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Clarification of the mechanism of clopidogrel-mediated drug–drug interaction in a clinical cassette small-dose study and its prediction based on in vitro information

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Abbreviations:

AUC, area under the concentration–time curve; C_{\max} , maximum plasma concentration; CYP, cytochrome P450; DDI, drug–drug interaction; OATP, organic anion-transporting polypeptide;

K_i , inhibition constant; k_a , first order absorption rate constant; t_{\max} , time to C_{\max}

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Abstract

Clopidogrel is reported to be associated with cerivastatin-induced rhabdomyolysis, and clopidogrel and its metabolites are capable of inhibiting CYP2C8 and OATP 1B1 in vitro. The objective of the present study was to identify the mechanism of clopidogrel-mediated drug–drug interactions (DDIs) on the pharmacokinetics of OATP1B1 and/or CYP2C8 substrates in vivo. A clinical cassette small-dose study using OATPs, CYP2C8, and OATP1B1/CYP2C8 probe drugs (pitavastatin, pioglitazone, and repaglinide, respectively) with or without the coadministration of either 600 mg rifampicin (an inhibitor for OATPs), 200 mg trimethoprim (an inhibitor for CYP2C8), or 300 mg clopidogrel was performed, and the area under the concentration–time curve (AUC) ratios (AUCRs) for probe substrates were predicted using static model.

Clopidogrel increased the AUC of pioglitazone (2.0-fold) and repaglinide (3.1-fold), but did not significantly change the AUC of pitavastatin (1.1-fold). In addition, the AUC of pioglitazone M4, a CYP2C8-mediated metabolite of pioglitazone, was reduced to 70% of the control by coadministration of clopidogrel. The predicted AUCRs using the mechanism-based inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide were similar to the observed AUCRs, and the predicted AUCR (1.1) of repaglinide using only the inhibition of OATP1B1 did not reach the observed AUCR (3.1). In conclusion, a single 300 mg of clopidogrel mainly inhibits CYP2C8-mediated metabolism by clopidogrel acyl- β -glucuronide, but its effect on the pharmacokinetics of OATP1B1 substrates is negligible. Clopidogrel is expected to have an effect not only on CYP2C8 substrates, but also dual CYP2C8/OATP1B1 substrates as seen in the case of repaglinide.

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Introduction

Clopidogrel is a potent antiplatelet agent, and is widely used to prevent blood clots in patients with stroke, myocardial infarction, cardiovascular disease, and coronary artery disease. It is a prodrug, and about 90% of the absorbed dose is rapidly hydrolyzed by a hepatic carboxylase to an inactive carboxylic acid metabolite and then is secondarily metabolized to a glucuronide conjugate, while the remaining clopidogrel is metabolized to an active thiol metabolite through inactive intermediate 2-oxo-clopidogrel by hepatic cytochrome P450s (CYPs) (Hagihara et al., 2009; Zhu et al., 2013). Recently, epidemiological research using the FDA Adverse Event Reporting System (AERS) found that the use of clopidogrel or gemfibrozil was highly associated with rhabdomyolysis caused by cerivastatin (Floyd et al., 2012). In the case of gemfibrozil, it was already reported that gemfibrozil greatly increased the plasma concentration of cerivastatin because gemfibrozil 1-O- β -glucuronide inhibited CYP2C8-mediated metabolism irreversibly and the organic anion-transporting polypeptide (OATP) 1B1-mediated hepatic uptake of cerivastatin (Backman et al., 2002; Shitara et al., 2004; Kudo et al., 2013). However, there is no clinical evidence for the pharmacokinetic interaction of clopidogrel with cerivastatin, and a study of any drug–drug interaction (DDI) between clopidogrel and cerivastatin is problematic because cerivastatin is not available as a drug for clinical study. Moreover, clopidogrel and its metabolites inhibit not only CYPs-mediated metabolism (CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4), but also OATP1B1 in vitro, and their in vitro inhibition constants (K_i) values were considered to affect the pharmacokinetic interactions of coadministered drugs (Hagihara et al. 2008; Nishiya et al., 2009a; Nishiya et al., 2009b; Tamraz et al., 2013; Tornio et al., 2014). The effects of clopidogrel on the pharmacokinetics of CYP2B6 substrates and CYP2C19 substrates clearly include that the plasma concentrations of efavirenz, omeprazole, and sibutramine are increased by a mechanism-based inhibition of CYP2B6, CYP2C19, and both, respectively (Bae et al., 2011; Chen et al., 2009; Jiang et al., 2013). However, the clopidogrel-mediated DDIs with OATP1B1 or OATP1B1/CYPs substrates are not consistent. Although

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rosuvastatin, simvastatin and fluvastatin are substrates of OATP1B1, clopidogrel increased the plasma concentration of rosuvastatin (1.7-fold for AUC), but did not change those of simvastatin and fluvastatin (Ayalasomayajula et al., 2007; Itkonen et al., 2015; Pinheiro et al., 2012).

To identify directly the mechanism of clopidogrel-mediated DDIs with OATP1B1 and CYP2C8 in vivo in humans, we planned a cassette small-dose study with specific probes and specific inhibitors of CYP2C8 and/or OATP1B1, which are associated with the hepatic clearance of cerivastatin. Pitavastatin and pioglitazone are clinically applicable probe drugs for OATP1B1 and CYP2C8, respectively. Pitavastatin is a sensitive OATP1B1 probe with high oral absorption and minimum metabolism (Aquilante et al., 2013; Mukhtar et al., 2005; Shitara et al., 2006; US FDA, 2012), and pioglitazone is metabolized mainly by CYP2C8, and to a lesser extent by CYP3A4, and CYP2C9 is not significant in the elimination of pioglitazone (<10%)(Jaakkola et al., 2006). Repaglinide is a multisubstrate of CYP2C8/CYP3A4 and OATP1B1 like cerivastatin (Kajosaari et al., 2005a; Niemi et al., 2005; Säll et al., 2012), and showed similar DDI patterns with the same inhibitors, cyclosporine A and gemfibrozil (Backman et al., 2002; Honkalammi et al., 2011a; Kajosaari et al., 2005b; Mück et al., 1999). A recent clinical study found that clopidogrel increased the plasma concentration of repaglinide, and clopidogrel acyl- β -glucuronide is a mechanism based-inhibitor of CYP2C8 in vitro (Tornio et al., 2014). Thus, in the present clinical study, a cassette consisted of pitavastatin, pioglitazone, and repaglinide as a probe drug for OATP1B1, CYP2C8, and OATP1B1/CYP2C8 (cerivastatin), respectively. Each substrate was reported to have linear pharmacokinetics in range of 1–24 mg for pitavastatin [FDA (2009) Drug approval package: LIVALO (pitavastatin calcium), FDA application No., (NDA) 022363], 2–60 mg for pioglitazone [FDA (1999) Drug approval package: ACTOS (pioglitazone hydrochloride), FDA application No., (NDA) 021073], and 0.25–1 mg for repaglinide [PMDA (2016) Interview Form: SUREPOST (repaglinide)] (Table 1). Moreover, we

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confirmed that the unbound plasma concentration of each drug at the small-dose we used was far below the K_m values for OATPs or CYP2C8. Thus, we believe that potential nonlinearity concerns are minimal when we translate the effect from the dose given to the standard dose. In theory, if the linearity of pharmacokinetics of a substrate drug is maintained within the certain dose range, the extent of AUC increase should be the same at different doses of the substrate drug. Therefore, to avoid any DDIs among the probe cassette, a small dose (less than 20% of the clinical dose) of three probes was used in this study. Rifampicin and trimethoprim significantly increased the plasma concentration of specific OATP1B1 and CYP2C8 substrates, respectively (Maeda et al., 2011; Hruska et al., 2005; Tornio et al., 2008), and were therefore included as reference inhibitors in this study. We simultaneously administered a small-dose cassette—less than 20% of the clinical dose—of pitavastatin, pioglitazone, and repaglinide and investigated the effects of coadministration of either rifampicin, trimethoprim, or clopidogrel on the pharmacokinetics of these three probes in humans (Table 1 and Figure 1).

The evidence provided by our clinical study were further validated qualitatively by the prediction of DDI potential for each molecule with the use of a static model and *in vitro* K_i values of CYP2C8 and OATP1B1.

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Materials and methods

Participants and study design

Twenty-four healthy male Japanese volunteers participated in this clinical study. Each subject was physically normal and had no antecedent history of significant medical illness or hypersensitivity to any drugs, and a body mass index between 17.6 and 26.4 kg/m². The study protocol was approved by the Ethics Review Boards of Kyushu University, The University of Tokyo, Sugioka Memorial Hospital, and RIKEN. Written informed consent was obtained from all participants before their inclusion in the study. An open-label, parallel study with two phases was conducted, with a washout period of at least 1 week between the phases.

Participants were divided into three groups (eight subjects/group) (Figure 1). The probe drugs were prepared as follows: (1) by mixing 4 mg pitavastatin calcium (4 mg Livalo tablet, Kowa Company, Nagoya, Japan) with lactose up to total amounts of 4 g; (2) by mixing 15 mg pioglitazone hydrochloride (15 mg Actose tablet, Takeda Pharmaceutical Company, Osaka, Japan) with lactose up to total amounts of 4.5 g; (3) and by mixing 0.25 mg repaglinide (0.25 mg Surepost tablet, Sumitomo Dainippon Pharma Co, Osaka, Japan) with lactose up to total amounts of 1g. The small-dose cassette comprised 0.2 g of pitavastatin–lactose mixture, 0.3 g of pioglitazone–lactose mixture, and 0.4 g of repaglinide–lactose mixture; the final dose of pitavastatin, pioglitazone, and repaglinide was 0.2, 1, and 0.1 mg, respectively. In the only cassette phase, after overnight fasting, each participant in each group received a single small-dose cassette of pitavastatin, pioglitazone, and repaglinide followed by 150 mL of water. In the DDI phase, 600 mg rifampicin (4 × 150 mg Rifadin capsules, Daiichi Sankyo Co, Tokyo, Japan), 200 mg trimethoprim (2.5 g Baktar combination granules as sulfamethoxazole and trimethoprim, Shionogi & Co, Osaka, Japan) or 300 mg clopidogrel (4 × 75 mg Plavix tablets, Sanofi, Tokyo, Japan) was administered orally with a small-dose cassette for each group, respectively. Venous blood samples (7 mL) were collected before dosing and at 0.25, 0.5, 1,

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1.5, 2, 3, 4, 6, 8, 12, and 24 h postdosing. Plasma samples were separated immediately and stored at -80°C until analysis. This study was registered in the UMIN Clinical Trials Registry at <http://www.umin.ac.jp/ctr/index.htm> (UMIN000015430).

