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Naoki Katayama[1], Keiichi Odagiri[2,*], Akio Hakamata[1], Chiaki Kamiya[1], Shinya Uchida[3], Shimako Tanaka[3], Naoki Inui[1], Noriyuki Namiki[3], Koichiro Tatsumi[4], Hiroshi Watanabe[1*]

Author Affiliations:

1. Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

2. Hamamatsu University Hospital, Hamamatsu, Japan

3. Department of Pharmacy Practice and Science, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

4. Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan

*Corresponding author:

Keiichi Odagiri, MD, PhD

Center for Clinical Research, Hamamatsu University Hospital, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan

Tel.: +81-53-435-2006

E-mail: kodagiri@hama-med.ac.jp

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What is already known about this subject

- Selexipag is a safe, well-tolerated, long-acting oral IP receptor agonist that improves hemodynamic parameters in patients with pulmonary arterial hypertension and reduces the mortality and morbidity.
- The strong cytochrome CYP2C8 inhibitor gemfibrozil increased the $AUC_{0-\infty}$ of ACT-333679, an active metabolite of selexipag, by 11-fold. Thus, FDA warned that strong CYP2C8 inhibitors are contraindicated with selexipag because of a drug–drug interaction.
- The CYP2C8 inhibitor clopidogrel also increased the levels of ACT-333679, the active metabolite of selexipag by 2.7-fold in Europeans. However, the clinical impact of clopidogrel on the pharmacokinetics of selexipag and ACT-333679 has not been explored in the Japanese population.

What this study adds

- The $AUC_{0-\infty}$ of ACT-333679 was increased by 1.90-fold with the co-administration of clopidogrel without relevant effect on selexipag PK.
- When selexipag was administered 1 day after clopidogrel was discontinued, the increase in $AUC_{0-\infty}$ of ACT-333679 was 1.37-fold. suggesting that although the

inhibitory effect of clopidogrel on CYP2C8 was reduced, it persisted for at least 1 day after withdrawal.

– Selexipag and clopidogrel can be used concomitantly with dose adjustment or reducing the dosing frequency in Japanese clinical settings.

Abstract

Aims: The strong cytochrome P450 (CYP) 2C8 inhibitor gemfibrozil has been demonstrated to increase the area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) of ACT-333679, an active metabolite of selexipag, by 11-fold. Similarly as gemfibrozil, the CYP2C8 inhibitor clopidogrel also increases clopidogrel increased ACT-333679 concentration by 1.9-fold after a single loading dose (300 mg once daily) and 2.7-fold after repeated treatment with the maintenance dose (75 mg once daily) in Europeans. However, the effects of clopidogrel on the pharmacokinetics of selexipag and ACT-333679 have not been fully elucidated in Japanese population.

Methods: We investigated the effect of clopidogrel on the pharmacokinetics of selexipag and ACT-333679 in 14 healthy Japanese volunteers.

Results: The concomitant administration of clopidogrel with selexipag did not influence the maximum concentration (C_{max}) and $AUC_{0-\infty}$ of selexipag, whereas it significantly increased $AUC_{0-\infty}$ of ACT-333679 by approximately 1.90-fold (90% confidence interval (CI) 1.69–2.14) without changing C_{max}. When selexipag was administered 1 day after clopidogrel was discontinued, the increase in $AUC_{0-\infty}$ of ACT-333679 was 1.37-fold (90% CI 0.93–2.02), suggesting that although the inhibitory effect of clopidogrel on CYP2C8 was reduced, it persisted for at least 1 day

after withdrawal.

Conclusion: Our results demonstrated the impact of clopidogrel on the pharmacokinetics of selexipag and its active metabolite and suggested that selexipag should be carefully prescribed with clopidogrel with dose adjustment or reducing the dosing frequency in Japanese clinical settings.

Introduction

Selexipag [2-[4-[[5,6-di(phenyl)pyrazin-2-yl]-propan-2-ylamino]butoxy]-Nmethylsulfonylacetamide] is an orally available prostacyclin receptor (IP receptor) agonist that is chemically distinct from prostacyclin and that has been approved for the treatment of pulmonary arterial hypertension (PAH).[1]

Management of PAH remains challenging.[2] Intravenous prostacyclin I_2 therapy, while associated with decreased mortality, has practical limitations and requires significant lifestyle modifications. Selexipag is a safe, well-tolerated, long-acting oral IP receptor agonist that improves hemodynamic parameters in patients with PAH and reduces the mortality and morbidity of PAH. [3-7]

Selexipag is predominantly hydrolyzed to its active metabolite ACT-333679 by liver carboxylesterase-1 (CES-1, 77.0%), and a lesser extent by CES-2 (9.99%) ACT-333679 (Figure 1).[8] After oral administration, selexipag is rapidly absorbed and hydrolyzed to its pharmacologically active metabolite, ACT-333679, and it is also metabolized into inactive metabolites by cytochrome P450 (CYP) 3A4.[3] The oral bioavailability of selexipag is 49.4%.[3] ACT-333679 is metabolized mainly by CYP2C8 and to a lesser extent by CYP3A4, leading to the formation of hydroxylated and dealkylated products. Furthermore, the metabolism of ACT-333679 involves

CES and uridine diphosphate-glucuronosyltransferase (UGT) 1A3 and UGT2B7. Organic anion transporting polypeptide (OATP) 1B1 and OAPT1B3 also participate in their disposition.[9] Because both selexipag and ACT-333679 are mainly excreted via hepatic clearance, the exposure to selexipag and ACT-333679 is increased in patients with hepatic impairment.[10]

Both selexipag and ACT-333679 bind selectively with high affinity to the IP receptor.[11] In vitro, ACT-333679 exerted vasodilatory and anti-vascular remodeling effects.[12] Therefore, ACT-333679 has been identified as the major metabolite responsible for the effects of selexipag in humans.

