted for 8 hours before the oral drug administration in the morning and continued to fast for 2 hours thereafter. Samples were taken for PK analysis on days 16–21. Participants fasted for 2 hours before the evening doses of cyclosporine and continued to fast for 1 hour thereafter, and they were confined to the clinic until study day 21. On day 23, participants returned to the study site for safety assessments; end of study safety assessments were completed on day 25.

The sample size (16 participants so that at least 12 would complete the study) was selected to control the width of the confidence interval for the geometric mean ratio for fevipiprant AUC and C_{max} when given with and without cyclosporine. Further details may be found in the Supplemental Materials.

Key Inclusion and Exclusion Criteria. Men and women were admitted as study participants if they were aged 18–55 years and in good health, as determined by past medical history, physical examination, vital signs, electrocardiogram (ECG), and laboratory tests at screening and/or at first baseline visit. Further details on vital sign measurement are provided in the Supplemental Materials. Participants had to weigh between 60 and 90 kg and to have a body mass index within the range of 20–30 kg/m^2. Study participants gave written informed consent before any assessment took place, and they had to be able to communicate well with the investigator and to understand and comply with the requirements of the study. Exclusion criteria details are provided in the Supplemental Materials.

Pharmacokinetic Analyses. PK blood and urine samples were taken at prespecified time points from all participants and acidified pending analysis to avoid back-conversion of the AG metabolite to fevipiprant (details are provided in the Supplemental Materials). Validated liquid chromatography–mass

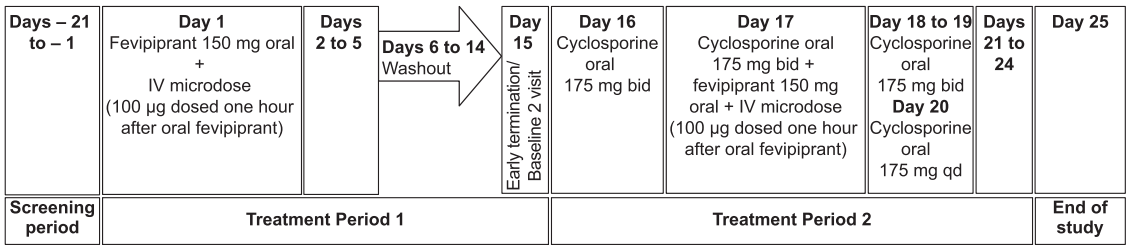

Fig. 2. Study design.

spectrometry/mass spectrometry (LC-MS/MS) methods were used to measure the plasma concentrations of unlabeled fevipiprant (given orally) and labeled fevipiprant (given as intravenous microdose) using the transitions of mass to charge ratio (m/z) 427 to m/z 145 and of m/z 431 to m/z 149, respectively. The MS/MS transition used for the acyl glucuronide metabolite of (unlabeled) fevipiprant was m/z 603 to m/z 427. The MS/MS transition used for the measurement of cyclosporine was m/z 1219.9 to m/z 1202.9. Further details of the LC-MS/MS methods may be found in the Supplemental Materials. The plasma concentrations of the AG metabolite of unlabeled fevipiprant and the blood concentrations of cyclosporine were measured by validated LC-MS/MS methods. The concentrations of unlabeled fevipiprant and its major AG metabolite in urine were determined using qualified LC-MS/MS methods. Concentrations below the lower limits of quantification (LLOQ) were reported as "zero," and missing data were labeled as such in the bioanalytical data reports.

The following PK measures of fevipiprant were determined using the actual recorded sampling times and noncompartmental method(s) with Phoenix WinNonlin (Version 6.4): C_{max}, T_{max} (time to reach peak or maximum concentration following drug administration), AUC_{last}, AUC_{inf}, $t_{1/2}$ (terminal half-life), Vz/F (the apparent volume of distribution during the terminal phase following extravascular administration), CL, F, and CL/F from the plasma concentration time data. For the AG metabolite, C_{max}, T_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ were determined using noncompartmental analysis only after oral administration. The amount excreted into the urine of unlabeled fevipiprant and its AG metabolite was determined from the urine concentration and volume–time data. The renal clearance (CLr) of fevipiprant and its AG metabolite was determined as Ae/AUC from the same time period. The absolute oral bioavailability (F) was estimated as a ratio of the dose-normalized AUCs following oral and intravenous administration [$F = (AUC_{po}*DOSE_{iv})/(AUC_{iv}*DOSE_{po})$]. The linear trapezoidal rule was used for AUC calculation. Regression analysis of the terminal elimination phase for the determination of $t_{1/2}$ included at least three data points after C_{max}. If the adjusted R^2 (coefficient of determination) value of the regression analysis of the terminal phase was less than 0.75, no values were reported for $t_{1/2}$, AUC_{inf}, CL, V, or CL/F.

Key Safety and Tolerability Assessments. Safety assessments consisted of collecting all adverse events (AEs) and serious AEs with their severity and relationship to study drug. Laboratory evaluations included hematology, biochemistry, and urinalyses. Vital signs, physical condition, body weight, and standard 12-lead ECG were also assessed.

Statistical Analyses. Participants' data were analyzed according to the study treatments received for all analysis sets. The safety analysis set included all participants who received any study drug. The PK analysis set included all participants with at least one available valid PK concentration measurement, who received any study drug and with no protocol deviations that had an impact on PK data. For the primary endpoints, the log-transformed fevipiprant PK measures (AUC_{last}, AUC_{inf}, and C_{max}) were analyzed separately by a mixed-effects model, with treatment as a fixed effect and participant as random effect. The estimated mean and 90% confidence interval (CI) for treatment difference (fevipiprant plus cyclosporine vs. fevipiprant alone) were back-transformed to obtain a geometric mean ratio and 90% CI of the ratio. SAS software was used for all statistical analyses. Statistical analysis of the secondary endpoints is provided in the Supplemental Materials.

Results

Participants. Sixteen participants entered the study, of whom 13 (81.3%) completed Treatment Period 1. In total, 14 participants were male and 2 were female; 11 were Caucasian, 4 were black, and 1 of another race (not specified). Their mean age was 32.5 years (range: 20–52) with a mean weight of 77.7 kg (range: 64.5–88.7) and mean body mass index of 25.6 kg/m^2 (range: 21.6–30.0). Three participants discontinued for the following reasons: because of an AE ($n = 1$), being lost to follow-up ($n = 1$), or physician decision (positive drug screen on day 15; $n = 1$). All 13 participants who completed Treatment Period 1 entered Treatment Period 2 and completed the study. All 16 participants were included in the PK and the safety analysis sets.