Determination of plasma drug concentrations

The plasma concentrations of all compounds were measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). All sample pretreatment procedures were conducted on ice using an aluminum cooling block to prevent the back-conversion of clopidogrel acyl- β -glucuronide to clopidogrel carboxylic acid or clopidogrel (Silvestro et al., 2011).

For pitavastatin, pitavastatin lactone, pioglitazone, pioglitazone M4, repaglinide, repaglinide M1, repaglinide M4, clopidogrel, and 2-oxo-clopidogrel, an aliquot (100 μL) of plasma was mixed with 100 μL acetonitrile and 100 μL internal standards solution; the mixture was vigorously mixed for 10 min and centrifuged at $10,000 \times g$ for 10 min at 4°C . The supernatant was collected and an aliquot (5 μL) was injected into the LC–MS/MS system. To measure the concentration of rifampicin, trimethoprim, clopidogrel carboxylic acid, and clopidogrel acyl- β -glucuronide, an aliquot (20 μL) of plasma was mixed with 380 μL acetonitrile and 100 μL internal standards solution; the mixture was vigorously mixed for 10 min and centrifuged at $10,000 \times g$ for 10 min at 4°C . An aliquot (20 μL) of supernatant was diluted with 180 μL of the mixture of water/acetonitrile (50/50, v/v), except trimethoprim, which was diluted with the mixture of 0.1% formic acid in water/acetonitrile (70/30, v/v). An aliquot (5 μL) was injected into the LC–MS/MS system. Deuterated reference compounds of all compounds except rifampicin were used as internal standards. Chlorpropamide was used as an internal standard for rifampicin. Chromatography was performed on a reversed-phase column (Kintex C18 column; 2.1×100 mm, 2.6 μm ; Phenomenex, Torrance, CA) using

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gradient elution with a mobile phase of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). Phase B was maintained initially at 20% (5% for only trimethoprim) from 0 to 0.5 min. The percentage of phase B was increased linearly to 80% from 0.5 to 3.5 min and this was maintained up to 4.5 min; the column was then equilibrated with 20% (5% for only trimethoprim) of phase B for 1.5 min. The flow rate was 0.3 mL/min. The column and autosampler tray temperature was maintained at 40°C and 4°C, respectively. The mass spectrometer was operated in positive turbo-ion spray mode at the transitions (m/z) 422 to 290 for pitavastatin, 404 to 290 for pitavastatin lactone, m/z 357 to 134 for pioglitazone, 373 to 150 for pioglitazone M4, m/z 453 to 230 for repaglinide, 385 to 106 for repaglinide M1, m/z 469 to 246 for repaglinide M4, m/z 823 to 791 for rifampicin, 291 to 230 for trimethoprim, m/z 322 to 212 for clopidogrel, m/z 338 to 155 for 2-oxo-clopidogrel, m/z 308 to 198 for clopidogrel carboxylic acid, and m/z 484 to 308 for clopidogrel acyl- β -glucuronide. The limit of quantification was 0.005 ng/mL for repaglinide, 0.01 ng/mL for repaglinide M1 and repaglinide M4, 0.04 ng/mL for pitavastatin, pioglitazone, pioglitazone M4, clopidogrel and 2-oxo clopidogrel, 0.12 ng/mL for pitavastatin lactone, 9 ng/mL for clopidogrel carboxylic acid, 15 ng/mL for clopidogrel acyl- β -glucuronide, and 25 ng/mL for rifampicin, respectively, with acceptable precision (coefficient of variation < 20%) and accuracy (relative error < 20%).

Pharmacokinetics

C_{\max} and t_{\max} were observed directly from plasma concentration–time curves of probe drugs and metabolites. The AUC_{0-t} was calculated according to the linear trapezoidal rule, and extrapolation to infinity was determined by dividing the last measured concentration by k_{el} . The elimination rate constant (k_{el}) was determined by linear regression analysis of the log–linear part of the plasma drug concentration–time curve. The elimination log–linear part of

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the concentration–time curve was identified visually for each subject. $t_{1/2}$ was calculated by the division of 0.693 by k_{el} .

Statistical analysis

The pharmacokinetic variables between the two different phases were compared using a t test. $P < 0.05$ was considered significant.

DDI prediction

The DDI potential (R value) of OATP1B1-mediated and enzyme-mediated inhibition was calculated not only for the three inhibitors (rifampicin, trimethoprim, and clopidogrel), but also for the gemfibrozil based on the static model (EMDA, 2012; US FDA, 2012). Clopidogrel and gemfibrozil were highly associated with the expression of rhabdomyolysis caused by cerivastatin (Floyd et al., 2012). Gemfibrozil was not included in this clinical study. However, the mechanism of gemfibrozil-mediated DDI and clinical DDI data with cerivastatin have already found that gemfibrozil increased the plasma concentration of cerivastatin with the inhibition of CYP2C8 and OATP1B1 by gemfibrozil glucuronide (Backman et al., 2002; Shitara et al., 2004; Kudo et al., 2013). By contrast, to our knowledge, no pharmacokinetics-related DDI between clopidogrel and cerivastatin has been reported, therefore we compared the predicted DDI of cerivastatin by clopidogrel with that of gemfibrozil.

R value was determined using $R = (1 + [I]/K_i)$ and $R = [1 + (k_{inact} \times [I]) / \{ (K_I + [I]) \times K_{deg} \}]$ for reversible inhibition and mechanism-based inhibition, respectively. K_i is the in vitro inhibition constant, K_I is the inhibitor concentration needed to cause half of k_{inact} , k_{inact} is the maximum inactivation rate constant, and K_{deg} is the degradation rate constant (0.0330/h for CYP2C8 and 0.0193/h for CYP3A4), which is estimated by the half-life of the CYP enzyme (Backman et al., 2009; Rowland et al., 2011). $[I]$ represents the maximum unbound

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concentration in plasma ($[I_{u,max}] = f_u \times [I_{max}]$) for the enzyme inhibitor/metabolite inhibitor or the estimated maximum unbound concentration at the inlet to the liver ($[I_{u,in,max}]$) for the transporter inhibitor; f_u is the protein unbound fraction in plasma and $[I_{max}]$ is the maximum concentration in plasma. $[I_{u,in,max}]$ was calculated using the following equation:

$$[I_{u,in,max}] = f_u \times \left([I_{max}] + \frac{k_a \times F_a \times F_g \times Dose}{Q_h} \right) \quad (1)$$

where k_a is the absorption rate constant, F_a and F_g are the fraction absorbed and fraction available after intestinal metabolism of the oral dose, Dose is the dose of inhibitor, and Q_h is the hepatic blood flow rate (1500 mL/min). The k_a that was estimated by compartment analysis of clinical data was 0.52, 0.49, 1.22 and 3.50 /h for rifampicin, trimethoprim, clopidogrel and gemfibrozil, respectively. F_a and F_g were set to 1 (Ito et al., 1998). Plasma concentration-time profiles of a single oral dose of 600 mg rifampicin, 200 mg trimethoprim and 300 mg clopidogrel were obtained from our clinical study, and that of a single oral dose of 600 mg gemfibrozil was obtained from reported data (Honkalammi et al. 2011a). All in vitro inhibition data for inhibitors or their metabolites were derived from the University of Washington's Metabolism and Transport Drug Interaction Database (DIDB) (<http://www.druginteractioninfo.org>). In vitro K_i values were obtained directly from the reported data or IC_{50} values which should be equal to K_i values if the substrate concentration is well below its K_m value. The geometric mean of all the K_i or IC_{50} values for each inhibitor against the specific enzyme/transporter in various experimental conditions (substrate/cell systems) was used for the prediction of DDI potential by a static model (Table 4). For the calculation of the geometric mean of K_i for OATP1B1, K_i values against estrone-3-sulfate and sulfobromophthalein were not used because their K_i values were much different from those against other OATP1B1 substrates (Izumi et al., 2015). The quantitative prediction of the OATP1B1-mediated DDIs by PBPK model needs to be performed by using the inhibition data obtained with a clinically relevant probe (eg pitavastatin, pravastatin...), because the inhibition of OATP1B1 is substrate-dependent (Izumi et al., 2015).

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The maximum clinical transporter-/enzyme-mediated inhibitory effect of coadministered inhibitors including parent and/or metabolites was predicted as AUC ratio (AUCR) by the following equation (Ueda et al., 2001; Yoshida et al., 2012):

$$AUCR = \frac{AUC_i}{AUC} = 1 / \left[\frac{1}{\left(1 + \sum \frac{[I_{u,max}]}{K_{i,OATP1B1}}\right)} \times \left\{ \frac{f_{m,CYP2C8}}{\left(1 + \sum \frac{[I_{u,max}]}{K_{i,CYP2C8}}\right) \times \left(1 + \sum \frac{k_{inact,CYP2C8} \times [I_{u,max}]}{K_{deg,CYP2C8} \times \{K_{i,CYP2C8} + [I_{u,max}]\}}\right)} + (1 - f_{m,CYP2C8}) \right\} \right] \quad (2)$$

where AUC_i is the AUC in the presence of inhibitors, and $f_{m,CYP2C8}$ is the fraction of metabolic clearance of the substrate mediated by CYP2C8. In vitro $f_{m,CYP2C8}$ of pitavastatin, pioglitazone, and cerivastatin were reported to be 0.0, 0.68 and 0.61, respectively (Fujino et al., 2003; Jaakkola et al., 2006; Shitara et al., 2004). By contrast, $f_{m,CYP2C8}$ for repaglinide are various; 0.41–0.71 from in vitro experiments (Kajossari et al., 2005a; Säll et al., 2012) and 0.80–0.89 from in vivo data with modeling (Honkalammi et al., 2011b; Honkalammi et al., 2012; Kudo et al., 2013). We used in vitro $f_{m,CYP2C8}$ for the prediction in order to apply an unified method for all the substrates. The maximum value of in vitro $f_{m,CYP2C8}$ of repaglinide (0.71) was used as a worst case scenario which could provide the maximum extent of DDI with CYP2C8 inhibitor. In the case of metabolites, we used $[I_{u,max}]$ as the concentration of inhibitor for both the enzyme and transporter, because there is no information for the maximum unbound concentration of metabolites in the liver.

In equation 2, we assumed that OATP1B1 completely contributes to the uptake of OATP1B substrates because of the limited information for the contribution of OATP1B1 to the overall uptake of these substrates, therefore the extent of DDI predicted in this study is estimated when considering the maximum inhibition of OATP1B1. Relevant input parameters of substrates and inhibitors for DDI predictions are provided in Table 4 and 5.