Previous drug–drug interaction (DDI) studies with several CYP and OATP substrates have been reported. Selexipag did not influence the pharmacokinetics of warfarin (a CYP2C9 substrate) and exposure to midazolam (a CYP3A substrate), and lopinavir/ritonavir (an inhibitor of OATP1B1/1B3) did not affect the pharmacokinetis of selexipag and ACT-333679.[13-15] In contrast, Bruderer *et al.* reported a crucial DDI between selexipag and the strong CYP2C8 inhibitor and OATP1B1 inhibitor gemfibrozil. Gemfibrozil increased the area under the plasma concentration–time curve from 0 to infinity ($AUC_{0-\infty}$) of ACT-333679 by 11-fold.[16] A recent European study revealed that concomitant administration with the

CYP2C8 inhibitor clopidogrel also increased ACT-333679 exposure by 2.7-fold.[17] For this reason, the Pharmaceuticals and Medical Devices Agency has recommended a reduction of the dose or dosing frequency for selexipag when co-administered with clopidogrel. Although the extrapolation of foreign clinical trial data might be acceptable, reliable evidence in the Japanese population is needed. Furthermore, the influence of clopidogrel withdrawal on DDIs between clopidogrel and selexipag has not yet been explored. Therefore, this study aimed to clarify the effect of clopidogrel on the pharmacokinetics (PK) of selexipag and ACT-333679 after simultaneous administration and 1 day after withdrawal in Japanese volunteers

Methods

Ethics statement

The study protocol complied with the Declaration of Helsinki and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. The Ethics Committee of Hamamatsu University School of Medicine (1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka, Japan) approved the study in April 2018 (approved number 17-309). Written informed consent was provided by all participants. The study was registered at the UMIN Clinical Trials Registry (unique

identifier: UMIN 000032266).

Study participants

Healthy male volunteers participated in the study. Participants were recruited from June 2018 to March 2019 via open public recruiting. The inclusion criteria were age > 20 years, normal hemoglobin levels and platelet counts, normal liver and renal function, absence of any disease requiring treatment, absence of drug allergy and idiosyncrasy, and no dependence on drugs and/or alcohol.

Study design

The study was an open-label, nonrandomized, two-arm design, conducted at Hamamatsu University Hospital Translational Research Unit, Hamamatsu, Japan. The PK of selexipag without clopidogrel was evaluated in all subjects, after which participants were divided into two groups: an interaction study group and a withdrawal study group. Participants in the interaction study group were given one dose of 300 mg of clopidogrel (Plavix®, Sanofi-Aventis, France) on day 1, followed by 75 mg of the drug daily on days 2–4. On the morning of day 4, 200 μg of selexipag (Uptravi®, Nippon Shinyaku, Japan) were concomitantly administered with

clopidogrel after an overnight fast (Figure 2A), and the PK of selexipag and clopidogrel was evaluated. In the withdrawal study group, participants were given one dose of 300 mg of clopidogrel on day 1, followed by 75 mg daily on days 2–3 (Figure 2B). On the morning of day 4, 200 μg of selexipag alone without clopidogrel was administered after an overnight fast, and the PK of selexipag and clopidogrel was evaluated. The use of other drugs was not allowed from 1 week before each pharmacokinetic study.

Pharmacokinetic assessments

Serial blood samples were collected from each participant before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, and 48 h after selexipag administration. These samples were collected into EGTA 2Na-containing tubes and centrifuged at 3000 rpm for 10 min at 4°C. The samples were stored at −80°C until analysis.

The plasma concentrations of selexipag and its metabolite, ACT-333679, were determined using reversed-phase liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). An ultra-performance liquid chromatography system (ACQUITY UPLC I-Class; Waters Co., Milford, MA, USA) was connected to triple quadrupole tandem mass spectrometer (Xevo TQ-S; Waters Co.) coupled with an

electrospray ionization interface operated in the positive ion mode. Homo-sildenafil (10 ng) was added to plasma samples as an internal standard and extracted using solid-phase extraction (Oasis® HLB 96-well μElution Plate; Waters Co.). HPLC was performed using an analytical column (ZORBAX RRHD Eclipse plus C18 column, 2.1 mm × 50 mm, 1.8 μm, Agilent Technologies, Waldbronn, Germany) with the mobile phase (5 mM ammonium acetate:acetonitrile = 60/40) delivered at a flow rate of 0.4 mL/min. The mass transitions were as follows: m/z 497.1→302 for selexipag, m/z 420.2→302.2 for ACT-333679, and m/z 489.6→113.2 for homo-sildenafil. The lower limit of detection was 0.1 ng/mL for both selexipag and ACT-333679. The accuracies of the analyses ranged 91.3%–104% for selexipag and 81.4%–111% for ACT-333679. The precision was less than 9.2% for selexipag and less than 14% for ACT-333679.

The plasma concentrations of clopidogrel, clopidogrel carboxylic acid, and clopidogrel acyl glucuronide were determined using LC/MS. Briefly, clopidogrel-d3 (100 ng) as an internal standard and 0.1% formic acid (1000 μL) were added to the plasma sample (300 μL). After centrifugation at 10,000 rpm for 10 min, the supernatant was applied to a solid-phase extraction plate. The eluate (10 μL) was injected into the chromatographic system for analysis. LC was performed using an analytical column

(Symmetry C18; 5 μm, 2.1 mm × 150 mm, Waters Co.) with the mobile phase (0.1% formic acid/acetonitrile= 60/40) delivered at flow rate of 0.3 mL/min. The mass spectrometer (Micromass ZQ; Waters Co.) was operated in the positive ion mode at m/z 322.2 for clopidogrel, 308.2 for clopidogrel carboxylic acid, 484.2 for clopidogrel acyl glucuronide, and 327.2 for clopidogrel-d3. The limit of quantitation was 1 ng/mL.

Safety

The safety of subjects was monitored by recording supine systolic and diastolic blood pressure and pulse rates. Blood and biochemical examinations and adverse effects were also reported. The safety analysis was performed descriptively in each pharmacokinetic study.

Statistical analysis

Pharmacokinetic variables are presented as the arithmetic mean and standard deviation or the geometric mean and the geometric coefficient of variation, excluding the time to maximum plasma concentration (t_{max}) which is presented as the median and range. The pharmacokinetic variables were compared using the Wilcoxon signed rank test. P-values less than 0.05 were considered statistically significant.