Effect of Cyclosporine on the PK of Fevipiprant (Oral and Intravenous Doses). Peak concentrations of oral fevipiprant were seen 1.5 hours after the fevipiprant dose and 3 hours after the cyclosporine plus fevipiprant dose (Fig. 3; Table 1). The mean concentration-time data for fevipiprant with and without cyclosporine are provided in Supplemental Table 1. For C_{max}, the geometric mean ratio (90% CI) was 3.02 (2.38, 3.82) (Supplemental Table 2), indicating an approximately threefold increase in peak exposure of fevipiprant when coadministered with cyclosporine. The geometric mean ratios (90% CI) were 2.50 (2.17, 2.88) for AUC_{last} and 2.35 (1.99, 2.77) for AUC_{inf} (Supplemental Table 2), indicating that the effect of cyclosporine on fevipiprant total exposure is smaller than the effect on peak exposure. Oral clearance and volume of distribution of fevipiprant were reduced by approximately 50% upon coadministration with cyclosporine, whereas $t_{1/2}$ was similar (Table 1).

C_{max} values of the fevipiprant intravenous microdose were similar in the presence and absence of cyclosporine (Fig. 4; Table 2), with a geometric mean ratio for C_{max} (90% CI) of 1.04 (0.86, 1.25) (Supplemental Table 4). The mean concentration-time data for the fevipiprant intravenous microdose with and without cyclosporine are provided in Supplemental Table 3. T_{max} was typically at the first sampling time, i.e., 2 minutes after intravenous dosing. A $t_{1/2}$ and V for fevipiprant are not reported, as the terminal phase was not sufficiently covered in the concentration–time data, which is required to derive V. The $t_{1/2}$ after intravenous administration is expected to be identical to that derived from the oral data. AUC_{inf} and CL were estimated by noncompartmental analysis (Table 2) despite the limitations in describing the terminal phase. Because the captured concentration–time profiles cover a 1000-fold range in concentrations, the bias resulting from the incomplete representation of the AUC after concentrations dropped below the LLOQ of 20 pg/ml is considered to be small. Both AUC_{last} and estimated AUC_{inf} for fevipiprant intravenous microdose were approximately twofold higher in presence of cyclosporine (Table 2), with geometric mean ratios (90% CI) of 2.04 (1.83, 2.28) and 1.95 (1.76, 2.16), respectively (Supplemental Table 4).

Absolute Bioavailability of Fevipiprant. The combination of oral and intravenous microdose fevipiprant PK data were used to estimate its absolute bioavailability. Because of the limitations to deriving AUC_{inf} for the intravenous profiles, the absolute oral bioavailability for fevipiprant was based on comparison of dose normalized AUC_{last} (mean ± S.D.) and was 0.43 ± 0.09 and 0.53 ± 0.16 in the absence and presence of cyclosporine, respectively. These values are expected to be

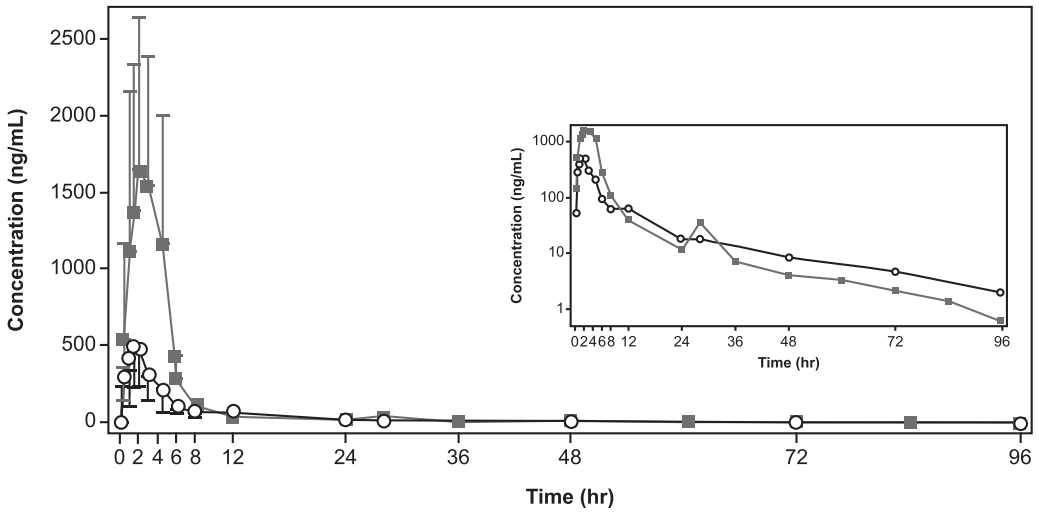

Fig. 3. Mean (S.D.) plasma concentration–time profiles of oral fevipiprant 150 mg in the presence (closed squares; $n = 13$) and absence (open circles; $n = 16$) of cyclosporine 175 mg twice daily, linear view and semilogarithmic view (inset).

slightly higher than the real values because the covered time interval is longer for the oral data. The absolute bioavailability of fevipiprant was also estimated based on comparison of $AUC_{0-11\ h}$ because most intravenous profiles could be measured up to 11 hours postdose. Estimates based on this comparison were (mean ± S.D.) 0.28 ± 0.05 and 0.48 ± 0.16 in the absence and presence of cyclosporine, respectively. Using this method, a larger fraction of the fevipiprant oral profile is ignored; therefore, this value is a minimal estimate of the real value, which is expected to be in the range defined by the assessments based on AUC_{last} and $AUC_{0-11\ h}$, i.e., approximately 0.3–0.4 in the absence and 0.5 in the presence of cyclosporine.

Effect of Cyclosporine on the PK of the Major AG Metabolite of Fevipiprant. No relevant change in exposure of the AG metabolite of oral fevipiprant was seen in the presence of cyclosporine (Table 3). Consequently, the metabolite-to-fevipiprant ratio of AUC_{last} decreased from 1.5 to 0.59 in presence of cyclosporine. The mean concentration–time data for the AG metabolite with and without cyclosporine are shown in Supplemental Table 1. Geometric mean ratios for PK measures for the AG metabolite are shown in Supplemental Table 5.

Effect of Cyclosporine on the Urinary Excretion of Fevipiprant and Its AG Metabolite. Coadministration with cyclosporine did not result in any relevant changes in the renal clearance of oral fevipiprant (Table 1) and its AG metabolite (Table 3). However, consistent with the higher exposure, the fraction of the dose excreted as unchanged fevipiprant into urine within 24 hours increased by 2.5-fold from 16.7% ± 3.55% to 40.9% ± 7.50% when coadministered with cyclosporine. There was only a slight increase in the amount of AG metabolite excreted into urine from 31.7 ± 6.08 to 37.3 ± 4.79 mg in the presence of cyclosporine (corresponding to approximately 15% or 18% of the fevipiprant dose).

Effect of Fevipiprant on the PK of Cyclosporine and Concentration-Effect Relationship of Cyclosporine. No relevant change in mean trough blood concentrations of cyclosporine was observed from day 17 in the morning (before administration of fevipiprant) to any time point after fevipiprant administration (mean trough blood concentrations were in the range of 45–54 ng/ml cyclosporine). This indicates the absence of a major effect of fevipiprant 150 mg on the PK of cyclosporine and that cyclosporine steady state had been achieved at the time of fevipiprant dosing.