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Results

Effects of rifampicin, trimethoprim, and clopidogrel on the pharmacokinetics of pitavastatin

The pharmacokinetics of pitavastatin (as a probe drug for OATPs) coadministered with rifampicin, trimethoprim, or clopidogrel was compared with its pharmacokinetics in the absence of coadministered drugs. Rifampicin increased the plasma concentration of pitavastatin, whereas trimethoprim and clopidogrel did not affect it (Figure 2). The area under the concentration–time curve from 0 to 24 h (AUC_{0-24h}) of pitavastatin increased 5.1-fold after rifampicin treatment, and coadministration of trimethoprim or clopidogrel did not significantly change this (Figure 2, Table 2). The AUC_{0-24h} of pitavastatin lactone did not change significantly with any coadministered drug (Table 2).

Effects of rifampicin, trimethoprim, and clopidogrel on the pharmacokinetics of pioglitazone

The pharmacokinetics of pioglitazone (as a probe drug for CYP2C8) coadministered with rifampicin, trimethoprim or clopidogrel was compared with its pharmacokinetics in the absence of coadministered drugs. Trimethoprim and clopidogrel increased the plasma concentration of pioglitazone, and decreased the plasma concentration of pioglitazone M4, which is a CYP2C8-dependent metabolite of pioglitazone (Figure 3). The AUC_{0-24h} of pioglitazone increased 1.3- and 2.0-fold, and the AUC_{0-24h} of pioglitazone M4 was reduced to 65% and 70% of the control by trimethoprim and clopidogrel, respectively (Table 2). Rifampicin did not change the AUC_{0-24h} of pioglitazone and pioglitazone M4 (Figure 3, Table 2).

Effects of rifampicin, trimethoprim, and clopidogrel on the pharmacokinetics of repaglinide

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The pharmacokinetics of repaglinide (as a probe drug for CYP2C8/OATP1B1) coadministered with rifampicin, trimethoprim, or clopidogrel was compared with its pharmacokinetics in the absence of coadministered drugs. The plasma concentration of repaglinide was increased by all interacting drugs (Figure 4). The AUC_{0-8h} of repaglinide was 2.8-, 2.0-, or 3.1-fold higher than that of the control when coadministered with rifampicin, trimethoprim, or clopidogrel, respectively (Figure 4, Table 2). The AUC_{0-2h} of repaglinide M1, a CYP3A4/CYP2C8-mediated metabolite of repaglinide, did not change significantly with any coadministered drug (Table 2). Repaglinide M4, a CYP2C8/CYP3A4-mediated metabolite of repaglinide, was below the lower limit of quantification (0.01 ng/mL) in all plasma samples.

Pharmacokinetics of rifampicin, trimethoprim, and clopidogrel

After a single 600 mg dose of rifampicin, the mean \pm SD peak plasma concentration (C_{max}) of rifampicin was 24.3 ± 5.5 $\mu\text{g/mL}$, and its AUC_{0-24h} was 140 ± 33 $\mu\text{g}\cdot\text{h/mL}$ (Figure 5, Table 3). After a single 200 mg dose of trimethoprim, the C_{max} of trimethoprim was 3.2 ± 1.0 $\mu\text{g/mL}$, and its AUC_{0-24h} was 28.2 ± 5.1 $\mu\text{g}\cdot\text{h/mL}$ (Figure 5, Table 3). After a single 300 mg dose of clopidogrel, the plasma concentrations of clopidogrel carboxylic acid and clopidogrel acyl- β -glucuronide were much higher, about 1000-fold those of clopidogrel and 2-oxo-clopidogrel (Figure 5). The C_{max} of clopidogrel, 2-oxo-clopidogrel, clopidogrel carboxylic acid, and clopidogrel acyl- β -glucuronide was 0.0033 ± 0.022 , 0.0055 ± 0.0028 , 10.4 ± 2.6 , and 5.83 ± 3.66 $\mu\text{g/mL}$, respectively (Table 3).

Calculation of R value and DDI prediction

The calculated R values of rifampicin, trimethoprim, clopidogrel, gemfibrozil and their metabolites are shown in Table 4. The predicted AUCRs and the observed AUCRs for pitavastatin, pioglitazone, repaglinide, and cerivastatin are summarized in Table 5.

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After a single 600 mg dose of rifampicin, the estimated R value using the in vitro inhibition data of OATP1B1 was 3.7, and there was a high likelihood of a potential DDI with the OATP1B1 substrate in vivo. The R values for CYP2C8 and CYP3A4 were less than 1.1, and there were no significant potential interactions with CYP2C8 and CYP3A4. The predicted AUCR using the inhibition of OATP1B1 by rifampicin was 3.7, 1.0, and 3.7, for pitavastatin, pioglitazone, and repaglinide, respectively.

After a single 200 mg dose of trimethoprim, the estimated R value using the in vitro inhibition data of CYP2C8 was 1.2, and there was a likelihood of a potential DDI with CYP2C8 substrate in vivo. The R values for OATP1B1 and CYP3A4 were less than 1.1, and there were no significant potential interactions with OATP1B1 and CYP3A4. The predicted AUCR using the inhibition of CYP2C8 by trimethoprim was 1.0 for pitavastatin, 1.13 for pioglitazone, and 1.14 for repaglinide, and these values were dependent on the $f_{m,CYP2C8}$; 0.00, 0.68, and 0.71 for pitavastatin, pioglitazone, and repaglinide, respectively.

After a single 300 mg dose of clopidogrel, the estimated R values for clopidogrel, 2-oxo clopidogrel and clopidogrel carboxylic acid were less than 1.1 for OATP1B1, CYP2C8, and CYP3A4, and there were no potential for a DDI in vivo. Clopidogrel acyl- β -glucuronide inhibited OATP1B1 with an R value of 1.1, and inhibited CYP2C8 in a mechanism-based manner with an R value of 10.3, and thus there was a likelihood and high likelihood of a potential DDI with CYP2C8 substrates in vivo. The predicted AUCR of pitavastatin and repaglinide using the in vitro inhibition data of OATP1B1 by clopidogrel acyl- β -glucuronide was 1.1. The AUCR is in a good agreement with the absence of significant DDI between clopidogrel and pitavastatin. However, the predicted AUCR of repaglinide did not reach the observed AUCR (3.1) despite the maximum inhibitory effect of OATP1B1. When considering only CYP2C8 or both OATP1B1 and CYP2C8 as clopidogrel acyl- β -glucuronide inhibition pathways for DDI, the predicted AUCRs were similar to the observed AUCRs; 2.6 vs 2.0 for pioglitazone, and 2.8–3.1 vs 3.1 for repaglinide.

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After a single 600 mg of gemfibrozil, the R values for the inhibition of OATP1B1 and CYP2C8 by gemfibrozil were less than 1.1, whereas those of gemfibrozil 1-O- β glucuronide were 1.4 and 22.1, respectively, and therefore, there was a high likelihood of a potential interaction with OATP1B1 and/or CYP2C8 substrates in vivo. The predicted AUCRs using the inhibition of OATP1B1 and the mechanism-based inhibition of CYP2C8 by gemfibrozil-1-O- β -glucuronide was 1.4, 4.0, and 4.3 for pitavastatin, pioglitazone, and repaglinide, respectively, indicating that they were comparable with the reported AUCRs of pioglitazone (3.2) and repaglinide (5.0).

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Discussion

To clarify the mechanisms of clopidogrel-mediated DDIs for OATP1B1 and CYP2C8, we performed a clinical cassette small-dose study using OATPs, CYP2C8, and OATP1B1/CYP2C8 probe drugs with rifampicin (an inhibitor for OATPs), trimethoprim (an inhibitor for CYP2C8), and clopidogrel, and these DDI results were validated qualitatively by the prediction of DDI potential for each molecule using a static model and in vitro K_i values.

In a clinical cassette small-dose study, rifampicin increased the AUC of pitavastatin (5.1-fold), repaglinide (2.8-fold), but did not change the AUC of pioglitazone. Trimethoprim and clopidogrel increased the AUC of pioglitazone (1.3-fold and 2.0-fold, respectively) and repaglinide (2.0-fold and 3.1-fold, respectively), but did not change the AUC of pitavastatin (Table 2). In addition, the AUC of a CYP2C8-mediated metabolite of pioglitazone (pioglitazone M4) was decreased to 65% and 70% of the control by trimethoprim and clopidogrel, respectively. Thus, given that the DDIs of trimethoprim and clopidogrel with other drugs were similar, the major mechanism of clopidogrel-mediated DDIs is the inhibition of CYP2C8, but not OATP1B1.

After a single 600 mg rifampicin, the predicted AUC increase of probe substrates because of inhibition of OATP1B1 well explained our observed DDI results, indicating rifampicin mainly inhibits the OATP1B1-mediated uptake and does not inhibit CYP2C8-mediated metabolism (Table 5). When coadministered with a single oral dose of 600 mg rifampicin, our observed AUC increase of pitavastatin (5.1-fold) at the small-dose (0.2 mg) is consistent with that of pitavastatin (5.7-fold) at the clinical dose (4 mg) (Chen et al., 2013).

After a single 200 mg dose of trimethoprim, the predicted AUCRs of probe substrates because of CYP2C8 inhibition was comparable to our observed AUCRs, indicating that trimethoprim mainly inhibits the CYP2C8-mediated metabolism and does not inhibit OATP-mediated uptake (Table 5). Moreover, our observed AUC increases of pioglitazone (1.3-fold at 1 mg dose) and repaglinide (2.0-fold at 0.1 mg dose) after coadministration of a single oral dose of 200 mg trimethoprim were comparable to those reported previously; that is, repeated 160 mg

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dosing with trimethoprim increased the AUCs of pioglitazone (1.4-fold at 15 mg dose) and repaglinide (1.6-fold at 0.25 mg dose) (Niemi et al., 2004; Tornio et al., 2008). In the case of a single oral dose of 300 mg clopidogrel, our observed AUCR of repaglinide (3.1-fold at 0.1 mg dose) was not so different from that reported (4.8-fold at 0.25 mg dose) (Tornio et al., 2014). Taken together, our clinical study using small-dose cassette dosing at less than 20% of the clinical dose was representative of what is observed with individual dosing without significant effect, if any, on the pharmacokinetic profiles of drugs in this cassette, and effectively described the DDIs that are mediated by the inhibition of OATP1B1 and/or CYP2C8.

