DMD Fast Forward. Published on August 24, 2009 as doi:10.1124/dmd.109.028498
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A possible mechanism of the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel

Katsunobu Hagihara, Miho Kazui, Atsushi Kurihara, Michiharu Yoshiike, Kokichi Honda, Osamu Okazaki, Nagy A. Farid and Toshihiko Ikeda.

Drug Metabolism & Pharmacokinetics Research Laboratories (K.H., M.K., A.K and, O.O.) and Clinical Data & Biostatistics Dep'ts.(M.Y. and K.H.), Daiichi Sankyo Co., Ltd., Tokyo, Japan; Eli Lilly and Company, Indianapolis, Indiana (N.A.F.); Yokohama College of Pharmacy, Yokohama, Japan (T.I.)

Running Title: bioactivation of prasugrel and clopidogrel

Address correspondence to:

Katsunobu Hagihara

Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-Ku, Tokyo, 140-8710, Japan

Tel: +81-3-3492-3131

Fax: +81-3-5436-8567

E-mail: hagihara.katsunobu.fc@daiichisankyo.co.jp

Text Pages: 22

Tables: 2

Figure: 9

References: 32

Abstract: 250 words

Introduction: 488 words

Discussion: 1001 words

Abbreviations: AUC, area under the concentration-time curve; prasugrel, 2-acetoxy-5-(α-cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine

ABSTRACT

The efficiency and interindividual variability in bioactivation of prasugrel and clopidogrel were quantitatively compared and the mechanisms involved were elucidated using twenty individual human liver microsomes. Prasugrel and clopidogrel are converted to their thiol-containing active metabolites through corresponding thiolactone metabolites. The formation rate of clopidogrel active metabolite was much lower and more variable (0.164±0.196 μL/min/mg protein, CV = 120%) compared to the formation of prasugrel active metabolite (8.68±6.64 μL/min/mg protein, CV = 76%). This was most likely attributable to the less efficient and less consistent formation of clopidogrel thiolactone metabolite (2.24±1.00 μL/min/mg protein, CV = 45%) compared to the formation of prasugrel thiolactone metabolite (55.2±15.4 μL/min/mg protein, CV = 28%). These differences may be attributed to the following factors. Clopidogrel was largely hydrolyzed to an inactive acid metabolite (approx. 90% of total metabolites analyzed) and the consumed clopidogrel concentrations were correlated to human carboxylesterase1 (hCE1) activity in each source of liver microsomes. In addition, 48% of the clopidogrel thiolactone metabolite formed was converted to an inactive thiolactone acid metabolite. The oxidation of clopidogrel to its thiolactone metabolite correlated with variable activities of CYP1A2, CYP2B6 and CYP2C19. In conclusion, clopidogrel's active metabolite was formed with less efficiency and higher variability than that of prasugrel. This difference in thiolactone formation was attributed to hydrolysis of clopidogrel and its thiolactone metabolite to inactive acid metabolites and to variability in CYP-mediated oxidation of clopidogrel to its thiolactone metabolite. This may contribute to the poorer and more variable active metabolite formation for clopidogrel than prasugrel.

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INTRODUCTION

Prasugrel and clopidogrel are thienopyridine antiplatelet agents. Prasugrel is shown to reduce the rate of thrombotic cardiovascular events and stent thrombosis in patients with acute coronary syndrome and those to be managed with percutaneous coronary intervention (Wiviott et al., 2007). Similarly, clopidogrel is used for the management of patients following percutaneous coronary intervention and stent placement (Braunwald et al., 2002; Schulman et al., 2004). Thienopyridines are prodrugs that are converted *in vivo* to pharmacologically active metabolites through corresponding thiolactone intermediates. The thiol-containing active metabolites inhibit platelet function by irreversibly binding to the platelet $P2Y_{12}$ ADP receptor (Niitsu et al., 2005; Savi et al., 2005; Algaier et al., 2008).

In animal models and aspirin-treated patients with stable coronary artery disease, prasugrel, a new thienopyridine $P2Y_{12}$ receptor antagonist, showed more potent antiplatelet activity with more rapid onset, and significantly reduced the cardiovascular events when compared with clopidogrel (Sugidachi et al., 2000; Niitsu et al., 2005; Jernberg et al., 2006; Wiviott et al., 2007). Such potent effect of prasugrel can be explained by higher exposure to the active metabolite of prasugrel than the active metabolite of clopidogrel (Brandt et al., 2007; Payne et al., 2007; Sugidachi et al., 2007) leading to a better pharmacodynamic (PD) response (Ernest et al., 2008; Farid et al., 2009).

