European Journal of Drug Metabolism and Pharmacokinetics (2019) 44:217–227
https://doi.org/10.1007/s13318-018-0521-7

ORIGINAL RESEARCH ARTICLE

In Vitro Assessment of Potential for CYP-Inhibition-Based Drug–Drug Interaction Between Vonoprazan and Clopidogrel

Mitsuhiro Nishihara[1] · **Hitomi Yamasaki**[2] · **Richard Czerniak**[3] · **Helen Jenkins**[4]

Published online: 25 October 2018

Abstract

Background and Objectives It was recently proposed that CYP-mediated drug–drug interactions (DDIs) of vonoprazan with clopidogrel and prasugrel can attenuate the antiplatelet actions of the latter two drugs. Clopidogrel is metabolized to the pharmacologically active metabolite H4 and its isomers by multiple CYPs, including CYP2C19 and CYP3A4. Therefore, to investigate the possibility of CYP-based DDIs, in vitro metabolic inhibition studies using CYP probe substrates or radiolabeled clopidogrel and human liver microsomes (HLMs) were conducted in this work.

Methods Reversible inhibition studies focusing on the effects of vonoprazan on CYP marker activities and the formation of the [^{14}C]clopidogrel metabolite H4 were conducted with and without pre-incubation using HLMs. Time-dependent inhibition (TDI) kinetics were also measured.

Results It was found that vonoprazan is not a significant direct inhibitor of any CYP isoforms ($IC_{50} \geq 16$ μM), but shows the potential for TDI of CYP2B6, CYP2C19, and CYP3A4/5. This TDI was weaker than the inhibition induced by the corresponding reference inhibitors ticlopidine, esomeprazole, and verapamil, based on the measured potencies (k_{inact}/K_I ratio and the $R2$ value). In a more direct in vitro experiment, vonoprazan levels of up to 10 μM (a 100-fold higher concentration than the plasma C_{max} of 75.9 nM after taking 20 mg once daily for 7 days) did not suppress the formation of the active metabolite H4 or other oxidative metabolites of [^{14}C]clopidogrel in a reversible or time-dependent manner. Additionally, an assessment of clinical trials and post-marketing data suggested no evidence of a DDI between vonoprazan and clopidogrel.

Conclusions The body of evidence shows that the pharmacodynamic DDI reported between vonoprazan and clopidogrel is unlikely to be caused by the inhibition of CYP2B6, CYP2C19, or CYP3A4/5 by vonoprazan.

Key Points

Vonoprazan is a potassium-competitive acid blocker drug approved in Japan for multiple indications.

It causes weak time-dependent inhibition of CYP2B6, CYP2C19, and CYP3A4/5, but it is not expected to cause CYP-based interactions at clinical doses and concentrations.

Vonoprazan did not exhibit significant inhibitory effects on the formation of the active metabolite of [^{14}C]clopidogrel, as $IC_{50} > 10$ μM, which is > 100× the plasma C_{max} of vonoprazan in a clinical setting. Thus, the recently reported pharmacodynamic interaction between vonoprazan and clopidogrel is not likely to be CYP mediated, and the reason for the pharmacodynamic interaction is unknown at this time.

An assessment of clinical trials and post-marketing data suggested no evidence of drug–drug interactions between vonoprazan and clopidogrel.

✉ Mitsuhiro Nishihara
mitsuhiro.nishihara@takeda.com

1 Global Drug Metabolism and Pharmacokinetics, Takeda Pharmaceuticals International Co., 35 Landsdowne Street, Cambridge, MA 02139, USA

2 Drug Disposition and Analysis, Research Division, Axcelead Drug Discovery Partners, Inc., Fujisawa, Kanagawa, Japan

3 Quantitative Clinical Pharmacology, Takeda Pharmaceuticals International Co., Cambridge, MA, USA

4 d3 Medicine, A Certara Company, London, UK

1 Introduction

Vonoprazan fumarate ("vonoprazan"), 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamine monofumarate, is an orally active potassium-competitive acid blocker (P-CAB) that has been approved in Japan for the treatment of gastroesophageal reflux disease, peptic ulcer, gastric ulcer, erosive esophagitis, and reflux esophagitis, and for use as an adjunct in *Helicobacter pylori* eradication [1, 2]. Nonclinical studies have established that vonoprazan has a rapid, potent, stable, and long-lasting acid-inhibitory effect resulting from the reversible inhibition of the gastric proton pump via competition with K^+ binding on the luminal surface of H^+,K^+-ATPase [3, 4]. These findings were also confirmed clinically, as vonoprazan caused rapid and profound suppression of gastric acid secretion in healthy male subjects over a 24-h period [5, 6].

The biotransformation of vonoprazan has been investigated in nonclinical [7, 8] and clinical studies [9], and four major metabolites, M-I, M-II, M-III, and M-IV-Sul, have been identified. None show pharmacological activity. Previous in vitro studies indicated that multiple metabolizing enzymes, including cytochrome P450 (CYP) 3A4, CYP2B6, CYP2C19, CYP2D6, and a non-CYP enzyme, sulfotransferase (SULT) 2A1, are involved in the primary metabolism of vonoprazan. CYP3A4 predominantly contributes to the metabolism of vonoprazan to the metabolites M-I, M-III, and *N*-demethylated vonoprazan (a presumed metabolite), while SULT2A1 transforms vonoprazan to vonoprazan *N*-sulfate, and CYP2C9 mediates the formation of M-IV-Sul from vonoprazan *N*-sulfate [8]. The contribution of CYP2C19 to the metabolism of vonoprazan was found to be small, so the influence of CYP2C19 genotype status on the pharmacokinetics of vonoprazan is considered to be minimal [10]. The involvement of multiple metabolic pathways could also help minimize the effects of coadministered CYP inhibitors or inducers on the pharmacokinetics of vonoprazan.

An assessment of clinical trials and post-marketing data suggested that, as yet, there is no evidence of a drug–drug interaction (DDI) between vonoprazan and clopidogrel or prasugrel (data on file). However, in a recent article, Kagami et al. reported that vonoprazan was an inhibitor of not only CYP3A4 but also CYP2C19, and suggested that a CYP-mediated DDI between vonoprazan and clopidogrel or prasugrel attenuated their antiplatelet function [11]. However, no evidence of any pharmacokinetic changes was provided, and the concentrations of clopidogrel, prasugrel, and their active thiol metabolites (H4 and R-138727 [(2*Z*)-{1-[(1*RS*)-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-sulfanylpiperidin-3-ylidene}ethanoic acid], respectively) were not measured to obtain direct evidence of metabolism/PK-based DDI in that study.

The objective of the present study was to investigate the potential for DDI between vonoprazan and clopidogrel from the point of view of their metabolism. The ability of vonoprazan to inhibit the major CYP enzymes (namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) in vitro in a direct (reversible) or time-dependent (irreversible) manner was investigated using pooled human liver microsomes (HLMs) with or without pre-incubation to determine the potential of vonoprazan to cause DDIs with other concomitantly administered CYP-substrate drugs. In a more direct experiment, the inhibitory effect of vonoprazan on the in vitro metabolism of [^{14}C]clopidogrel in HLMs was investigated. A partial summary of these studies was communicated recently in a Letter to the Editor in *Clinical Pharmacology and Therapeutics* [12].

