DRUG INTERACTIONS

Effect of gemfibrozil and rifampicin on the pharmacokinetics of selexipag and its active metabolite in healthy subjects

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AIMS

Based on *in vitro* data, there is evidence to suggest that cytochrome P450 (CYP) 2C8 is involved in the metabolism of selexipag and its active metabolite, ACT-333679. The present study evaluated the possible pharmacokinetic interactions of selexipag with gemfibrozil, a strong CYP2C8 inhibitor, and rifampicin, an inducer of CYP2C8.

METHODS

The study consisted of two independent parts, each conducted according to an open-label, randomized, crossover design. The pharmacokinetics and safety of selexipag and ACT-333679 were studied following single-dose administration either alone or in the presence of multiple-dose gemfibrozil (part I) or rifampicin (part II) in healthy male subjects.

RESULTS

Gemfibrozil had comparatively small effects on selexipag (less than 2-fold difference in any pharmacokinetic variable) but, with respect to ACT-333679, increased the maximum plasma concentration (C_{max}) 3.6-fold [90% confidence interval (CI) 3.1, 4.3] and the area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) 11.1-fold (90% CI 9.2, 13.4). The marked increased exposure to ACT-333679, which mediates the majority of the pharmacological activity of selexipag, was accompanied by significantly more adverse events such as headache, nausea and vomiting. Coadministration of rifampicin increased the C_{max} of selexipag 1.8-fold (90% CI 1.4, 2.2) and its $AUC0_{-\infty}$ 1.3-fold (90% CI 1.1, 1.4); its effects on ACT-333679 were to increase its C_{max} 1.3-fold (90% CI 1.1, 1.6), shorten its half-life by 63% and reduce its $AUC0_{-\infty}$ by half (90% CI 0.45, 0.59).

CONCLUSION

Concomitant administration of selexipag and strong inhibitors of CYP2C8 must be avoided, whereas when coadministered with inducers of CYP2C8, dose adjustments of selexipag should be envisaged.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Selexipag, a prostacyclin receptor agonist marketed for the treatment of pulmonary arterial hypertension, is enzymatically hydrolysed to form the active metabolite, ACT-333679. Based on *in vitro* data, both compounds have been found to be substrates of cytochrome P450 (CYP) 2C8 and CYP3A4.
- Selexipag and ACT-333679 are substrates of organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3. In addition, selexipag is a substrate of P-glycoprotein, and its active metabolite is a substrate of breast cancer resistance protein.

WHAT THIS STUDY ADDS

- Concomitant administration of selexipag and gemfibrozil resulted in a marked increase in the exposure to ACT-333679, associated with a significant increase in the incidence and/or intensity of typical prostacyclin-related adverse events. Concomitant administration of selexipag and strong inhibitors of CYP2C8 must be avoided.
- Concomitant administration of rifampicin decreased exposure to ACT-333679. The clinical efficacy of selexipag is mediated mainly by ACT-333679 and, therefore, an increase in the dose of selexipag should be considered when administered concomitantly with CYP2C8 inducers.

Introduction

Pulmonary arterial hypertension (PAH) is a disease characterized by a progressive increase in pulmonary artery pressure and pulmonary vascular resistance, ultimately leading to right heart failure and death [1, 2]. Epoprostenol, a prostacyclin receptor agonist, was the first drug to be approved for the treatment of PAH. Therapy with epoprostenol requires a chronic indwelling catheter, reconstitution of the drug and operation of an infusion pump, and carries the risk of serious bacteraemia [3, 4]. Therefore, other prostanoids have been developed, such as treprostinil for continuous subcutaneous infusion [5], inhalation [6] and oral administration [7]; iloprost for inhalation [8] and beraprost for oral administration [9]. The short half-life and poor oral bioavailability of the prostanoids led to the search for other compounds acting on the prostacyclin receptor. Selexipag (NS-304, ACT-293987) was the first orally active, selective, long-acting, nonprostanoid prostacyclin receptor agonist approved for the treatment of PAH [10–14].

Selexipag is hydrolysed by carboxylesterases to its active metabolite, ACT-333679. Both selexipag and ACT-333679 bind selectively and with high affinity to the prostacyclin receptor [10]. *In vitro*, ACT-333679 is approximately 37-fold more potent than selexipag in activating the human prostacyclin receptor and is present at 3- to 4-fold higher levels than the parent drug at steady state in humans after oral selexipag administration [15]. Therefore, ACT-333679 is the major contributor to the drug effect in humans. The structures of both compounds have been published previously [16]. An absolute bioavailability study in healthy subjects showed that the clearance of selexipag is $17.9 \, l \, h^{-1}$, the volume of distribution 11.7 l and the absolute bioavailability 49.4% [17]. Following single- and multiple-dose oral administration of selexipag to healthy subjects, the pharmacokinetics (PK) of selexipag and ACT-333679 were dose proportional [16, 18]. The time to maximum concentration was within 2.5 h and 4 h for selexipag and ACT-333679, respectively, and the apparent terminal half-life ($t_{1/2}$) varied from 0.8 h to 2.5 h for selexipag and from 6.2 h to 13.5 h for ACT-333679 [15]. Steady-state conditions are reached within 3 days and no relevant accumulation in plasma, either of parent compound or ACT-333679, occurred at steady state [18].