The pharmacologically active metabolites of thienopyridines have been shown to be produced through cytochromes P450 (CYP)-mediated oxidation of their corresponding thiolactones as shown in Figure 1 (Kurihara et al., 2005; Rehmel et al., 2006). Clopidogrel has two competing metabolic pathways (Figure 1), with the primary pathway leading to the formation of the inactive clopidogrel acid metabolite (Caplain et al., 1999). The clopidogrel inactive acid metabolite is formed through ester hydrolysis by hepatic human carboxylesterase (hCE) 1 (Tang et al., 2006). The minor pathway in clopidogrel metabolism that yields the active metabolite requires two sequential CYP-dependent steps. The first is the formation of a thiolactone from clopidogrel by CYP1A2, CYP2B6 and CYP2C19 and the second is the subsequent formation of the active metabolite from the thiolactone by CYP2B6, CYP2C9, CYP2C19 and CYP3A4 (Kurihara et al., 2005). On the other hand, the bioactivation of prasugrel to its active metabolite requires the hydrolysis of the ester by carboxylesterases, which occurs during the absorption process, to form the thiolactone followed by oxidation by CYP2B6, CYP2C9, CYP2C19 and CYP3A4 (Rehmel et al., 2006; Farid et al., 2007a; Williams et al., 2008) (Figure 1) to form prasugrel's active metabolite. The hydrolysis step of prasugrel mediated by hCE1 and hCE2 is very rapid, such that prasugrel is not detected in human or animal plasma after oral administration even at early time points (Farid et al., 2007a; Smith et al., 2007; Williams et al., 2008; Hagihara et al., 2009).

This study was performed to directly compare the efficiency and variability in the formation of the active metabolites from prasugrel and clopidogrel and to elucidate the detailed mechanisms of the differences using individual human liver microsomes.

Materials and Methods

Materials.

Prasugrel (Effient™ in the US and Efient® in EU), prasugrel thiolactone metabolite (R-95913), prasugrel active metabolite (R-138727), clopidogrel (Plavix®/Iscover®), clopidogrel acid and the internal standard R-135766, shown in Figure 1b, were synthesized by Ube Industries, Ltd. (Ube, Japan). Clopidogrel thiolactone metabolite, clopidogrel thiolactone acid and clopidogrel active metabolite were synthesized by Daiichi Sankyo Co., Ltd. Purity of these compounds ranged from 89 to 100%. Individual human liver microsomes were purchased from the Human & Animal Bridging Research Organization (Tokyo, Japan), where each activity of CYP or hCE1 was determined. A derivatizing reagent, 3'-methoxyphenacyl bromide (MPBr) was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan).

Metabolism of prasugrel, clopidogrel thiolactone metabolite and clopidogrel in human liver microsomes.

Each mixture (total volume: 99 μL) in duplicate contained potassium phosphate buffer (29.5 mM, pH 7.4), β-NADP (2.5 mM), G-6-P (25 mM), G-6-PDH (0.5 units/mL) and $MgCl_2$ (10 mM), glutathione (5 mM) and human liver microsome (1 mg protein/mL). Each mixture was preincubated at 37°C for 5 min, and 1 μL of prasugrel, clopidogrel thiolactone metabolite or clopidogrel (1 μM, 1 μM or 50 μM, respectively, as final concentrations) was added to the respective mixture, which was incubated at 37°C for 15 min for prasugrel and clopidogrel thiolactone metabolite or 30 min for clopidogrel. At each time point, 100 μL of the incubation mixture was mixed with 200 μL of acetonitrile, 2 μL of 500 mM MPBr acetonitrile solution and 100 μL of 100 ng/mL

R-135766 acetonitrile solution to stop the reaction and derivatize the thiol moiety of prasugrel active metabolite or clopidogrel active metabolite. After storage at room temperature for 10 min, the mixture was centrifuged (15,000 × g, 3 min, 4°C). A 10 μL-aliquot of the supernatant fraction was subjected to LC-MS/MS analysis.

Assay procedure for prasugrel and clopidogrel metabolites.