Statistical analyses were performed using GraphPad Prism Version 6 (GraphPad Software Inc., San Diego, CA, USA).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries at http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY. [18]

Results

Study participant characteristics

Fourteen participants were enrolled in this study. The study had two parts: interaction and withdrawal studies. After baseline PK for selexipag was evaluated, participants were assigned to either the interaction (clopidogrel was concomitantly administered with selexipag on the day of the pharmacokinetic study; age range, 22–25 years; body mass index range, 18.0–26.6 kg/m^2) or withdrawal study group (clopidogrel was discontinued 1 day before the pharmacokinetic study; age range, 21–26 years; body mass index range, 18.6–28.1 kg/m^2).

Effect of clopidogrel on selexipag PK

In the interaction study, the concomitant administration of clopidogrel with selexipag had no relevant effect on the PK of selexipag regarding both the maximum concentration (C_{max}) and $AUC_{0-\infty}$ (Figure 3A). Conversely, clopidogrel significantly increased $AUC_{0-\infty}$ of ACT-333679 by 1.90-fold, whereas C_{max} was unchanged (Figure 3B). Clopidogrel did not influence t_{max} for either selexipag or ACT-333679. Clopidogrel prolonged the ACT-33679 elimination half-life ($t_{1/2}$), whereas $t_{1/2}$ for selexipag was not affected. In the withdrawal study, after baseline PK for selexipag was evaluated, clopidogrel was administered for 3 days to six participants because one participant was excluded because of a protocol violation. Following the withdrawal of clopidogrel for 24 h, selexipag was administered without clopidogrel. As shown in Figure 4A, there was no difference in the selexipag PK regarding C_{max} and $AUC_{0-\infty}$ between the baseline period and after the withdrawal of clopidogrel. t_{max} and $t_{1/2}$ of selexipag were also unchanged versus the baseline value. Conversely, clopidogrel increased $AUC_{0-\infty}$ of ACT-333679 by 1.37-fold even after the withdrawal of clopidogrel for 24 h, whereas C_{max} was unchanged (Figure 4B). Detailed pharmacokinetic variables are presented in Tables 1 and 2.

Plasma concentration of clopidogrel and its metabolites

In the interaction study, the plasma clopidogrel concentration was detected in two participants (Figure 5A). The plasma concentrations of clopidogrel carboxylic acid and clopidogrel acyl glucuronide peaked at 1 h after the concomitant administration of clopidogrel with selexipag (Figure 5B–C). In the withdrawal study, neither clopidogrel nor clopidogrel Acyl-β-D-glucuronide were detected in plasma (Figure 5D and F). Only clopidogrel carboxylic acid was detected, but its concentration was considerably lower than that in the interaction study (Figure 5E). The detailed pharmacokinetic variables of clopidogrel and its metabolites are described in Table 3.

Safety

No serious adverse events or adverse events, including excessive blood pressure decreases, leading to study drug discontinuation were observed.

Discussion

In this study, we initially investigated the effect of clopidogrel, an inhibitor of CYP2C8, on the PK of selexipag and ACT-333679 in Japanese volunteers and clarified

the effects of the discontinuation of clopidogrel treatment for 24 h on these DDIs. The results demonstrated that a clinically used dose of clopidogrel increased $AUC_{0-\infty}$ of ACT-333679 by 1.90-fold, and the magnitude of the impact of clopidogrel on ACT-333679 was weaker in Japanese subjects than that in European subjects.[17] We also revealed that the impact of clopidogrel on the PK of selexipag and ACT-333679 decreased after the withdrawal of clopidogrel for 24 h.

DDIs involving CYP inhibition are clinically important because of the increase in serious adverse event risk caused by increased concentrations of CYP substrates. More than 50 CYP family enzymes have been identified in humans, and CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 are involved in the metabolism of more than 90% of all drugs.[19] Among these CYPs, CYP2C8 metabolizes more than 100 drugs, and many drugs, including gemfibrozil and clopidogrel, have been identified as CYP2C8 inhibitors.[20] Indeed, a previous DDI study that evaluated the impact of the strong CYP2C8 inhibitor gemfibrozil on the PK of selexipag and ACT-333679 revealed that gemfibrozil increased the ACT-333679 $AUC_{0-\infty}$ by approximately 11-fold, whereas it has a small effect on the selexipag plasma concentration.[16]

Clopidogrel is a prodrug. When clopidogrel is administered orally, up to 90% of the

dose is hydrolyzed by CES-1 to its inactive metabolite clopidogrel carboxylic acid and subsequently glucuronidated to clopidogrel acyl-β-D-glucuronide by UGT2B7.[20, 21] The remaining clopidogrel is converted to its active metabolite via two-step oxidation, primarily by CYP2C19 and CYP3A4.[22] Clopidogrel acyl-β-D-glucuronide acts as a mechanism-based inhibitor of CYP2C8.[20] Clinical DDI studies illustrated that clopidogrel significantly increased the plasma concentration of CYP2C8 substrates such as montelukast, pioglitazone, and repaglinide.[23-25]

Genetic polymorphism of CYP enzymes can affect their enzymatic activities. Three alleles, namely CYP2C8*2, *3, and *4, are major variants associated with decreased enzymatic activity.[20] The variant allele frequencies of CYP2C8 have obvious ethnic differences; that is, the Japanese population has extremely low frequencies of these three alleles (<1% of Japanese) compared with those in Caucasian and African-American populations.[26, 27] Therefore, the results of DDI studies conducted overseas cannot always be extrapolated to Japanese populations. Thus, we conducted the current clinical DDI study targeting Japanese subjects and revealed that concomitant administration of clopidogrel with selexipag significantly increased $AUC_{0-\infty}$ of ACT-333679 by 1.90-fold without relevant effects on that of selexipag. We are convinced that clopidogrel can act as an inhibitor based on its effect on ACT-

333679 levels in Japanese subjects.

