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Comparison of Human Cytochrome P450 Inhibition by the Thienopyridines Prasugrel, Clopidogrel, and Ticlopidine

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Summary: Differences in the inhibition of cytochrome P450 activities among thienopyridine antiplatelet agents, ticlopidine, clopidogrel, prasugrel, and the metabolites, 2-oxo-clopidogrel, clopidogrel acid metabolite, deacetylated metabolite of prasugrel (R-95913) and the pharmacologically active metabolites of clopidogrel and prasugrel, were examined using recombinant cytochromes P450 and fluorescent probe substrates. Ticlopidine and clopidogrel inhibited CYP2B6 with IC_{50} values of 0.0517 ± 0.0323 μM and 0.0182 ± 0.0069 μM, respectively, and inhibited CYP2C19 with IC_{50} values of 0.203 ± 0.124 μM and 0.524 ± 0.160 μM, respectively. Ticlopidine also inhibited CYP2D6 (IC_{50} of 0.354 ± 0.158 μM). In contrast, 2-oxo-clopidogrel, prasugrel and R-95913 were much weaker inhibitors of CYP2B6, CYP2C19 and CYP2D6. The inhibitory effects of all the compounds tested were much weaker on the isoforms other than those indicated above. The active metabolites of clopidogrel and prasugrel and clopidogrel acid metabolite also did not affect the activities of the P450s examined.

Keywords: prasugrel; antiplatelet; thienopyridine; cytochrome P450; active metabolite

Introduction

The thienopyridines, ticlopidine and clopidogrel, have become standard drugs for the management of patients following percutaneous coronary intervention and stent placement.[1,2] Thienopyridines are prodrugs that are converted *in vivo* to pharmacologically active metabolites that inhibit platelet function after irreversibly binding to the platelet ADP $P2Y_{12}$ receptor.[3,4]

Treatment with ticlopidine is associated with a slightly higher risk of adverse effects such as hepatotoxicity, neutropenia and thrombotic thrombocytopenic purpura than clopidogrel.[5] In the global market, clopidogrel is being used predominantly due to its relatively faster onset of action and a lower incidence of adverse effects compared with ticlopidine.[5]

In animal models, prasugrel, a new thienopyridine $P2Y_{12}$ receptor antagonist, showed more potent antiplatelet activity with more rapid onset than clopidogrel and ticlopidine.[3,6] Recently, Jernberg *et al*. reported that prasugrel achieved greater inhibition of platelet aggregation than clopidogrel in aspirin-treated patients with stable coronary artery disease.[7] Prasugrel's antiplatelet effect *in vivo* is greater than that of ticlopidine and clopidogrel which emanates from the more efficient generation of its active metabolite compared to the other thienopyridines.[8]

Pharmacologically active metabolites of thienopyridines have been shown to be produced through cytochromes P450 (CYP)-mediated oxidation of their thiolactone derivatives as shown in **Figure 1**.[11–13] Clopidogrel is oxidized by CYP1A2, CYP2B6 and CYP2C19 to form 2-oxo-clopidogrel, and production of 2-oxo-ticlopidine is also catalyzed by CYPs.[9,12,14] On the other hand, production of the deacetylated metabolite of prasugrel (R–95913) is catalyzed by esterases.[8,10] The esterase-mediated hydrolysis step is very rapid both *in vitro* and *in vivo*, such that prasugrel is not detected in human plasma even at early time points after oral administration.[10,15] Thus, for the three thienopyridines, five meta-

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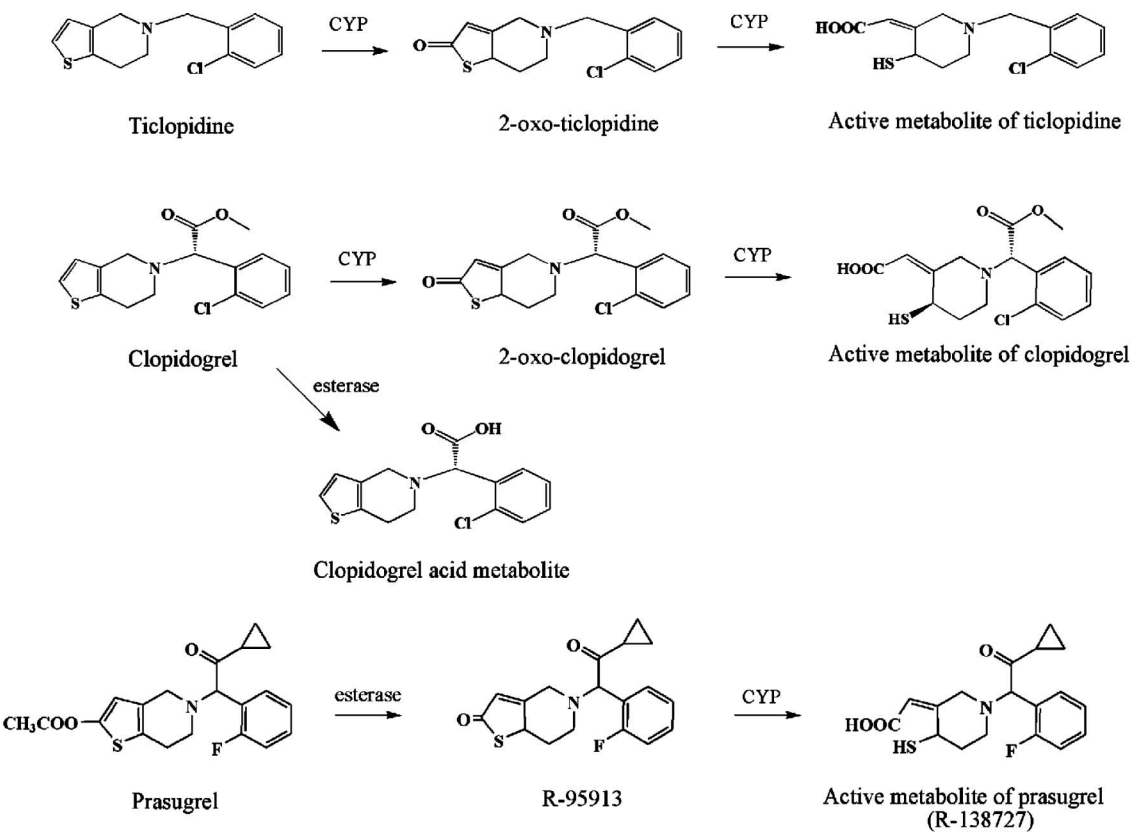

