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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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**Running Title Page**

Running title: Mechanism of clopidogrel-mediated drug–drug interaction

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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; ka, first order absorption rate constant; $t_{max}$, time to $C_{max}$

**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 clopiodogrel. 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

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

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/m$^2$. 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,

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 ×*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 ×*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-meidated 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):

$$AUCR = \frac{AUC_{\mathrm{i}}}{AUC} =$$

$$1 \Big/ \left[ \frac{1}{\left(1+\sum \frac{[I_{\mathrm{u,in,max}}]}{K_{\mathrm{i,OATP1B1}}}\right)} \times \left\{ \frac{f_{\mathrm{m,CYP2C8}}}{\left(1+\sum \frac{[I_{\mathrm{u,max}}]}{K_{\mathrm{i,CYP2C8}}}\right) \times \left(1+\sum \frac{k_{\mathrm{inact,CYP2C8}} \times [I_{\mathrm{u,max}}]}{K_{\mathrm{deg,CYP2C8}} \times \{K_{\mathrm{I,CYP2C8}} + [I_{\mathrm{u,max}}]\}}\right)} + \left(1 - f_{\mathrm{m,CYP2C8}}\right) \right\} \right]$$

(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 ± SD peak plasma concentration ($C_{max}$) of rifampicin was 24.3 ± 5.5 μg/mL, and its $AUC_{0–24h}$ was 140 ± 33 μg·h/mL (Figure 5, Table 3). After a single 200 mg dose of trimethoprim, the $C_{max}$ of trimethoprim was 3.2 ± 1.0 μg/mL, and its $AUC_{0–24h}$ was 28.2 ± 5.1 μg·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 μg/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.

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 clopidogorel 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 μM, $[I_{u,max}]/K_i < 0.1$; Table 4). Clopidogrel acyl-β-glucuronide inhibits OATP1B1 competitively with an in vitro $K_i$ value of 10.9 μM in coincubation conditions, and inhibits CYP2C8 time-dependently with a maximum inactivation rate constant ($k_{inact}$) of 2.8 /h and inhibitor concentration of 9.9 μM 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 coincubation 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 μg/mL) of rifampicin is lower than that in our study (24 μg/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 ($[I_{u,in,max}]/K_i$) 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-

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.

**Author Contributions**

Participated in research design: Kim, Yoshikado, Ieiri, Maeda, Kimura, Irie, Kusuhara, and Sugiyama

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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**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 coadminstered 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 *AUC*s (d). RIF, rifampicin; TRM, trimethoprim; CPD, clopidogrel; ***$P < 0.0005$ vs. Control.

**Figure 3.** Pharmacokinetics of a single 1 mg pioglitazone when coadminstered 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 *AUC*s 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 coadminstered 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 *AUC*s (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

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; Sanifi. 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 | *P* value | Control | Trimethoprim 600 mg | *P* value | Control | Clopidogrel 300 mg | *P* 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 |

Data are presented as mean ± 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 |
| $C_{max}$ (μg/mL) | 24.3 ± 5.5 | 3.20 ± 0.96 | 0.0033 ± 0.0022 | 0.0055 ± 0.0028 | 10.4 ± 2.6 | 5.83 ± 3.66 |
| $t_{max}$ (h) | 1.31 ± 0.88 | 1.69 ± 1.25 | 0.88 ± 0.58 | 0.75 ± 0.27 | 1.31 ± 0.75 | 2.06 ± 0.62 |
| $t_{1/2}$ (h) | 3.34 ± 0.63 | 8.58 ± 1.83 | 4.85 ± 3.89 | 7.20 ± 3.38 | 8.28 ± 1.41 | 5.25 ± 1.06 |
| $AUC_{0-t}$ (μg·h/mL) | 140 ± 33 | 28.2 ± 5.1 | 0.0061 ± 0.0037 | 0.012 ± 0.004 | 41.0 ± 7.0 | 29.1 ±14.9 |
| $AUC_{0-\infty}$ (μg·h/mL) | 142 ± 34 | 33.4 ± 7.1 | 0.0068 ± 0.0045 | 0.013 ± 0.003 | 44.3 ± 8.0 | 30.0 ± 15.3 |