2 Materials and Methods

2.1 Chemicals

Vonoprazan fumarate (vonoprazan) was synthesized by Takeda Pharmaceutical Company Limited (Tokyo, Japan). Phenacetin, acetaminophen, α-naphthoflavone, furafylline, ticlopidine hydrochloride, diclofenac sodium salt, 4′-hydroxydiclofenac, tranylcypromine hydrochloride, 1′-hydroxybufuralol maleate, quinidine, 1′-hydroxymidazolam, testosterone, 6β-hydroxytestosterone, verapamil hydrochloride, and esomeprazole magnesium hydrate were obtained from Merck KGaA (Darmstadt, Germany). Bupropion hydrochloride, hydroxybupropion, gemfibrozil 1-*O*-β-glucuronide, (*S*)-mephenytoin, 4′-hydroxymephenytoin, bufuralol hydrochloride, paroxetine hydrochloride, 2-oxo-clopidogrel hydrochloride (mixture of diastereomers), and clopidogrel carboxylic acid hydrochloride were from Toronto Research Chemicals Inc. (North York, ON, USA). Paclitaxel, midazolam, and clopidogrel sulfate were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 6α-Hydroxypaclitaxel was from Corning (Corning, NY, USA). Montelukast sodium was from LKT Laboratories (St. Paul, MN, USA). Sulfaphenazole was from Santa Cruz Biotechnology (Dallas, TX, USA). Tienilic acid was from Cayman Chemical (Ann Arbor, MI, USA). Ketoconazole was from Tokyo Chemical Industry (Tokyo, Japan). [^{14}C]clopidogrel was from Moravek, Inc. (Brea, CA, USA).

The other reagents used in the study were commercially available in analytical grade.

2.2 Biological Materials

The pooled human liver microsomes (HLMs) from 50 donors of mixed gender (lot nos. 1210153 and 1410013)

were commercially available from Sekisui Xenotech, LLC (Kansas City, KS, USA).

2.3 CYP Inhibition Study With or Without Pre-incubation

The concentrations and identities of the marker substrates and positive control inhibitors as well as the incubation times for each CYP reaction are shown in Table 1.

For the direct inhibition study, the standard incubation mixtures consisted of 0.1 M Na_2HPO_4–KH_2PO_4 phosphate buffer (pH 7.4), 0.2 mg protein/mL HLMs, 0.05 mM EDTA, the NADPH-generating system (5 mM $MgCl_2$, 5 mM glucose 6-phosphate, 0.5 mM β-$NADP^+$, and 1.0 unit/mL glucose-6-phosphate dehydrogenase), and a marker substrate in the presence or absence of inhibitor (vonoprazan or a positive control substance) in a final volume of 1000 µL. The concentrations of vonoprazan in the incubation mixture were set at 1, 3, 10, or 30 µM based on the maximum solubility in this reaction solution. After a pre-warming period of 5 min at 37 °C, the reaction was initiated by adding the NADPH-generating system and conducted at 37 °C. The reaction was terminated by adding 1 mL of acetonitrile.

For the time-dependent inhibition study, the incubation mixtures were pre-warmed at 37 °C for 5 min without the NADPH-generating system and the marker substrate. The NADPH-generating system was then added, and the incubation mixtures were pre-incubated at 37 °C for 30 min. After that, the reaction was initiated by adding the marker substrate and conducted at 37 °C. The final concentrations of the incubation mixtures and the incubation times were the same as in the direct inhibition study. After the incubation, the reaction was terminated by adding 1 mL of acetonitrile. All incubations in a series of in vitro studies including the following studies were carried out in duplicate, and no significant difference between the duplicate samples was observed.

2.4 Time-Dependent Inhibition Kinetics for CYP2B6, CYP2C19, and CYP3A4/5

The time-dependent inhibition study was conducted by the dilution method to determine the kinetic parameters, the maximum inactivation rate constant (k_{inact}), and the inactivator concentration for half the inactivation rate k_{inact} (K_I) for CYP2B6, CYP2C19, and CYP3A4/5 [13]. The first incubation mixtures consisted of 0.1 M Na_2HPO_4–KH_2PO_4 phosphate buffer (pH 7.4), 0.2 mg protein/mL HLMs, 0.05 mM EDTA, the NADPH-generating system, and various concentrations of vonoprazan or a positive control substance (ticlopidine for CYP2B6, esomeprazole for CYP2C19, or verapamil for CYP3A4/5) in a final volume of 1000 µL. After a pre-warming period of 5 min at 37 °C, the reaction was initiated by adding the NADPH-generating system and conducted for 2, 5, 10, 15, or 30 min (pre-incubation step).

After each pre-incubation time, a 100-µL aliquot was taken from the first incubation mixture and added to the second incubation mixture containing 0.1 M Na_2HPO_4–KH_2PO_4 phosphate buffer (pH7.4), and the NADPH-generating system, which was pre-warmed for 5 min, and then the substrate was added immediately to initiate the reaction. The final volume was 1000 µL. After incubation at 37 °C for 20 min (CYP2B6), 30 min (CYP2C19), or 5 min (CYP3A4/5), the reaction was terminated by adding 1 mL of acetonitrile.

2.5 Inhibition of [^{14}C]Clopidogrel Metabolism by Vonoprazan

The incubation mixtures consisted of 50 mM KH_2PO_4–K_2HPO_4 phosphate buffer (pH 7.4), 1 mg protein/mL HLMs, 5 mM glutathione (GSH), the NADPH-generating system, and 10 µM [^{14}C]clopidogrel in the presence or absence of inhibitor (vonoprazan or esomeprazole) in a

Table 1 Summary of experimental conditions and analytical methods for direct and time-dependent CYP-inhibition assays

Enzyme	Substrate	Conc. (µM)	Incubation (min)	Direct inhibitor (conc. in µM)	Time-dependent inhibitor (conc. in µM)	Metabolite	Mass transition monitored (*m/z*)
CYP1A2	Phenacetin	30	30	α-Naphthoflavone (0.2)	Furafylline (0.3)	Acetaminophen	152 → 110
CYP2B6	Bupropion	100	30	Ticlopidine (1)	Ticlopidine (0.1)	Hydroxybupropion	256 → 167
CYP2C8	Paclitaxel	10	20	Montelukast (1)	Gemfibrozil 1-*O*-β-glucuronide (10)	6α-Hydroxypaclitaxel	871 → 525
CYP2C9	Diclofenac	5	5	Sulfaphenazole (5)	Tienilic acid (1)	4′-Hydroxydiclofenac	312 → 231
CYP2C19	(*S*)-Mephenytoin	20	30	Tranylcypromine (25)	*S*-fluoxetine (10)	4′-Hydroxymephenytoin	235 → 150
CYP2D6	Bufuralol	10	20	Quinidine (1)	Paroxetine (0.3)	1′-Hydroxybufuralol	278 → 186
CYP3A4/5	Midazolam	5	5	Ketoconazole (0.5)	Verapamil (10)	1′-Hydroxymidazolam	342 → 324
	Testosterone	50	10			6β-Hydroxytestosterone	305 → 269

conc. concentration

final volume of 300 µL. After pre-incubating for 0 min or 30 min at 37 °C, the reaction was initiated by adding [^{14}C] clopidogrel solution and conducted for 30 min at 37 °C. The reaction was terminated by adding 150 µL of acetonitrile. Each analytical sample was centrifuged at 1500×*g* for 10 min at 4 °C to obtain the supernatant, which was used as the HPLC sample.