Fig. 1. Biotransformation of thienopyridine-type $P2Y_{12}$ receptor antagonists, ticlopidine, clopidogrel and prasugrel, to pharmacologically active metabolites
CYP: cytochrome P450

bolic processes in **Figure 1** are catalyzed by cytochrome P450.

Being a substrate for a cytochrome P450 indicates that the substrate may also be a competitive inhibitor of cytochrome P450 isoforms. In fact, potent inhibition of the activities of cytochrome P450 has been shown *in vitro*: CYP2B6, CYP2C19 and CYP2D6 by ticlopidine[14,17,21,22]; CYP2C19 by 2-oxo-ticlopidine[17]; CYP2B6 and CYP2C19 by clopidogrel.[22] On the other hand, R-95913 did not inhibit CYP1A2, and inhibited CYP2C9, CYP2C19, CYP2D6, and CYP3A with K_i values ranging from 7.2 μM to 82 μM. These K_i values significantly exceed maximum circulating concentrations of R-95913 of 265 nM, obtained after a 10 mg prasugrel therapeutic dose, by 27- to 309-fold.[13] Thus R-95913 would not affect the metabolic clearance of drugs metabolized by these cytochromes. R-95913 was found to be a weak inhibitor of CYP2B6 *in vitro*.[28] The active metabolite of prasugrel did not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A *in vitro*.[13] Clinically, ticlopidine is known to inhibit the CYP2C19-mediated metabolism of phenytoin[23] and omeprazole.[24,25] It has also been reported that both clopidogrel and ticlopidine significantly inhibit CYP2B6-catalyzed bupropion hydroxylation in the clinical setting, reducing the mean exposure to hydroxybupropion by 52% and 84%, respectively.[26] In humans, prasugrel was found to be a weak inhibitor of CYP2B6.[29]

To estimate the potential of clinical drug-drug interactions with prasugrel, it is of importance to compare the inhibitory effects of R-95913, to which prasugrel is rapidly hydrolyzed *in vivo*, with those of other thienopyridine drugs and their metabolites, on the activities of cytochromes P450. In this study, the differences in the inhibitory effects of thienopyridines and of clopidogrel acid metabolite, 2-oxo-clopidogrel and R-95913 on the activities of various cytochromes P450 using fluorometric microtiter plate assays were examined. We also examined whether the pharmacologically active metabolites of clopidogrel and prasugrel are inhibitors of CYP activities. We determined the IC_{50} values without preincubation in the presence of both NADPH and the thienopyridines, and therefore, this paper is focused on direct inhibition by the thienopyridines.

Materials and Methods

Materials: Clopidogrel, clopidogrel acid metabolite, 2-oxo-clopidogrel, prasugrel, thiolactone metabolite of prasugrel (R-95913), the pharmacologically active

metabolites of clopidogrel and prasugrel and R-135766 for the internal standard were synthesized at Ube Industries, Ltd. (Ube, Japan). Ticlopidine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The structures of these compounds are shown in **Figure 1**. Assay kits for the inhibition of cytochrome P450 including fluorescent probe substrates and typical chemical inhibitors: CYP1A2, 3-cyano-7-ethoxycoumarin and furafylline; CYP2A6, coumarin and tranylcypromine; CYP2B6, 7-ethoxy-4-trifuluoromethylcoumarin and tranylcypromine; CYP2C8, dibenzylfluorescein and quercetin; CYP2C9, 7-methoxy-4-trifluoromethylcoumarin and sulfaphenazole; CYP2C19, 3-cyano-7-ethoxycoumarin and tranylcypromine; CYP2D6, 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin and quinidine; CYP2E1, 7-methoxy-4-trifluoromethylcoumarin and diethyldithiocarbamic acid; CYP3A4, 7-benzyloxyquinoline and ketoconazole were purchased from Gentest Corporation (Woburn, MA, USA). All other reagents and solvents were commercially available and of the purist grade.