In vitro experiments have shown that: (i) selexipag and ACT-333679 undergo oxidative metabolism by the cytochrome P450 (CYP) enzymes, CYP2C8 and CYP3A4; (ii) UDP-glucuronosyltransferase (UGT) enzymes, UGT1A3 and UGT2B7, are involved in the metabolism of ACT-333679; (iii) both selexipag and ACT-333679 are substrates of the organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3; and (iv) selexipag is a substrate of P-glycoprotein (P-gp), and its active metabolite is a substrate of breast cancer resistance protein [15]. The present study was designed to investigate the effects of gemfibrozil, a strong inhibitor of CYP2C8 [19, 20] and rifampicin, an inducer of CYP2C8 [21, 22], on the PK of selexipag and ACT-333679 in healthy male subjects.

Methods

Subject eligibility

Subjects were eligible to participate in the study if they were male, between 18 and 55 years of age and with a body mass index (BMI) between $18.0 \, kg \, m^{-2}$ and $28.0 \, kg \, m^{-2}$. All subjects were healthy, based on examinations performed during the screening visit, with no history of significant disease and not taking any medications, including over-the-counter drugs and herbal medicines such as St John's wort. Further, subjects were not enrolled if they were allergic/hypersensitive to study drugs or were otherwise judged to be unsuitable to participate in the study. Prior to any study-related procedures, all subjects signed the informed consent form after receiving a full explanation of the study.

Clinical study design

The clinical study was approved by the Ethikkommission II, Bismarckallee 8–12, 23795 Bad Segeberg, Germany, on 10 May 2016, reference number 055/16 II, and by the Federal Institute for Drugs and Medical Devices, the German health authorities. The study was conducted in accordance with the Declaration of Helsinki principles, International Council for Harmonization and Good Clinical Practice guidelines, and applicable regulations and laws.

The study consisted of two parts that were conducted according to an open-label, randomized, two-treatment,

two-way crossover design. In parts I and II, subjects received a single dose of 400 μg selexipag (UPTRAVI®, 200 μg film-coated tablet, Actelion Pharmaceuticals Ltd) at least 30 min before a light breakfast either alone (treatment A) or concomitantly with gemfibrozil (GEVILON®, 600 mg film-coated tablet, Pfizer Ltd) (treatment B, part I) or rifampicin (EREMFAT®, 600 mg film-coated tablet, RIEMSER Pharma GmbH) (treatment B, part II). During treatment B in part I, subjects received 600 mg gemfibrozil twice daily on days 1 to 9 in the morning and evening, at least 30 min prior to breakfast and dinner. On the morning of day 4, selexipag 400 μg was administered concomitantly. In part II, once-daily 600 mg rifampicin was administered at least 30 min before a light breakfast on days 1 to 9, with concomitant selexipag 400 μg on the morning of day 7.

Serial blood samples for the analysis of selexipag and ACT-333679 were collected in lithium–heparin tubes for 72 h or 144 h (treatment B, part I only) after administration of selexipag. In order to prevent degradation of selexipag and ACT-333679 in the plasma, exposure to light was minimized and sample preparation was conducted under yellow light.

Based on standard deviations (SDs) observed for area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) and maximum observed plasma concentration (C_{max}) in a previous study [23], a sample size of 16, and assuming a geometric mean ratio of 1.00, it was estimated that the 90% confidence interval (CI) of the geometric mean ratio (treatment B/treatment A) would be 0.64 to 1.56 for the $AUC_{0-\infty}$ and 0.62 to 1.62 for the C_{max} of selexipag, and 0.68 to 1.47 and 0.73 to 1.36 for the $AUC_{0-\infty}$ and C_{max}, respectively, of ACT-333679. In order to compensate for any potential dropouts, in each part of the study 20 subjects were enrolled.

Analysis of selexipag and ACT-333679 concentrations in plasma

The concentrations of selexipag and its active metabolite in the plasma were measured using a previously described validated liquid chromatography method with tandem mass spectrometry (LC–MS/MS) [23] with slight modifications. To 50 μl of plasma, 200 μl of internal standard (stable isotope-labelled selexipag and ACT-333679) solution was added. After vortex mixing and centrifugation, the supernatant was evaporated under nitrogen and the residue reconstituted in 100 μl acetonitrile/water (1:1 v/v), vortex mixed, and 20 μl of the supernatant was injected onto the LC–MS/MS. The chromatographic system consisted of a pump, autosampler, degasser and analytical column (Kinetex C18, 50 × 3.0 mm ID, 2.6 μm (Phenomenex, Brechbühler, Schlieren, Switzerland). Mobile phases consisted of water containing 0.1% formic acid (v/v) and acetonitrile containing 0.1% formic acid (v/v). Mass spectrometric detection (API 5000, AB Sciex, Brugg, Switzerland) was performed with a turbo ion spray operating in positive-ion mode at 600°C.

For both analytes, the lower limit of quantification (LLOQ) was 0.01 ng ml^{-1} and the method was linear from 0.01 ng ml^{-1} up to 20.0 ng ml^{-1}. The concentrations of the analytes were calculated by the internal standardization method – i.e. using the peak area ratio of analyte to internal standard.