2.6 Analytical Procedures

For the CYP inhibition and kinetic studies, a 100-µL aliquot of methanol/ultrapure water (1:1, v/v) and a 100-µL aliquot of each internal standard solution were added to the reaction mixture after adding the acetonitrile. Deuterium-labeled metabolites were used for internal standards. Each analytical sample was centrifuged at 1600×*g* for 10 min at 4 °C to obtain the supernatant. Undiluted supernatant or supernatant diluted with ultrapure water (analytical samples) were analyzed using validated high-performance liquid chromatography (HPLC)–tandem mass spectrometry (LC-MS/MS) methods.

The samples were injected into a Quattro micro tandem mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an Alliance 2695 or 2795 liquid chromatograph (Waters Corp.), as well as other equivalent LC–MS/MS systems that use validated analytical methods. A Symmetry C18 column (2.1 × 100 mm; 3.5 µm, Waters Corp.) with a column temperature of 40 °C was used for chromatographic separation, and elution was carried out at a flow rate of 0.25 mL/min using a linear gradient for the mobile phase, which consisted of a mixture of A (acetic acid/acetonitrile, 1:1000, v/v) and B (acetic acid/ultrapure water, 1:1000, v/v). The eluate was introduced into the MS/MS system in the electrospray, positive-ion mode. Transitions shown in Table 1 were used in the selected reaction-monitoring method to quantify the specific metabolites.

[^{14}C]clopidogrel and its metabolites in the in vitro incubation mixture were analyzed by HPLC (Shimadzu Corp., Kyoto, Japan) with an on-line flow scintillation analyzer (PerkinElmer Inc., Waltham, MA, USA). The HPLC conditions were as follows. The column was a CAPCELL PAK C18 MGII (5 µm, 250 × 4.6 mm I.D.; Shiseido Co., Ltd., Tokyo, Japan). The column temperature and the flow rate were 40 °C and 1.0 mL/min, respectively. Mobile phase A was 10 mM ammonium formate/acetonitrile/formic acid (900:100:2, v/v), and mobile phase B was 10 mM ammonium formate/acetonitrile/formic acid (100:900:2, v/v/v). The time program for the gradient elution was as follows: the concentration of mobile phase (B) was linearly increased from 15 to 100% over a period of 20 min, then held at 100% for 5 min, and then cycled back to the initial conditions.

2.7 Data Analysis

All calculations were performed with Microsoft Excel 2003 or 2010 (Microsoft Corp., Redmond, WA, USA) except for the calculations described below. IC_{50}, K_I, and k_{inact} values were determined by nonlinear least-squares regression analysis using WNL5 Classic Modeling (pharmacodynamic model 103—simple E_{max} inhibitory effect model or pharmacodynamic model 102—simple E_{max} model) along with Phoenix WinNonlin 6.2 or 6.3 (Certara LP, Princeton, NJ, USA) using the following equation:

$$k_{obs} = k_{obs\ [I=0]} + \frac{k_{inact} \times [I]}{K_I + [I]}, \quad (1)$$

where $k_{obs\ [I=0]}$ is the apparent inactivation rate constant (k_{obs}) at 0 µM of inhibitor and [I] is the mean concentration of the inhibitor.

In the inhibition study using [^{14}C]clopidogrel with HLMs, "diastereomers of 2-oxo-clopidogrel" and "H4 and its isomers" were evaluated as individual groups due to the difficulties involved with separating the diastereomer peaks and the isomer peaks in the radiochromatogram. The relative activity was calculated as the formation ratios of "diastereomers of 2-oxo-clopidogrel" and "H4 and its isomers" in the sample with the inhibitor when that in the control sample with 0 and 30 minutes pre-incubation is regarded as 100%.

3 Results

3.1 Direct Inhibition of CYP Activities by Vonoprazan

The reversible inhibitory effects of vonoprazan on the marker activities of seven CYP isoforms are presented in Table 2. Vonoprazan reversibly inhibited the activities of CYP2B6 and CYP3A4/5 (midazolam 1′-hydroxylation) with IC_{50} values of 16 µM and 29 µM, respectively. At 30 µM, it also suppressed the activities of CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation) to 64.3%, 61.3%, and 61.3%, respectively, of the control activities; IC_{50} values were > 30 µM. Vonoprazan did not substantially inhibit other CYP activities at concentrations of up to 30 µM.

3.2 Time-Dependent Inhibition of CYP Activities by Vonoprazan

The time-dependent inhibitory effects of vonoprazan on the marker activities of the seven CYP isoforms are also presented in Table 2. In the study with pre-incubation, vonoprazan inhibited the activities of CYP2B6, CYP2C19, CYP3A4/5 (midazolam 1′-hydroxylation), and CYP3A4/5 (testosterone 6β-hydroxylation), with IC_{50} values of

Table 2 IC_{50} values of vonoprazan for marker enzyme activities in human liver microsomes

Enzyme	Marker reaction	IC_{50} (μM)	
		Without pre-incubation	With pre-incubation
CYP1A2	Phenacetin *O*-deethylation	> 30	> 30
CYP2B6	Bupropion hydroxylation	16	2.6
CYP2C8	Paclitaxel 6α-hydroxylation	> 30	> 30
CYP2C9	Diclofenac 4′-hydroxylation	> 30	> 30
CYP2C19	(*S*)-Mephenytoin 4′-hydroxylation	> 30	13
CYP2D6	Bufuralol 1′-hydroxylation	> 30	> 30
CYP3A4/5	Midazolam 1′-hydroxylation	29	10
CYP3A4/5	Testosterone 6β-hydroxylation	> 30	9.8

The inhibitory effects of vonoprazan were investigated with or without pre-incubation for 30 min in the presence of the NADPH-generating system. Each value represents the mean of duplicate determinations. *IC_{50}* half-maximal inhibitory concentration, *CYP* cytochrome P450

2.6 μM, 13 μM, 10 μM, and 9.8 μM, respectively. Vonoprazan did not show substantial inhibition of other CYP activities at concentrations of up to 30 μM. Due to the large shifts in IC_{50} for vonoprazan with respect to the activities of CYP2B6, CYP2C19, and CYP3A4/5 upon pre-incubation, the TDI kinetics were assessed (Fig. 1). The TDI parameters (k_{inact}, K_I, k_{inact}/K_I) for vonoprazan and the positive controls ticlopidine, esomeprazole, and verapamil—the known time-dependent inactivators of CYP2B6, CYP2C19, and CYP3A4/5, respectively [14, 15]—are shown in Table 3.