Microtiter plate assays for inhibition of human cytochrome P450: Studies on the inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 were carried out in duplicate three times using the assay kits for inhibition of cytochrome P450.[27] Each experiment was conducted following the manufacturer's assay protocol. The final concentrations of the test compounds (ticlopidine, clopidogrel, prasugrel, clopidogrel acid metabolite, 2-oxo-clopidogrel, R-95913, clopidogrel active metabolite and R-138727) were set at 0.00, 0.0229, 0.0686, 0.206, 0.617, 1.85, 5.56, 16.7 and 50.0 μM except for the combination of cytochromes P450 and inhibitors described below. For the inhibition of CYP2B6 by ticlopidine and clopidogrel, the final concentrations of these three test compounds were 0.00, 0.000229, 0.000686, 0.00206, 0.00617, 0.0185, 0.0556, 0.167 and 0.500 μM. For the inhibition of CYP2C19 by ticlopidine and clopidogrel, the final concentrations of these three test compounds were 0.00, 0.00229, 0.00686, 0.0206, 0.0617, 0.185, 0.556, 1.67 and 5.00 μM. The typical chemical inhibitors of cytochrome P450 isoforms indicated in the Material Section were used as positive controls. All the test compounds and typical chemical inhibitors were dissolved in acetonitrile. After preincubation of the mixture containing 16.25 μM NADP, 825 μM $MgCl_2$, 825 μM glucose 6-phosphate, 0.4 units/mL glucose 6-phosphate dehydrogenase (final concentrations) and the test compounds for 10 min at 37°C, an enzyme reaction was started by adding the cytochrome P450 and its fluorescent probe substrate. After incubation for 15 min (CYP1A2), 25 min (CYP2A6), 30 min (CYP2B6, CYP2C19, CYP2D6 and CYP3A4), 40 min (CYP2C8 and CYP2E1) or 45 min (CYP2C9), the reaction was terminated by adding 0.5 M Tris Base and acetonitrile or 2 N sodium hydroxide. Fluorescence of the reaction products was measured by means of a fluorescent plate scanner, Biolumin 960 (Molecular Dynamics Inc., Sunnyvale, CA, USA) controlled with the computer software Xperiment Biolumin 960 (Molecular Dynamics Inc.) and SPECTRA MAX GEMINI EM (Molecular Devices Corp.) controlled using SOFT max PRO (ver. 4.3, Molecular Devices Corp.). The excitation/emission filters used for the assays were 405/450 nm (CYP1A2), 390/450 nm (CYP2A6), 405/535 nm (CYP2B6), 485/535 nm (CYP2C8), 405/535 nm (CYP2C9), 405/450 nm (CYP2C19), 390/450 nm (CYP2D6), 405/535 nm (CYP2E1) and 405/535 nm (CYP3A4). For clopidogrel active metabolite and R-138727, they were above or as follows: 410/460 nm (CYP1A2), 390/460 nm (CYP2A6), 409/530 nm (CYP2B6), 485/538 nm (CYP2C8), 409/530 nm (CYP2C9), 410/460 nm (CYP2C19), 390/460 nm (CYP2D6), 410/538 nm (CYP2E1) and 409/530 nm (CYP3A4).

Stability of clopidogrel active metabolite and R-138727 in the assay system: Clopidogrel active metabolite and R-138727 were dissolved in acetonitrile. After preincubation of the mixture containing 16.25 μM NADP, 825 μM $MgCl_2$, 825 μM glucose 6-phosphate, 0.4 units/mL glucose 6-phosphate dehydrogenase and 5 μM test compounds (final concentrations) for 10 min at 37°C, an enzyme reaction was started by adding human liver microsomes (final concentration: 1 mg/mL). After incubation for 0, 15, 30 and 45 min, the reaction was terminated by adding acetonitrile, and the active metabolites were derivatized with 1 μmol of methoxyphenacyl bromide. The assays were performed following the methods previously reported.[33,34] R-135766 was used as the internal standard. Separation of the analytes by HPLC was conducted using an Alliance2690 Separations Module (Waters Co., Milford, MA). Mass spectra were determined using a Quattro LC MS/MS system (Micromass Ltd., Milford, MA) in the positive ion detection mode using an ESI-interface. The lower limit of quantification was set at 1.6 nM for clopidogrel active metabolite or 8 nM for R-138727. Data acquisition and analysis were performed using MassLynx software (Version 4.0, Micromass Ltd.).

Data analysis: The fluorescence value in each well after subtracting that of the blank well was regarded as the net activity of each cytochrome P450. To the blank well, stop solution was added prior to the initiation of the reaction. The v/v_0 value, which is the ratio of the activity in the well containing the inhibitor (the activity of the sample well: v) to that in the control well without any of the inhibitor (the activity of control well: v_0), was calculated and rounded off to three significant figures using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA). Some of strongly inhibited samples

showed smaller fluorescence values than those of the blank, and thus they are expressed as zero. For each cytochrome P450, each of the three inhibition curves was prepared in duplicate, and the IC_{50} value was calculated from each curve according to Equation 1. The mean IC_{50} values were expressed to three significant figures, and the corresponding standard deviation values were rounded off to the same decimal places. When one or more IC_{50} values out of three were greater than 50.0 μM, which was the highest concentration of each test compound added, those values were treated as 50.0 μM to calculate the mean IC_{50} value, which was described with a mark "$>$" without the standard deviation.