The assays were performed following the methods previously reported (Takahashi et al., 2006; Farid et al., 2007b). Separation of the analytes by HPLC was achieved using an Alliance2690 Separations Module (Waters Co., Milford, MA, USA). Mass spectra were obtained using a Quattro LC MS/MS system (Micromass Ltd., Milford, MA, USA) in the positive ion detection mode using an ESI-interface. The transitions *m/z* 498 → 348 for derivatized prasugrel active metabolite, *m/z* 548 → 206 for R-135766, *m/z* 374 → 206 for prasugrel, *m/z* 332 → 149 for prasugrel thiolactone metabolite, *m/z* 504 → 354 for derivatized clopidogrel active metabolite, *m/z* 322 → 212 for clopidogrel, *m/z* 338 → 183 for clopidogrel thiolactone metabolite, *m/z* 308 → 198 for clopidogrel acid metabolite and *m/z* 324 → 278 for clopidogrel thiolactone acid metabolite were monitored using the multiple reaction monitoring (MRM) mode. The lower limit of quantification for each compound was 1.6 nM. Data acquisition and analysis were performed using MassLynx software (Version 4.0, Micromass Ltd.).

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Multiple linear regression analysis

Multiple linear regression analyses for each enzyme activity were performed using Microsoft Office Excel 2003 (SP2, Microsoft Corp.) and JMP 6.0.3 (SAS Institute Japan Co., Ltd., Tokyo, Japan). Each estimate and its 95% confidence interval of the intercept (a) and slope (bi) were calculated by the least squares method.

$Y = a + bi \times Xi$

where Y is estimate of the metabolite concentration, bi is partial regression coefficient for each enzyme, and Xi is each enzyme activity. A P value of 0.05 or less obtained from t-test where each slope was assumed to be zero was considered statistically significant.

Calculation of metabolite formation ratio and formation rate divided by substrate concentration (V/S)

The metabolite formation ratio was calculated by dividing concentrations of the metabolites by those of the total metabolites analyzed, and is expressed as a percentage. The formation rate (V) was calculated by dividing the metabolite concentration by the incubation time and protein concentrations, and is expressed in units of pmol/min/mg protein. The formation rate divided by the substrate concentration (V/S) is expressed in units of μL/min/mg protein. These values are expressed as the mean ± standard deviation throughout the Results section.

Statistical analyses

Variation and magnitude of the V/S values for the formation of thiolactones and active metabolites from prasugrel and clopidogrel were compared by F test statistic and Welch test statistic using SAS system release 8.2 software. Level of significance was set at 0.05.

Results

CYPs and hCE1 activities in individual human liver microsomes. Variable activities of cytochrome P450 (CYP) isoforms and hCE1 in individual human liver microsomes (N = 20) used in this experiment are shown in Table 1. The coefficient of variation (CV) values of the enzyme activities ranged from 36% to 148% (Table 1).

Comparison of V/S for the formation of active metabolites from prasugrel and clopidogrel

The efficiency of the formation of the active metabolites between prasugrel and clopidogrel was compared (Figure 2). Prasugrel active metabolite was formed with lower individual variability for most of the enzyme activities. The V/S values for prasugrel active metabolite formation (8.68±6.64 μ L/min/mg protein) were significantly higher than those for the formation of clopidogrel active metabolite (0.164±0.196 μL/min/mg protein) ($p < 0.0001$, Welch test). The mean V/S for the formation of prasugrel active metabolite was approximately 53-fold higher than that for the formation of clopidogrel active metabolite. Variability in the formation of prasugrel active metabolite (CV 76%), was similar to those (CV 86%) observed in previous experiment (Rehmel et al., 2006), and was significantly less than that for the formation of clopidogrel's active metabolite (CV 120%) ($p < 0.0001$, F test). The formation of both prasugrel active metabolite and clopidogrel active metabolite was not detected in one human liver microsomal sample (HLM-009-1), and this was considered to be due to the lower CYP activities in this sample than those in the other samples.

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Comparison of V/S for the formation of thiolactone intermediates from prasugrel and clopidogrel. The efficiency of the formation of the thiolactone intermediates of prasugrel and clopidogrel were compared (Figure 3). Since the thiolactone intermediates are subsequently metabolized to the active metabolites (for prasugrel and clopidogrel), the sum of the thiolactone and the active and inactive thiolactone acid (for clopidogrel only) metabolites were used to calculate V/S of prasugrel thiolactone metabolite and clopidogrel thiolactone metabolite. The V/S for the formation of prasugrel thiolactone metabolite (55.2±15.4 μL/min/mg protein) was significantly higher than that for the formation of clopidogrel thiolactone metabolite (2.24±1.00 μL/min/mg protein) ($p < 0.0001$, Welch test). The mean V/S for the formation of prasugrel thiolactone metabolite was about 25-fold higher than that of clopidogrel thiolactone metabolite. Variation was significantly higher in the formation of clopidogrel thiolactone metabolite (CV = 45%) compared with that of prasugrel thiolactone metabolite (CV = 28%) ($p < 0.0001$, F test).