3.3 Evaluation of the Drug–Drug Interaction Based on Guidance and Guidelines

Furthermore, to evaluate the degree of drug–drug interaction, *R*1 and *R*2 values (the predicted ratio of the inhibited drug's area under the curve (AUC) in the presence and absence of an inhibitor for basic models of direct inhibition and TDI, respectively) were calculated as shown below (these equations are also used in the U.S. Food and Administration (FDA) draft guidance [16], the European Medicines Agency (EMA) guideline [17], and the Pharmaceuticals and Medical Devices Agency (PMDA) draft guideline [18]):

$$R1 = 1 + \frac{I_{max},u}{K_I} \tag{2}$$

$$R2 = \frac{k_{obs} + k_{deg}}{k_{deg}} \tag{3}$$

$$k_{obs} = \frac{k_{inact} \times 50 \times I_{max},u}{K_I + 50 \times I_{max},u} \tag{4}$$

$$I_{max},u = C_{max} \times f_{u,p}(\text{unbound plasma protein binding ratio}) \tag{5}$$

where K_I is $IC_{50}/2$ and the nonspecific binding to microsomes is 1.0 when calculated using a physicochemical property, k_{obs} is the apparent inactivation rate constant of CYP as calculated using Eq. 4, and the values of k_{deg} for CYP2B6, CYP2C19, and hepatic CYP3A4/5 are 0.00036 min^{-1}, 0.00045 min^{-1}, and 0.00032 min^{-1}, respectively [19, 20]. $I_{max,u}$ is the unbound inhibitor concentration in plasma as calculated using Eq. 5. The value of C_{max} was 0.0759 μM for vonoprazan 20 mg after 7 days of repeat dosing [5], 2.74 μM for ticlopidine 250 mg after a single dose [21], 2.65 μM for esomeprazole 20 mg after 5 days of repeat dosing [22], and 0.139 μM for verapamil 80 mg after a single dose [23], while the value of $f_{u,p}$ was 0.135 for vonoprazan [7], 0.02 for ticlopidine [24], 0.03 for esomeprazole [14], and 0.069 for verapamil [25].

Since the IC_{50} values for the direct inhibition of CYP2B6 and CYP3A4/5 were 16 and 29 μM, respectively, the calculated *R*1 values were less than the guidance criteria (1.02) for these CYPs. The *R*2 values are also shown in Table 3.

3.4 Inhibitory Effects of Vonoprazan and Esomeprazole on the Metabolism of [^{14}C] Clopidogrel in Human Liver Microsomes

The inhibitory effects of vonoprazan and esomeprazole on the metabolism of [^{14}C]clopidogrel in vitro were examined at concentrations of 0.03, 0.1, 0.3, 1, 3, and 10 μM (vonoprazan) or 1, 3, 10, and 30 μM (esomeprazole) with or without pre-incubation for 30 min with HLMs in the presence of the NADPH-generating system, and the results are shown in Table 4. The experimental samples were analyzed promptly after being prepared. For all samples, the recoveries with the pretreatment were 100 ± 10%, and no significant difference was observed between the duplicate samples and during a series of sequential analyses.

Clopidogrel was metabolized to a variety of metabolites in the presence of the NADPH-generating system and GSH. Seven of the metabolites were characterized by LC/MS analysis (data not shown). The metabolites identified were clopidogrel carboxylic acid, two diastereomers of 2-oxo-clopidogrel, and the active metabolite H4 and its three isomers (H1, H2, and H3) (data not shown). The clopidogrel carboxylic acid was formed independently of NADPH. The diastereomers of 2-oxo-clopidogrel as well as H4 and its isomers could not be fully resolved in the radiochromatogram. Therefore, "diastereomers of 2-oxo-clopidogrel" and "H4 and its isomers" were evaluated as

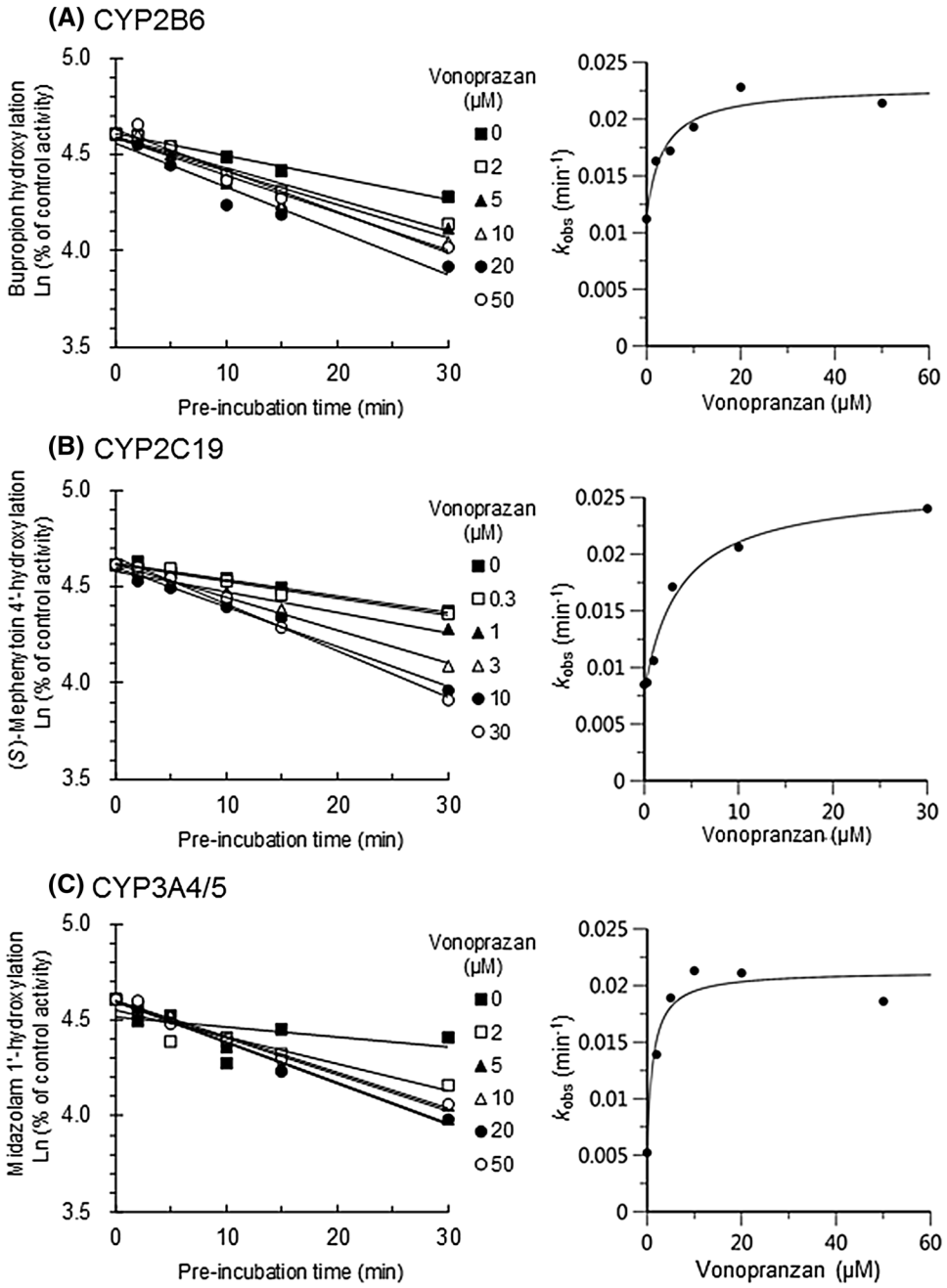

Fig. 1 Time-dependent inhibition (TDI) kinetics for the activities of CYP2B6, CYP2C19, and CYP3A4/5 inhibited by vonoprazan in human liver microsomes. The *left panels* show the time- and concentration-dependent inhibition of the activities of CYP2B6 (**a**), CYP2C19 (**b**), and CYP3A4/5 (**c**) by vonoprazan in HLMs, and the *right panels* show the corresponding plots of k_{obs} (the apparent inactivation rate constant of CYP) against the vonoprazan concentration, which were used to estimate the TDI kinetic parameters. Each value shown is the mean of duplicate determinations