Data are presented as mean ± 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); $AUC_{0-\infty}$, 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 + ($k_{inact}$ × [*I*])/{($K_I$ + [*I*]) × $K_{deg}$}] 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) | $f_u$ | $I_{u,max}$ (μM) | $I_{u,in,max}$ (μM) | OATP1B1 | | | CYP2C8 | | | CYP3A4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $K_i$ or $IC_{50}$ (μM) | n | R | $K_i$ or $IC_{50}$ (μM) | n | R | $K_i$ or $IC_{50}$ (μM) | 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 μM $k_{inact}$, 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 μM $k_{inact}$, 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 μM (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 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rifampicin 600 mg | | Trimethoprim 200 mg | | Clopidogrel 300 mg | | | | Gemfibrozil 600 mg | | | |
| Name | $f_{m,CYP2C8}$[a] | Observed AUCR | Predicted AUCR | Observed AUCR | Predicted AUCR | Observed AUCR | Predicted AUCR | | | Observed AUCR | Predicted AUCR | | |
| | | | OATP1B1[b] | | 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).

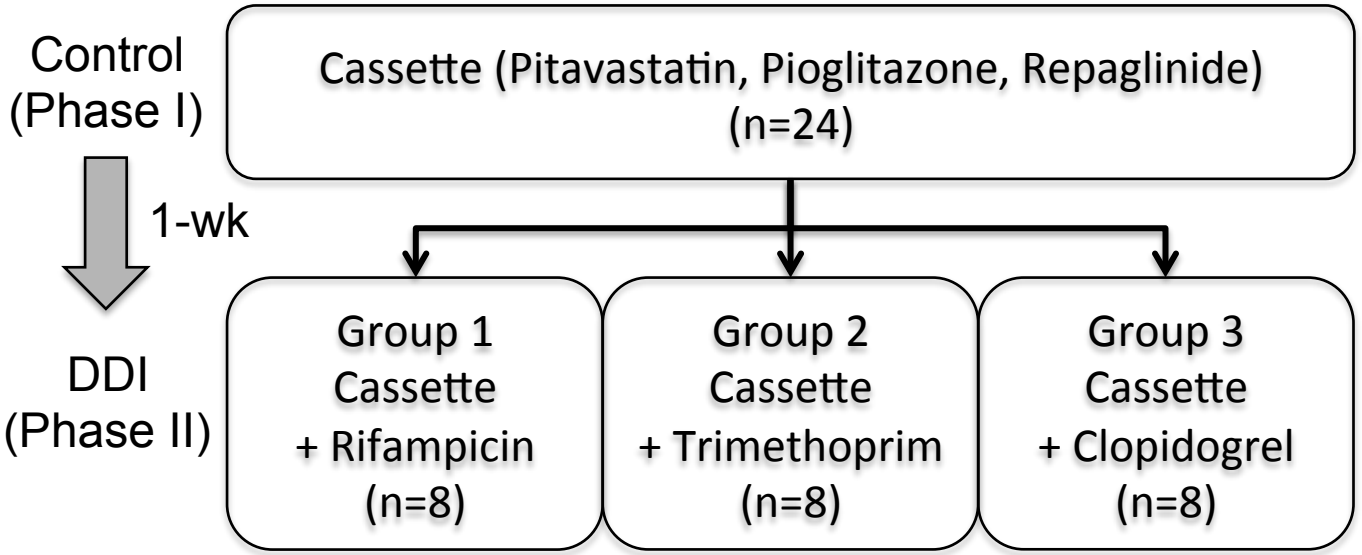
[b] Inhibition pathways for the prediction of AUCR

[c] Aquilante et al. (2012)