The performance of the method was monitored using quality control samples of known concentrations. Precision [percentage coefficient of variation (% CV)] of the assay was ≤5.6% for selexipag and ≤6.7% for ACT-333679. The interassay accuracy ranged from −3.2% to 1.8% for selexipag and from −2.1% to 3.8% for ACT-333679.

PK calculations

Noncompartmental analysis of selexipag and ACT-333679 plasma concentrations was performed using Phoenix WinNonlin (version 6.4, Pharsight Corporation, Mountain View, CA, USA). The measured individual plasma concentrations of selexipag and ACT-333679 were used to obtain C_{max} and the time to reach C_{max} (t_{max}) directly. AUC from zero until the last quantifiable concentration (AUC_{0-t}) was calculated according to the linear trapezoidal rule, using the measured concentration–time values above the LLOQ. $AUC_{0-\infty}$ was calculated by combining AUC_{0-t} and AUC_{extra}. AUC_{extra} represents an extrapolated value obtained by C_t/λ_z, where C_t is the last plasma concentration measured above the LLOQ and λ_z represents the terminal elimination rate constant determined by log-linear regression analysis of the measured plasma concentrations of the terminal elimination phase. The $t_{1/2}$ of selexipag and its metabolite was calculated as: ln $2/\lambda_z$.

Statistical analysis of PK variables

PK variables were analysed, providing geometric means and corresponding 95% CIs for AUC, C_{max} and $t_{1/2}$, whereas the median and range are shown for t_{max}.

The effects of gemfibrozil and rifampicin on the AUC, C_{max} and $t_{1/2}$ of selexipag and ACT-333679 were explored using the ratio of the geometric means and 90% CI of selexipag plus gemfibrozil or rifampicin (treatment B) as the test treatment vs. selexipag alone (treatment A) as the reference treatment. The log-transformed values were analysed by mixed-effects models, including treatment, sequence and period as fixed effects and subject as random effect. Differences between treatments for t_{max} were explored using the Wilcoxon signed-rank test, providing the median differences and corresponding 90% CIs.

Safety

The safety of the subjects was monitored by the recording of vital signs [supine systolic and diastolic blood pressure (SBP, DBP) and pulse rate] and 12-lead electrocardiogram (ECG), physical examination, clinical chemistry and haematology tests and adverse events (AEs) reporting. Safety results were analysed descriptively by treatment. For this, in each part, treatment B was divided into treatment B1, corresponding to gemfibrozil or rifampicin alone – i.e. day 1 to the morning of day 4 (gemfibrozil) or day 7 (rifampicin), and treatment B2, corresponding to gemfibrozil or rifampicin plus selexipag.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [24], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [25, 26].

Results

Gemfibrozil

All 20 enrolled subjects completed part I of the study as per protocol and were evaluable for PK. Of the enrolled subjects, 18 were Caucasian and two Asian. The mean age was 28.8 years (range 18–54 years) and mean BMI was 23.82 kg m^{-2} (range 18.1–27.9 kg m^{-2}).

Single-dose mean selexipag plasma concentrations increased when coadministered with gemfibrozil (Figure 1). Based on geometric mean ratios, the increase was 1.4-fold (90% CI 1.1, 1.7) and 2.0-fold (90% CI 1.7, 2.3) for C_{max} and $AUC_{0-\infty}$, respectively. No effect on t_{max} was observed but $t_{1/2}$ was 50% longer in the presence of gemfibrozil (Table 1). When compared with selexipag, the gemfibrozil-induced increase in the plasma concentrations of the active metabolite, ACT-333679, was more pronounced (Figure 1). Together, a 3.6-fold (90% CI 3.1, 4.3) increase in C_{max} and a doubling of $t_{1/2}$ resulted in an 11.1-fold (90% CI 9.2, 13.4) increase in the $AUC_{0-\infty}$ for ACT-333679. Coadministration of gemfibrozil also delayed attainment of peak ACT-333679 concentrations by 3.5 h (Table 1). A graph showing individual changes in the $AUC_{0-\infty}$ for selexipag and ACT-333679 for the two treatments is presented in Figure 2.

Rifampicin

Nineteen out of 20 enrolled subjects completed part II of the study as per protocol and were included in the PK analysis. One subject withdrew informed consent during treatment B1 (rifamipicin treatment alone) after completing treatment A. At the time of withdrawing consent, the subject had ongoing AEs of a moderate hypoesthesia and a mild ear discomfort. Of the enrolled subjects, 19 were Caucasian and one African American. The mean age at screening was 35.7 years (range 18–54 years) and the mean BMI was 24.41 kg m^{-2} (range 18.4–27.9 kg m^{-2}).

Single-dose mean selexipag plasma concentrations increased when coadministered with rifampicin (Figure 3). Based on geometric mean ratios, the increase was 1.76-fold (90% CI 1.44, 2.15) for C_{max} and 1.25-fold for $AUC_{0-\infty}$ (90% CI 1.11, 1.41). No effect on median t_{max} was observed but $t_{1/2}$ was 34% shorter in the presence of rifampicin (Table 2). Figure 3 illustrates that, when compared with selexipag alone, the mean plasma concentrations of ACT-333679 were initially slightly higher but from 2 h after administration onwards, the mean plasma concentrations of ACT-333679 were lower and decreased below the LLOQ earlier. Although a 1.3-fold (90% CI 1.1, 1.6) increase in the C_{max} of ACT-333679 was observed when compared with selexipag alone, total exposure to the metabolite ($AUC_{0-\infty}$) decreased by half (90% CI 0.45, 0.59). In the presence of rifampicin, the C_{max} of the metabolite was attained 2 h earlier and the $t_{1/2}$ was 63% shorter (Table 2). A graph with individual changes in the $AUC_{0-\infty}$ for selexipag and ACT-333679 for the two treatments is shown in Figure 4.