Metabolism of clopidogrel in individual human liver microsomes. The V values of clopidogrel acid, clopidogrel thiolactone metabolite, clopidogrel thiolactone acid and clopidogrel active metabolite generated from clopidogrel are shown in Figure 4. Clopidogrel was largely hydrolyzed to the carboxylic acid metabolite (metabolite formation ratio of 92.19±3.81%). The substrate concentrations consumed after incubation of clopidogrel in human liver microsomes were best correlated with hCE1 activity ($R^2 = 0.4028$, Figure 5). The results show that high hCE1 activity possibly contributes to lower efficiency of the formation of clopidogrel thiolactone metabolite

and subsequent active metabolite as this activity would divert the compound to an alternate inactive pathway.

The metabolite formation from clopidogrel thiolactone metabolite in individual human liver microsomes. The active metabolite formation from clopidogrel thiolactone metabolite was evaluated since clopidogrel undergoes two sequential steps of CYP oxidation to form the active metabolite *via* a thiolactone intermediate. The results demonstrated that approximately 48% of the clopidogrel thiolactone metabolite was also hydrolyzed to an inactive thiolactone acid as shown in Table 2.

Correlation between CYP activities and concentrations of clopidogrel thiolactone metabolite formed. The correlation between the activities of CYP isoforms (Kurihara et al., 2005) and clopidogrel thiolactone metabolite formation rates was investigated. The coefficients of correlation of thiolactone metabolite formation from clopidogrel, calculated as the sum of thiolactone, thiolactone acid and active metabolite, with the activities of CYP1A2, CYP2B6 and CYP2C19 were 0.4058, 0.3380 and 0.1300, respectively (Figure 6).

Multiple linear regression analyses for clopidogrel thiolactone metabolite formation. To confirm that clopidogrel thiolactone metabolite formation is mediated by three CYP isoforms, CYP1A2, CYP2B6 and CYP2C19, in twenty individual liver microsomes used in this experiment, we evaluated whether the concentration of clopidogrel thiolactone metabolite formed in each liver microsome can be estimated by their respective three CYP-isoform activities. Using the values of the observed

concentration of clopidogrel thiolactone metabolite (sum of clopidogrel thiolactone, clopidogrel thiolactone acid and clopidogrel active metabolite) and CYP activities (CYP1A2, CYP2B6 and CYP2C19) in each liver microsome sample, coefficients for each CYP-isoform activity and an intercept best for estimating the concentration of clopidogrel thiolactone metabolite were calculated by multiple linear regression analyses. The multiple regression equation and the determination coefficient r^2 were as follows: Y (estimate of clopidogrel thiolactone metabolite concentration) = 2117.91 + 15.907 × CYP1A2 activity + 243.369 × CYP2B6 activity + 12.397 × CYP2C19 activity, r^2 = 0.5003. By using the equation above, the observed clopidogrel thiolactone metabolite concentrations were well correlated with the estimated concentrations (coefficient of correlation by single linear regression between observed and estimated concentrations, R^2 = 0.5792, Figure 7), demonstrating that CYP1A2, CYP2B6 and CYP2C19 contributed to clopidogrel thiolactone metabolite formation from clopidogrel in each human liver microsome used in this experimental system.

Correlation between CYP activities and prasugrel active metabolite formation. The correlation between the activities of CYP isoforms involved (Rehmel et al., 2006) and prasugrel active metabolite formation was investigated. The coefficients of correlation with the activities of CYP2B6, CYP2C9, CYP2C19 and CYP3A4 were 0.3734, 0.4884, 0.2716 and 0.1497, respectively (Figure 8). The coefficients were not so high for each CYP-isoform probably due to its multi-isoform mediated reaction. On the other hand, according to multiple linear regression analysis, the observed prasugrel active metabolite concentrations were well correlated with the concentrations estimated by the CYP2B6, CYP2C9, CYP2C19 and CYP3A4 activities (coefficient of

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correlation by single linear regression between observed and estimated concentrations, R^2 = 0.8563, Figure 9), demonstrating that CYP2B6, CYP2C9, CYP2C19 and CYP3A4 contributed to prasugrel active metabolite formation in individual human liver microsomes used in this experimental system.