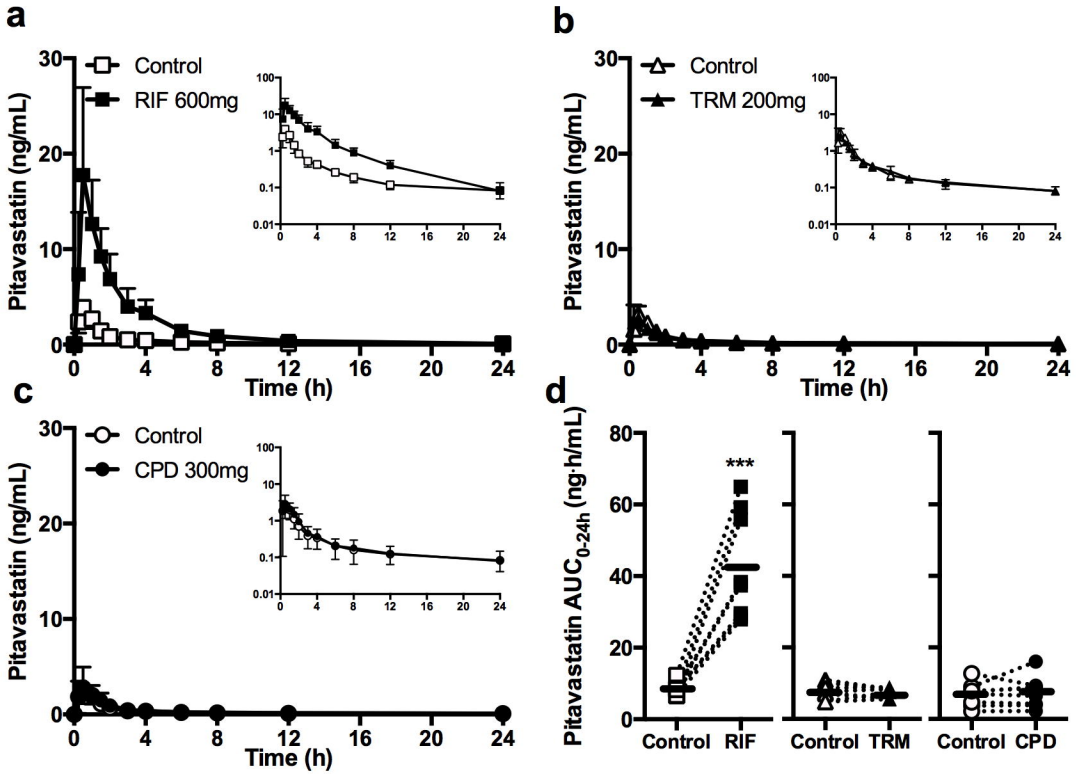
[d] Honkalammi et al. (2011a)

[e] Backman et al. (2002)