Table 3 Time-dependent inhibitory parameters for vonoprazan and the positive controls in relation to the activities of CYP2B6, CYP2C19, and CYP3A4/5

	Inhibitor	k_{inact} (min^{-1})	K_I (μM)	k_{inact}/K_I (min^{-1} × μM^{-1})	*R*2
CYP2B6	Vonoprazan	0.0115	3.50	0.00329	5.08
	Ticlopidine	0.127	0.201	0.632	330
CYP2C19	Vonoprazan	0.0182	3.67	0.00496	5.95
	Esomeprazole	0.0547	2.58	0.0212	74.7
CYP3A4/5	Vonoprazan	0.0161	1.22	0.0132	15.9
	Verapamil	0.0441	1.60	0.0276	32.8

k_{inact} and K_I are the maximum inactivation rate constant and the inactivator concentration for half k_{inact} in the kinetic analysis, respectively. Each value represents the mean of duplicate determinations. *R*2 is the predicted ratio of the inhibited drug's area under the curve (AUC) in the presence and absence of an inhibitor for basic models of enzyme TDI [16–18]

Table 4 Inhibitory effects of vonoprazan and esomeprazole on the metabolism of [^{14}C]clopidogrel in human liver microsomes in vitro

	Conc. (μM)	Without pre-incubation		With pre-incubation	
		Formation of diastereomers of 2-oxo-clopidogrel (% w.r.t. control)	Formation of H4 and its isomers (% w.r.t. control)	Formation of diastereomers of 2-oxo-clopidogrel (% w.r.t. control)	Formation of H4 and its isomers (% w.r.t. control)
Control	0	100.0	100.0	100.0	100.0
Vonoprazan	0.03	99.0	100.0	100.0	103.8
	0.1	102.1	100.0	99.1	101.9
	0.3	105.2	98.5	102.7	105.8
	1	106.2	97.1	108.0	94.2
	3	105.2	86.8	107.1	82.7
	10	109.3	83.8	103.6	73.1
Esomeprazole	1	99.0	98.5	104.5	105.8
	3	103.1	97.1	107.1	100.0
	10	107.2	86.8	107.1	90.4
	30	115.5	70.6	111.6	71.2

[^{14}C]clopidogrel (10 μM) and the inhibitor were incubated with human liver microsomes (1 mg protein/mL) in the presence of the NADPH-generating system and GSH at 37 °C for 30 min with or without pre-incubation of the inhibitor and microsomes for 30 min. Each value represents the mean of duplicate determinations. Diastereomers of 2-oxo-clopidogrel as well as H4 and its isomers are metabolites of clopidogrel. % w.r.t. control is % with respect to the metabolite formation in the control sample

Conc. concentration

two individual groups for quantitation purposes. The formation of each diastereomer of 2-oxo-clopidogrel as well as H1, H2, H3, and H4 in the mass chromatogram did not change in the presence of vonoprazan or esomeprazole.

In the study without pre-incubation, vonoprazan and esomeprazole did not exhibit a significant inhibitory effect on the formation of H4 and its isomers from [^{14}C]clopidogrel; IC_{50} values were greater than 10 μM and 30 μM, the highest tested concentrations (these concentrations suppressed activity to ≥ 83.8% and ≥ 70.6% of the control activity, respectively). Neither vonoprazan nor esomeprazole showed substantial inhibition of the formation of diastereomers of 2-oxo-clopidogrel.

In the study with pre-incubation, vonoprazan or esomeprazole again did not exhibit a significant inhibitory effect on the formation of H4 and its isomers; IC_{50} values were greater than 10 μmol/L and 30 μmol/L, the highest tested concentrations (these concentrations suppressed activity to ≥ 73.1% and ≥ 71.2% of the control activity, respectively). The inhibitory effect of vonoprazan at the tested concentrations on the formation of H4 and its isomers was negligible and similar to that seen without pre-incubation of vonoprazan, except at a concentration of 10 μM, in which case the inhibitory effect with pre-incubation was somewhat higher (26.9% reduction in activity) than it was without pre-incubation (16.2% reduction in activity). However, the IC_{50} value of vonoprazan with or without pre-incubation was greater than 10 μM, and the time-dependent inhibitory effect of vonoprazan on the formation of H4 and its isomers was considered to be weak. Neither vonoprazan nor esomeprazole showed substantial inhibition of the formation of diastereomers of 2-oxo-clopidogrel.

4 Discussion

Clopidogrel is a thienopyridine prodrug that is metabolized into active thiols through two CYP-dependent steps. In the first step, it is converted into a sulfenic acid; it is then reduced to the final thiol derivative (H4) [26, 27], the pharmacologically active metabolite, which is the only isomer responsible for antiplatelet activity in humans [28]. The major metabolic pathways of clopidogrel are shown in Fig. 2 [27, 29, 30]. About 90% of the absorbed clopidogrel is hydrolyzed by esterases into an inactive form, clopidogrel carboxylic acid, and the remaining 10% is oxidized initially by CYP1A2, CYP2B6, and CYP2C19 into the intermediate metabolite 2-oxo-clopidogrel, and then to the thiolactone sulfoxide by CYP2C19 and CYP3A4 and, to a lesser extent, by CYP2B6 and CYP2C9. The active metabolite, H4, is finally formed in a reducing step [27, 31, 32]. In another study, CYP3A4 was found to be the main CYP isoform involved in the formation of H4 from 2-oxo-clopidogrel (approximately 50% of the H4 is generated by CYP3A4) [33]. The inhibitory effects of vonoprazan on these major CYP isoforms were investigated in this study.

Upon studying the direct inhibition of the major CYPs by vonoprazan in HLMs, we found that vonoprazan directly inhibited CYP2B6, CYP2C19, CYP2D6, and

CYP3A4/5 (midazolam 1′-hydroxylation and testosterone 6β-hydroxylation) in a concentration-dependent manner with IC_{50} values of 16, > 30, > 30, 29, and > 30 μM, respectively. In healthy volunteers, following oral administration of vonoprazan at a dose of 20 mg (the maximum clinical dose) once daily for 7 days, the maximum plasma concentration (C_{max}) of vonoprazan was observed to be low, 26.2 ng/mL (76 nM) [5]. Considering the $f_{u,p}$ value of 0.135 [7], the calculated $R1$ values for these CYPs were less than the guidance criterion (1.02), indicating that interaction due to direct inhibition is unlikely.