The relationship of trough concentrations of cyclosporine to the observed change in oral fevipiprant C_{max} was explored graphically (Fig. 5). There was no apparent trend for an increasing effect on fevipiprant C_{max} with increasing trough concentrations of cyclosporine.

Safety and Tolerability. Overall, administration of fevipiprant alone and fevipiprant plus cyclosporine showed no major or novel safety signals. There were 20 AEs reported in nine patients: headache ($n = 3$), nausea ($n = 2$), alanine aminotransferase (ALT) elevation ($n = 1$), back pain ($n = 1$), catheter site hematoma ($n = 1$), contact dermatitis ($n = 1$), dizziness ($n = 1$), dysgeusia ($n = 1$), flushing ($n = 1$), muscle fatigue ($n = 1$), pharyngitis ($n = 1$), upper respiratory tract infection ($n = 1$), chlamydial urethritis ($n = 1$), vessel puncture site pain ($n = 1$), and vomiting ($n = 1$). A breakdown of the safety data by treatment period may be found in the Supplemental Materials (Supplemental Table 6).

TABLE 1

Pharmacokinetic measures for oral fevipiprant with and without coadministration of cyclosporine

Data are arithmetic means ± S.D. (CV%) [n]. CV% = Coefficient of variation (%) = S.D./mean*100.

Measure (Unit)	Fevipiprant 150 mg Oral, $n = 16$	Cyclosporine 175 mg Twice Daily + Fevipiprant 150 mg Oral, $n = 13$
C_{max} (ng/ml)	724 ± 207 (28.5) [$n = 16$]	2270 ± 809 (35.7) [$n = 13$]
AUC_{last} (h*ng/ml)	3210 ± 601 (18.7) [$n = 16$]	8110 ± 1980 (24.4) [$n = 13$]
AUC_{inf} (h*ng/ml)	3330 ± 718 (21.5) [$n = 15$]	7900 ± 1980 (25.1) [$n = 10$]
T_{max} (h)[a]	1.50 (0.50–4.50) [$n = 16$]	3.00 (0.92–4.50) [$n = 13$]
CL/F (l/h)	47.0 ± 10.3 (22.0) [$n = 15$]	20.2 ± 5.71 (28.2) [$n = 10$]
Vz/F (l)	1090 ± 585 (53.6) [$n = 15$]	446 ± 256 (57.4) [$n = 10$]
$t_{1/2}$ (h)	17.5 ± 13.5 (77.1) [$n = 15$]	14.9 ± 6.46 (43.4) [$n = 10$]
CL_r (l/h)	9.49 ± 1.25 (13.2) [$n = 16$]	8.45 ± 2.39 (28.3) [$n = 13$]

[a]For T_{max}, data are median (minimum–maximum) [n].

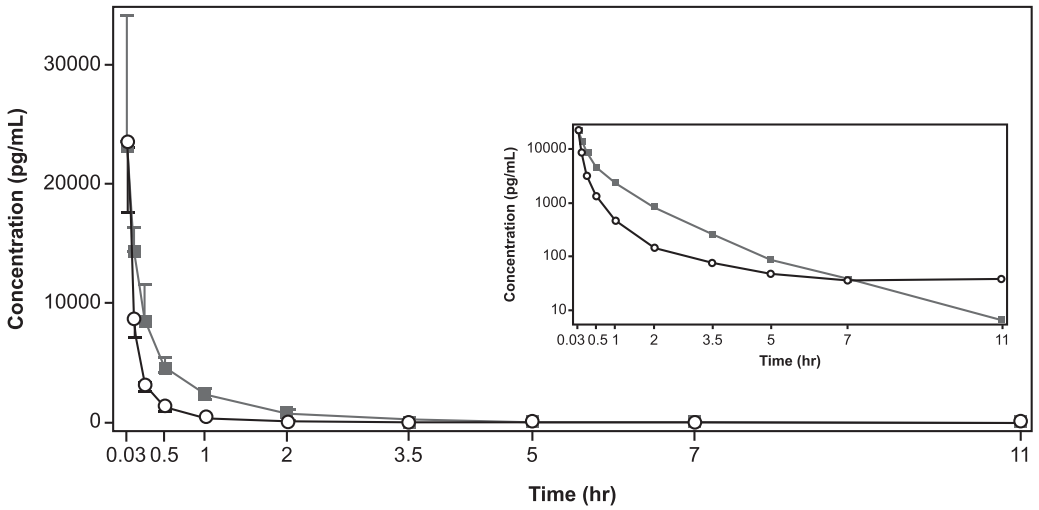

Fig. 4. Mean (S.D.) plasma concentration–time profiles of labeled fevipiprant after intravenous dosing of 100 μg in the presence (closed squares; $n = 13$) and absence (open circles; $n = 16$) of cyclosporine 175 mg twice daily, linear view and semilogarithmic view (inset).

Ten AEs were suspected to be related to study medication: eight related to cyclosporine, one to fevipiprant (mild postural dizziness), and one to one or other, or both study treatments (mild headache); it was not possible to distinguish which. All reported AEs were of mild intensity except one AE (pharyngitis), which was of moderate intensity. The participant who experienced increased ALT discontinued the study; this AE was not suspected to be related to study drug. Apart from the elevation in ALT in one participant, no clinically significant changes were seen in laboratory parameters, vital signs, or ECG parameters. There were no serious AEs or deaths.

Discussion

The aim of this study was to assess how cyclosporine, by inhibiting OATP1B3-mediated liver uptake and P-gp–mediated efflux in the intestine and liver, affects the PK of oral fevipiprant. The inclusion of an intravenous microdose of stable isotope-labeled fevipiprant provided major additional learnings without the need for more participants or a longer study duration; the absolute bioavailability of fevipiprant was derived, and the mechanistic DDI understanding increased, by the ability to differentiate between systemic and presystemic effects of cyclosporine. These learnings would be reflected in a potential future fevipiprant drug label in two sections: 1) the absolute bioavailability and dependence on active transport as part of the PK section and 2) the assessment of the need for dose adjustment in presence of OATP1B3 or P-gp inhibitors in the drug interaction section.

In the absence of cyclosporine, the PK properties of fevipiprant, including exposure to its major metabolite, were consistent with earlier studies (Erpenbeck et al., 2016b, 2017; Pearson et al., 2017). Coadministration of cyclosporine increased the C_{max} of oral fevipiprant threefold and the AUC_{inf} by 2.35-fold. While fevipiprant concentrations shortly after intravenous dosing were similar, the AUC of intravenous-administered fevipiprant was twofold higher in the presence of cyclosporine, corresponding to a twofold decrease in systemic clearance. The approximately 1.2-fold stronger effect on oral as compared with intravenous exposure (2.35/2, i.e., ~1.2) can be attributed to the effect of cyclosporine on absorption and/or first-pass elimination of fevipiprant. This combined effect was small and indicates that the inhibition of intestinal P-gp has only a minor or no impact. Early clinical investigations in healthy participants indicated a dose-proportional PK of fevipiprant over a twofold dose range (Erpenbeck et al., 2016b), whereas later more comprehensive data in patients demonstrated dose-proportional PK over a 10-fold dose range (unpublished data). Therefore, the magnitude of drug interaction observed here is also relevant for other oral fevipiprant dose levels such as 450 mg, which was also tested in patient trials.