After oral administration of clopidogrel, two major circulating inactive metabolites, clopidogrel carboxylic acid and clopidogrel acyl- β -glucuronide, were at around 1000-fold higher exposure levels than clopidogrel and 2-oxo-clopidogrel (Table 3). Clopidogrel and 2-oxo-clopidogrel are reported to inhibit OATP1B1, CYP3A4, and CYP2C8 in vitro; however, on account of their low plasma concentration ($[I_{u,max}]/K_i < 0.01$), they predicted a remote possibility of DDIs with a single oral dose of 300 mg clopidogrel (Table 4). Even though plasma concentration of clopidogrel carboxylic acid was high, interaction is not possible because it has a weak in vitro inhibitory effect on OATP1B1, CYP2C8, and CYP3A4 ($K_i > 100 \mu\text{M}$, $[I_{u,max}]/K_i < 0.1$; Table 4). Clopidogrel acyl- β -glucuronide inhibits OATP1B1 competitively with an in vitro K_i value of $10.9 \mu\text{M}$ in incubation conditions, and inhibits CYP2C8 time-dependently with a maximum inactivation rate constant (k_{inact}) of $2.8 /\text{h}$ and inhibitor concentration of $9.9 \mu\text{M}$ needed to cause half of k_{inact} (K_I) (Tornio et al., 2014). The estimated R value for the inhibition of OATP1B1 and the mechanism-based inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide was 1.1 and 10.3, respectively, suggesting a high likelihood of a potential DDI with CYP2C8 substrates in vivo. When coadministered with a single oral dose of 300 mg clopidogrel, the predicted AUCRs using the mechanism-based inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide with $[I_{u,max}]$ of metabolite and

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$f_{m,CYP2C8}$ of substrate were similar with the observed AUCRs; 2.6 vs 2.1 for pioglitazone, and 2.8 vs 3.1 for repaglinide. Considering the accumulation of metabolite in hepatocytes, the maximum unbound concentration in the liver of clopidogrel acyl- β -glucuronide is expected to be higher than $[I_{u,max}]$, indicating that the predicted AUCR in this study may be underestimated. When considering only the inhibition of OATP1B1 in cocubation conditions with clopidogrel acyl- β -glucuronide, the predicted AUCR of pitavastatin (a probe of OATP1B1) was 1.1, which may be an overestimate because we assumed that OATP1B1 completely contributes to the uptake of OATP1B1 substrates. Nevertheless, the predicted AUCR of pitavastatin was in a good agreement with the absence of a significant DDI between clopidogrel and pitavastatin in present clinical study, indicating that the inhibition of OATP1B1 by clopidogrel and its metabolites is negligible, and the preincubation effect in OATP1B1-mediated uptake is small, if any. Furthermore, the predicted AUCR of repaglinide (a substrate of OATP1B1/CYP2C8) using only the inhibition of OATP1B1 by clopidogrel acyl- β -glucuronide was 1.1 in an assumption (complete contribution of OATP1B1 to repaglinide uptake), and 1.05 in 49.6% contribution of OATP1B1 to repaglinide uptake (Gertz et al., 2013). It did not reach the observed AUCR (3.1) of repaglinide, also suggesting that the main mechanism of clopidogrel-mediated DDI is the inhibition of CYP2C8, not OATP1B1.

In this clinical study, all subjects were Japanese, and it was reported that the ratio of hepatic OATP function in Japanese to that in Caucasians was 0.67 (Tomita et al., 2013). The lower contribution of OATP-mediated uptake in Japanese may reduce the magnitude of the DDI with OATP substrates. By contrast, if an inhibitor is also a substrate of OATPs (that can act as a competitive inhibitor), the inhibitor concentration in plasma and in the liver will be increased and decreased, respectively, and that may increase the magnitude of OATP1B1 inhibition, in Japanese. The effect of rifampicin (given as a single oral dose, 600 mg) was also examined for pitavastatin in the United States although the ethnicity of the subjects was not reported (Prueksaritanont et al., 2014). The AUCR of pitavastatin was 5.2, which is similar to

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that in our study (5.1); however, the C_{max} (10 $\mu\text{g/mL}$) of rifampicin is lower than that in our study (24 $\mu\text{g/mL}$) in Japanese. Taken together, it is difficult to conclude the impact of ethnic difference on the DDI involving OATP1B1. In the case of CYP2C8, CYP2C8*3 occurs more frequently in Caucasians, and its functional effect is substrate dependent, e.g. decreased metabolic capacity for paclitaxel and arachidonic acid, and increased metabolic capacity for pioglitazone, repaglinide, and rosiglitazone (Zanger et al., 2013). Garcia-Martin et al. reported the intraethnic variability of CYP2C8*3, demonstrating statistically significant differences in allele frequency among Caucasian Europeans and also among Caucasian Americans, which is likely to be related to their various ethnicity (Garcia-Martin et al., 2006). Thus, the ethnic differences of the pharmacokinetics of CYP2C8 substrate have been unclear. We have considered that the ethnic difference for OATP1B1 seems to impact the magnitude of DDI, but the magnitude of the impact on repaglinide is small (if any) because the hepatic clearance of repaglinide is mediated not simply by OATP1B1 alone, but by both OATP1B1 and CYP2C8, and the passive permeability accounts for about 40% of the total hepatic uptake of repaglinide (Varma et al., 2013).

Taken together, the major mechanism of clopidogrel-mediated DDIs with probe substrates is the mechanism-based inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide, and the inhibition of OATP1B1 is negligible for a single dose of 300 mg clopidogrel in vivo. Our result is consistent with the lack of clinically relevant OATP1B1-mediated DDI of clopidogrel with simvastatin and fluvastatin, although it causes an increase in the AUC of repaglinide (5.0-fold) mainly via the inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide (Ayalasomayajula et al., 2007; Itkonen et al., 2015; Tornio et al., 2014). A clinical study found that a single oral dose of 300 mg clopidogrel increased the AUC of rosuvastatin (1.7-fold) in patients with coronary heart disease (Pinheiro et al., 2012). Rosuvastatin is relatively hydrophilic, with lower passive permeability and lower oral absorption (50%) than fluvastatin, simvastatin, pitavastatin, and cerivastatin, which are lipophilic and have high oral absorption

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(98%, 65–85%, 80%, and 98%, respectively) (Mukhtar et al., 2005; Shitara et al., 2006). It is taken up into the liver by some uptake transporters including OATP1B1, OATP1B3, OATP2B1, and Na⁺/taurocholate cotransporting polypeptide (NCTP) and excreted into bile and intestinal lumen by the breast cancer resistance protein (BCRP) (Ho et al., 2006; Kitamura et al., 2008). Polymorphisms of OATP1B1, BCRP, and NTCP had significant effects on the pharmacokinetics of rosuvastatin (Keskitalo et al., 2009; Lou et al., 2014; Pasanen et al., 2007). By contrast, the pharmacokinetics of pitavastatin were affected by OATP1B1 polymorphism, but not by BCRP polymorphism (Ieiri et al., 2007; Oh et al., 2013). Clopidogrel inhibits BCRP with an in vitro K_i of 63 μM (Elsby et al., 2016). The maximum theoretical gastrointestinal concentration ($[I_g]$) of 300 mg clopidogrel can be calculated by dividing the dose [mol] by 250 mL, and was 3729 μM . The estimated R-value for intestinal BCRP ($[I_g]/K_i$) and hepatic BCRP ($[I_{u,\text{in,max}}]/K_i$) was 59 and <0.01 , respectively, and these data ($[I_g]/K_i >10$) suggest that the inhibition of intestinal BCRP by clopidogrel will cause a DDI in vivo (EMDA, 2012; US FDA, 2012). Thus, pitavastatin is a more sensitive probe substrate of OATP1B1 in vivo than rosuvastatin, and the DDI between rosuvastatin and clopidogrel would be more associated with the inhibition of BCRP in the intestine than the inhibition of OATP1B1 in the liver.

We found that a single dose of 300 mg clopidogrel mainly inhibited CYP2C8-mediated metabolism and increased plasma concentration of not only CYP2C8 substrates, but also dual OATP1B1/CYP2C8 substrates, whereas the effect of clopidogrel on the pharmacokinetics of OATP1B1 substrate was negligible. Gemfibrozil and clopidogrel are highly associated with cerivastatin-induced rhabdomyolysis (Floyd et al., 2012), and they inhibited CYP2C8 irreversibly and hepatic OATP1B1 reversibly through their glucuronide conjugates (Shitara et al., 2004; Tamraz et al., 2013; Floyd et al. 2012). According to reported in vitro inhibition data, they showed similar inhibitory effects on OATP1B1 and CYP2C8 (Table 4). However, the unbound maximum plasma concentration of gemfibrozil 1-O- β -

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glucuronide at a single oral dose of 600 mg gemfibrozil was about 5-fold higher than that of clopidogrel acyl- β -glucuronide at a single oral dose of 300 mg clopidogrel. The calculated R values for the mechanism-based inhibition of CYP2C8 and the inhibition of OATP1B1 were 22.1 and 1.39 by gemfibrozil 1-O- β -glucuronide, and 10.3 and 1.11 by clopidogrel acyl- β -glucuronide, respectively. The maximum predicted AUCRs of repaglinide calculated using equation 2 were 4.3 and 3.1 by gemfibrozil and clopidogrel, respectively, and this suggests that clopidogrel-mediated DDI is milder than gemfibrozil-mediated DDI. The plasma AUC of repaglinide was increased 5.0-fold by a single dose of 600 mg gemfibrozil, and 3.1-fold by a single dose of 300 mg clopidogrel, and these clinical DDIs were consistent with the predicted DDIs (Table 5). The maximum predicted AUCR (3.3-fold) of cerivastatin by a single dose of 600 mg gemfibrozil was validated using the reported AUCR (3.9-fold) (Backman et al., 2002). In this study, the maximum predicted AUCR (2.5-fold) of cerivastatin by a single dose of 300 mg clopidogrel suggested that clopidogrel may also increase the risk of rhabdomyolysis in cerivastatin users, though its DDI effect is somewhat smaller than gemfibrozil.

In conclusion, in a clinical cassette small-dose study using OATP1B1, CYP2C8, and OATP1B1/CYP2C8 probe drugs [pitavastatin and pioglitazone, and repaglinide (instead of cerivastatin), respectively] and inhibitors (rifampicin, trimethoprim, or clopidogrel), we clarified that the major mechanism of clopidogrel-mediated DDIs in vivo is the mechanism-based inhibition of CYP2C8 by clopidogrel acyl- β -glucuronide, and the inhibition of OATP1B1 by the clinical dose of clopidogrel is negligible. These results suggest that clopidogrel is expected to have an effect not only on CYP2C8 substrates, but also dual CYP2C8/OATP1B1 substrates as seen in the case of repaglinide and predicted for cerivastatin, but the effect of clopidogrel on the pharmacokinetics of OATP1B1 substrates is negligible.

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Author Contributions

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Conducted experiments: Kim, and Kimura

Contributed new reagents or analytic tools: Kim, Yoshikado, Ieiri, Maeda, Kimura, Irie, Kusuhara, and Sugiyama

Performed data analysis: Kim, Yoshikado, Ieiri, Maeda, Kimura, Irie, Kusuhara, and Sugiyama.

Wrote or contributed to the writing of the manuscript: Kim, Yoshikado, Ieiri, Maeda, Kimura, Irie, Kusuhara, and Sugiyama

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References

Aquilante CL, Kosmiski LA, Bourne DW, Bushman LR, Daily EB, Hammond KP, Hopley CW, Kadam RS, Kanack AT, Kompella UB, Le M, Predhomme JA, Rower JE and Sidhom MS (2013) Impact of the CYP2C8*3 polymorphism on the drug-drug interaction between gemfibrozil and pioglitazone. *Br J Clin Pharmacol* **75**:217-226.