After the mid-2000s, glucuronide metabolites such as gemfibrozil 1-O-β- glucuronide attracted attention as causes of DDIs based on time-dependent inhibition, and the time-dependent CYP2C8 inhibitory effect of clopidogrel acyl-β-D-glucuronide was secondly identified in 2014.[23, 28] Previous clinical DDI studies indicated that inhibition of CYP2C8 by gemfibrozil occurs within 1 h after oral administration, with the effect persisting for at least 48 h after drug discontinuation.[29, 30] In the current study, concomitant administration of clopidogrel with selexipag significantly increased $AUC_{0-\infty}$ of ACT-333679 by approximately 1.90-fold. After the withdrawal of clopidogrel for 24 h, $AUC_{0-\infty}$ of ACT-333679 was increased by 1.37-fold, suggesting the inhibitory effect of clopidogrel acyl-β-D-glucuronide on CYP2C8 could persist for at least 24 h after the withdrawal of clopidogrel although the impact on $AUC_{0-\infty}$ of ACT-333679 was attenuated time-dependently. This result agrees with the half-life of the CYP2C8 which estimated to be 22 ± 6 h. [30]

Clopidogrel carboxylic acid, which is a precursor of clopidogrel acyl-β-D-glucuronide, was detected in all blood samples. These findings reflect that all participants of this study were administered clopidogrel. The inhibitory effect of clopidogrel on CYP2C8 persisted for 1 day after clopidogrel discontinuation when clopidogrel acyl-β-D-

glucuronide was not detected in the withdrawal study. The findings suggest that clopidogrel acyl-β-D-glucuronide could inhibit CYP2C8 irreversibly; however, the activity of CYP2C8 could be recovered, at least partially, in the absence of clopidogrel acyl-β-D-glucuronide.

Our study had several study limitations. First, UGT2B7 is involved in the glucuronidation of ACT-333679; however, we did not evaluate the influence of competitive inhibition of UGT2B7 by clopidogrel carboxylic on ACT-333679 concentrations. Furthermore, no previous study assessed the effect of UGT2B7 inhibition on selexipag PK. Further studies, such as those for assessing PBPK models and human DDIs, are required to reveal this issue. Second, we did not assess CYP2C8 polymorphisms in our participants. Polymorphisms of CYP enzymes have great effects on their enzymatic activities and the impact of CYP inhibitors.[31] Axelsen *et al.* assessed the effect of the CYP2C8 genotype on DDIs between selexipag and clopidogrel. They found that the effect of clopidogrel on ACT-333679 was higher in the *1/*3 group than in the wild type group. However, AUC of ACT-333679 was comparable among the *1/*1, *1/*3 and *1/*4 alleles, because the baseline ACT-333679 concentration was lower in the *1/*3 group than in the other group.[17]

Previous reports indicated that the frequencies of the CYP2C8*3 and P404A variant alleles, which are associated with reduced enzymatic activity were extremely low in the Japanese subjects (<0.001 and <0.007%, respectively).[26, 32] This ethnic difference in the variant allele frequencies of CYP2C8, especially CYP2C8*3, could explain the difference of magnitude of the impact of clopidogrel-selexipag DDIs between European and Japanese populations. Third, a previous study reported that clopidogrel had a greater impact on repaglinide $AUC_{0-\infty}$ at the 300-mg loading dose than at the maintenance dose (75 mg once daily).[23] We did not assess the difference of the inhibitory effect on CYP2C8 between the loading and maintenance doses of clopidogrel. However, a recent clopidogrel-selexipag DDI study, which assessed the effect of clopidogrel (300 mg single dose or 75 mg once daily) on selexipag (200 μg twice daily) indicated that the impact of the maintenance dose of clopidogrel on ACT-333679 was equivalent to, or stronger than that of the loading dose. [17]

In conclusion, the concomitant administration of clopidogrel with selexipag significantly increased $AUC_{0-\infty}$ of ACT-333679 by 1.90-fold; however, the impact of clopidogrel on the PK of ACT-333679 was small compared with that of gemfibrozil. We also revealed that the magnitude of the impact of DDIs between clopidogrel and

selexipag was weaker in our Japanese population than previously observed in European research. Our results support that selexipag and clopidogrel can be used concomitantly with careful monitoring and appropriate dose adjustment or reduction of the dosing frequency in Japanese clinical settings.

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Contributors

All authors have critically reviewed the manuscript, agreed to be accountable fir aspects of the work and given final approval of the version to be published. In addition, N.K. made substantial contributions to the data acquisition, design, analysis, interpretation and wrote the manuscript. K.O. made substantial contributions to the design, data acquisition, acquisition of funding and wrote the manuscript. A.H. made substantial contributions to the design and data acquisition. C.K. made substantial contributions to the data acquisition. S.U. made substantial

contributions to the data acquisition, analysis, interpretation and wrote the manuscript. S.T. made substantial contributions to the analysis and interpretation. N.I. wrote the manuscript. N.N. made substantial contributions to the analysis and interpretation. K.T. made substantial contributions to conception, design. H.W. made substantial contributions to conception, design, acquisition of funding and wrote the manuscript, and had direct clinical responsibility for subject including in the study.

Conflict of interest statement

Keiichi Odagiri received a lecture fee from Nippon-Shinyaku. Koichiro Tatsumi received lecture fees from Actelion Pharmaceuticals and Nippon Boehringer Ingelheim. Hiroshi Watanabe received lecture fees from Daiichi-Sankyo, Pfizer, and Bayer and advisory board fees from Nippon-Shinyaku. The other authors have no conflicts of interest to declare.

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Data availability statement

The data that support the findings of this study have not been made openly available.

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Table 1. Comparison of the pharmacokinetic variables of selexipag and ACT-333679 in the control (selexipag alone) and inhibitory phases (selexipag plus 75 mg of clopidogrel on day 4 after pretreatment with 300 mg of clopidogrel on day 1 followed by 75 mg per day on days 2–3)

	Arithmetic mean (standard deviation) Geometric mean (geometric coefficient of variation)		Geometric mean ratio (90% confidence interval)	P value
Variable	Control phase	Inhibitory phase		
Selexipag				
C_{max} (ng/mL)	5.55 (1.32) 5.38 (0.24)	4.80 (1.96) 4.43 (0.41)	0.822 (0.67–1.01)	0.219
t_{max} (h)	1.50 (1.00–2.00)	1.50 (1.00–2.00)	NA	0.484
$t_{1/2}$ (h)	0.596 (0.18) 0.577 (0.29)	0.715 (0.20) 0.690 (0.28)	1.20 (1.01–1.41)	0.109
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	10.97 (3.11) 10.50 (0.28)	10.03 (3.67) 9.36 (0.37)	0.892 (0.77–1.04)	0.297
$AUC_{0\text{-}\infty}$ (ng·h/mL)	10.97 (3.11) 10.50 (0.28)	10.04 (3.67) 9.37 (0.37)	0.891 (0.77–1.04)	0.297
CL/F (L/h)	20.12 (8.17) 19.04 (0.41)	23.20 (11.09) 21.36 (0.48)	1.12 (0.96–1.31)	0.157
ACT-333679				
C_{max} (ng/ml)	5.24 (1.55) 5.04 (0.30)	5.36 (2.28) 4.90 (0.43)	0.972 (0.82–1.16)	0.813
t_{max} (h)	2.00 (2.00–2.00)	2.00 (2.00–4.00)	NA	1.000