Despite the approximate two-fold reduction in clearance, no increase in the terminal half-life of fevipiprant was observed with coadministration of cyclosporine. The likely explanation is a reduced distribution in the presence of cyclosporine, as the terminal half-life depends on the ratio of distribution to clearance. Because cyclosporine reduced CL/F and Vz/F to a similar extent, the terminal half-life of fevipiprant remained largely unchanged. Mechanistically, the reduced hepatic uptake caused by inhibition of OATP1B3 can explain the lower volume of distribution of fevipiprant in the presence of cyclosporine. In a rat ADME study with radiolabeled fevipiprant, the liver showed the highest exposure to drug-related radioactivity (unpublished data), suggesting that liver uptake may be a major determinant of the distribution of

TABLE 2

Pharmacokinetic measures for labeled intravenous fevipiprant with and without coadministration of cyclosporine

Data are arithmetic means ± S.D. (CV%) [n]. CV% = Coefficient of variation (%) = S.D./mean*100.

Measure (Unit)	Fevipiprant 100 μg Intravenous, $n = 16$	Cyclosporine 175 mg Twice Daily + Fevipiprant 100 μg intravenous, $n = 13$
C_{max} (pg/ml)[a]	23,600 ± 6030 (25.5) [$n = 16$]	25,000 ± 8500 (34.1) [$n = 13$]
AUC_{last} (h*pg/ml)	5040 ± 774 (15.3) [$n = 16$]	10,300 ± 2160 (20.9) [$n = 13$]
AUC_{inf} (h*pg/ml)	5360 ± 959 (17.9) [$n = 15$]	10,400 ± 2150 (20.7) [$n = 13$]
CL (l/h)	19.2 ± 3.32 (17.3) [$n = 15$]	9.97 ± 1.97 (19.7) [$n = 13$]

[a] T_{max} was typically at the first sampling time, i.e., 2 min (0.033 h) after intravenous dosing.

TABLE 3

Pharmacokinetic measures for the AG metabolite of fevipiprant (unlabeled, derived from oral fevipiprant) with and without coadministration of cyclosporine

Data are arithmetic means ± S.D. (CV%) [n]; CV% = Coefficient of variation (%) = S.D./mean*100.

Measure (Unit)	Fevipiprant 150 mg Oral, n = 16	Cyclosporine 175 mg Twice Daily + Fevipiprant 150 mg Oral, n = 13
C_{max} (ng/ml)	1180 ± 333 (28.3) [n = 16]	1330 ± 369 (27.7) [n = 13]
AUC_{last} (h*ng/ml)	6650 ± 1450 (21.8) [n = 16]	6720 ± 1470 (21.9) [n = 13]
M/P[a]	1.5	0.59
AUC_{inf} (h*ng/ml)	6880 ± 1580 (22.9) [n = 16]	6800 ± 1510 (22.3) [n = 12][b]
T_{max} (h)[c]	2.0 (0.92–6.0) [n = 16]	3.0 (1.5–4.5) [n = 13]
$t_{1/2}$ (h)	18.9 ± 13.0 (68.9) [n = 16]	13.3 ± 6.96 (52.4)[n = 12][b]
CL_r	6.07 ± 1.02 l/h (16.7) [19.4]	6.29 ± 1.22 (19.4) [n = 13]

[a]Molar metabolite-to-parent (M/P) ratio for mean AUC_{last} considering the molecular weight difference (426 g/mol for fevipiprant and 602 g/mol for the AG metabolite).
[b]AUC_{inf} and $t_{1/2}$ were reported when R^2 (coefficient of determination in the regression analysis) adjusted value of the terminal elimination phase was >0.75.
[c]For T_{max}, data are median (minimum–maximum) [n].

fevipiprant. This change in distribution points to inhibition of liver uptake by OATP1B3 as the predominant mechanism for the effect of cyclosporine.

While in the presence of cyclosporine fevipiprant exposure was increased, little change in exposure to the AG metabolite was observed, and consequently, the metabolite:fevipiprant ratio for AUC_{last} was decreased 2.5-fold from 1.5 to 0.59 by cyclosporine (Table 3). Again, this suggests that reduced uptake into the liver, the major site of fevipiprant glucuronidation, is the primary mechanism of the observed cyclosporine effect. Otherwise, the metabolite: fevipiprant ratio would not decrease so strongly because glucuronidation, as a high-capacity system, is not easily saturated (Williams et al., 2004).

The renal clearance of oral fevipiprant and its AG metabolite were not affected by cyclosporine. However, because of the higher systemic exposure to fevipiprant in the presence of cyclosporine, renal excretion contributed 2.5-fold more to the elimination of fevipiprant (40.9% vs. 16.7% of the dose excreted into urine).

There was no apparent relationship between the change in oral fevipiprant C_{max} and trough concentrations of cyclosporine; i.e., within the covered range, higher cyclosporine trough concentrations were not linked to a stronger drug interaction (Fig. 5). Therefore, the degree of drug interaction may not be greater at higher cyclosporine oral doses that provide larger exposures than investigated in this study.

The terminal half-life of the intravenous microdose could not be derived because concentrations dropped below the LLOQ of 20 pg/ml by 23 hours postdose for most profiles. Concentrations shortly after dosing were approximately 1000-fold above LLOQ, suggesting that most of the AUC of the intravenous microdose was captured (extrapolated fraction of AUC_{inf} based on mean data was ≤6%, both with and without cyclosporine).

The disposition of transporter substrates such as fevipiprant is complex, making prospective predictions of exposure changes with inhibitors of transporter activity challenging (Poirier et al., 2009a,b; Jamei et al., 2014; Taskar et al., 2020). Part of the challenge is that active transport processes influence absorption, clearance, and tissue distribution, making it difficult to derive clean PK input parameters for modeling from oral data only. Combined intravenous and oral data obtained in this study in the presence and absence of a transporter inhibitor allow differentiation between systemic and presystemic processes and provide more robust parameters. This supports the development of more reliable, physiologically- based PK models. These can be used to predict untested case scenarios, such as transporter DDI effects at steady state, and in the case of fevipiprant, the impact of other OATP1B3 inhibitors on its PK.