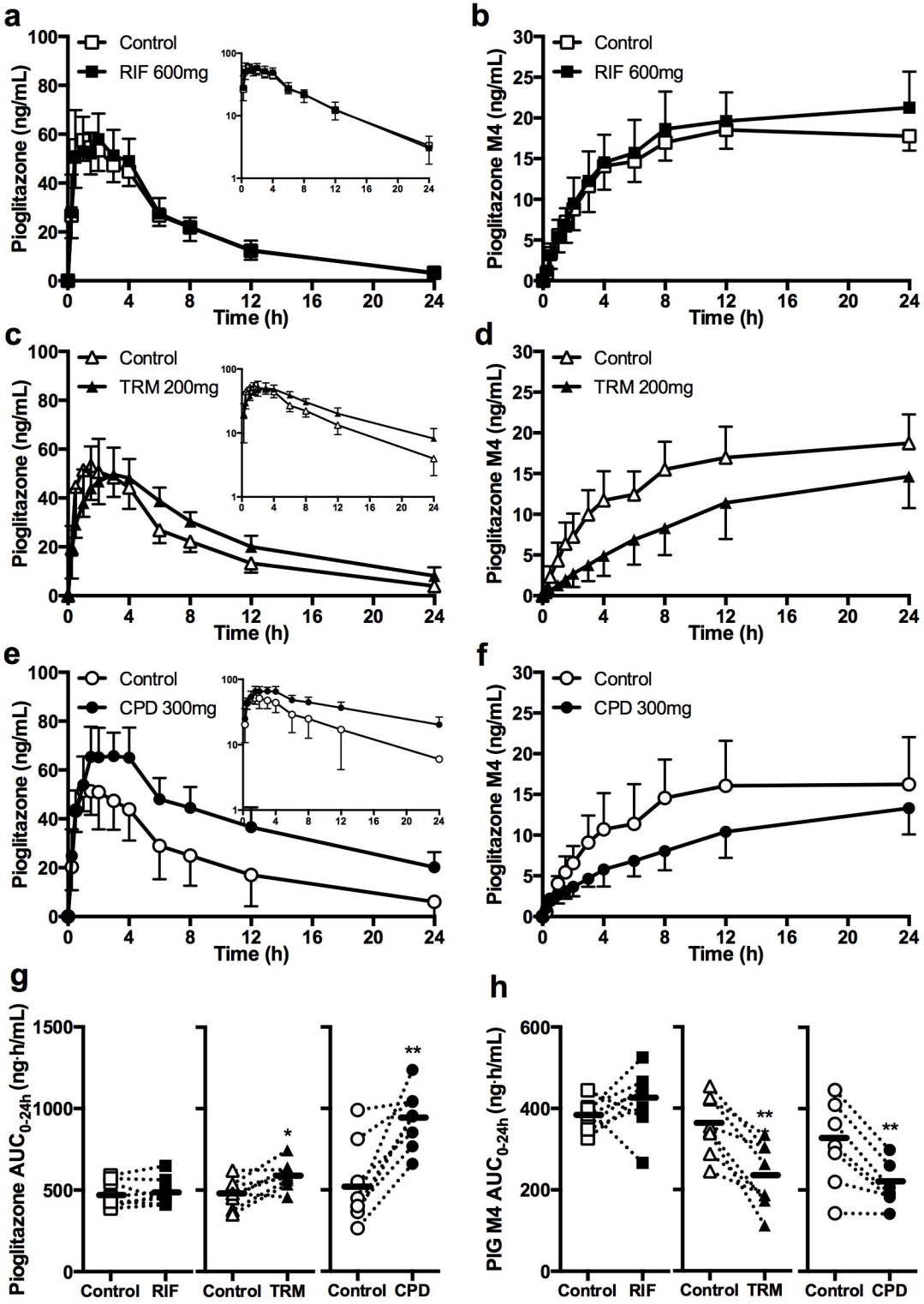
Fig. 1


Control
(Phase I)
Cassette (Pitavastatin, Pioglitazone, Repaglinide)
(n=24)
1-wk
DDI
(Phase II)
Group 1
Cassette
+ Rifampicin
(n=8)
Group 2
Cassette
+ Trimethoprim
(n=8)
Group 3
Cassette
+ Clopidogrel
(n=8)