$t_{1/2}$ (h)	3.53 (0.95) 3.40 (0.27)	5.56 (1.13) 5.47 (0.20)	1.61 (1.31–1.98)	0.016
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	24.68 (11.09) 22.64 (0.45)	43.80 (14.31) 41.40 (0.33)	1.83 (1.59–2.10)	0.016
$AUC_{0\text{-}\infty}$ (ng·h/mL)	25.07 (11.30) 23.00 (0.45)	46.52 (16.15) 43.69 (0.35)	1.90 (1.69–2.14)	0.016

Data are presented as the arithmetic mean with standard deviation and the geometric mean with the geometric coefficient of variation, excluding t_{max} which is presented as the median and range. The geometric mean ratios between the two phases are presented with 90% confidence interval. C_{max}, peak plasma concentration; t_{max}, time-to-maximum plasma concentration; $t_{1/2}$, elimination half-life; CI, confidence interval; AUC, area under the plasma concentration-time curve; CL/F, clearance; NA, not available.

Table 2. Comparison of the pharmacokinetic variables of selexipag and ACT-333679 in the control (selexipag alone) and inhibitory phases (selexipag alone on day 4 after oral pretreatment with 300 mg of clopidogrel on day 1 followed by 75 mg per day on days 2–3)

	Arithmetic mean (standard deviation) Geometric mean (geometric coefficient of variation)		Geometric mean ratio (90% confidence interval)	P value
Variable	Control phase	Inhibitory phase		
Selexipag				
C_{max} (ng/mL)	6.04 (2.97) 5.51 (0.49)	6.19 (0.98) 6.13 (0.16)	1.11 (0.76–1.63)	0.563
t_{max} (h)	1.50 (1.00–2.00)	1.50 (1.00–2.00)	NA	0.424
$t_{1/2}$ (h)	0.782 (0.15) 0.770 (0.19)	0.813 (0.16) 0.800 (0.19)	1.04 (0.90–1.19)	0.844
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	13.53 (6.44) 12.34 (0.48)	14.23 (3.72) 13.85 (0.26)	1.12 (0.71–1.79)	0.688
$AUC_{0\text{-}\infty}$ (ng·h/mL)	13.55 (6.44) 12.34 (0.48)	14.23 (3.72) 13.85 (0.26)	1.12 (0.71–1.79)	0.688
CL/F (L/h)	17.67 (7.70) 16.21 (0.44)	14.83 (3.63) 14.44 (0.24)	0.891 (0.56–1.42)	0.563
ACT-333679				
C_{max} (ng/mL)	6.80 (3.06) 6.38 (0.45)	7.90 (2.38) 7.64 (0.30)	1.20 (0.99–1.45)	0.219
t_{max} (h)	3.50 (2.00–4.00)	3.00 (2.00–3.00)	NA	0.345

$t_{1/2}$ (h)	5.38 (2.88) 4.68 (0.54)	6.11 (1.31) 6.00 (0.21)	1.28 (0.77–2.14)	0.688
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	55.16 (32.49) 48.78 (0.59)	69.65 (25.53) 66.59 (0.37)	1.37 (0.97–1.93)	0.156
$AUC_{0\text{-}\infty}$ (ng·h/mL)	59.87 (36.22) 52.10 (0.61)	75.44 (31.59) 71.26 (0.42)	1.37 (0.93–2.02)	0.156

Data are presented as the arithmetic mean with standard deviation and the geometric mean with the geometric coefficient of variation, excluding t_{max} which is presented as the median and range. The geometric mean ratios between the two phases are presented with 90% confidence interval. C_{max}, peak plasma concentration; t_{max}, time-to-maximum plasma concentration; $t_{1/2}$, elimination half-life; CI, confidence interval; AUC, area under the plasma concentration-time curve; CL/F, clearance; NA, not available.

Table 3. Pharmacokinetic variables of clopidogrel and its metabolites clopidogrel carboxylic acid clopidogrel acyl-ß-D-glucuronide in interaction study

	Arithmetic mean (SD) Geometric mean (95% CI)
Variable	Interaction study
Clopidogrel	
C_{max} (ng/mL)	0.335 (0.58) 0.334 (1.73)
t_{max} (h)	0.00 (0.00–1.00)
$t_{1/2}$ (h)	n.c.
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	n.c.
$AUC_{0\text{-}U}$ (ng·h/mL)	n.c.
Clopidogrel carboxylic acid	
C_{max} (ng/ml)	1623 (182.6) 1614 (0.11)
t_{max} (h)	1.00 (0.50–1.50)
$t_{1/2}$ (h)	9.58 (1.93) 9.41 (0.20)
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	7136 (1454) 7005 (0.20)
$AUC_{0\text{-}A}$ (ng·h/mL)	8216 (1752) 8057 (0.21)
Clopidogrel acyl-ß-D-glucuronide	
C_{max} (ng/ml)	338.8 (64.8) 333.5 (0.19)
t_{max} (h)	1.00 (1.00–1.50)
$t_{1/2}$ (h)	4.28 (2.44) 3.77 (0.57)
$AUC_{0\text{-}24\ h}$ (ng·h/mL)	1127 (257.3) 1101 (0.23)
$AUC_{0\text{-}\infty}$ (ng·h/mL)	1171 (293.7) 1140 (0.25)

Data are presented as the arithmetic mean with standard deviation and the geometric mean with the geometric coefficient of variation, excluding t_{max} which is

presented as the median and range. Clopidogrel was detected in plasma in only two participants in the first part. C_{max}, peak plasma concentration; t_{max}, time-to-maximum plasma concentration; ; $t_{1/2}$, elimination half-life; AUC, area under the plasma concentration-time curve; CL/F, clearance; n.c., not calculated.

Figure Legends

Figure 1. Structures of selexipag and ACT-333679.