There was no effect of fevipiprant 150 mg on the trough concentrations of cyclosporine, which is consistent with expectations based on available drug interaction data for fevipiprant as a perpetrator (Poller et al., 2019). Overall, administration of fevipiprant alone and fevipiprant plus cyclosporine was well tolerated with no unexpected or novel AEs. Most AEs suspected to be drug-related were attributed to cyclosporine. These findings are consistent with previous studies showing that fevipiprant was safe and well tolerated at single and multiple oral doses up to 1800 mg/day (Erpenbeck et al., 2017). The exposure change observed in this study will guide fevipiprant dosing recommendations in presence of OATP1B3 inhibitors in the context of the safety profile of fevipiprant in any potential future drug label.

The addition of a labeled intravenous microdose in this study allowed investigation of the absolute bioavailability and absolute clearance

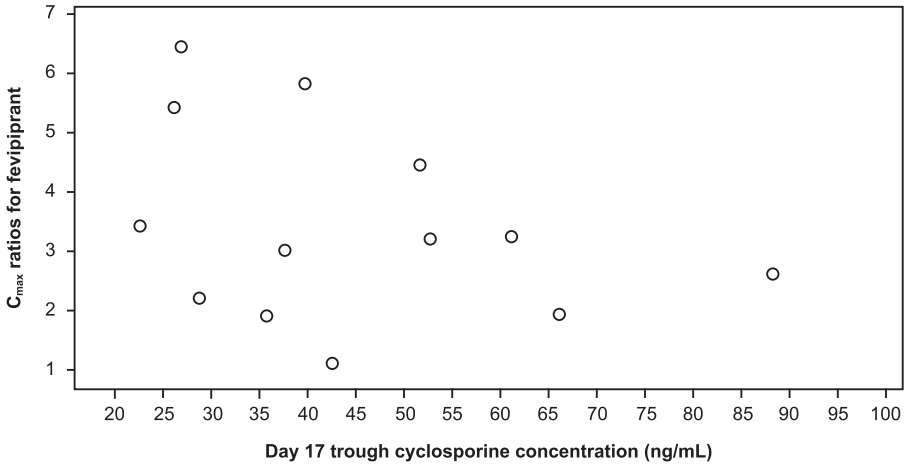

Fig. 5. Scatter plot of oral fevipiprant ratio (Treatment Period 2/Treatment Period 1) for C_{max} with different cyclosporine concentrations. On the x-axis is the trough cyclosporine concentration on day 17, before the coadministration with fevipiprant. Only study participants with data from both study periods are included.

without the need to conduct an intravenous toxicology program and a separate intravenous clinical trial. Conventionally, absolute bioavailability studies are crossover studies involving 6–12 participants; the microdose approach negates the need for a separate study with the further advantage that intravenous and oral dose data from the same participants at the same time avoids variability. Furthermore, use of a microdose simplifies formulation work because of the small amounts involved, and there are fewer potential safety concerns.

Concomitant oral dosing of unlabeled and intravenous microdosing of labeled compound in humans has previously been used to determine the absolute bioavailability as well as the oral and intravenous PK of the human immunodeficiency virus protease inhibitor nelfinavir (Sarapa et al., 2005); dabrafenib, a BRAF inhibitor (Denton et al., 2013); saxagliptin, a dipeptidyl peptidase-4 inhibitor; and dapagliflozin, a sodium glucose cotransporter-2 inhibitor (Boulton et al., 2013). In all these studies, the compound given as intravenous microdose was radiolabeled with ^{14}C and quantified by accelerator mass spectrometry. Furthermore, a double tracer technique using oral ^{14}C-radiolabeled tofogliflozin simultaneously administered with ^{13}C-stable isotope–labeled tofogliflozin has been used successfully in an oral human ADME study to obtain additional information on the intravenous PK of the compound (Schwab et al., 2013). Intravenous microdosing for PK analysis has been accepted as a method by major health authorities (FDA Center for Drug Evaluation and Research, 2012; Boulton et al., 2013).

Administering the microdose after the oral dose circumvents the potential problem of PK nonlinearity at microdose levels (Lappin et al., 2006), as the (labeled) microdose enters the body while the unlabeled compound given orally is present in the therapeutic drug concentration range. We observed little variability in the intravenous microdose data, suggesting good data quality.

Analytical interferences between the unlabeled 1500-fold higher oral dose and the labeled microdose were avoided by careful selection of the positions of labeling (Fig. 1) and the transitions used in the LC-MS/MS analyses. With the MS/MS transitions given in the experimental part, the spillover of the unlabeled compound into the signal of the labeled compound was reduced to only 3 ppm of the unlabeled signal (Gu et al., 2012), which is negligible in the present context. The internal standard used in the LC-MS/MS analyses was fevipiprant labeled with five deuteriums and one ^{13}C in the 4-methanesulfonyl-2-trifluoromethylbenzyl part of the molecule; no interference with the intravenous-dosed–labeled fevipiprant is expected.

In this study, a single-sequence design without randomization was used. This is common in DDI studies and accepted by health authorities because the risk for bias of PK endpoints is considered small when investigated drugs do not display changes in clearance with time.

In conclusion, coadministration of cyclosporine increased oral fevipiprant AUC_{inf} and C_{max} by 2.35- and threefold, respectively. The use of an intravenous microdose allowed estimation of the absolute bioavailability of fevipiprant (0.3–0.4) and differentiation between a small (approximately 1.2-fold) presystemic effect of cyclosporine and a larger (approximately twofold) effect on systemic elimination of fevipiprant. The effect seems to be mainly by inhibition of OATP1B3-mediated hepatic uptake of fevipiprant. Additional mechanistic learnings from the intravenous data did not require any extra study participants or a separate trial. Although significant DDI was observed, administration of fevipiprant alone and fevipiprant plus cyclosporine was well tolerated with no novel AEs observed.

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Data sharing statement

Novartis will not provide access to patient-level data if there is a reasonable likelihood that individual patients could be reidentified. Phase 1 studies, by their nature, present a high risk of patient reidentification; therefore, patient individual results for phase 1 studies cannot be shared. In addition, clinical data, in some cases, have been collected subject to contractual or consent provisions that prohibit transfer to third parties. Such restrictions may preclude granting access under these provisions. Where codevelopment agreements or other legal restrictions prevent companies from sharing particular data, companies will work with qualified requestors to provide summary information where possible.

Authorship Contributions

Participated in research design: Weiss, Erpenbeck, Cain, Vemula, Elbast, Zollinger.

Conducted experiments: Elbast.

Performed data analysis: Weiss, Umehara, Vemula.

Wrote or contributed to the writing of the manuscript: All authors.