Safety

No serious AEs or AEs leading to study drug discontinuation were reported.

In part I, following administration of selexipag on day 1 in treatment A, the only frequently reported AE was headache (14 subjects, 70.0%). One subject reported headache during the days when gemfibrozil was administered alone (treatment B1). Following concomitant administration of selexipag and gemfibrozil on day 4 in treatment B2, headache was reported in 18/20 (90%) subjects. In treatment B2 (selexipag and gemfibrozil), all subjects experienced at least

Selexipag — ACT-333679
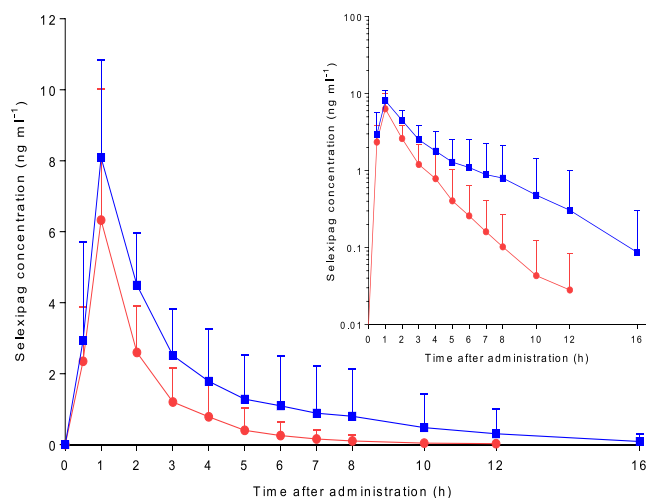
(●) 400 μg selexipag alone; (■) 600 mg gemfibrozil twice daily

Figure 1

Arithmetic mean (± standard deviation) plasma concentration–time profile of selexipag and ACT-333679 after administration of 400 μg selexipag alone and with 600 mg gemfibrozil twice daily; $n = 20$
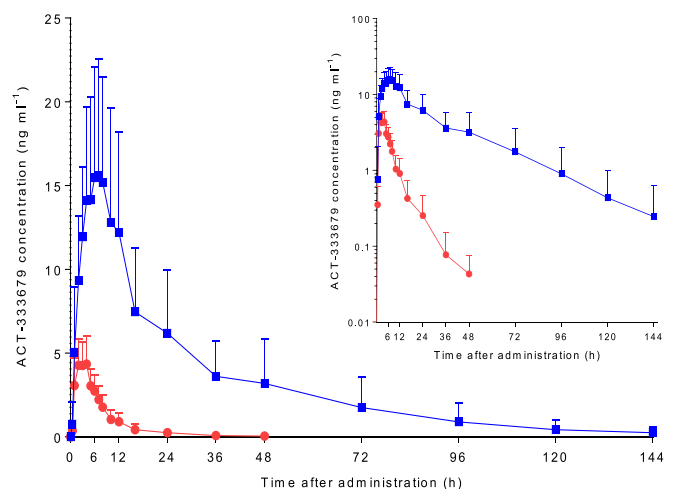
Table 1

Summary of the pharmacokinetic (PK) variables of selexipag and its active metabolite, ACT-333679, after administration of 400 µg selexipag alone (treatment A) and with 600 mg gemfibrozil twice daily (treatment B), and geometric mean ratios for the comparison of treatments ($n = 20$)

	Treatment A	Treatment B	Geometric mean ratio Treatment B/A
Selexipag			
C_{max} [ng ml^{-1}]	5.57	7.71	1.39
	4.40, 7.04	6.49, 9.17	1.12, 1.72
t_{max} [h]	1.00	1.00	0.00
	0.50, 1.00	0.50, 2.00	0.00, 0.01
$AUC_{0-\infty}$ [ng*h ml^{-1}]	10.47	20.47	1.96
	8.40, 13.05	16.17, 25.92	1.65, 2.32
$t_{1/2}$ [h]	1.66	2.50	1.51
	1.34, 2.05	2.12, 2.96	1.21, 1.87
ACT-333679			
C_{max} [ng ml^{-1}]	4.74	17.21	3.63
	4.07, 5.51	14.18, 20.89	3.06, 4.31
t_{max} [h]	3.02	7.02	3.54
	1.00, 4.02	3.00, 12.00	2.56, 4.60
$AUC_{0-\infty}$ [ng*h ml^{-1}]	35.93	398.44	11.090
	30.71, 42.04	303.72, 522.71	9.20, 13.36
$t_{1/2}$ [h]	11.04	24.03	2.18
	8.87, 13.73	21.12, 27.35	1.75, 2.72