The potential of vonoprazan to exert TDI of the seven major CYP isoforms was also evaluated using the IC_{50} shift approach [34]. After a 30-min pre-incubation period, vonoprazan showed increased inhibition of the metabolism of bupropion, (*S*)-mephenytoin, midazolam, and testosterone, which suggests that it has TDI potential towards CYP2B6, CYP2C19, and CYP3A4/5, and has the potential to affect the metabolism of clopidogrel. Therefore, the TDI potency of vonoprazan towards CYP2B6, CYP2C19, and CYP3A4/5 was examined in more detail by measuring k_{inact} and K_I

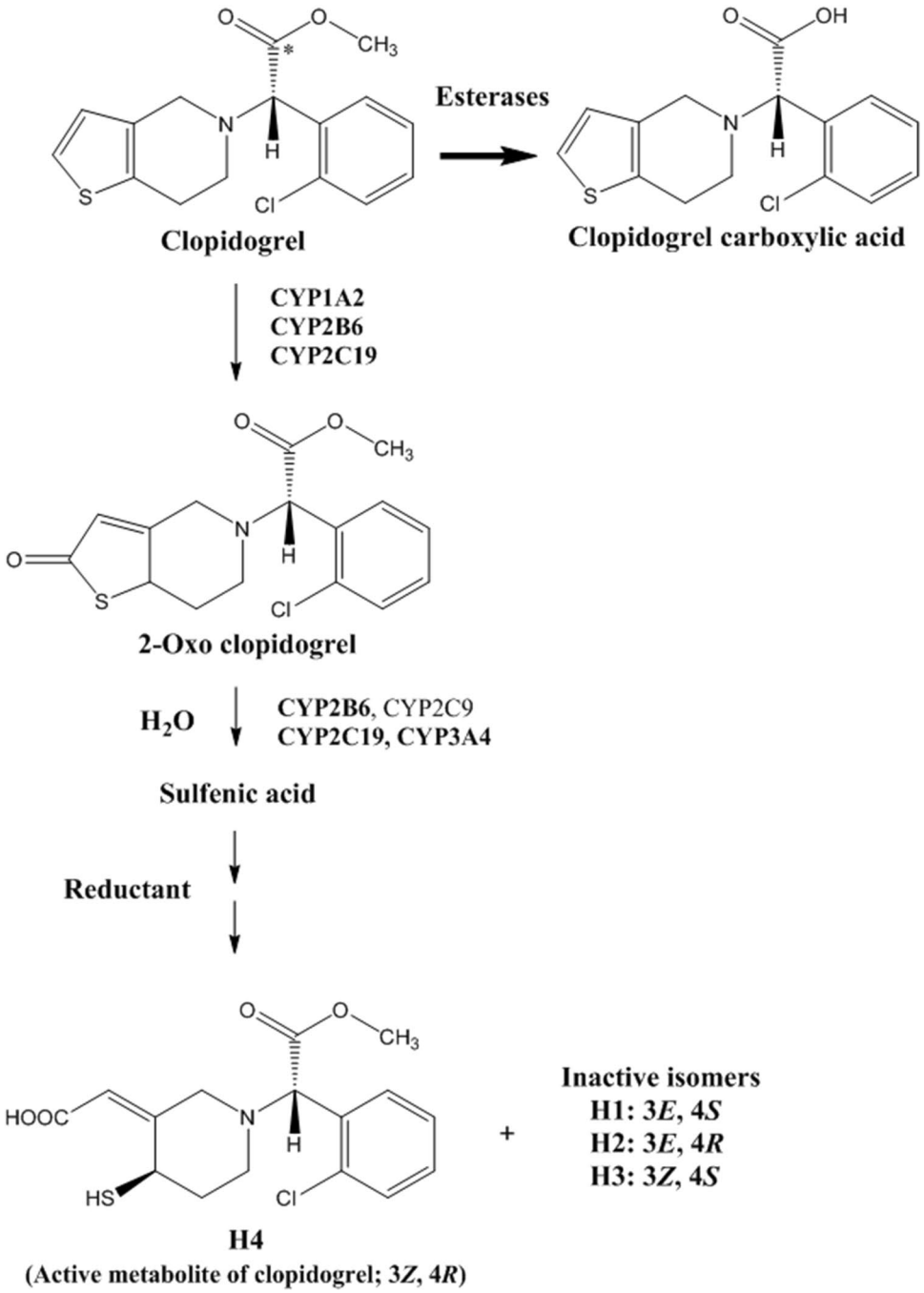

Fig. 2 Metabolic pathways of clopidogrel to pharmacologically active metabolites. *Asterisk* indicates the radiolabeled position. *CYP* cytochrome P450

and comparing the results with those obtained for positive controls (ticlopidine, esomeprazole, and verapamil). The results, including the *R*2 values calculated according to the latest DDI guidance or the guidelines from the FDA, EMA, or PMDA are presented in Table 3. The *R*2 values of vonoprazan were more than the guidance criteria (1.25) for CYP2B6, CYP2C19, and CYP3A4/5, but were 65-fold, 13-fold, and 2.1-fold lower than those of ticlopidine, esomeprazole, and verapamil, respectively. These results indicate that vonoprazan is capable of the TDI of CYP2B6, CYP2C19, and CYP3A4/5, but its potency in this regard is lower than those of the corresponding positive controls [13]. In actuality, the influence of the positive controls on the pharmacokinetics of coadministrated drugs has been found to be limited in clinical studies, and led to just a few fold change in exposure at therapeutic doses in most cases; ticlopidine, esomeprazole, and verapamil (positive controls for assessing CYP2B6, CYP2C19, and CYP3A4/5 activity, respectively) were reported to increase the mean area under the plasma concentration vs time curve (AUC) of bupropion (a typical CYP2B6 substrate) by 61% [35], that of diazepam (a typical CYP2C19 substrate) by 81% [36], and that of midazolam (a typical CYP3A4/5 substrate) by 191% [37], respectively. Since vonoprazan is a 2- to 65-fold weaker inhibitor than these positive controls, the TDI of CYP2B6, CYP2C19, or CYP3A4/5 by vonoprazan is unlikely to cause a clinically significant DDI.

Additionally, since vonoprazan has inhibitory effects on CYP2B6, CYP2C19, and CYP3A4/5, which are also the enzymes involved in the metabolism of vonoprazan itself [8], autoinhibition would be expected to result in the accumulation of vonoprazan on repeat dosing. However, our clinical data showed only minor accumulation (accumulation index < 1.2) following 10-40 mg QD dosing for 7 days [5]. In addition, no clinically significant mutual pharmacokinetic interactions have been reported between clarithromycin (a substrate and inhibitor of CYP3A4/5) and vonoprazan following triple therapy with vonoprazan–amoxicillin–clarithromycin in a repeat-dose setting [38]. It is known that amoxicillin is not a CYP3A4/5 inhibitor [39]. In the present study, exposure to vonoprazan and clarithromycin was observed to increase moderately while exposure to the major metabolites of vonoprazan decreased, whereas exposure to hydroxyclarithromycin—a metabolite generated by CYP3A4/5 [40]—actually increased. In other words, while the metabolism of vonoprazan was inhibited by clarithromycin (a CYP3A4/5 inhibitor), vonoprazan did not inhibit the metabolism of clarithromycin by CYP3A4/5. These results suggest that vonoprazan is not a CYP3A4/5 inhibitor in vivo. The cause of the increase in exposure to clarithromycin was not elucidated.