Ayalasomayajula SP, Vaidyanathan S, Kemp C, Prasad P, Balch A and Dole WP (2007) Effect of clopidogrel on the steady-state pharmacokinetics of fluvastatin. *J Clin Pharmacol* **47**: 613-619.

Backman JT, Kyrklund C, Neuvonen M and Neuvonen PJ (2002) Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* **72**:685-691.

Backman JT, Honkalammi J, Neuvonen M, Kurkinen KJ, Tornio A, Niemi M and Neuvonen PJ (2009) CYP2C8 activity recovers within 96 hours after gemfibrozil dosing: estimation of CYP2C8 half-life using repaglinide as an in vivo probe. *Drug Metab Dispos* **37**: 2359-2366.

Bae JW, Jang CG and Lee SY (2011) Effects of clopidogrel on the pharmacokinetics of sibutramine and its active metabolites. *J Clin Pharmacol* **51**:1704-1711.

Boman G and Ringberger VA (1974) Binding of rifampicin by human plasma proteins. *Eur J Clin Pharmacol* **7**:369-373.

Chen BL, Chen Y, Tu JH, Li YL, Zhang W, Li Q, Fan L, Tan ZR, Hu DL, Wang D, Wang LS, Ouyang DS and Zhou HH (2009) Clopidogrel inhibits CYP2C19-dependent hydroxylation of omeprazole related to CYP2C19 genetic polymorphisms. *J Clin Pharmacol* **49**:574-581.

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Chen Y, Zhang W, Huang WH, Tan ZR, Wang YC, Huang X and Zhou HH (2013) Effect of a single-dose rifampicin on the pharmacokinetics of pitavastatin in healthy volunteers. *Eur J Clin Pharmacol* **69**:1933-1938.

Elsby R, Martin P, Surry D, Sharma P and Fenner K (2016) Solitary inhibition of the breast cancer resistance protein efflux transporter results in a clinically significant drug-drug interaction with rosuvastatin by causing up to a 2-fold increase in statin exposure. *Drug Metab Dispos* **44**:398-408.

EMA (2012) Guideline on the Investigation of Drug Interactions. *Committee for Human Medicinal Products (CHMP)*.

Floyd JS, Kaspera R, Marcianti KD, Weiss NS, Heckbert SR, Lumley T, Wiggins KL, Tamraz B, Kwok PY, Totah RA and Psaty BM (2012) A screening study of drug-drug interactions in cerivastatin users: an adverse effect of clopidogrel. *Clin Pharmacol Ther* **91**:896-904.

Fujino H, Yamada I, Shimada S, Yoneda M and Kojima J (2003) Metabolic fate of pitavastatin, a new inhibitor of HMG-CoA reductase: human UDP-glucuronosyltransferase enzymes involved in lactonization. *Xenobiotica* **33**:27-41.

García-Martín E, Martínez C, Ladero JM and Agúndez JA (2006) Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. *Mol Diagn Ther* **10**:29-40.

Gertz M, Cartwright CM, Hobbs MJ, Kenworthy KE, Rowland M, Houston JB and Galetin A (2013) Cyclosporine inhibition of hepatic and intestinal CYP3A4, uptake and efflux

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transporters: application of PBPK modeling in the assessment of drug-drug interaction potential. *Pharm Res* **30**:761-780.

Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA and Ikeda T (2009) A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos* **37**:2145-2152.

Hagihara K, Nishiya Y, Kurihara A, Kazui M, Farid NA and Ikeda T (2008) Comparison of human cytochrome P450 inhibition by the thienopyridines prasugrel, clopidogrel, and ticlopidine. *Drug Metab Pharmacokinet* **23**:412-420.

Honkalammi J, Niemi M, Neuvonen PJ and Backman JT (2011a) Mechanism-based inactivation of CYP2C8 by gemfibrozil occurs rapidly in humans. *Clin Pharmacol Ther* **89**:579-586.

Honkalammi J, Niemi M, Neuvonen PJ and Backman JT (2011b) Dose-dependent interaction between gemfibrozil and repaglinide in humans: strong inhibition of CYP2C8 with subtherapeutic gemfibrozil doses. *Drug Metab Dispos* **39**:1977-1986.

Honkalammi J, Niemi M, Neuvonen PJ and Backman JT (2012) Gemfibrozil is a strong inactivator of CYP2C8 in very small multiple doses. *Clin Pharmacol Ther* **91**:846-855.

Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y and Kim RB (2006) Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130**:1793-1806.

DMD#70276

Hruska MW, Amico JA, Langae TY, Ferrell RE, Fitzgerald SM and Frye RF (2005) The effect of trimethoprim on CYP2C8 mediated rosiglitazone metabolism in human liver microsomes and healthy subjects. *Br J Clin Pharmacol* **59**:70-79.

Ieiri I, Suwannakul S, Maeda K, Uchimar H, Hashimoto K, Kimura M, Fujino H, Hirano M, Kusuhara H, Irie S, Higuchi S and Sugiyama Y (2007) SLCO1B1 (OATP1B1, an uptake transporter) and ABCG2 (BCRP, an efflux transporter) variant alleles and pharmacokinetics of pitavastatin in healthy volunteers. *Clin Pharmacol Ther* **82**:541-547.

Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M and Backman JT (2015) Clopidogrel has no clinically meaningful effect on the pharmacokinetics of the organic anion transporting polypeptide 1B1 and cytochrome P450 3A4 substrate simvastatin. *Drug Metab Dispos* **43**:1655-1660.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H and Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50**:387-412.

Izumi S, Nozaki Y, Maeda K, Komori T, Takenaka O, Kusuhara H and Sugiyama Y (2015) Investigation of the impact of substrate selection on in vitro organic anion transporting polypeptide 1B1 inhibition profiles for the prediction of drug-drug interactions. *Drug Metab Dispos* **43**:235-247.

Jaakkola T, Laitila J, Neuvonen PJ and Backman JT (2006) Pioglitazone is metabolised by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin Pharmacol Toxicol* **99**:44-51.

DMD#70276

Jiang F, Desta Z, Shon JH, Yeo CW, Kim HS, Liu KH, Bae SK, Lee SS, Flockhart DA and Shin JG (2013) Effects of clopidogrel and itraconazole on the disposition of efavirenz and its hydroxyl metabolites: exploration of a novel CYP2B6 phenotyping index. *Br J Clin Pharmacol* **75**:244-253.

Kajosaari LI, Laitila J, Neuvonen PJ and Backman JT (2005a) Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. *Basic Clin Pharmacol Toxicol* **97**:249-256.

Kajosaari LI, Niemi M, Neuvonen M, Laitila J, Neuvonen PJ and Backman JT (2005b) Cyclosporine markedly raises the plasma concentrations of repaglinide. *Clin Pharmacol Ther* **78**:388-399.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ and Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **86**:197-203.

Kitamura S, Maeda K, Wang Y and Sugiyama Y (2008) Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. *Drug Metab Dispos* **36**:2014-2023.

Kudo T, Hisaka A, Sugiyama Y and Ito K (2013) Analysis of the repaglinide concentration increase produced by gemfibrozil and itraconazole based on the inhibition of the hepatic uptake transporter and metabolic enzymes. *Drug Metab Dispos* **41**:362-371.

Lou XY, Zhang W, Wang G, Hu DL, Guo D, Tan ZR, Zhou HH, Chen Y and Bao HH (2014) The effect of Na⁺/taurocholate cotransporting polypeptide (NTCP) c.800C > T polymorphism on rosuvastatin pharmacokinetics in Chinese healthy males. *Pharmazie* **69**:775-779.

DMD#70276

Maeda K, Ikeda Y, Fujita T, Yoshida K, Azuma Y, Haruyama Y, Yamane N, Kumagai Y and Sugiyama Y (2011) Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. *Clin Pharmacol Ther* **90**:575-581.

Mück W, Mai I, Fritsche L, Ochmann K, Rohde G, Unger S, Johne A, Bauer S, Budde K, Roots I, Neumayer HH and Kuhlmann J (1999) Increase in cerivastatin systemic exposure after single and multiple dosing in cyclosporine-treated kidney transplant recipients. *Clin Pharmacol Ther* **65**:251-261.

Mukhtar RY, Reid J and Reckless JP (2005) Pitavastatin. *Int J Clin Pract* **59**:239-252.

Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivistö KT and Neuvonen PJ (2005) Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* **77**:468-478.

Niemi M, Kajosaari LI, Neuvonen M, Backman JT and Neuvonen PJ (2004) The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. *Br J Clin Pharmacol* **57**:441-447.

Nishiya Y, Hagihara K, Ito T, Tajima M, Miura S, Kurihara A, Farid NA and Ikeda T (2009a) Mechanism-based inhibition of human cytochrome P450 2B6 by ticlopidine, clopidogrel, and the thiolactone metabolite of prasugrel. *Drug Metab Dispos* **37**:589-593.

DMD#70276

Nishiya Y, Hagihara K, Kurihara A, Okudaira N, Farid NA, Okazaki O and Ikeda T (2009b) Comparison of mechanism-based inhibition of human cytochrome P450 2C19 by ticlopidine, clopidogrel, and prasugrel. *Xenobiotica* **39**:836-843.

Oh ES, Kim CO, Cho SK, Park MS and Chung JY (2013) Impact of ABCC2, ABCG2 and SLCO1B1 polymorphisms on the pharmacokinetics of pitavastatin in humans. *Drug Metab Pharmacokinet* **28**:196-202.

Pasanen MK, Fredrikson H, Neuvonen PJ and Niemi M (2007) Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **82**:726-733.

Pinheiro LF, Franca CN, Izar MC, Barbosa SP, Bianco HT, Kasma SH, Mendes GD, Povia RM and Fonseca FA (2012) Pharmacokinetic interactions between clopidogrel and rosuvastatin: effects on vascular protection in subjects with coronary heart disease. *Int J Cardiol* **158**:125-129.

Prueksaritanont T, Chu X, Evers R, Klopfer SO, Caro L, Kothare PA, Dempsey C, Rasmussen S, Houle R, Chan G, Cai X, Valesky R, Fraser IP and Stoch SA (2014) Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. *Br J Clin Pharmacol* **78**:587-598.

Rowland Yeo K, Walsky RL, Jamei M, Rostami-Hodjegan A and Tucker GT (2011) Prediction of time-dependent CYP3A4 drug-drug interactions by physiologically based pharmacokinetic modeling: impact of inactivation parameters and enzyme turnover. *Eur J Pharm Sci* **43**:160-173.