Treatment A: selexipag (400 µg single dose); treatment B: selexipag (400 µg single dose) + gemfibrozil (600 mg twice daily). Data for PK variables are presented as the geometric mean [95% confidence interval (CI)], and for t_{max} the median (range). Data for the ratio are presented as the geometric means and 90% CI, except for t_{max}, for which the median difference and 90% CI are presented. $AUC_{0-\infty}$, area under the plasma concentration–time curve from zero to infinity; C_{max}, maximum plasma concentration; $t_{1/2}$, apparent terminal half-life; t_{max}, time to reach C_{max}
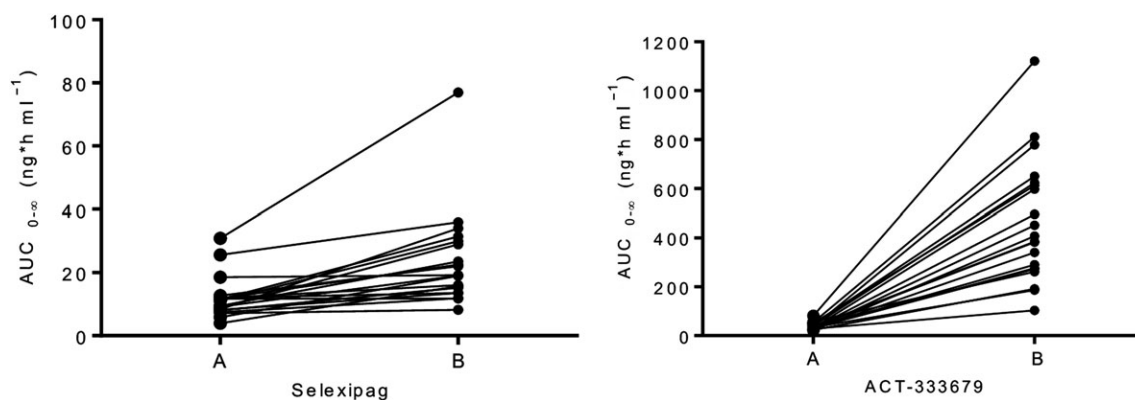
Figure 2

Individual area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) values (ng*h ml^{-1}) of selexipag and ACT-333679, after administration of 400 µg selexipag alone (A) and with 600 mg gemfibrozil twice daily (B); $n = 20$

one AE, compared with 75% in treatment A (selexipag) and 15% in treatment B1 (gemfibrozil). No AEs of nausea and vomiting were reported following administration of selexipag or gemfibrozil alone. However, the majority of subjects treated with the combination of selexipag and gemfibrozil reported nausea (15/20 subjects, 75%) and vomiting (12/20 subjects, 60%) (Table 3). Most reported AEs were of mild or moderate intensity. Two subjects had severe intensity AEs (headache) during treatment B2. All but two

AEs (dyspepsia and nasopharyngitis in treatment B2) were considered by the investigator to be treatment related. All reported AEs resolved by the end of the study.

In part II, following administration of selexipag on day 1 in treatment A, the most frequently reported AE was headache (eight subjects, 40.0%). Other AEs included nausea and limb discomfort (two subjects each, 10.0%). Following concomitant administration of selexipag and rifampicin on day 7 in treatment B2, headache was the
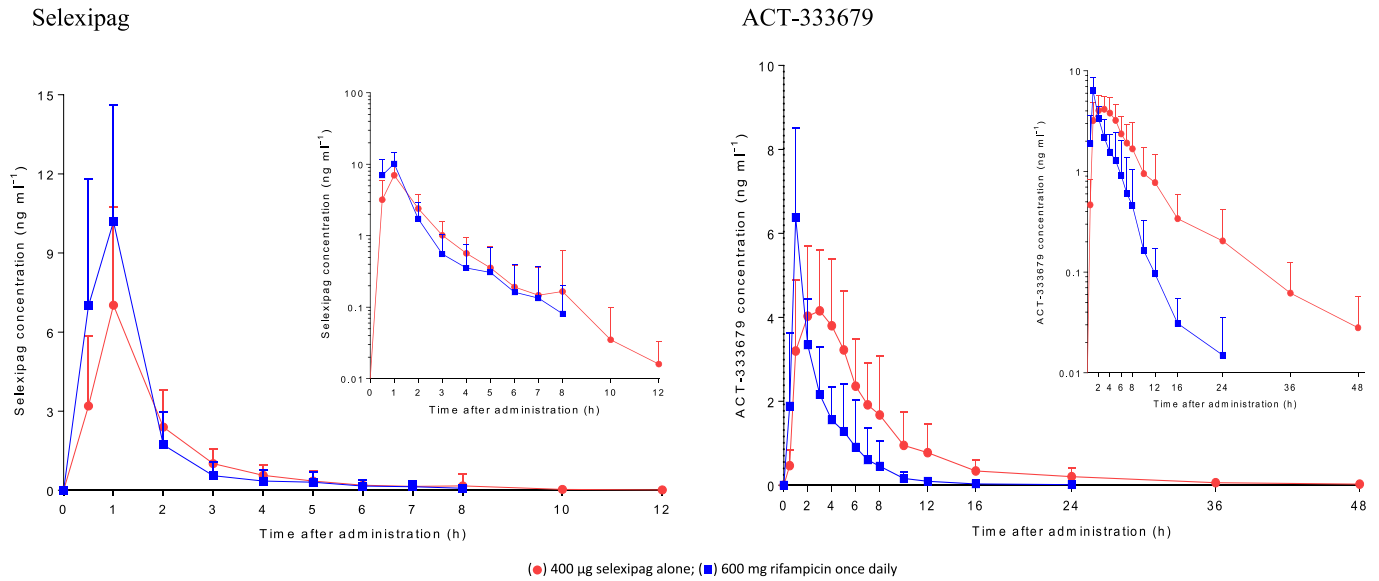
Selexipag   ACT-333679