Equation 1:

$$IC_{50} = (50\% - \text{Low } \%) \times (\text{High conc.} - \text{Low conc.}) / (\text{High } \% - \text{Low } \%) + \text{Low conc.}$$

where Low % is the highest % inhibition less than 50%, Low conc. is the concentration of the test substance at the Low %, High % is the lowest % inhibition greater than 50%, and High conc. is the concentration of the test substance at the High %. The detailed information is described in the following web site.
http://www.bdbiosciences.com/discovery_labware/products/display_product.php?keyID=528

Results

Inhibitory effects of thienopyridines on activities of cytochromes P450: The inhibitory effects of the test compounds (ticlopidine, clopidogrel, prasugrel, clopidogrel acid metabolite, 2-oxo-clopidogrel, R-95913, clopidogrel active metabolite and R-138727) on the formation of the metabolites of the CYP-specific fluorescent substrates were examined using a high throughput method. To ensure the reliability of these experiments, typical chemical inhibitors of cytochromes P450 were used as positive controls. For each cytochrome P450, the v/v_0 values were plotted against the logarithmic concen-

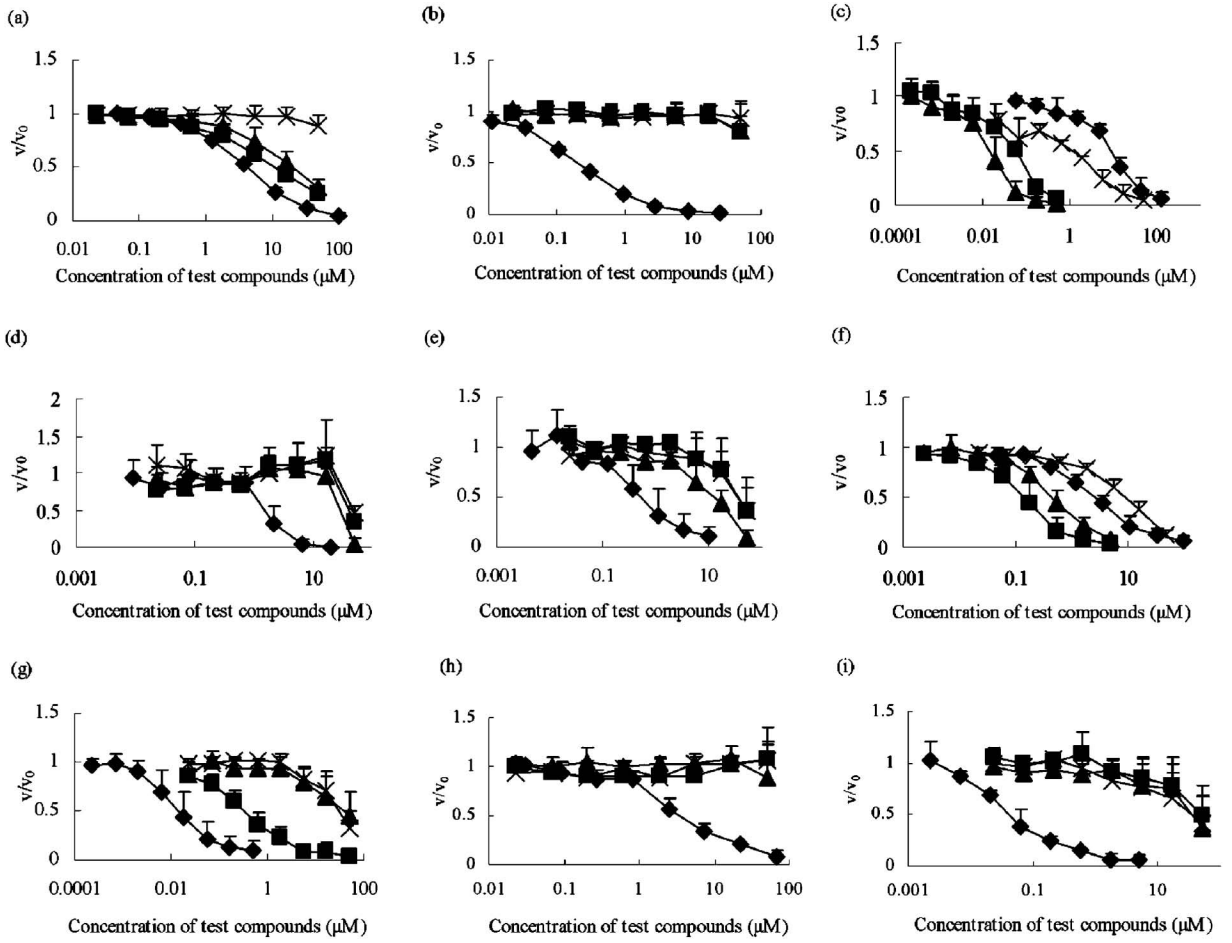

Fig. 2. Inhibitory effects of ticlopidine, clopidogrel and prasugrel on various cytochromes P450
Microsomes overexpressing CYP1A2 (a), CYP2A6 (b), CYP2B6 (c), CYP2C8 (d), CYP2C9 (e), CYP2C19 (f), CYP2D6 (g), CYP2E1 (h) or CYP3A4 (i) were co-incubated at 37°C with ticlopidine (■), clopidogrel (▲), prasugrel (×) or each typical inhibitor (◆). All test compounds and typical chemical inhibitors were dissolved in acetonitrile. Each mean v/v_0 value was calculated from each of three duplicate experiments, and the mean of three values and the standard deviation are expressed as the mean+standard deviation.