Furthermore, the inhibitory effect of vonoprazan on the in vitro metabolism of [^{14}C]clopidogrel with HLMs was studied to obtain direct evidence for a DDI between vonoprazan and clopidogrel. The inhibitory effect of esomeprazole was also examined as a reference. Both compounds inhibited the formation of H4 and its isomers in a concentration-dependent manner, albeit weakly, even at the maximum tested concentration of 10 μM for vonoprazan and 30 μM for esomeprazole. The formation of diastereomers of 2-oxo-clopidogrel was not inhibited by either vonoprazan or esomeprazole (Table 4). These results imply that CYP3A4 is heavily involved in the formation of H4 from 2-oxo-clopidogrel, but not in the formation of 2-oxo-clopidogrel from clopidogrel.

In the repeated-dose study of the effects of vonoprazan 10 mg for 7 days and esomeprazole 20 mg for 5 days, C_{max} was found to be 35.3 nM [5] and 2.65 μM [22], respectively. Since vonoprazan did not appear to inhibit H4 formation even at concentrations more than 100-fold the plasma C_{max}, its inhibitory effects on the metabolism of clopidogrel are likely to be very weak at clinical doses. Meanwhile, the plasma C_{max} of esomeprazole was comparable to the concentration tested in this study, so esomeprazole would be expected to show more inhibition of clopidogrel activation than vonoprazan would. However, the opposite pharmacodynamic findings have been reported by Kagami et al. Since their report did not include any pharmacokinetic data from that clinical study, the reason for the attenuation of clopidogrel activity in their study is unknown, but it is unlikely to be related to the inhibition of these CYPs by vonoprazan.

5 Conclusions

A DDI mediated by CYP metabolism is not expected to be a major contributor to the attenuation of the antiplatelet activity of clopidogrel upon coadministration with vonoprazan.

Taken together, these findings suggest that the observed pharmacodynamic drug interaction of clopidogrel is unlikely to be due to the inhibition of CYP2B6, CYP2C19, or CYP3A4/5 by vonoprazan, meaning that further clinical assessment is needed.

Acknowledgements The authors would like to thank Suresh K. Balani of Global Drug Metabolism and Pharmacokinetics, the Global Vonoprazan Project Team members at Takeda, Hideki Hirabayashi of Drug Metabolism and Pharmacokinetics Research Laboratories, and Junzo Takahashi for their contributions to these studies.

Author contributions MN and HY mainly wrote the manuscript. MN, HY, RC and HJ designed the research and analyzed the data. HY performed the research.

Compliance with Ethical Standards

Conflict of interest All the authors are employees of or have retired from working for Takeda Pharmaceutical Company Limited. The draft manuscript was prepared by Axcelead. The authors declare no other conflicts of interest.

Ethical approval All studies were performed according to the applicable institutional guidelines.

Funding All studies reported here were supported and conducted by Takeda Pharmaceutical Company Limited.

References

1. Ashida K, Sakurai Y, Hori T, Kudou K, Nishimura A, Hiramatsu N, Umegaki E, Iwakiri K. Randomised clinical trial: vonoprazan, a novel potassium-competitive acid blocker, vs. lansoprazole for the healing of erosive oesophagitis. Aliment Pharmacol Ther. 2016;43:240–51.
2. Murakami K, Sakurai Y, Shiino M, Funao N, Nishimura A, Asaka M. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for *Helicobacter pylori* eradication: a phase III, randomised, double-blind study. Gut. 2016;65:1439–46.
3. Shin JM, Inatomi N, Munson K, Strugatsky D, Tokhtaeva E, Vagin O, Sachs G. Characterization of a novel potassium-competitive acid blocker of the gastric H, K-ATPase, 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamine monofumarate (TAK-438). J Pharmacol Exp Ther. 2011;339(2):412–20.
4. Martinucci I, Blandizzi C, Bodini G, Marabotto E, Savarino V, Marchi S, de Bortoli N, Savarino E. Vonoprazan fumarate for the management of acid-related diseases. Expert Opin Pharmacother. 2017;18(11):1145–52.
5. Jenkins H, Sakurai Y, Nishimura A, Okamoto H, Hibberd M, Jenkins R, Yoneyama T, Ashida K, Ogama Y, Warrington S. Randomised clinical trial: safety, tolerability, pharmacokinetics and pharmacodynamics of repeated doses of TAK-438 (vonoprazan), a novel potassium-competitive acid blocker, in healthy male subjects. Aliment Pharmacol Ther. 2015;41:636–48.
6. Sakurai Y, Mori Y, Okamoto H, Nishimura A, Komura E, Araki T, Shiramoto M. Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects—a randomised open-label cross-over study. Aliment Pharmacol Ther. 2015;42:719–30.
7. Kogame A, Takeuchi T, Nonaka M, Yamasaki H, Kawaguchi N, Bernards A, Tagawa Y, Morohashi A, Kondo T, Moriwaki T, Asahi S. Disposition and metabolism of TAK-438 (vonoprazan fumarate), a novel potassium-competitive acid blocker, in rats and dogs. Xenobiotica. 2017;47(3):255–66.
8. Yamasaki H, Kawaguchi N, Nonaka M, Takahashi J, Morohashi A, Hirabayashi H, Moriwaki T, Asahi S. In vitro metabolism of TAK-438, vonoprazan fumarate, a novel potassium-competitive acid blocker. Xenobiotica. 2017;47(12):1027–34.
9. Yoneyama T, Teshima K, Jinno F, Kondo T, Asahi S. A validated simultaneous quantification method for vonoprazan (TAK-438F) and its 4 metabolites in human plasma by the liquid chromatography-tandem mass spectrometry. J Chromatogr B. 2016;1015:42–9.
10. Sakurai Y, Nishimura A, Kennedy G, Hibberd M, Jenkins R, Okamoto H, Yoneyama T, Jenkins H, Ashida K, Irie S, Täubel J. Safety, tolerability, pharmacokinetics, and pharmacodynamics of single rising TAK-438 (vonoprazan) doses in healthy male Japanese/non-Japanese subjects. Clin Transl Gastroenterol. 2015;6:e94.
11. Kagami T, Yamade M, Suzuki T, Uotani T, Hamaya Y, Iwaizumi M, Osawa S, Sugimoto K, Umemura K, Miyajima H, Furuta T. Comparative study of effects of vonoprazan and esomeprazole on antiplatelet function of clopidogrel or prasugrel in relation to CYP2C19 genotype. Clin Pharmacol Ther. 2018;103(5):906–13.
12. Nishihara M, Czerniak R. CYP-mediated drug–drug interaction is not a major determinant of attenuation of anti-platelet function of clopidogrel by vonoprazan. Clin Pharmacol Ther. 2018;104(1):31–2.
13. Obach RS, Walsky RL, Venkatakrishnan K. Mechanism-based inactivation of human cytochrome P450 enzymes and the prediction of drug–drug interactions. Drug Metab Dispos. 2007;35(2):246–55.
14. Zvyaga T, Chang SY, Chen C, Yang Z, Vuppugalla R, Hurley J, Thorndike D, Wagner A, Chimalakonda A, Rodrigues AD. Evaluation of six proton pump inhibitors as inhibitors of various human cytochrome P450: focus on cytochrome P450 2C19. Drug Metab Dispos. 2012;40(9):1698–711.
15. Grimm SW, Einolf HJ, Hall SD, He K, Lim HK, Ling KHJ, Lu C, Nomeir AA, Seibert E, Skordos KW, Tonn GR, Van Horn R, Wang RW, Wong YN, Yang TJ, Obach RS. The conduct of in vitro studies to address time-dependent inhibition of drug-metabolizing enzymes: a perspective of the pharmaceutical research and manufacturers of America. Drug Metab Dispos. 2009;37(7):1355–70.
16. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Draft guidance for Industry. In vitro metabolism- and transporter-mediated drug–drug interaction studies. Silver Spring, MD: FDA; 2017. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581965.pdf. Accessed 15 Sep 2018.
17. European Medicines Agency. Guideline on the investigation of drug interactions. London: EMA; 2013. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf. Accessed 15 Sep 2018.
18. Ministry of Health, Labour and Welfare, Japan. Guideline of drug interaction studies for drug development and appropriate provision of information. Tokyo, Japan: Ministry of Health; 2018. https://www.pmda.go.jp/files/000225191.pdf. Accessed 15 Sep 2018.
19. Shirasaka Y, Sager JE, Lutz JD, Davis C, Isoherranen N. Inhibition of CYP2C19 and CYP3A4 by omeprazole metabolites and their contribution to drug–drug interactions. Drug Metab Dispos. 2013;41(7):1414–24.
20. Yang J, Liao M, Shou M, Jamei M, Yeo KR, Tucker GT, Rostami-Hodjegan A. Cytochrome p450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. Curr Drug Metab. 2008;9:384–93.
21. Lu WJ, Huang JD, Lai ML. The effects of ergoloid mesylates and ginkgo biloba on the pharmacokinetics of ticlopidine. J Clin Pharmacol. 2006;46:628–34.
22. Andersson T, Hassan-Alin M, Hasselgren G, Röhss K, Weidolf L. Pharmacokinetic studies with esomeprazole, the (*S*)-isomer of omeprazole. Clin Pharmacokinet. 2001;40(6):411–26.
23. Maeda K, Takano J, Ikeda Y, Fujita T, Oyama Y, Nozawa K, Kumagai Y, Sugiyama Y. Nonlinear pharmacokinetics of oral quinidine and verapamil in healthy subjects: a clinical microdosing study. Clin Pharmacol Ther. 2011;90(2):263–70.
24. Hardman JG, Limbird LE, Gilman AG. Goodman & Gilman's The pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill; 2001. p. 2013.