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A study of the effect of cyclosporine on fevipiprant pharmacokinetics and its absolute bioavailability using an intravenous microdose approach

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Online data supplement

Materials and methods

Study design

The sample size (16 participants so that at least 12 would complete the study, allowing for a 20% dropout rate) was selected to control the width of the confidence interval for the geometric mean ratio for fevipiprant area under the curve (AUC) and maximum plasma concentration (C_{max}) when given with and without cyclosporine. A 2-3 fold increase in AUC was expected. The relative half-width of the confidence interval was the upper limit of the 90% confidence interval for the geometric mean divided by the estimated geometric mean. With the chosen sample size of 12 completers and assuming an intra-subject (coefficient of variation) CV 20% for AUC based on previous clinical experience, the relative half-width is 1.14 (0.133 in log scale). The lower and upper 90% confidence intervals (CI) for the geometric mean ratio was evaluated as geometric mean ratio*exp(+/-0.133). The 90% CI for different geometric mean ratios for pharmacokinetic (PK) parameters are shown in the table below.

Table Expected upper and lower 90% CI for different geometric mean ratios with sample size of 12 completers

Geometric mean ratio	Lower 90% CI	Upper 90% CI
2	1.75	2.28
2.5	2.19	2.86
3	2.63	3.43

Inclusion criteria

Subjects eligible for inclusion in this study had to fulfill all of the following criteria:

- Written informed consent was obtained before any assessment was performed.
- Healthy male and female subjects 18 to 55 years of age (inclusive), and in good health as determined by past medical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and/or first baseline visit as indicated.
- Subjects were required to weigh between 60 and 90 kg (inclusive) to participate in the study, and were required to have a body mass index (BMI) within the range of 20 - 30 kg/m^2. BMI = Body weight (kg) / $[Height (m)]^2$.
- Subjects had to be able to communicate well with the investigator, and were able to understand and comply with the requirements of the study.
- At screening and first baseline visit, vital signs (systolic and diastolic blood pressure and pulse rate) were assessed in the sitting position after the participant had rested for at least three minutes, and again after three minutes in the standing position. Sitting vital signs were required to be within the following ranges:
    - oral body temperature between 35.0-37.5 °C
    - systolic blood pressure, 90-139 mm Hg
    - diastolic blood pressure, 50-89 mm Hg
    - pulse rate, 40 - 90 bpm

  If vital signs were out-of-range, the Investigator was permitted to obtain two additional readings for the respective parameter(s) so that up to three consecutive assessments were made, with the participant seated quietly for approximately five minutes preceding each repeat assessment. At least the last reading was required to be within the ranges provided above in order for the participant to qualify. Subjects were excluded if their

standing vital signs (relative to sitting) showed findings, which, in the opinion of the Investigator, were associated with clinical manifestation of postural hypotension (i.e. absence of any other cause). The Investigator carefully considered enrolling subjects with either a > 20 mm Hg decrease in systolic or a >10 mm Hg decrease in diastolic blood pressure, accompanied by a > 20 bpm increase in heart-rate (comparing standing to sitting results).

Exclusion criteria

Subjects fulfilling any of the following criteria were not eligible for inclusion in this study:

- Use of other investigational drugs at the time of enrollment, or within 5 half-lives of initial dosing, or within 30 days of initial dosing, whichever was longer; or longer if required by local regulations
- History of clinically significant electrocardiogram (ECG) abnormalities, or any of the following ECG abnormalities at screening or first baseline visit:
    - PR > 200 msec
    - QRS complex > 120 msec
    - QTcF > 450 msec (males)
    - QTcF > 460 msec (females)
- History or presence of long QT syndrome or other clinically significant ECG abnormalities, e.g. arrhythmia or tachycardia.
- History or presence of malignancy of any organ system, treated or untreated.
- Pregnant or nursing (lactating) women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test.
- Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant. Women were considered post-menopausal and not of child-bearing

potential if they had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks prior to screening. In the case of oophorectomy alone, only when the reproductive status of the woman was been confirmed by follow up hormone level assessment was she considered not of child-bearing potential.

- Smokers (use of tobacco products in the previous three months). Smokers were defined as any subject who reported tobacco use and/or who had a urine cotinine ≥ 500 ng/ml at screening or first baseline.
- Usage of any prescription drugs and/or herbal supplements within four weeks prior to initial dosing, and/or over-the-counter (OTC) medication, dietary supplements (vitamins included) within two weeks prior to initial dosing. If needed, (i.e. an incidental and limited need) acetaminophen, was acceptable, but was required to be documented in the Concomitant medications / Significant non-drug therapies page of the CRF.
- Donation or loss of 400 mL or more of blood within eight weeks prior to initial dosing, or longer if required by local regulation.
- Plasma donation within four weeks prior to initial dosing.
- Hemoglobin levels below normal ranges of local laboratory for males and females, respectively at screening and first baseline visit.
- Significant illness or infection which was not resolved within two weeks prior to initial dosing.
- Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
- History of multiple and recurring allergies or allergies/hypersensitivities or allergy/hypersensitivity to the investigational compounds/compound class being used in

this study (e.g. cyclosporine or any of its ingredients and DP_2 antagonists).

- History of any food allergies.
- Any surgical or medical condition which significantly altered the absorption, distribution, metabolism, or excretion of drugs, or which jeopardized the subject in case of participation in the study. The Investigator was required to make this determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following:
    - Inflammatory bowel disease, peptic ulcers, gastrointestinal including rectal bleeding within 12 months prior to screening;
    - History of major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
    - Pancreatic injury or pancreatitis within 12 months prior to screening;
    - Liver disease or liver injury as indicated by abnormal liver function tests. Alanine aminotransferase (ALT), asparate aminotransferase (AST), gamma-glutamyl transferase (γ-GT), alkaline phosphatase and serum bilirubin were tested.
    - ALT or AST > 2.0 X the upper limit of normal (ULN) or total bilirubin > 1.3 X ULN at screening or at the first baseline visit
    - γ-GT or alkaline phosphatase >1.5 X ULN at screening or at the first baseline visit
    - Any elevation above ULN of more than one parameter of ALT, AST, γ-GT, alkaline phosphatase or serum bilirubin at screening or at the first baseline visit excluded a subject from participation in the study

If necessary, laboratory testing was to be repeated on one occasion (as soon as possible) prior to randomization, to rule out any laboratory error.

    - History or presence of impaired renal function as indicated by clinically significantly abnormal creatinine or blood urea nitrogen (BUN) and/or urea values, or abnormal urinary constituents (e.g., albuminuria)
    - Evidence of urinary obstruction or difficulty in voiding at screening or at the first baseline visit
- History of immunodeficiency diseases or active disease, including a positive HIV (e.g., chemiluminescence assay and MultiSpot) test result.
- A positive Hepatitis B surface antigen or Hepatitis C test result.
- Any vaccination with live-attenuated vaccines within two months prior to screening.
- History of drug or alcohol abuse within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during screening and first baseline visit.

No additional exclusions were applied by the investigator, in order to ensure that the study population was representative of all eligible subjects.