Figure 2. Study design. PK, pharmacokinetics; P.O, per os.

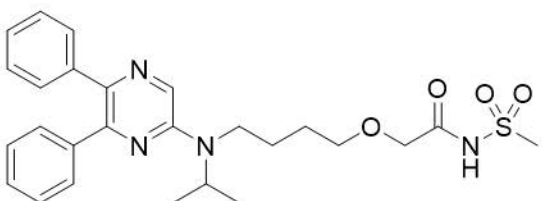
Figure 3. The effect of clopidogrel (300 mg on day 1 followed by 75 mg per day on days 2–4) on the plasma concentrations of selexipag (A) and ACT-333679 (B) in the interaction study. The pharmacokinetic study was conducted on day 4. Data are presented as geometric means with 90% confidence intervals. h, hour(s)

Figure 4. The effect of clopidogrel (300 mg on day 1 followed by 75 mg per day on days 2–3) on the plasma concentrations of selexipag (A) and ACT-333679 (B) in the withdrawal study. The pharmacokinetic study was conducted 1 day after clopidogrel discontinuation. Data are presented as geometric means with 90% confidence intervals. h, hour(s)

Figure 5. The mean plasma concentration-time profiles of clopidogrel (A, D), clopidogrel carboxylic acid (B, E), and clopidogrel acyl-β-D-glucuronide (C, F) in the

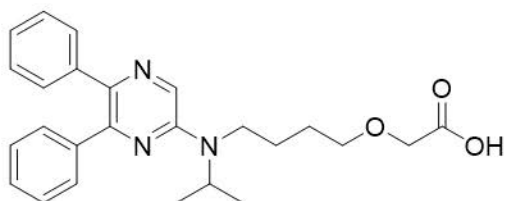
interaction (A–C, n = 7) and withdrawal studies (D–E, n = 6). In the interaction study, the plasma concentration of clopidogrel could be detected in only two participants. Data are presented as geometric means with 90% confidence intervals. h, hour(s)