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Discussion

This study was performed to directly and quantitatively compare the efficiency and variability in the formation of the active metabolites from thienopyridines, prasugrel and clopidogrel, and to clarify the detailed mechanisms of the differences using human liver microsomes. In this study, substrate concentration of clopidogrel was set at relatively high level (50 μM) in view of the analytical sensitivity of clopidogrel active metabolite. This might cause underestimate of intrinsic clearance of clopidogrel active metabolite formation. The V/S values (0.164±0.196 μL/min/mg protein) obtained in this experimental system, however, corresponded to the V_{max}/K_m value (0.27 μL/min/mg protein, K_m = 44 μM) reported for pooled (N = 10) human liver microsomes (Kurihara et al., 2005). The V/S value for prasugrel active metabolite formation (8.68±6.64 μL/min/mg protein) from 1 μM of prasugrel was also consistent with the V_{max}/K_m values reported previously (5.8 to 12 μL/min/mg protein) (Rehmel et al., 2006). Accordingly, it was considered reasonable to compare the V/S values for metabolite formation from 1 μM of prasugrel and 50 μM of clopidogrel. Additionally, the time points and protein concentrations selected were also considered to be appropriate. In this study, prasugrel and clopidogrel were incubated for 15 min and 30 min, respectively, in human liver microsomes with a protein concentration of 1 mg/mL. Our preliminary results showed that formation of active metabolites from prasugrel and clopidogrel was linear up to 30 min and 60 min, respectively, and up to 1 mg/mL and 2 mg/mL, respectively, of protein concentrations in human liver microsomes (data not shown).

Consistent with the clinical observations (Payne et al., 2007; Wallentin et al., 2008), prasugrel active metabolite was generated more efficiently and less variably than clopidogrel active metabolite in individual human liver microsomes. We expected that these differences arose from those in their thiolactone formation as it has been reported that prasugrel thiolactone metabolite is formed by hydrolysis while clopidogrel needs CYP oxidation to form its thiolactone intermediate (Kazui et al., 2005; Kurihara et al., 2005; Williams et al., 2008). According to the results, prasugrel thiolactone metabolite formation level was higher with more consistency compared with that of clopidogrel thiolactone metabolite. In *in vivo* situation, prasugrel is wholly converted to prasugrel thiolactone metabolite by esterases including hCE1 and hCE2 and in part subsequently to prasugrel active metabolite in the intestine, and therefore prasugrel thiolactone metabolite and prasugrel active metabolite, not prasugrel itself, flow into the liver, while clopidogrel is not metabolized to its thiolactone metabolite in the intestine (Kazui et al., 2005; Williams et al., 2008; Hagihara et al., 2009). In this study, prasugrel was not completely converted to prasugrel thiolactone metabolite in human liver microsomes probably due to lower expression level of hCE2 protein compared with the intestine (Imai, 2006; Taketani et al., 2007). This could indicate that more efficient formation of prasugrel thiolactone metabolite may occur *in vivo* than in the *in vitro* experimental system.

To clarify the mechanism of the less efficient and more variable formation of the thiolactone metabolite from clopidogrel, the metabolism was investigated using individual human liver microsome samples. Reflecting the previous clinical results (Caplain et al., 1999), clopidogrel was largely converted to its inactive acid metabolite by hCE1 which competes with the oxidation of clopidogrel to form the thiolactone

metabolite. Since it has been reported that there is relatively large interindividual variability in hCE1 activity for some esterified drugs in human liver microsomes (Takahashi et al., 2008; Yang et al., 2009), clopidogrel thiolactone metabolite formation level might change depending on individual hCE1 activities. Clopidogrel thiolactone metabolite was also hydrolyzed to an inactive thiolactone acid (48%), which additionally contributes to the lower concentrations of clopidogrel thiolactone metabolite available to be metabolized to clopidogrel active metabolite. The esterases involved in this inactivation pathway have not been identified yet.