DMD#70276

Säll C, Houston JB and Galetin A (2012) A comprehensive assessment of repaglinide metabolic pathways: impact of choice of in vitro system and relative enzyme contribution to in vitro clearance. *Drug Metab Dispos* **40**:1279-1289.

Shitara Y, Hirano M, Sato H and Sugiyama Y (2004) Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)- mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* **311**:228-236.

Shitara Y and Sugiyama Y (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacol Ther* **112**:71-105.

Tamraz B, Fukushima H, Wolfe AR, Kaspera R, Totah RA, Floyd JS, Ma B, Chu C, Marciante KD, Heckbert SR, Psaty BM, Kroetz DL and Kwok PY (2013) OATP1B1-related drug-drug and drug-gene interactions as potential risk factors for cerivastatin-induced rhabdomyolysis. *Pharmacogenet Genomics* **23**:355-364.

Tomita Y, Maeda K and Sugiyama Y (2013) Ethnic variability in the plasma exposures of OATP1B1 substrates such as HMG-CoA reductase inhibitors: a kinetic consideration of its mechanism. *Clin Pharmacol Ther* **94**:37-51.

Tornio A, Niemi M, Neuvonen PJ and Backman JT (2008) Trimethoprim and the CYP2C8*3 allele have opposite effects on the pharmacokinetics of pioglitazone. *Drug Metab Dispos* **36**:73-80.

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Tornio A, Filppula AM, Kailari O, Neuvonen M, Nyrönen TH, Tapaninen T, Neuvonen PJ, Niemi M and Backman JT (2014) Glucuronidation converts clopidogrel to a strong time-dependent inhibitor of CYP2C8: a phase II metabolite as a perpetrator of drug-drug interactions. *Clin Pharmacol Ther* 96:498-507.

Silvestro L, Gheorghe M, Iordachescu A, Ciuca V, Tudoroni A, Rizea Savu S and Tarcomnicu I (2011) Development and validation of an HPLC-MS/MS method to quantify clopidogrel acyl glucuronide, clopidogrel acid metabolite, and clopidogrel in plasma samples avoiding analyte back-conversion. *Anal Bioanal Chem* 401:1023-1034.

Ueda K, Kato Y, Komatsu K and Sugiyama Y (2001) Inhibition of biliary excretion of methotrexate by probenecid in rats: quantitative prediction of interaction from in vitro data. *J Pharmacol Exp Ther* 297:1036-1043.

US Food and Drug Administration (2012) Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. *Center for Drug Evaluation and Research (CDER)*.

Varma MV, Lai Y, Kimoto E, Goosen TC, El-Kattan AF, Kumar V (2013) Mechanistic modeling to predict the transporter- and enzyme-mediated drug-drug interactions of repaglinide. *Pharm Res* 30:1188-1199.

Wijkström A and Westerlund D (1983) Plasma protein binding of sulphadiazine, sulphamethoxazole and trimethoprim determined by ultrafiltration. *J Pharm Biomed Anal* 1:293-299.

DMD#70276

Yoshida K, Maeda K and Sugiyama Y (2012) Transporter-mediated drug-drug interactions involving OATP substrates: predictions based on in vitro inhibition studies. *Clin Pharmacol Ther* **91**:1053-1064.

Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* **138**:103-141.

Zhu HJ, Wang X, Gawronski BE, Brinda BJ, Angiolillo DJ and Markowitz JS (2013) Carboxylesterase 1 as a determinant of clopidogrel metabolism and activation. *J Pharmacol Exp Ther* **344**:665-672.

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Footnotes

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Figure Legends

Figure 1. Study design. A cassette was consisted with 0.2 mg pitavastatin, 1 mg pioglitazone and 0.1 mg repaglinide; 600 mg rifampicin, 200 mg trimethoprim and 300 mg clopidogrel were coadministered with the cassette dose to each group, respectively.

Figure 2. Pharmacokinetics of a single 0.2 mg pitavastatin when coadministered with a single dose of 600 mg rifampicin, 200 mg trimethoprim or 300 mg clopidogrel in 8 healthy volunteers. Mean plasma concentration-time profiles of pitavastatin with rifampicin (a), trimethoprim (b) and clopidogrel (c) and individual pitavastatin AUCs (d). RIF, rifampicin; TRM, trimethoprim; CPD, clopidogrel; *** $P < 0.0005$ vs. Control.

Figure 3. Pharmacokinetics of a single 1 mg pioglitazone when coadministered with a single dose of 600 mg rifampicin, 200 mg trimethoprim or 300 mg clopidogrel in 8 healthy volunteers. Mean plasma concentration-time profiles of pioglitazone and its metabolite M4 with rifampicin (a and b), trimethoprim (c and d) and clopidogrel (e and f) and individual AUCs of pioglitazone (g) and its metabolite M4 (h). RIF, rifampicin; TRM, trimethoprim; CPD, clopidogrel; * $P < 0.05$ vs. Control, ** $P < 0.005$ vs. Control.

Figure 4. Pharmacokinetics of a single 0.1 mg repaglinide when coadministered with a single dose of 600 mg rifampicin, 200 mg trimethoprim or 300 mg clopidogrel in 8 healthy volunteers. Mean plasma concentration-time profiles of repaglinide with rifampicin (a), trimethoprim (b) and clopidogrel (c) and individual repaglinide AUCs (d). RIF, rifampicin; TRM, trimethoprim; CPD, clopidogrel; * $P < 0.05$ vs. Control, ** $P < 0.005$ vs. Control, *** $P < 0.0005$ vs. Control.

Figure 5. Mean plasma concentration-time profiles of rifampicin, trimethoprim, and clopidogrel and its three metabolites after a single dose of 600 mg rifampicin (a), 200 mg

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trimethoprim (b) and 300 mg clopidogrel (c), respectively. CPD, clopidogrel; 2-oxo CPD, 2-oxo clopidogrel; CPD acid, clopidogrel carboxylic acid; CPD-glu, clopidogrel acyl- β -glucuronide.

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Table 1. Summary for all probe drugs and inhibitors in cassette small dose clinical study.

Drugs	Enzyme/Transporter^a	Clinical dose (mg)	Used dose (mg) (Single oral)
Cassette of probe substrates			
Pitavastatin	OATP1B1	1 ~ 4 ^b	0.2
Pioglitazone	CYP2C8	15 ~ 30 ^c	1
Repaglinide	OATP1B1/CYP2C8	0.25 ~ 1 ^d	0.1
Inhibitors			
Rifampicin	OATPs	450 ~ 600 ^e	600
Trimethoprim	CYP2C8	80 ~ 320 ^f	200
Clopidogrel	OATP1B1/CYP2C8	75 ~ 300 ^g	300

^a Elimination pathway for drug-drug interaction in this study

^b FDA (2009) Drug approval package: LIVALO (pitavastatin calcium), FDA application No., (NDA) 022363

^c FDA (1999) Drug approval package: ACTOS (pioglitazone hydrochloride), FDA application No., (NDA) 021073

^d PMDA (2016) Interview Form: SUREPOST (repaglinide) (https://www.pmda.go.jp/PmdaSearch/iyakuDetail/ResultDataSetPDF/430574_6164001M1216_1_07)

^e RIFADIN (rifampicin) JAPAN PMDA drug label, revised April 2015; Daiichi-Sankyo Co. Accessed via PMDA website (www.pmda.go.jp) on 13 April 2016.

^f Baktar (sulfamethoxazole and trimethoprim) JAPAN PMDA drug label, revised February 2014; Shionogi & Co. Accessed via PMDA website (www.pmda.go.jp) on 13 April 2016.

^g Plavix (clopidogrel bisulfate) JAPAN PMDA drug label, revised March 2016; Sanofi. Accessed via PMDA website (www.pmda.go.jp) on 13 April 2016.

Table 2. Pharmacokinetic variables of pitavastatin, pioglitazone, repaglinide and their metabolite in eight healthy volunteers when coadministered with a single dose of 600 mg rifampicin, 200 mg trimethoprim or 300 mg clopidogrel.

Variables	Control	Rifampicin 600 mg	<i>P</i> value	Control	Trimethoprim 600 mg	<i>P</i> value	Control	Clopidogrel 300 mg	<i>P</i> value
Pitavastatin									
C_{max} (ng/mL)	3.97 ± 1.31	18.7 ± 8.0	0.0011**	3.26 ± 1.35	2.98 ± 1.58	0.6301	2.78 ± 1.62	2.86 ± 2.13	0.8840
t_{max} (h)	0.56 ± 0.18	0.56 ± 0.18	1.0000	0.56 ± 0.18	0.66 ± 0.40	0.5040	0.56 ± 0.18	0.56 ± 0.18	1.0000
AUC _{0-24h} (ng·h/mL)	8.45 ± 1.75	42.7 ± 15.0	0.0002***	7.69 ± 2.20	7.26 ± 1.45	0.5150	6.84 ± 3.32	7.45 ± 4.34	0.5779
Pitavastatin lactone									
C_{max} (ng/mL)	2.82 ± 0.74	2.16 ± 0.43	0.0353*	2.77 ± 0.84	2.68 ± 0.68	0.7366	3.10 ± 0.97	2.55 ± 0.68	0.0570
t_{max} (h)	1.00 ± 0.00	0.81 ± 0.26	0.0796	1.13 ± 0.44	1.13 ± 0.44	1.0000	1.00 ± 0.00	1.31 ± 0.37	0.0492*
AUC _{0-24h} (ng·h/mL)	14.2 ± 3.6	12.6 ± 3.0	0.2616	13.4 ± 3.9	13.5 ± 2.3	0.9242	15.6 ± 4.1	15.9 ± 6.4	0.8652
Pioglitazone									
C_{max} (ng/mL)	65.0 ± 11.0	65.6 ± 11.8	0.8775	59.6 ± 13.0	54.2 ± 11.0	0.2426	58.4 ± 10.8	72.0 ± 12.3	0.0041**
t_{max} (h)	1.06 ± 0.56	1.81 ± 1.39	0.1635	1.63 ± 1.27	2.44 ± 0.82	0.1415	1.50 ± 0.80	2.69 ± 1.10	0.0460*
AUC _{0-24h} (ng·h/mL)	475 ± 81	486 ± 82	0.5859	477 ± 87	591 ± 84	0.0096*	529 ± 248	938 ± 179	0.0015**
Pioglitazone M4									
C_{max} (ng/mL)	19.6 ± 1.5	22.0 ± 3.7	0.0897	19.3 ± 3.1	14.6 ± 3.9	0.0050*	17.2 ± 5.5	13.3 ± 3.2	0.0040**
t_{max} (h)	16.0 ± 6.8	20.0 ± 7.4	0.2002	20.5 ± 6.6	24.0 ± 0.0	0.1755	16.0 ± 6.8	24.0 ± 0.0	0.0123*
AUC _{0-24h} (ng·h/mL)	382 ± 37	421 ± 76	0.2725	359 ± 72	233 ± 79	0.0007**	328 ± 109	221 ± 58	0.0013**
Repaglinide									
C_{max} (ng/mL)	2.36 ± 0.88	5.82 ± 1.66	0.0007**	2.04 ± 0.80	2.88 ± 1.14	0.0332*	2.18 ± 0.92	2.79 ± 1.19	0.0254*
t_{max} (h)	0.50 ± 0.23	0.53 ± 0.21	0.7849	0.56 ± 0.18	0.56 ± 0.40	1.0000	0.66 ± 0.40	0.88 ± 0.58	0.1334
AUC _{0-8h} (ng·h/mL)	2.94 ± 1.28	7.64 ± 2.86	0.0005***	2.71 ± 1.05	4.93 ± 1.72	0.0044**	4.01 ± 2.58	10.0 ± 5.2	0.0088*
Repaglinide M1									
C_{max} (ng/mL)	0.10 ± 0.03	0.13 ± 0.05	0.1333	0.08 ± 0.03	0.08 ± 0.02	0.7150	0.08 ± 0.04	0.07 ± 0.03	0.7748
t_{max} (h)	0.69 ± 0.26	0.50 ± 0.00	0.0796	0.66 ± 0.30	0.88 ± 0.58	0.2133	0.75 ± 0.38	0.94 ± 0.50	0.4758
AUC _{0-2h} (ng·h/mL)	0.12 ± 0.03	0.14 ± 0.04	0.3876	0.09 ± 0.04	0.09 ± 0.03	0.9036	0.09 ± 0.05	0.10 ± 0.04	0.4110