(●) 400 μg selexipag alone; (■) 600 mg rifampicin once daily

Figure 3

Arithmetic mean (± standard deviation) plasma concentration–time profile of selexipag and ACT-333679 after administration of 400 μg selexipag alone and with 600 mg rifampicin once daily; $n = 19$

Table 2

Summary of pharmacokinetic (PK) variables of selexipag and its active metabolite, ACT-333679, after administration of 400 μg selexipag alone (treatment A) and with 600 mg rifampicin once daily (treatment B), and geometric mean ratios for the comparison of treatments ($n = 19$)

	Treatment A	Treatment B	Geometric mean ratio Treatment B/A
Selexipag			
C_{max} [ng ml^{-1}]	6.30	11.14	1.76
	5.04, 7.87	9.49, 13.08	1.44, 2.15
t_{max} [h]	1.00	1.00	−0.01
	0.95, 1.02	0.50, 1.00	−0.24, 0.00
$AUC_{0-\infty}$ [ng*h ml^{-1}]	10.98	13.83	1.25
	9.05, 13.34	11.78, 16.24	1.11, 1.41
$t_{1/2}$ [h]	1.93	1.27	0.66
	1.46, 2.55	1.04, 1.54	0.52, 0.83
ACT-333679			
C_{max} [ng ml^{-1}]	4.74	6.18	1.30
	3.99, 5.64	5.20, 7.35	1.07, 1.57
t_{max} [h]	3.00	1.00	−2.00
	0.98, 8.00	1.00, 6.00	−2.50, −1.49
$AUC_{0-\infty}$ [ng*h ml^{-1}]	31.50	16.26	0.52
	25.50, 38.92	13.65, 19.38	0.45, 0.59
$t_{1/2}$ [h]	11.87	4.39	0.37
	9.70, 14.54	3.54, 5.44	0.29, 0.469

Treatment A: selexipag (400 μg single dose); treatment B selexipag (400 μg single dose) + rifampicin (600 mg once daily). Data for PK variables are presented as the geometric mean [95% confidence interval (CI)], and for t_{max} the median (range). Data for the ratio are presented as the geometric means and 90% CI, except for t_{max}, for which the median difference and 90% CI are presented. $AUC_{0-\infty}$, area under the plasma concentration–time curve from zero to infinity; C_{max}, maximum plasma concentration; $t_{1/2}$, apparent terminal half-life; t_{max}, time to reach C_{max}
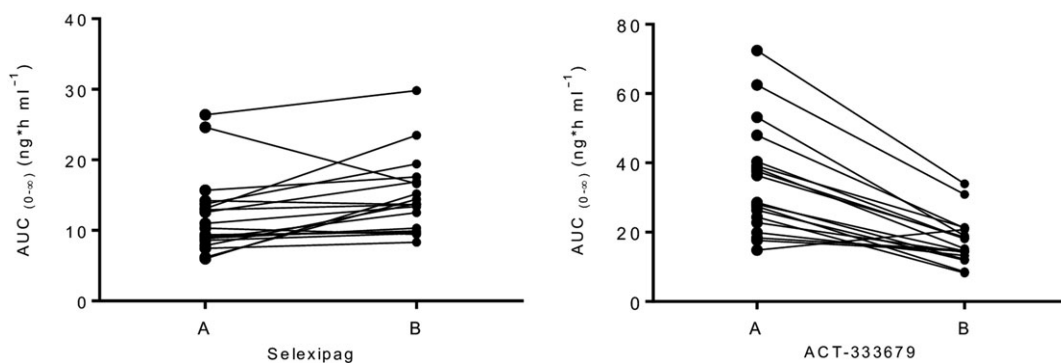
Figure 4

Individual area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) values (ng*h ml^{-1}) of selexipag and ACT-333679, after administration of 400 µg selexipag alone (A) and with 600 mg rifampicin once daily (B); $n = 19$

Table 3

Summary of adverse events (AEs) reported during part I by frequency

Preferred term	Treatment A ($n = 20$)			Treatment B1 ($n = 20$)			Treatment B2 ($n = 20$)			Overall ($n = 20$)		
	AEs	n	%	AEs	n	%	AEs	n	%	AEs	n	%
Number of subjects with at least one AE		15	75		3	15		20	100		20	100
Number of different AEs	3			2			11			12		
Total number of AEs	17			3			74			94		
Headache	15	14	70	1	1	5	20	18	90	36	18	90
Nausea							18	15	75	18	15	75
Vomiting							23	12	60	23	12	60
Pain in extremity	1	1	5				4	3	15	5	3	15
Diarrhoea				2	2	10	1	1	5	3	3	15
Myalgia							2	2	10	2	2	10
Pain in jaw							2	2	10	2	2	10
Presyncope							1	1	5	1	1	5
Dyspepsia							1	1	5	1	1	5
Arthralgia							1	1	5	1	1	5
Neck pain	1	1	5							1	1	5
Nasopharyngitis							1	1	5	1	1	5

Treatments: A = selexipag; B1 = gemfibrozil; B2 = gemfibrozil + selexipag. Data are expressed as AE (number of adverse events), n (number of subjects experiencing at least one AE) and % (percentage of subjects with at least one AE)

most frequently reported AE (five subjects, 26.3%) followed by nausea (two subjects, 10.5%). There was a tendency for fewer subjects reporting AEs after coadministration of rifampicin *vs.* selexipag alone (Table 4). All reported AEs were of mild or moderate intensity. All but two AEs (in treatment B1) were considered by the investigator to be treatment related.