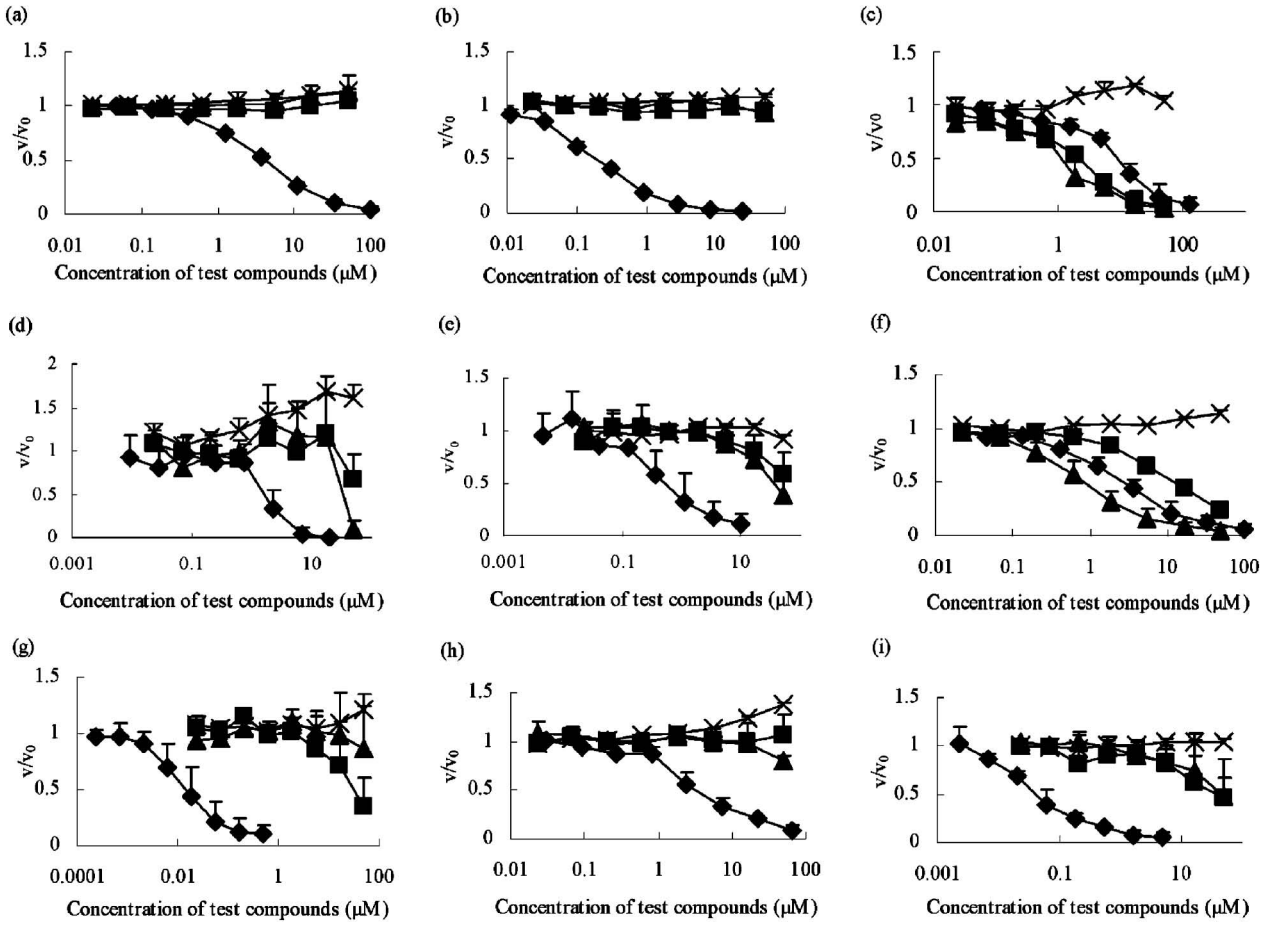

Fig. 3. Inhibitory effects of R-95913, 2-oxo-clopidogrel and clopidogrel acid metabolite on various cytochromes P450
Microsomes overexpressing CYP1A2 (a), CYP2A6 (b), CYP2B6 (c), CYP2C8 (d), CYP2C9 (e), CYP2C19 (f), CYP2D6 (g), CYP2E1 (h) or CYP3A4 (i) were co-incubated at 37°C with R-95913 (■), 2-oxo-clopidogrel (▲), clopidogrel acid metabolite (×) or each typical inhibitor (◆). All test compounds and typical chemical inhibitors were dissolved in acetonitrile. Each mean v/v_0 value was calculated from each of three duplicate experiments, and the mean of three values and the standard deviation are expressed as the mean + standard deviation.

trations of the test compounds and the typical chemical inhibitor (**Figs. 2–4**). The IC_{50} values calculated for the test compounds in each enzyme reaction are summarized in **Table 1**. As shown in **Figures 2** to **4**, the activities of all the cytochromes P450 investigated were inhibited by the typical chemical inhibitors: CYP1A2 by furafylline; CYP2A6, CYP2B6 and CYP2C19 by tranylcypromine; CYP2C8 by quercetin; CYP2C9 by sulfaphenazole; CYP2D6 by quinidine; CYP2E1 by diethyldithiocarbamic acid; CYP3A4 by ketoconazole.