selexipag

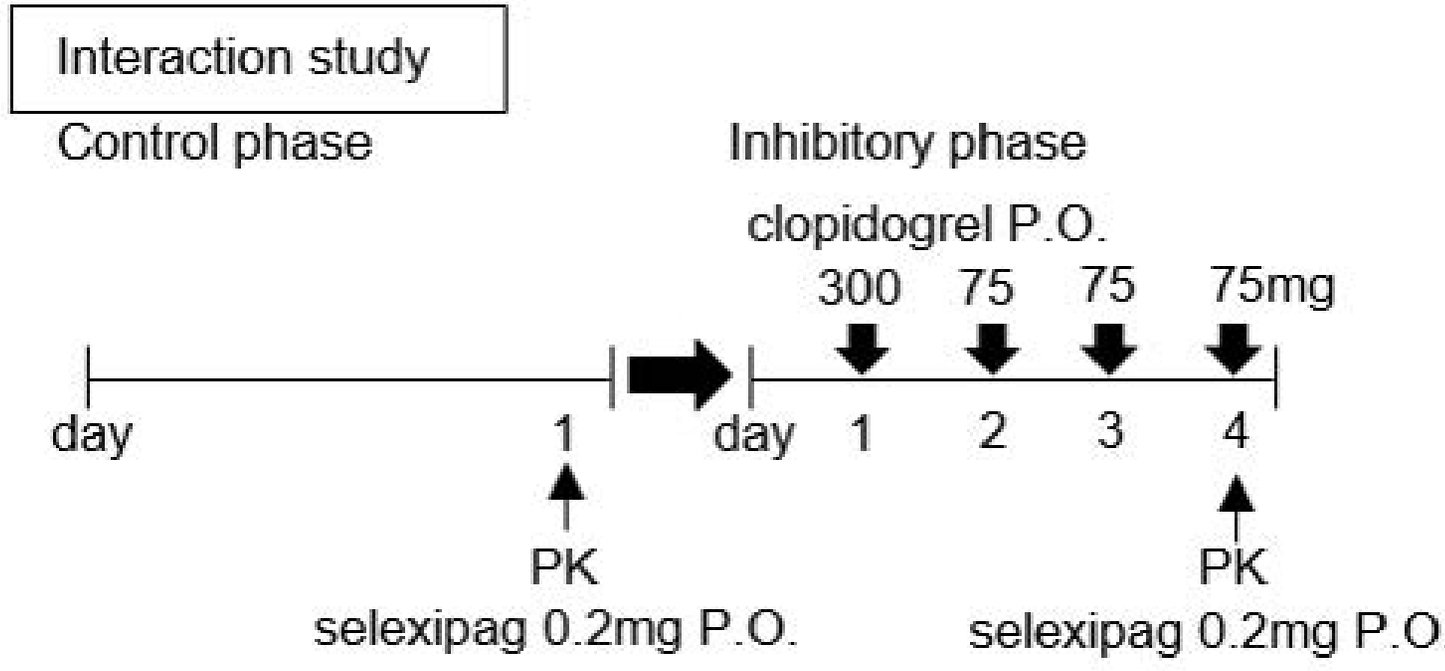


hydrolysis

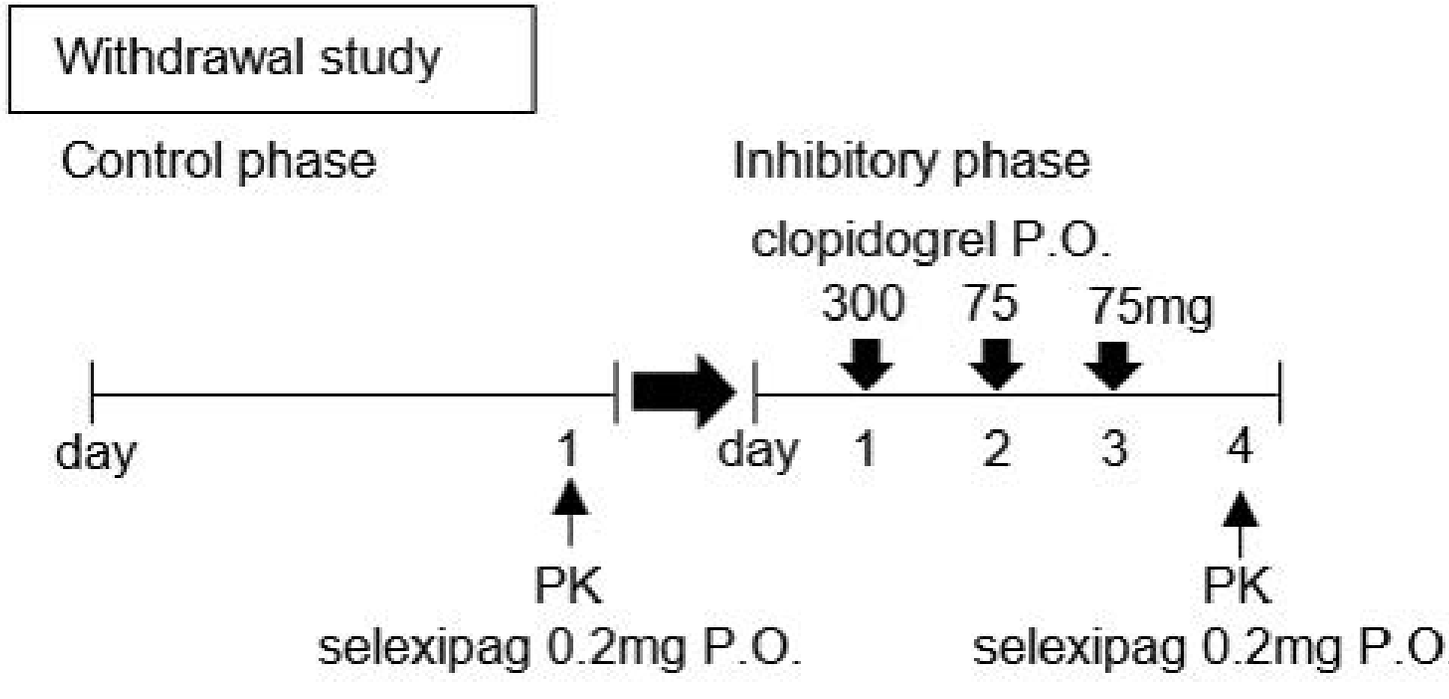
ACT-333679

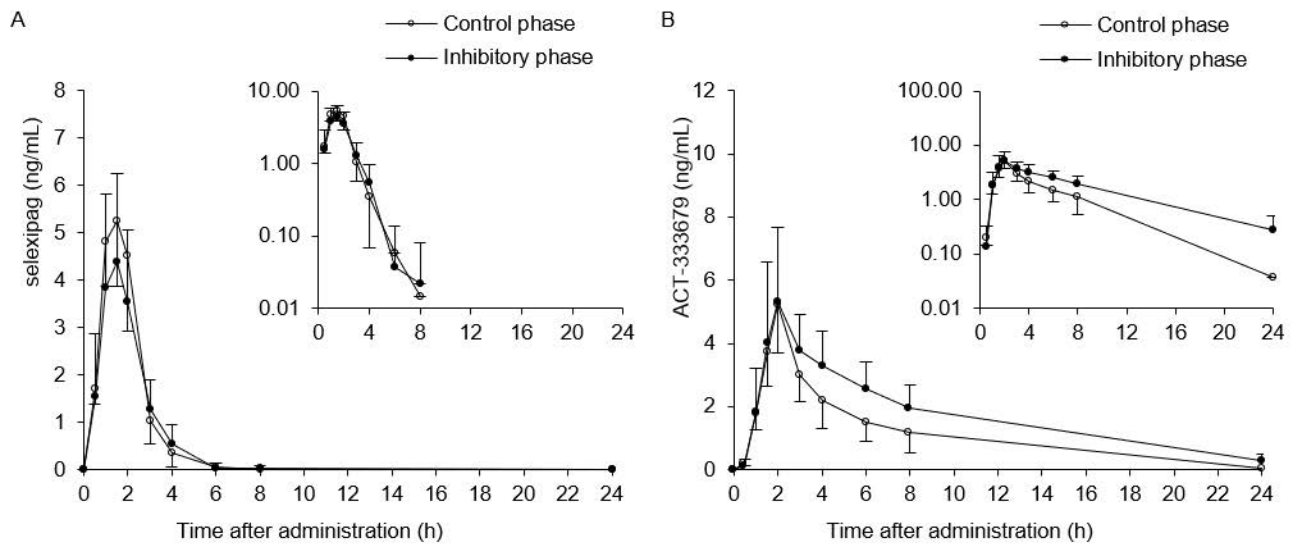


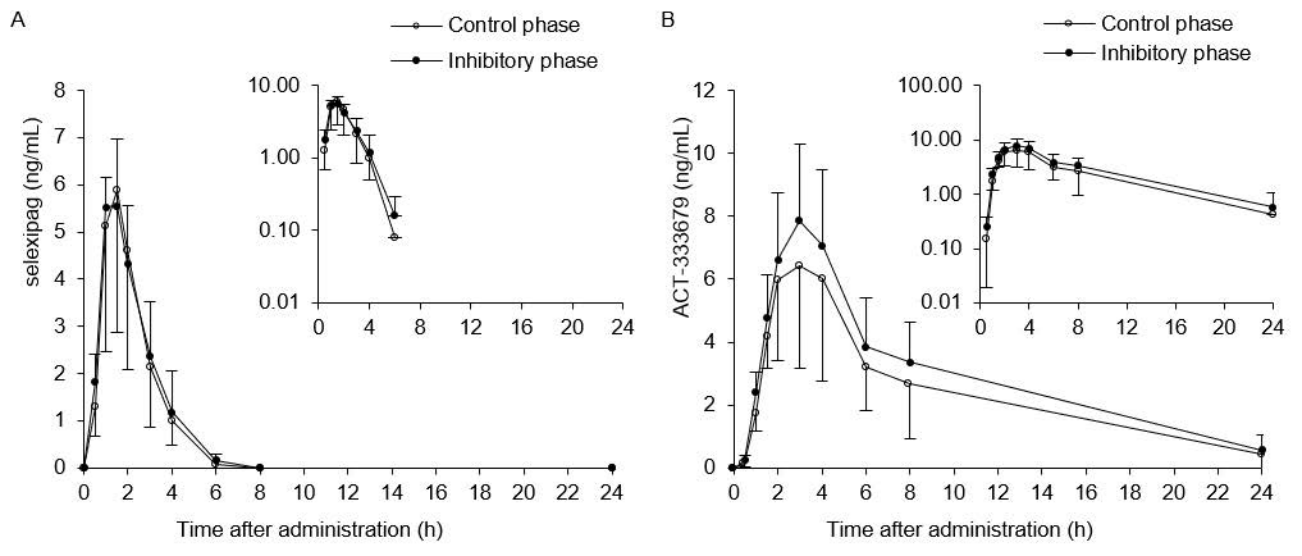
A


Interaction study
Control phase
Inhibitory phase
clopidogrel P.O.
300
75
75
75mg
day
1
day
1
2
3
4
PK
PK
selexipag 0.2mg P.O.
selexipag 0.2mg P.O.