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Data are presented as mean \pm SD. In all plasma samples, the concentration of repaglinide M4 was below the lower limit of quantification (0.01 ng/mL).

C_{\max} , peak plasma concentration; t_{\max} , time to C_{\max} ; AUC_{0-t} , area under the plasma concentration–time curve from 0 to t.

* $P < 0.05$ vs. Control, ** $P < 0.005$ vs. Control, *** $P < 0.0005$ vs. Control.

Table 3. Pharmacokinetic variables of rifampicin, trimethoprim, and clopidogrel and its metabolites (2-oxo clopidogrel, clopidogrel carboxylic acid and clopidogrel acyl- β -glucuronide).

Variables	Rifampicin 600 mg	Trimethoprim 200 mg	Clopidogrel 300 mg			
			clopidogrel	2-oxo clopidogrel	Clopidogrel carboxylic acid	Clopidogrel acyl- β -glucuronide
C_{\max} ($\mu\text{g/mL}$)	24.3 \pm 5.5	3.20 \pm 0.96	0.0033 \pm 0.0022	0.0055 \pm 0.0028	10.4 \pm 2.6	5.83 \pm 3.66
t_{\max} (h)	1.31 \pm 0.88	1.69 \pm 1.25	0.88 \pm 0.58	0.75 \pm 0.27	1.31 \pm 0.75	2.06 \pm 0.62
$t_{1/2}$ (h)	3.34 \pm 0.63	8.58 \pm 1.83	4.85 \pm 3.89	7.20 \pm 3.38	8.28 \pm 1.41	5.25 \pm 1.06
AUC _{0-t} ($\mu\text{g}\cdot\text{h/mL}$)	140 \pm 33	28.2 \pm 5.1	0.0061 \pm 0.0037	0.012 \pm 0.004	41.0 \pm 7.0	29.1 \pm 14.9
AUC _{0-∞} ($\mu\text{g}\cdot\text{h/mL}$)	142 \pm 34	33.4 \pm 7.1	0.0068 \pm 0.0045	0.013 \pm 0.003	44.3 \pm 8.0	30.0 \pm 15.3

Data are presented as mean \pm SD.

C_{\max} , peak plasma concentration; t_{\max} , time to C_{\max} ; $t_{1/2}$, elimination half-life; AUC_{0-t}, area under the plasma concentration-time curve from 0 to t (24 h for rifampicin, trimethoprim, clopidogrel carboxylic acid and clopidogrel acyl- β -glucuronide, 8 h for clopidogrel, and 12 h for 2-oxo clopidogrel); AUC_{0- ∞} , area under the plasma concentration-time curve from 0 to infinity.

Table 4. Summary of input parameters and prediction of drug-drug interactions by static model.

R value was determined using $R = (1 + [I]/K_i)$ and $R = [1 + (k_{inact} \times [I]) / \{(K_I + [I]) \times K_{deg}\}]$ for reversible inhibition and mechanism-based inhibition, respectively. $[I]$ represents $[I_{u,max}]$ for the inhibition of CYP2C8, and $[I_{u,in,max}]$ for the inhibition of OATP1B1 of parent drug. In the case of metabolites, $[I_{u,max}]$ was used as $[I]$ regardless inhibition pathway. K_i values were the geometric mean of all in vitro K_i (or IC_{50}) values that were derived from the University of Washington's Metabolism and Transport Drug Interaction Database (DIDB) (<http://www.druginteractioninfo.org>) for each inhibitor against the specific enzyme/transporter in various experimental conditions. The figures in parentheses represent minimum value and maximum value of all reported data, and n is the number of reports.

Inhibitors	Dose (mg)	k_a (/h)	f_u	$I_{u,max}$ (μ M)	$I_{u,in,max}$ (μ M)	OATP1B1			CYP2C8			CYP3A4		
						K_i or IC_{50} (μ M)	n	R	K_i or IC_{50} (μ M)	n	R	K_i or IC_{50} (μ M)	n	R
Rifampicin	600	0.52 ^a	0.11 ^b	3.28 ^a	3.75 ^a	1.39 (0.30–50)	47	3.70	30.2	1	1.11	N.A. (18.5, >240)	3	<1.18
Trimethoprim	200	0.49 ^a	0.50 ^c	5.50 ^a	7.37 ^a	>100	1	<1.07	27.3 (7.6–122)	11	1.20	>600	3	<1.01
Clopidogrel	300	1.22 ^a	0.02 ^d	0.0002 ^a	0.25 ^a	3.95	1	1.06	12.3 (3.4–53.6)	3	<1.01	K_i , 87 μ M k_{inact} , 3.18 1/h	1	<1.01
2-oxo clopidogrel	NA	NA	0.02 ^d	0.0003	NA	8.18	1	<1.00	11.6 (4.2, 32)	2	<1.01	6.37 (3.1, 13.1)	2	<1.01
Clopidogrel carboxylic acid	NA	NA	0.06 ^d	2.03	NA	>100	1	<1.02	233 (136, 400)	2	<1.01	N.A. (356, >400)	2	<1.01
Clopidogrel acyl- β -glucuronide	NA	NA	0.1 ^d	1.21	NA	10.9	1	1.11	K_i , 9.9 μ M k_{inact} , 2.82 1/h	1	10.3	N.A. (27, >340)	2	<1.05

Gemfibrozil	600	3.50 ^e	0.0065 ^f	0.69 ^e	1.30 ^e	32.2 (4.0–252)	32	1.04	45.6 (9.3–120)	21	1.02	260 (174–406)	4	<1.01
Gemfibrozil 1-O- β -glucuronide	NA	NA	0.115 ^f	5.55 ^e	NA	14.2 (7.9–24)	4	1.39	K_i , 26.7 μ M (10–52) k_{inact} , 4.05 1/h (1.3–13)	9	22.1	267	1	1.02

NA, not applicable.

^a Data from the present cassette small-dose study.

^b Boman and Ringberger. (1974)

^c Wijkström et al. (1983)

^d Tornio et al. (2014)

^e Honkalammi et al. (2011a)

^f Shitara et al. (2004)

Table 5. Predicted and observed AUC ratios (AUCRs) with rifampicin, trimethoprim, clopidogrel and gemfibrozil based upon inhibition of OATP1B1 and/or CYP2C8.

Observed AUCRs are presented as mean (95% confidence interval), and predicted AUCRs were calculated by equation (2), the detail data used for this calculation are presented in Table 4.

Substrates		Coadministered drugs											
Name	$f_{m,CYP2C8}^a$	Rifampicin 600 mg		Trimethoprim 200 mg		Observed AUCR	Clopidogrel 300 mg			Observed AUCR	Gemfibrozil 600 mg		
		Observed AUCR	Predicted AUCR OATP1B1 ^b	Observed AUCR	Predicted AUCR CYP2C8 ^b		OATP1B1 ^b	CYP2C8 ^b	OATP1B1/ CYP2C8 ^b		OATP1B1 ^b	CYP2C8 ^b	OATP1B1/ CYP2C8 ^b
Pitavastatin	0.00	5.10 (3.89, 6.31)	3.70	0.99 (0.80, 1.18)	1.00	1.09 (0.87, 1.31)	1.11	1.00	1.11	-	1.39	1.00	1.39
Pioglitazone	0.68	1.03 (0.95, 1.11)	1.00	1.26 (1.11, 1.42)	1.13	2.01 (1.56, 2.46)	1.00	2.59	2.59	3.2 ^c (2.7, 3.8)	1.39	2.85	3.97
Repaglinide	0.71	2.78 (2.05, 3.53)	3.70	1.99 (1.45, 2.52)	1.14	3.09 (2.12, 4.06)	1.11	2.78	3.09	5.0 ^d (4.2, 5.8)	1.39	3.11	4.32
Cerivastatin	0.61	-	3.70	-	1.11	-	1.11	2.23	2.47	3.9 ^e (2.8, 4.9)	1.39	2.39	3.33

-, not reported.

^a In vitro data; pitavastatin (Fujino et al., 2003), pioglitazone (Jaakkola et al., 2006), repaglinide (Kajossari et al., 2005a), cerivastatin (Shitara et al., 2004).