In this study, IC_{50} values of below 1 μM, which is often considered as criteria of cytochrome P450 inhibition in the screening of drug candidates, 1–50 μM and over 50 μM, which is maximum concentration in this experimental system, were defined as strong, moderate and weak inhibition, respectively. Ticlopidine strongly inhibited the reactions catalyzed by CYP2B6, CYP2C19 and CYP2D6 with IC_{50} values of 0.0517 ± 0.0323 μM, 0.203 ± 0.124 μM and 0.354 ± 0.158 μM, respectively (**Fig. 2** and **Table 1**). Clopidogrel also strongly inhibited the reactions catalyzed by CYP2B6 and CYP2C19 with IC_{50} values of 0.0182 ± 0.0069 μM and 0.524 ± 0.160 μM, respectively (**Fig. 2** and **Table 1**). In addition, 2-oxo-clopidogrel inhibited the activity of CYP2C19 strongly (IC_{50} of 0.995 ± 0.450 μM) and that of CYP2B6 moderately (IC_{50} of 1.30 ± 0.14 μM). Prasugrel and R-95913 inhibited CYP2B6 moderately, IC_{50} of 1.19 ± 0.27 μM and 2.30 ± 0.75 μM, respectively, with weaker effects on CYP2C19 (IC_{50} of 10.4 ± 3.9 μM and 13.7 ± 2.7 μM, respectively) compared with ticlopidine and clopidogrel (IC_{50} values of 0.203 ± 0.124 μM and 0.524 ± 0.160 μM, respectively), and on CYP2D6 (IC_{50} of 34.3 ± 16.8 μM and > 36.7 μM, respectively) compared with ticlopidine (IC_{50} of 0.354 ± 0.158 μM). Ticlopidine and clopidogrel moderately inhibited CYP1A2 with IC_{50} values of 12.4 ± 6.2 and 24.3 ± 11.0 μM, respectively, while no inhibition of this enzyme was observed for the 2-oxo-clopidogrel, prasugrel or R-95913 (**Figs. 2, 3** and **Table 1**).

All the compounds tested weakly to moderately inhibited CYP2C9 (IC_{50} values of > 13.4 μM), CYP2C8