B

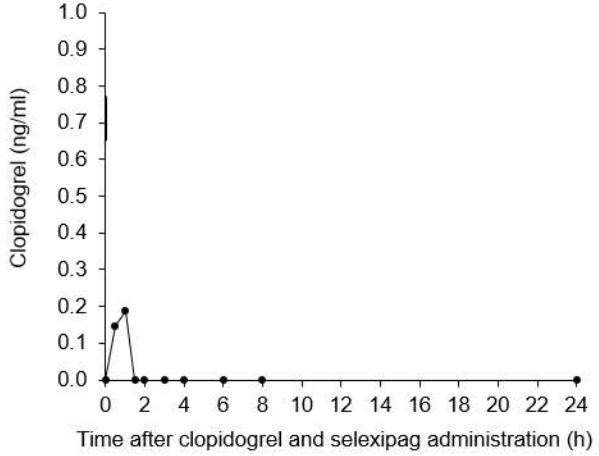

Withdrawal study
Control phase
Inhibitory phase
clopidogrel P.O.
300
75
75mg
day
1
day
1
2
3
4
PK
PK
selexipag 0.2mg P.O.
selexipag 0.2mg P.O.


A
Control phase
Inhibitory phase
selexipag (ng/mL)
Time after administration (h)
B
Control phase
Inhibitory phase
ACT-333679 (ng/mL)
Time after administration (h)


A
Control phase
Inhibitory phase
selexipag (ng/mL)
Time after administration (h)
B
Control phase
Inhibitory phase
ACT-333679 (ng/mL)
Time after administration (h)

Interaction study

A



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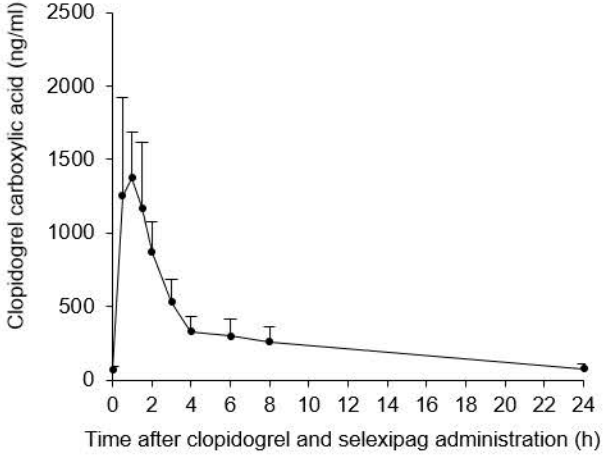

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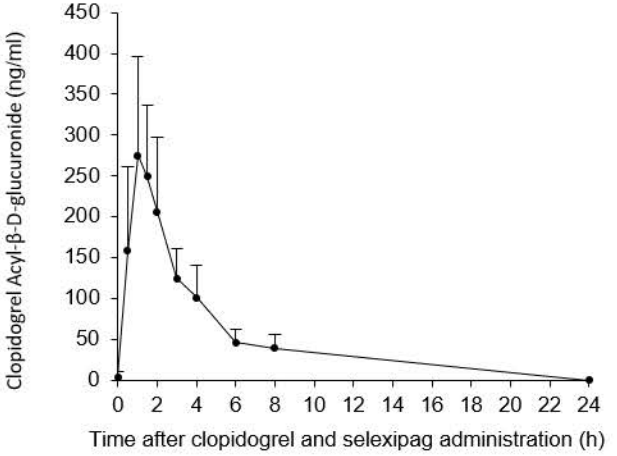
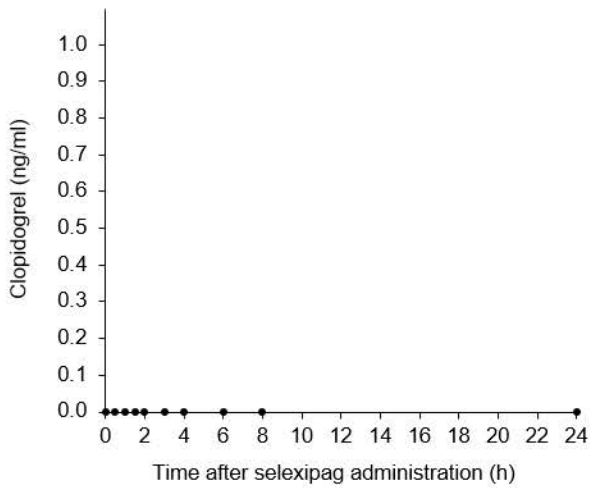
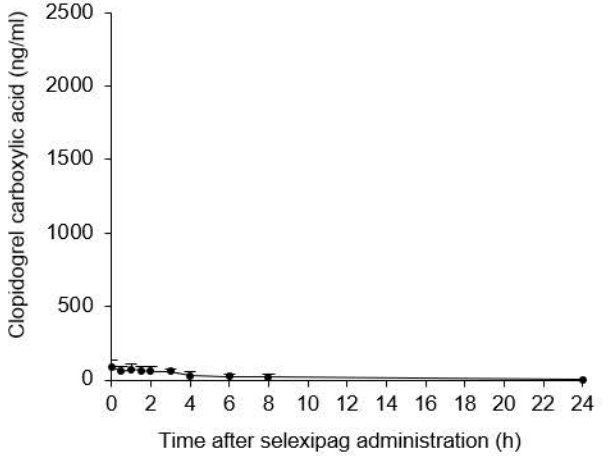


Withdrawal study

D



E



F