^b Inhibition pathways for the prediction of AUCR

^c Aquilante et al. (2012)

^d Honkalammi et al. (2011a)

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^e Backman et al. (2002)

Fig. 1

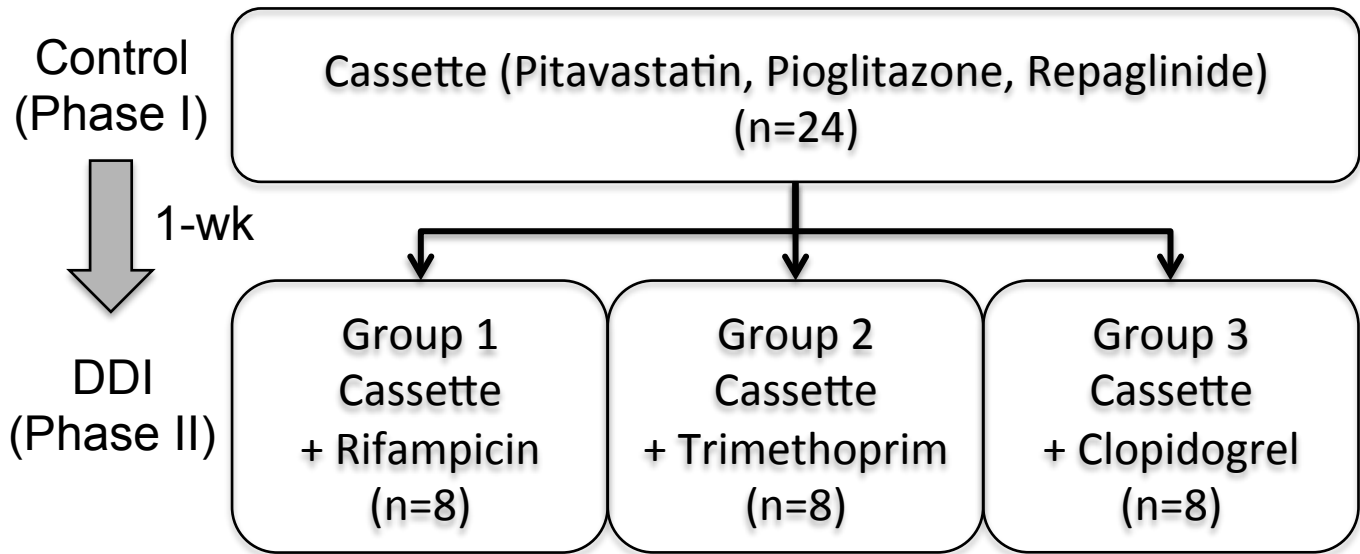


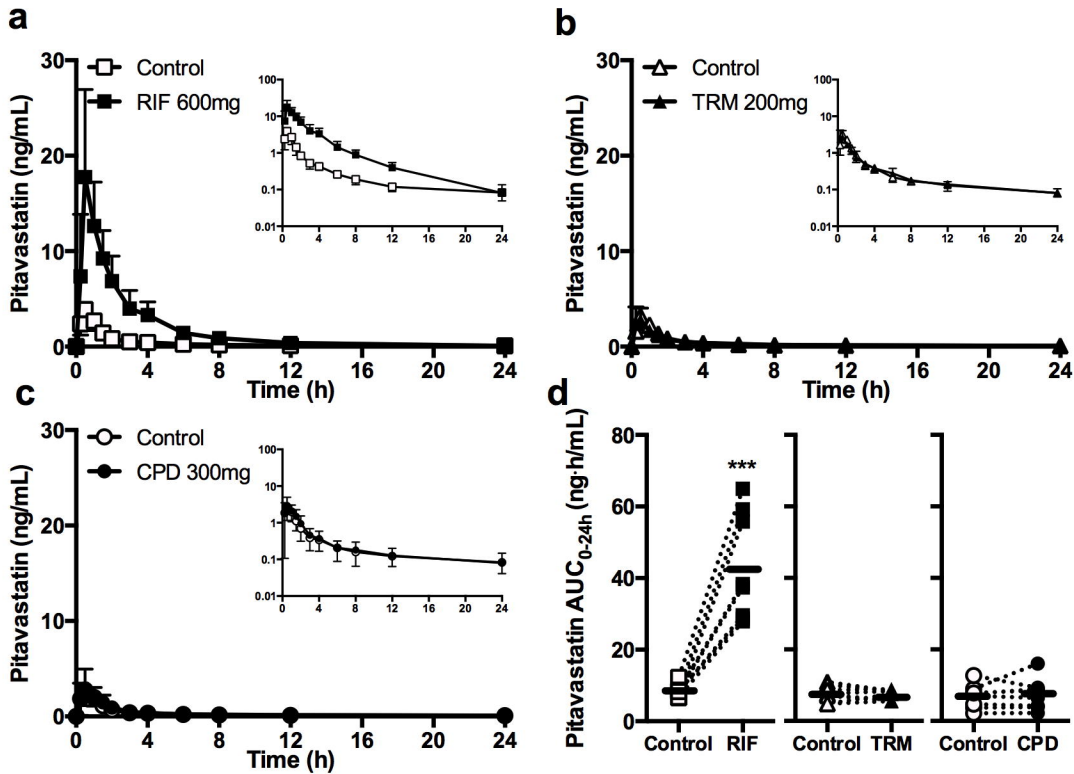
Fig. 2

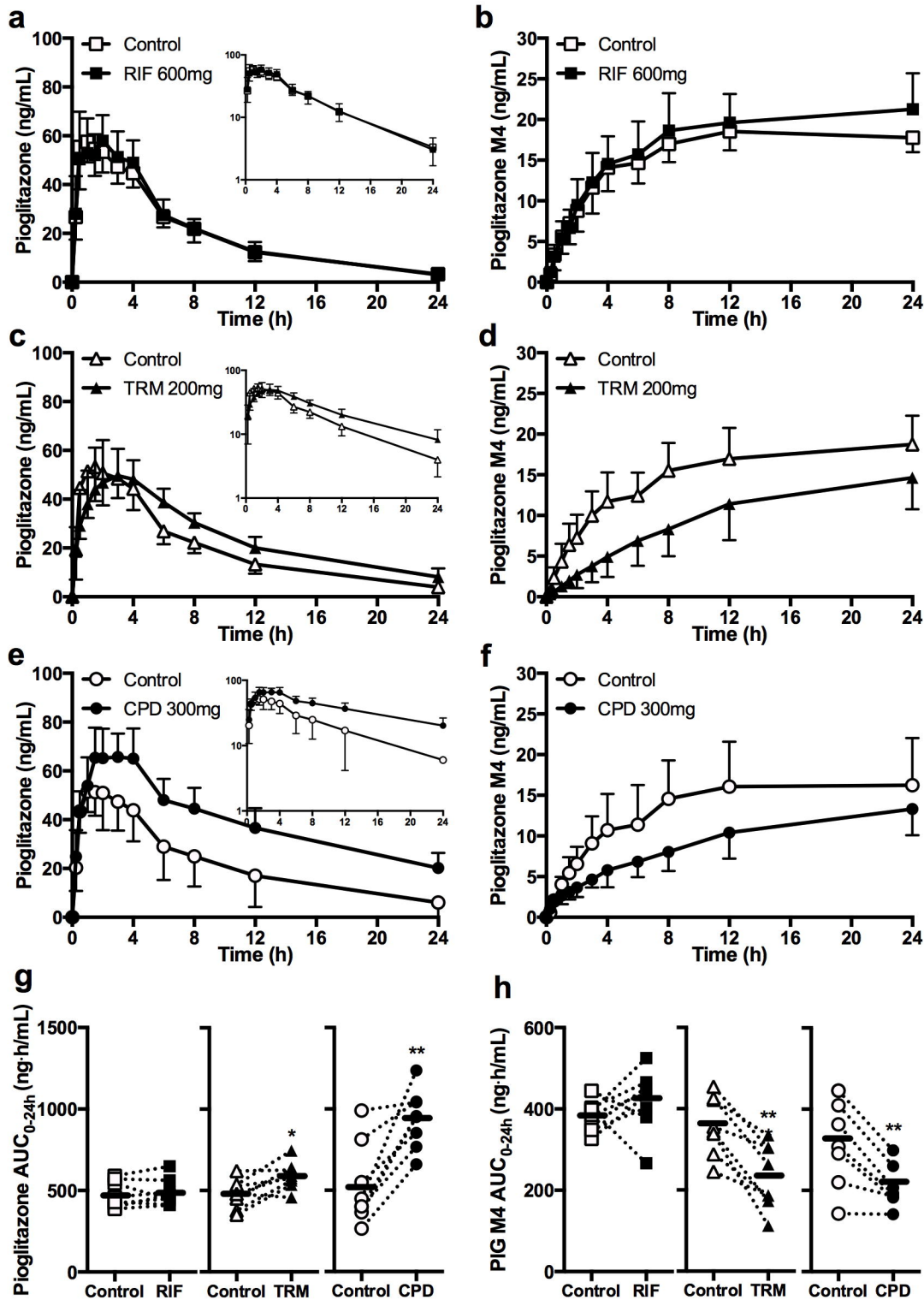
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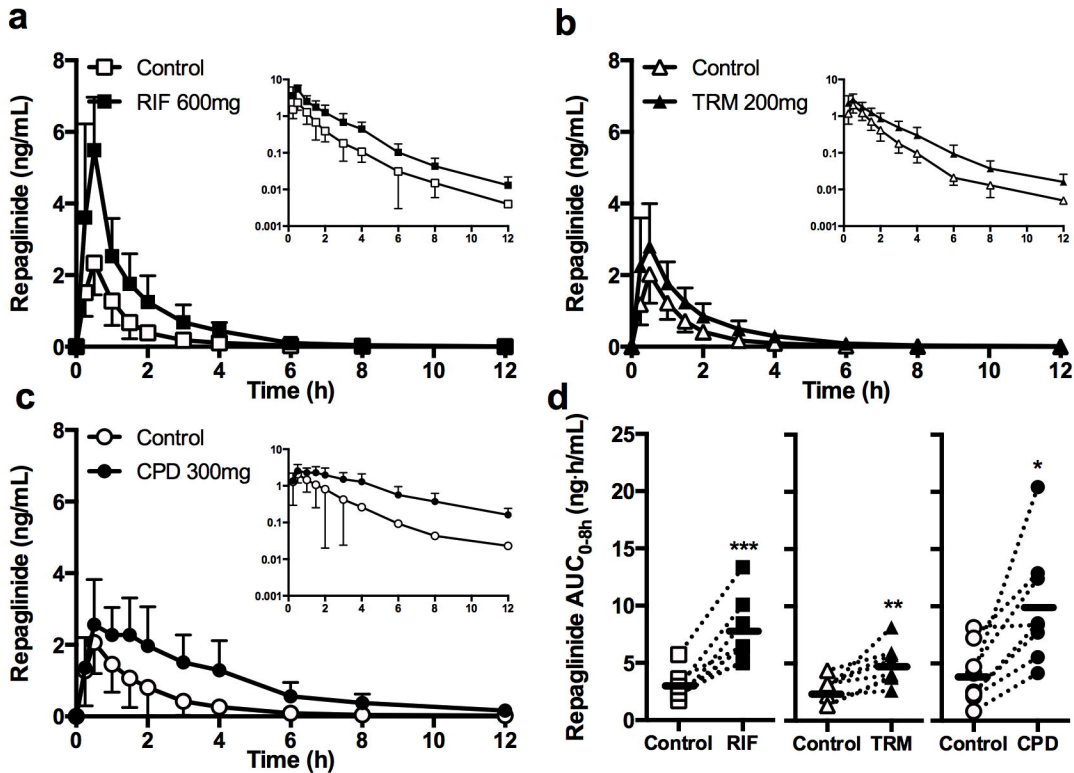
Fig. 4

Fig. 5