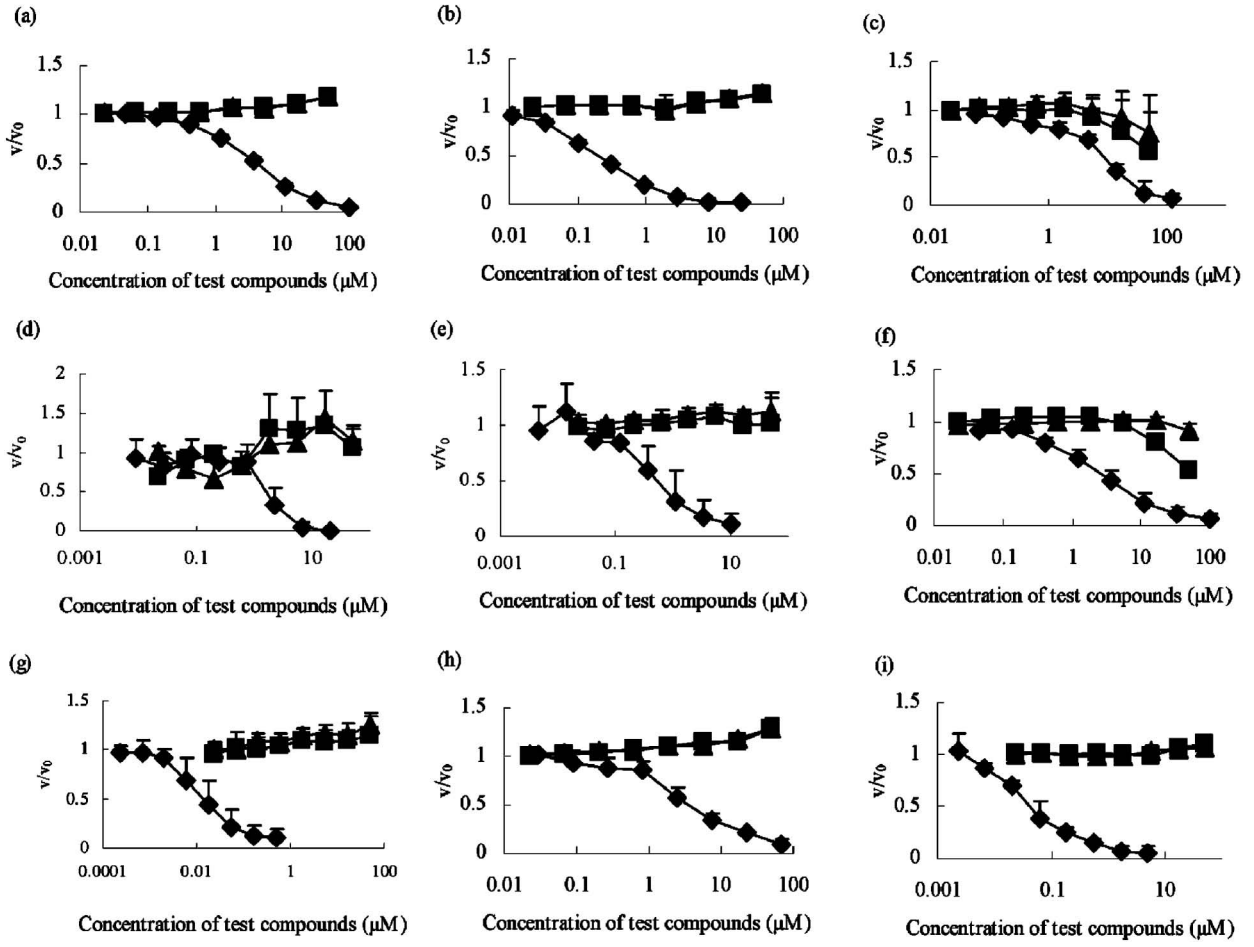

Fig. 4. Inhibitory effects of clopidogrel active metabolite and R-138727 on various cytochromes P450
Microsomes overexpressing CYP1A2 (a), CYP2A6 (b), CYP2B6 (c), CYP2C8 (d), CYP2C9 (e), CYP2C19 (f), CYP2D6 (g), CYP2E1 (h) or CYP3A4 (i) were co-incubated at 37°C with clopidogrel active metabolite (■), R-138727 (▲) or each typical inhibitor (◆). All test compounds and typical chemical inhibitors were dissolved in acetonitrile. Each mean v/v_0 value was calculated from each of three duplicate experiments, and the mean of three values and the standard deviation are expressed as the mean + standard deviation.

(IC_{50} values of $>33.2\ \mu M$), and CYP3A4 (IC_{50} values of $>34.6\ \mu M$) and the inhibition of CYP2A6 and CYP2E1 was negligible (**Figs. 2, 3** and **Table 1**). Clopidogrel acid metabolite did not show inhibitory effects on any CYP isoforms examined (**Fig. 3** and **Table 1**).

Inhibitory effects of the pharmacologically active metabolites of clopidogrel and prasugrel on activities of cytochromes P450: The inhibitory effects of the pharmacologically active metabolites of clopidogrel and prasugrel on cytochromes P450 were evaluated. The active metabolite of ticlopidine was not evaluated since it could not be obtained. The results show that the active metabolites of clopidogrel and prasugrel negligibly inhibited the activities of all the cytochromes P450 as shown in **Figure 4** and **Table 1**. These two active metabolites were stable (over 90% remained) for 45 min in the condition similar to this assay system (**Fig. 5**).

Discussion

In this study, we performed the assays to measure the potential for the thienopyridines and their respective thiolactones to inhibit a cytochrome P450 activity using a well-established high throughput method using a microtiter plate and isoform-specific fluorescent probe substrates.[27] We determined the IC_{50} values without preincubation in the presence of both NADPH and the thienopyridines.

As shown in **Table 1**, ticlopidine strongly inhibited CYP2B6, CYP2C19 and CYP2D6 activities, and clopidogrel also showed strong inhibition of CYP2B6 and CYP2C19 activities. On the other hand, the inhibitory effects of 2-oxo-clopidogrel, prasugrel and R-95913 on these cytochromes P450 were weak to almost moderate. Extent of the inhibitory effects of ticlopidine, clopidogrel and R-95913 on cytochromes P450 in this study was comparable with those in the previous literatures,[13,22]

Table 1. IC_{50} values of test compounds in inhibiting each isoform of cytochrome P450

Compound	CYP isoform								
	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Positive control	4.50 (0.90)	0.225 (0.009)	9.92 (0.59)	1.91 (0.82)	0.782 (0.430)	3.07 (0.98)	0.0212 (0.0204)	4.11 (1.71)	0.0516 (0.0117)
Ticlopidine	12.4 (6.2)	>49.1 –	0.0517 (0.0323)	43.9 (1.5)	>39.5 –	0.203 (0.124)	0.354 (0.158)	>50.0 –	>40.3 –
Clopidogrel	24.3 (11.0)	>50.0 –	0.0182 (0.0069)	33.2 (0.8)	13.4 (7.7)	0.524 (0.160)	>37.4 –	>50.0 –	>40.5 –
Prasugrel	>50.0 –	>50.0 –	1.19 (0.27)	>45.2 –	>36.5 –	10.4 (3.9)	34.3 (16.8)	>50.0 –	>34.6 –
R–95913	>50.0 –	>50.0 –	2.30 (0.75)	>46.7 –	>44.0 –	13.7 (2.7)	>36.7 –	>50.0 –	>36.0 –
2-oxo-clopidogrel	>50.0 –	>50.0 –	1.30 (0.14)	33.9 (8.8)	>40.6 –	0.995 (0.450)	>41.1 –	>50.0 –	>40.8 –
Clopidogrel acid metabolite	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –
Clopidogrel active metabolite	>50.0 –	>50.0 –	>35.1 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –
R–138727	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –	>50.0 –

The IC_{50} value in μM was calculated from each mean v/v_0 value obtained in each of three duplicate experiments. The mean of the three IC_{50} values and the standard deviation are reported.