Pharmacokinetic analyses

At each collection time 1.5 mL of plasma (from 3 mL of blood collected with K3EDTA as anticoagulant) was transferred into a VACUETTE FC Mixtube for acidification to avoid back-conversion of the AG metabolite to fevipiprant. Two aliquots were frozen and stored at ≤ -70°C until analysis. From the 24 h urine pools 1 mL aliquots were transferred into a VACUETTE FC Mixtubes for acidification and then frozen and stored at ≤ -70°C until analysis.

In Treatment Period 1 blood was collected for PK analysis of oral fevipiprant on at 0h, 0.25h, 0.5h, 1h, 1.5h 2h 3h 4.5h 6h 8h 12h post dose on Day 1, 24h and 28h post dose (Day 2), 48h post dose (Day 3) and 72h post dose (Day 4) and on Day 5. PK blood collection for the fevipiprant IV microdose (dosed 1 h after the oral dose) was collected at 0.03h, 0.12h, 0.5h 1h

2h 3.5h 5h 7h 11h post IV dose on Day 1, 23h and 27h post dose on Day 2, 47h post dose on Day 3 and 71h post dose on Day 4 and 95 h post dose on Day 5. Urine collection for PK analysis took place on Day 1 (24 h pool).

In Treatment Period 2 blood was collected for PK analysis of oral fevipiprant on at 0h, 0.25h, 0.5h, 1h, 1.5h, 2h, 3h, 4.5h, 6h, 8h, 12h post dose on Day 17, 24h, 28h, 36h post dose on Day 18 and at 48h and 60h post dose on Day 19, and 72h and 84h post-dose on Day 20 and at 96h post-dose on Day 21. PK blood collection for the fevipiprant IV microdose (dosed 1 h after the oral dose) was collected at 0.03h, 0.12h, 0.5h 1h 2h 3.5h 5h 7h 11h post dose on Day 17, 23h, 27h, 35h post dose (Day 18) and at 47h and 59h post-dose on Day 19, and 71h and 83h post-dose on Day 20 and at 95h post-dose on Day 21. PK blood collection for cyclosporine took place pre cyclosporine dose on Day 16 (evening dose only), Days 17 and 18 both doses. Urine collection for PK analysis took place on Day 17 (24 h pool).

LC-MS/MS materials and methods for oral fevipiprant and its AG metabolite

Samples were prepared as follows: 50 µL of plasma sample were added and mixed with 120 µL of water 2% acetic acid. The mixture was loaded on Isolute SLE-96 well plate (200 mg). The plate was then eluted twice with 300 µL of tert-butyl-methyl ether (MTBE). The solvent was evaporated to dryness under nitrogen stream at a temperature of 50ºC. The dry residue was reconstituted with 250 µL of 0.2% formic acid in methanol/water (50/50; v/v). The plate was sealed, mixed and then centrifuged for 5 min at a temperature of 4ºC. A volume of 10 µL was injected into the LC-MS/MS system.

Lower limit of quantification (LLOQ), and inter- and intra-day variability were: 1.0 ng/mL (LLOQ) and 400 ng/mL (upper limit of quantification; ULOQ) for fevipiprant and 0.480 ng/mL (LLOQ) and 192 (ULOQ) ng/mL for the AG metabolite.

Sample analysis was performed on a LC-MS/MS system consisting of an API 4000 triple quadrupole mass spectrometer equipped with a TurboIonSpray™ interface (Applied Biosystems). The MS system was connected to a LC-20AC XR auto-sampler (Shimadzu) and to a LC-20AD XR pump (Shimadzu). Chromatographic separations were performed at a flow rate of 0.500 mL/min on Zorbax Rapid Resolution HT SB-C_{18} column (50 mm × 2.1 mm, 1.8 µm). A binary gradient with a mobile phase consisting of 0.2% formic acid in water (A) and methanol (B) was used for the LC-separation. The elution gradient program was as follows: time (min), (% mobile phase B): (0, 20) (2.5, 70) (2.6, 95) (3.0, 95) (3.1, 20) (4.25, 20). The column temperature was maintained at 60°C using a column heater. The system was operated in electrospray positive ionization using MRM mode. The other MS conditions were as follows: turbo ion spray 5500 V; source temperature 650°C; collision activated dissociation 8; curtain gas 10 psi; Gas1 60 psi; Gas2 40 psi; entrance potential 12 V (compound-1 and [internal standard] ISTD); dwell time 60 ms (compound-1 and ISTD); collision energy 60 eV (fevipiprant and ISTD) and 36 eV (AG metabolite and ISTD); declustering potential 94 V, collision cell exit potential 11 V (fevipiprant and ISTD) and 25 eV (AG metabolite and ISTD).

Statistical analysis of secondary endpoints

For fevipiprant and AG metabolite PK data and parameters, descriptive summary statistics were provided by treatment and visit/sampling time point. Summary statistics included mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum and maximum. Concentrations below LLOQ were treated as zero in summary statistics. A geometric mean was not reported if the dataset included zero values.

Results

Table S1 Mean concentration-time data for oral fevipiprant and its AG metabolite without cyclosporine and with cyclosporine

	Sampling Time	Without cyclosporine		With cyclosporine 175 mg	
		Fevipiprant (ng/mL)	AG metabolite (ng/mL)	Fevipiprant (ng/mL)	AG metabolite (ng/mL)
Fevipiprant 150 mg oral + fevipiprant 100µg IV		(n = 16)	(n = 16)	(n = 13)	(n = 13)
	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.0473 (0.170)
	0.25	52.9 (118)	2.36 (4.29)	146 (218)	8.42 (11.1)
	0.5	289 (324)	90.3 (126)	540 (632)	152 (187)
	1	416 (312)	429 (436)	1120 (1030)	588 (506)
	1.5	492 (272)	814 (540)	1380 (953)	820 (612)
	2	495 (267)	907 (488)	1660 (990)	934 (588)
	3	306 (164)	791 (261)	1550 (838)	1010 (472)
	4.5	214 (153)	580 (354)	1170 (824)	988 (317)
	6	95.2 (38.2)	297 (139)	286 (148)	421 (197)
	8	63.2 (23.2)	159 (54.9)	109 (41.8)	170 (64.2)
	12	64.2 (21.6)	106 (28.1)	39.7 (19.1)	66.7 (27.2)
	24	18.0 (9.12)	43.3 (13.1)	11.6 (3.42)	22.5 (5.76)
	28	18.4 (8.02)	44.4 (19.9)	36.4 (12.7)	30.5 (10.3)
	36	–	–	7.16 (2.68)	12.9 (4.88)

	48	8.41 (5.15)	18.2 (10.0)	4.06 (1.67)	7.45 (4.16)
	60	–	–	3.33 (2.74)	4.91 (4.31)
	72	4.69 (5.26)	9.85 (11.1)	2.14 (1.74)	3.88 (3.58)
	84	–	–	1.37 (2.79)	2.46 (4.58)
	96	1.96 (2.98)	4.52 (6.18)	0.601 (1.80)	1.52 (3.53)