25. Xu L, Chen Y, Pan Y, Skiles GL, Shou M. Prediction of human drug–drug interactions from time-dependent inactivation of CYP3A4 in primary hepatocytes using a population-based simulator. Drug Metab Dispos. 2009;37(12):2330–9.
26. Dansette PM, Levent D, Hessani A, Mansuy D. Bioactivation of clopidogrel and prasugrel: factors determining the stereochemistry of the thiol metabolite double bond. Chem Res Toxicol. 2015;28(6):1338–45.
27. Zhang H, Lau WC, Hollenberg PF. Formation of the thiol conjugates and active metabolite of clopidogrel by human liver microsomes. Mol Pharmacol. 2012;82(2):302–9.
28. Tuffal G, Roy S, Lavisse M, Brasseur D, Schofield J, Touchard ND, Savi P, Bremond N, Rouchon MC, Hurbin F, Sultan E. An improved method for specific and quantitative determination of the clopidogrel active metabolite isomers in human plasma. Thromb Haemost. 2011;105:696–705.
29. Takahashi M, Pang H, Kawabata K, Farid NA, Kurihara A. Quantitative determination of clopidogrel active metabolite in human plasma by LC-MS/MS. J Pharm Biomed Anal. 2008;48:1219–24.
30. Shaw SA, Balasubramanian B, Bonacorsi S, Cortes JC, Cao K, Chen BC, Dai J, Decicco C, Goswami A, Guo Z, Hanson R, Humphreys WG, Lam PYS, Li W, Mathur A, Maxwell BD, Michaudel Q, Peng L, Pudzianowski A, Qiu F, Su S, Sun D, Tymiak AA, Vokits BP, Wang B, Wexler R, Wu DR, Zhang Y, Zhao R, Baran PS. Synthesis of biologically active piperidine metabolites of clopidogrel: determination of structure and analyte development. J Org Chem. 2015;80:7019–32.
31. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos. 2010;38(1):92–9.
32. Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA, Ikeda T. A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. Drug Metab Dispos. 2009;37(11):2145–52.
33. Liu C, Chen Z, Zhong K, Li L, Zhu W, Chen X, Zhong D. Human liver cytochrome P450 enzymes and microsomal thiol methyltransferase are involved in the stereoselective formation and methylation of the pharmacologically active metabolite of clopidogrel. Drug Metab Dispos. 2015;43:1632–41.
34. Burt HJ, Galetin A, Houston JB. IC_{50}-based approaches as an alternative method for assessment of time-dependent inhibition of CYP3A4. Xenobiotica. 2010;40(5):331–43.
35. Turpeinen M, Tolonen A, Uusitalo J, Jalonen J, Pelkonen O, Laine K. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. Clin Pharmacol Ther. 2005;77:553–9.
36. Andersson T, Hassan-Alin M, Hasselgren G, Röhss K. Drug interaction studies with esomeprazole, the (*S*)-isomer of omeprazole. Clin Pharmacokinet. 2001;40(7):523–37.
37. Backman JT, Olkkola KT, Aranko K, Himberg JJ, Neuvonen PJ. Dose of midazolam should be reduced during diltiazem and verapamil treatments. Br J Clin Pharmacol. 1994;37:221–5.
38. Sakurai Y, Shiino M, Okamoto H, Nishimura A, Nakamura K, Hasegawa S. Pharmacokinetics and safety of triple therapy with vonoprazan, amoxicillin, and clarithromycin or metronidazole: a phase 1, open-label, randomized, crossover study. Adv Ther. 2016;33:1519–35.
39. Niwa T, Morimoto M, Hirai T, Hata T, Hayashi M, Imagawa Y. Effects of penicillin-based antibiotics, amoxicillin, ampicillin, and piperacillin, on drug-metabolizing activities of human hepatic cytochromes P450. J Toxicol Sci. 2016;41(1):143–6.
40. Suzuki A, Iida I, Hirota M, Akimoto M, Higuchi S, Suwa T, Tani M, Ishizaki T, Chiba K. CYP isoforms involved in the metabolism of clarithromycin in vitro: comparison between the identification from disappearance rate and that from formation rate of metabolites. Drug Metab Pharmacokin. 2003;18(2):104–13.