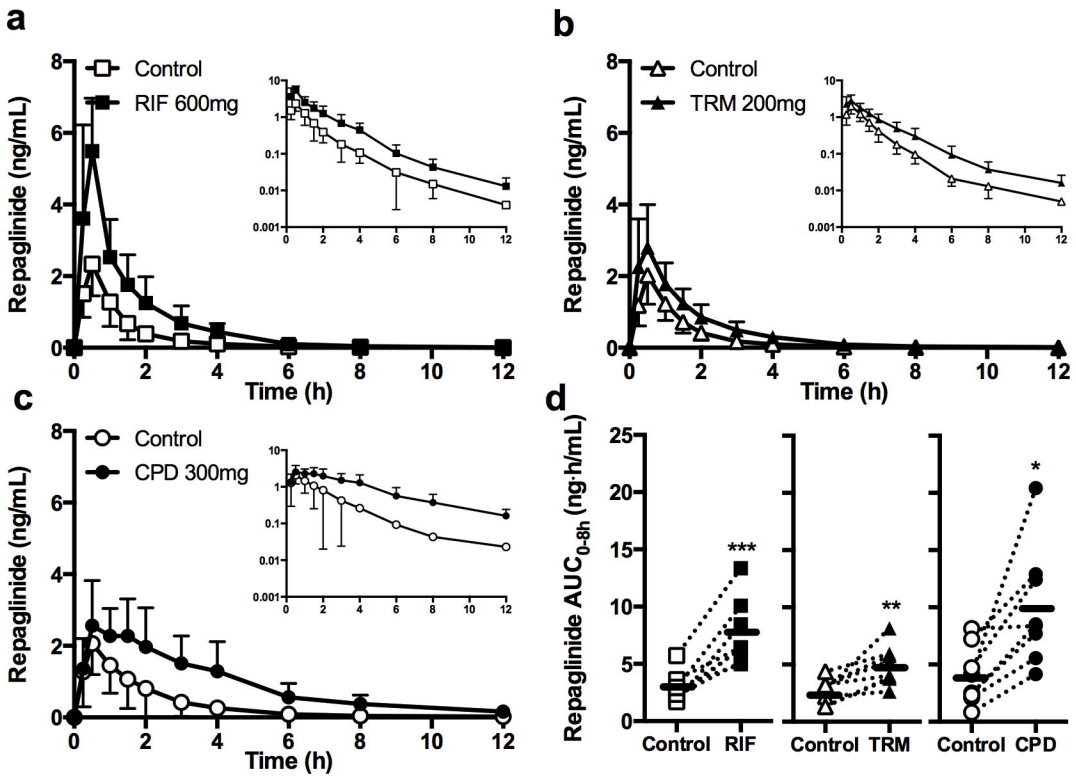
# Fig. 2


a
Control
RIF 600mg
Pitavastatin (ng/mL)
Time (h)
b
Control
TRM 200mg
Pitavastatin (ng/mL)
Time (h)
c
Control
CPD 300mg
Pitavastatin (ng/mL)
Time (h)
d
Pitavastatin AUC0-24h (ng·h/mL)
***
Control RIF
Control TRM
Control CPD

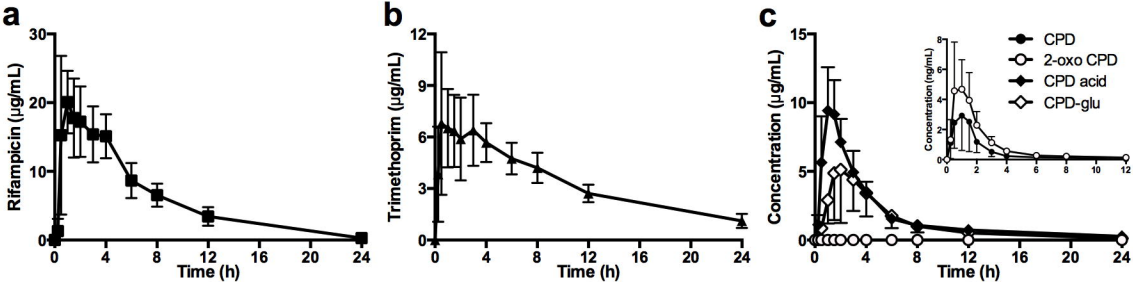
# Fig. 3


a
Control
RIF 600mg
Pioglitazone (ng/mL)
Time (h)
b
Control
RIF 600mg
Pioglitazone M4 (ng/mL)
Time (h)
c
Control
TRM 200mg
Pioglitazone (ng/mL)
Time (h)
d
Control
TRM 200mg
Pioglitazone M4 (ng/mL)
Time (h)
e
Control
CPD 300mg
Pioglitazone (ng/mL)
Time (h)
f
Control
CPD 300mg
Pioglitazone M4 (ng/mL)
Time (h)
g
Pioglitazone AUC0-24h (ng·h/mL)
Control RIF
Control TRM
Control CPD
h
PIG M4 AUC0-24h (ng·h/mL)
Control RIF
Control TRM
Control CPD

# Fig. 4


a
Control
RIF 600mg
Repaglinide (ng/mL)
Time (h)
b
Control
TRM 200mg
Repaglinide (ng/mL)
Time (h)
c
Control
CPD 300mg
Repaglinide (ng/mL)
Time (h)
d
Repaglinide AUC0-8h (ng·h/mL)
***
**
*
Control RIF
Control TRM
Control CPD

# Fig. 5


a
Rifampicin (μg/mL)
Time (h)
b
Trimethoprim (μg/mL)
Time (h)
c
Concentration (μg/mL)
Time (h)
CPD
2-oxo CPD
CPD acid
CPD-glu
Concentration (ng/mL)