Data are mean ± SD

Table S2 Geometric mean ratio (test/reference) and 90% confidence intervals for PK parameters of oral fevipiprant

Parameter	Treatment	n*	Adjusted Geometric mean	Comparison	Treatment Comparison[#] Geometric mean ratio	(90% CI)
C_{max} (ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	2100.93	Test vs Reference	3.02	(2.38, 3.82)
	Fevipiprant 150 mg oral and 100 µg IV	16	696.76			
AUC_{last} (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	7885.36	Test vs Reference	2.50	(2.17, 2.88)
	Fevipiprant 150 mg oral and 100 µg IV	16	3155.39			
AUC_{inf} (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	10	7659.86	Test vs Reference	2.35	(1.99, 2.77)
	Fevipiprant 150 mg oral and 100ug IV	15	3263.71			

Test: Cyclosporine 175mg b.i.d + fevipiprant 150mg oral and 100 µg IV; Reference: Fevipiprant 150mg oral and 100 µg IV.[#]Geometric mean ratio and 90% CI are back transformed from log scale. *Number of evaluable subjects.

Table S3 Mean concentration-time data for fevipiprant 100 µg IV without and with cyclosporine

	Fevipiprant 100µg IV (pg/mL)		
Fevipiprant 100µg IV	Scheduled Sampling Time (h)	Without cyclosporine (n = 16)	With cyclosporine 175 mg b.i.d. (n = 13)
	0.03	23600 (6030)	23200 (11000)
	0.12	8660 (1490)	14400 (1970)
	0.25	3240 (516)	8720 (2890)
	0.5	1350 (348)	4700 (789)
	1	480 (124)	2350 (551)
	2	146 (39.4)	841 (313)
	3.5	77.2 (23.9)	270 (115)
	5	48.2 (11.9)	89.4 (34.8)
	7	36.3 (8.26)	39.3 (12.2)
	11	39.6 (15.9)	6.65 (13.0)
	23	2.01 (8.03)	0.00 (0.00)
	27	4.34 (9.34)	3.99 (9.77)
	35	–	0.00 (0.00)
	47	0.00 (0.00)	0.00 (0.00)
	59	–	0.00 (0.00)
	71	0.00 (0.00)	0.00 (0.00)
	83	–	0.00 (0.00)
	95	0.00 (0.00)	0.00 (0.00)

Data are mean ± SD

Table S4 Geometric mean ratio (test/reference) and 90 percent confidence intervals for PK parameters of IV administered fevipiprant ([$^{13}C_2{}^{15}N_2$]fevipiprant)

					Treatment Comparison[#]	
Parameter	**Treatment**	**n***	**Adjusted Geometric mean**	**Comparison**	**Geometric mean ratio**	**(90% CI)**
Cmax (pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150mg oral and 100 µg IV	13	23780.39	Test vs Reference	1.04	(0.86, 1.25)
	Fevipiprant 150mg oral and 100 µg IV	16	22908.72			
AUClast (h*pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	10201.76	Test vs Reference	2.04	(1.83, 2.28)
	Fevipiprant 150 mg oral and 100 µg IV	16	4989.97			
AUCinf (h*pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150mg oral and 100 µg IV	13	10395.08	Test vs Reference	1.95	(1.76, 2.16)
	Fevipiprant 150mg oral and 100 µg IV	15	5340.58			

Test: Cyclosporine 175mg b.i.d + fevipiprant 150mg oral and 100 µg IV; Reference: Fevipiprant 150mg oral and 100 µg IV. [#]Geometric mean ratio and 90% CI are back transformed from log scale. *Number of evaluable subjects.

Table S5 Geometric mean ratio (test/reference) and 90% confidence intervals for PK parameters for the AG metabolite

Parameter	Treatment	n*	Adjusted Geometric mean	Comparison	Treatment Comparison[#] Geometric mean ratio	Treatment Comparison[#] (90% CI)
C_{max} (ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	1314.91	Test vs Reference	1.16	(1.01, 1.35)
	Fevipiprant 150 mg oral and 100 µg IV	16	1129.80			
AUC_{last} (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	6634.48	Test vs Reference	1.02	(0.92, 1.13)
	Fevipiprant 150 mg oral and 100 µg IV	16	6506.48			
AUC_{inf} (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	11	6697.04	Test vs Reference	1.00	(0.88, 1.12)
	Fevipiprant 150 mg oral and 100ug IV	16	6713.60			

Test: Cyclosporine 175mg b.i.d. + Fevipiprant 150mg oral and 100 µg IV. Reference: Fevipiprant 150mg oral and 100ug IV. n* = Number of evaluable subjects data considered.
[#]Geometric mean ratio and 90% CI are back transformed from log scale.

Table S6 Incidence of adverse events by preferred term (Safety analysis set)

EPOCH	**Treatment 1**	**Treatment 2**		**All treatments**
	Fevipiprant 150mg oral and 100ug IV	Cyclosporine 175mg b.i.d	Cyclosporine 175mg b.i.d + Fevipiprant 150mg oral and 100ug IV.	
Preferred term	N=16 n (%)	N=13 n (%)	N=13 n (%)	N=16 n (%)
Number of subjects with at least one AE	5 (31.3)	1 (7.7)	5 (38.5)	9 (56.3)
Headache	0	0	3 (23.1)	3 (18.8)
Nausea	0	1 (7.7)	1 (7.7)	2 (12.5)
Alanine aminotransferase increased	1 (6.3)	0	0	1 (6.3)
Back pain	0	0	1 (7.7)	1 (6.3)
Catheter site hematoma	1 (6.3)	0	0	1 (6.3)
Dermatitis contact	1 (6.3)	0	0	1 (6.3)
Dizziness postural	1 (6.3)	0	0	1 (6.3)
Dysgeusia	0	0	1 (7.7)	1 (6.3)
Flushing	0	0	1 (7.7)	1 (6.3)

Muscle fatigue	0	0	1 (7.7)	1 (6.3)
Pharyngitis	0	0	1 (7.7)	1 (6.3)
Upper respiratory tract infection	0	0	1 (7.7)	1 (6.3)
Urethritis chlamydial	0	0	1 (7.7)	1 (6.3)
Vessel puncture site pain	1 (6.3)	0	0	1 (6.3)
Vomiting	0	0	1 (7.7)	1 (6.3)

n = number of subjects with at least one AE (preferred term); N = number of subjects studied within treatment. A participant with multiple AEs is counted only once in the “at least one AE” row. A participant with multiple AEs with the same preferred term is counted only once for that preferred term and treatment. Preferred terms are sorted in descending frequency, as reported in the “All treatments” column. An AE starting in one treatment and continuing into the next treatment is counted in the earlier treatment only. In Treatment 2, an AE starting before combination (cyclosporine + fevipiprant) treatment is counted under cyclosporine treatment. All other AEs are counted under combination (cyclosporine + fevipiprant) treatment.
AE: adverse event