No clinically relevant mean changes from baseline in clinical laboratory variables, vital signs or ECG parameters were observed.

One subject randomized to treatment sequence B–A in part I had a vasovagal reaction on day 4 in treatment B2 (gemfibrozil + selexipag). The subject had an ongoing AE of headache at the time of the vasovagal reaction. The vital signs measured at the time of this reaction showed an SBP of 85 mmHg (predose on day 4: 122 mmHg), DBP of 59 mmHg (predose on day 4: 86 mmHg) and pulse rate of 46 bpm (predose on day 4: 56 bpm). The AE was mild in intensity and considered by the investigator to be treatment related. The AE resolved in 10 min without sequelae.

Table 4

Summary of adverse events (AEs) reported during part II by frequency

Preferred term	Treatment A (n = 20)			Treatment B1 (n = 20)			Treatment B2 (n = 19)			Overall (n = 20)		
	AEs	n	%	AEs	n	%	AEs	n	%	AEs	n	%
Number of subjects with at least one AE		8	40		4	20		6	31		12	60
Number of different AEs	5			8			3			13		
Total number of AEs	16			8			9			33		
Headache	8	8	40				5	5	26.3	13	9	45
Nausea	2	2	10				2	2	10.5	4	4	20
Vomiting	3	1	5				2	1	5.3	5	2	10
Limb discomfort	2	2	10							2	2	10
Hypoesthesia				1	1	5				1	1	5
Somnolence				1	1	5				1	1	5
Flatulence				1	1	5				1	1	5
Back pain				1	1	5				1	1	5
Pain in extremity	1	1	5							1	1	5
Fatigue				1	1	5				1	1	5
Peripheral oedema				1	1	5				1	1	5
Ear discomfort				1	1	5				1	1	5
Generalized pruritus				1	1	5				1	1	5

Treatments: A = selexipag; B1 = rifampicin; B2 = rifampicin + selexipag. Data are expressed as AE (number of adverse events), n (number of subjects experiencing at least one AE) and % (percentage of subjects with at least one AE)

Discussion

Based on *in vitro* studies, the metabolism of selexipag is driven by initial hydrolysis to its active metabolite, ACT-333679. In addition, both CYP2C8 and CYP3A4 are involved in the metabolism of selexipag and ACT-333679. The metabolism of ACT-333679 also involves UGT1A3 and -2B7 [15].

The aim of the present study was to investigate the effect of gemfibrozil, a strong inhibitor of CYP2C8, (part I) and the effect of rifampicin, an inducer of CYP2C8, (part II) on the PK of selexipag and ACT-333679. The study results provide important information on the contribution of the CYP2C8 pathway to the disposition of selexipag and its active metabolite, ACT-333679.

The results of part I showed that gemfibrozil had a comparatively small effect on the exposure to selexipag (less than 2-fold on any PK variable) but increased the $AUC_{0-\infty}$ of ACT-333679 by 11-fold.

The results of the drug–drug interaction (DDI) study with selexipag and lopinavir/**ritonavir**, a strong inhibitor of OATP1B1 and OATP1B3, CYP3A4 and P-gp, showed a 2-fold increase in exposure to selexipag in the presence of lopinavir/ritonavir [23]. The nature of this increase (increase in C_{max} and AUC but no significant change in $t_{1/2}$) suggests that the interaction was the result of inhibition of OATP1B1, OATP1B3 or P-gp rather than of CYP3A4. The increase in selexipag exposure (2.0-fold increase in AUC, 1.4-fold

increase in C_{max}, and 1.5-fold increase in $t_{1/2}$) in the presence of gemfibrozil could be explained by the inhibitory effect of gemfibrozil on CYP2C8 and also by its effect on OATP1B1, as gemfibrozil is also a moderate inhibitor of the latter [27].

The results of the DDI study with selexipag and lopinavir/ritonavir [23] showed only a minor change in C_{max} and no change in the AUC for ACT-333679, indicating the low dependency of ACT-333679 on CYP3A4 enzymes and OATP transporters. Therefore, the pronounced effect of gemfibrozil on AUC, C_{max} and $t_{1/2}$ of ACT-333679 is most likely due to the inhibition of CYP2C8 and indicates the importance of the CYP2C8 pathway in the disposition of ACT-333679.

Strong *in vivo* interactions of gemfibrozil with CYP2C8 substrates are due to mechanism-based irreversible inactivation of CYP2C8 by the gemfibrozil 1-O-β-glucuronide metabolite. CYP2C8 activity can only be regained by *de novo* synthesis of the enzyme, and the full CYP2C8 activity recovers gradually within 3–4 days after cessation of gemfibrozil at the clinically used therapeutic dose of 600 mg twice daily [22, 28].