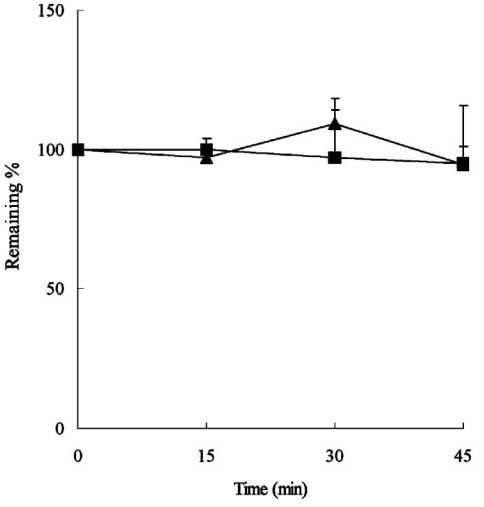

Fig. 5. Stability of clopidogrel active metabolite and R-138727 in the assay system

Clopidogrel active metabolite (■) or R-138727 (▲) (final concentration: 5 μM) was preincubated at 37°C with 16.25 μM NADP, 825 μM $MgCl_2$, 825 μM glucose 6-phosphate, 0.4 units/mL glucose 6-phosphate dehydrogenase (final concentrations) for 10 min. After adding human liver microsomes (final concentration: 1 mg/ml protein) without glutathione, the mixture was incubated for 0, 15, 30 and 45 min. Each residual ratio to the concentration at 0 min was calculated. The data are expressed as the mean + standard deviation.

even though ticlopidine was a weaker inhibitor in some articles likely due to the difference of the experimental conditions.[14,16,17,23)]

Consistent with the results obtained in the present study, clinical trials by Turpeinen *et al.*[26)] reported that both clopidogrel and ticlopidine significantly inhibit CYP2B6-catalyzed hydroxylation of a monocyclic antismoking and antidepressant drug, bupropion. In contrast, prasugrel was recently shown to have much less of an effect of CYP2B6 in humans compared to that previously reported with clopidogrel and ticlopidine.[29)]

The maximum concentrations at the inlet to the liver ($I_{inlet,\,max}$) in a clinical setting, estimated as maximum plasma concentrations in portal vein, of ticlopidine, clopidogrel and R-95913 are 18.2 μM, 8.05 μM and 1.94 μM, respectively, which were calculated using the equation as follows: $I_{inlet,\,max} = C_{max} + (ka \times Dose \times fa/Q_H)$, where the C_{max} values of ticlopidine, clopidogrel and R-95913 are 2540 nM, 11.7 nM and 265 nM, respectively, after oral dosing of ticlopidine, clopidogrel and prasugrel at doses of 250 mg, 75 mg and 10 mg, respectively.[28,32)] The ratio of $I_{inlet,\,max}$ to IC_{50} of R-95913 is much lower than those of ticlopidine and clopidogrel for CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP2D6, even though there is no remarkable difference regarding

CYP2A6, CYP2C8, CYP2E1 and CYP3A4, indicating that the effect of prasugrel on pharmacokinetics of concomitantly administered drugs would be predicted to be lower than those of ticlopidine and clopidogrel.

Previous studies showed that 2-oxo-clopidogrel is not formed in human intestinal S9 and only at a very low level in human liver S9.[8] In addition, a recent report confirmed that carboxylesterase 1, the primary carboxylesterase in human liver, is the enzyme responsible for clopidogrel hydrolysis to its inactive acid metabolite.[30] Even though clopidogrel is substantially hydrolyzed to its acid metabolite, which circulates as a major metabolite in human plasma, clopidogrel acid metabolite showed no inhibitory effects on cytochromes P450. These data suggest that clopidogrel itself is the main moiety in terms of liver exposure and also the one possibly responsible for drug-drug interactions.

In contrast, prasugrel is rapidly hydrolyzed to R-95913 by carboxylesterase 2, which is predominant in the intestine, carboxylesterase 1, and/or other hydrolases. The rate of hydrolysis is at least 25 times faster with human carboxylesterase 2 than with carboxylesterase 1.[10] Previous studies showed that prasugrel may not be absorbed into the portal vein unmodified.[10] In addition, CYP3A, which accounts for about 80% of the total CYP content in human intestine, was shown to be one of main CYPs responsible for metabolism of R-95913 to R-138727.[13] Thus in the case of prasugrel, the main moieties in terms of liver exposure are R-95913 and R-138727. Prasugrel was rapidly hydrolyzed to R-95913 even in microsomes from insect cells used in this study (less than 1% of prasugrel remained in 10 min), just like the case *in vivo*, and therefore, the inhibitory effects of prasugrel and R-95913 were quite similar (**Table 1**).

It has been shown that the sulfhydryl group of the active metabolite of thienopyridines has a crucial role in antiplatelet activity, binding covalently to the cysteine residues of the ADP receptors on the platelets.[31] Therefore, we evaluated the inhibitory effects of the active metabolites of clopidogrel and prasugrel on the cytochromes P450 on the grounds that the sulfhydryl group has the potential of binding to cysteine residues of cytochrome P450. However, the inhibition of activities of cytochromes P450 by the active metabolites was negligible, indicating that the sulfhydryl group of these active metabolites is less likely to bind to these cytochrome P450 isoforms through disulfide bond formation. Richter *et al*. proposed previously that the mechanism based inhibition of CYP2B6 by clopidogrel could be caused by disulfide bond formation between the active metabolite and the enzyme protein.[22] The finding described above in the present study may exclude such possibility. In addition, CYP2B6 inhibition by clopidogrel was much more potent than that by 2-oxo clopidogrel (**Table 1**). It is quite likely that the mechanism based inhibition of CYP2B6 by clopidogrel is due to chemically reactive metabolites produced in the process of conversion of clopidogrel to the 2-oxo-clopidogrel, in which S-oxidation or epoxidation of the thiophene ring is believed to be involved, as in the case of mechanism based inhibition of CYP2C19 reported for ticlopidine, and as was discussed by Farid *et al*.[14,17,29]

In conclusion, the present results indicate that prasugrel would have a lower potential of metabolic drug-drug interaction compared to ticlopidine and clopidogrel.

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