In part II of the study, differential effects of rifampicin were observed, with a small increase in total exposure (AUC) to selexipag and a decrease in the AUC of ACT-333679. Inhibitory and inductive effects of rifampicin on the PK of the compounds have been also observed for other drugs such as bosentan [29] and repaglinide [30, 31].

Rifampicin is an inducer of CYP2C8 and UGT enzymes, a major inducer of CYP3A4 and a strong inhibitor of OATP1B1 and OATP1B3 [32, 33]. The 1.8-fold increase in the C_{max} of selexipag in the presence of rifampicin is likely to be mediated via inhibition of OATP1B1 and OATP1B3 by rifampicin. The shorter $t_{1/2}$ of selexipag in the presence of rifampicin is indicative of CYP enzyme induction but the inhibitory effects of rifampicin outweighed the inductive effects and led to a small increase in the AUC for selexipag. In the presence of rifampicin, the $t_{1/2}$ of ACT-333679 was significantly shortened (by 63%), resulting in a 50% reduction in exposure (AUC) to ACT-333679, which is likely to have been due to the inductive effect of rifampicin on CYP2C8. The initial small increase in the plasma concentrations of ACT-333679 (C_{max}), which was also observed after coadministration of lopinavir/ritonavir, is most likely mediated via inhibition of OATP1B1 and OATP1B3 by rifampicin. After cessation of rifampicin treatment, full CYP2C8 activity recovers gradually within 3–4 days [28].

In the present study, selexipag and rifampicin doses were taken simultaneously. The $t_{1/2}$ of rifampicin at a dose of 600 mg is 2.5 h [34], whereas the $t_{1/2}$ of CYP2C8 is 22–23 h *in vitro* [35], and *in vivo* [28], indicating that rifampicin-induced inhibition dissipates more rapidly than rifampicin-mediated CYP2C8 induction after discontinuation of rifampicin. Therefore, if rifampicin and selexipag are not taken simultaneously, the full effect of CYP2C8 induction on the PK of selexipag will most likely be larger than that observed in the present study. Taking into consideration the results of the DDI study with lopinavir/ritonavir [23], which showed a minor effect of OATP1B1 and OATP1B3 inhibition on the PK of ACT-333679, the full inductive effect of rifampicin on the PK of ACT-333679 (responsible for the majority of the drug effect) in such situations probably will not be markedly different from that observed in the present study.

In both parts I and II, the AEs reported in treatment A (selexipag) and treatment B2 (gemfibrozil or rifampicin + selexipag) were consistent with the known adverse drug reactions reported for selexipag (i.e. headache, diarrhoea, nausea, vomiting, jaw pain, myalgia, pain in the extremities, arthralgia and flushing) [15]. However, the incidence and/or intensity of AEs reported after concomitant administration of selexipag and gemfibrozil were significantly higher than those following the administration of selexipag alone, which is in line with the observed increase in exposure to the active metabolite ACT-333679, the major contributor to the effect of selexipag in humans.

In the pivotal phase 3 Prostacyclin (PG12) Receptor agonist In Pulmonary arterial HypertensiON (GRIPHON) study, selexipag was initiated in PAH patients at a dose of 200 µg twice daily, and the dose was increased weekly in twice-daily increments of 200 µg until the individual highest tolerated dose (HTD) was attained [12]. The maximum allowed dose of selexipag was 1600 µg twice daily [12, 36]. In this study, no statistically significant PK/pharmacodynamic relationship with important safety parameters such as vital signs could be established [37]. The occurrence of typical prostacyclin-related AEs, such as headache, nausea and vomiting, was lower in placebo- than in selexipag-treated patients. However, consistent with the concept of up-titration to the

individual HTD, no relevant difference could be determined between low and high exposures to selexipag and ACT-333679 [37]. In the present DDI study, the 11-fold increase in exposure to ACT-333679 in the presence of gemfibrozil was associated with a significant increase in the incidence and/or intensity of prostacyclin-related AEs. Concomitant treatment with gemfibrozil and selexipag in PAH patients would increase the exposure to ACT-333679 to levels higher than those at the highest allowed dose of 1600 µg twice daily, and up-titration of selexipag in these patients would probably not be possible. Therefore, concomitant treatment of selexipag and strong inhibitors of CYP2C8 should be avoided.

The effect of moderate inhibitors of CYP2C8 (e.g. clopidogrel, deferasirox, teriflunomide) [38–41] on the exposure to selexipag and ACT-333679 has not yet been studied but, based on the observed effect of gemfibrozil on the PK of ACT-333679 in the present study, a PK interaction between moderate inhibitors of CYPC28 and selexipag cannot be excluded. Therefore, caution is required when using these drugs concomitantly with selexipag.

In conclusion, there was an increased incidence and/or intensity of typical prostacyclin-related AEs when selexipag was administered concomitantly with gemfibrozil. Based on the observed interactions with gemfibrozil and rifampicin, concomitant administration of selexipag with strong inhibitors of CYP2C8 (e.g. gemfibrozil) must be avoided and dose adjustments should be considered when selexipag is administered with CYP2C8 inducers.

Competing Interests

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