According to the previous studies using expressed CYP isoforms (Kurihara et al., 2005), clopidogrel is metabolized by CYP1A2, CYP2B6 and CYP2C19 to form clopidogrel thiolactone metabolite. To confirm involvement of these CYP isoforms in individual human liver microsomes, a correlation study and multiple linear regression analysis were performed. The results showed a positive correlation between clopidogrel thiolactone metabolite formation and the activities of CYP1A2, CYP2B6 and CYP2C19. Variability in clopidogrel thiolactone metabolite formation *in vivo* may be related to changes in these enzyme activities associated with intrinsic and/or extrinsic factors. Recently, it has been reported that cigarette smoking is associated with enhanced platelet inhibition by clopidogrel (Bliden et al., 2008). Since smoking is generally known to induce CYP1A activity, this clinical observation is consistent with our results in this report. Moreover, a large number of clinical studies indicate involvement of CYP2C19 in the formation of clopidogrel active metabolite and in efficacy of clopidogrel (Hulot et al., 2006; Brandt et al., 2007; Trenk et al., 2008; Frere et al., 2008; Umemura et al., 2008; Collet et al., 2009; Mega et al., 2009). Our report showing a contribution of CYP2C19 in clopidogrel thiolactone

metabolite formation could support these clinical findings as there are known to be various polymorphisms in CYP2C19.

Prasugrel active metabolite formation was well correlated with the activities of CYP2B6, CYP2C9, CYP2C19 and CYP3A4, which is consistent with the previous report (Rehmel et al., 2006).

In conclusion, the formation of the active metabolite of clopidogrel was less efficient and less consistent than the formation of prasugrel active metabolite. This difference was attributed to decreased clopidogrel thiolactone metabolite concentrations due to the large hydrolytic inactivation to acid metabolites of both clopidogrel and its thiolactone metabolite, and the variable CYP isoform-mediated oxidation of clopidogrel. The hydrolysis pathways occur in the liver and compete directly with both the thiolactone metabolite formation and the formation of clopidogrel active metabollite from the thiolactone. By contrast, hydrolysis of prasugrel to its corresponding thiolactone occurs in the intestine during the absorption process, and thiolactone is metabolized to active metabolite in the intestine and in the liver in a single CYP-dependent step. These differences may help to explain the poorer and more variable response to clopidogrel compared to prasugrel in patients treated for acute coronary syndrome.

Acknowledgements

The activities of some CYP isoforms and hCEs were kindly analyzed by Dr. Satoshi Suzuki and his colleagues in Human & Animal Bridging Research Organization. The authors also thank Mary Pat Knadler, PhD, and Steven A. Wrighton, PhD, of Eli Lilly and Company and Takashi Izumi, PhD, of Daiichi Sankyo Co., Ltd. for their helpful comments and discussion on this study.

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FOOTNOTES

A portion of this work was presented at the scientific session of American Heart Association, New Orleans, LA, USA, in 2008.

The co-author Nagy A. Farid has retired from Eli Lilly and Company. Current email address: nagyfarid@gmail.com

FIGURE LEGENDS

Figure 1 Metabolic pathways of prasugrel and clopidogrel studied (a) and chemical structure of the internal standard R-135766 (b).

Figure 2 Comparison of V/S for the formation of the active metabolites from prasugrel (1 μM) and clopidogrel (50 μM), respectively, in individual human liver microsomes (N = 20).

Prasugrel active metabolite and clopidogrel active metabolite were not detected in HLM-009-1.

V/S: Formation rates divided by substrate concentrations.

$P < 0.0001$, prasugrel active metabolite vs clopidogrel active metabolite, F test and Welch test.

Figure 3 Comparison of V/S for the formation of the thiolactone metabolites from prasugrel (1 μM) and clopidogrel (50 μM), respectively, in individual human liver microsomes (N = 20).

V/S: Formation rates divided by substrate concentrations.

$P < 0.0001$, prasugrel thiolactone metabolite vs clopidogrel thiolactone metabolite, F test and Welch test.

Figure 4 Formation of clopidogrel acid, clopidogrel thiolactone metabolite, clopidogrel thiolactone acid and clopidogrel active metabolite from clopidogrel (50 μM) in individual human liver microsomes (N = 20).

The data are expressed as the mean of twenty results ± S.D.

Figure 5 Correlation between each enzyme activity and the consumed clopidogrel concentrations in individual human liver microsomes (N = 20).

Figure 6 Correlation between the activities of CYP1A2, CYP2B6 and CYP2C19 and thiolactone metabolite formation rates from clopidogrel in individual human liver microsomes (N = 20).

* Sum of V for the formation of thiolactone, thiolactone acid and active metabolite.

Figure 7 Correlation between estimated and observed clopidogrel thiolactone metabolite formation in individual human liver microsomes (N = 20).

CYP mediated metabolites are the sum of thiolactone, thiolactone acid and active metabolite. The estimated metabolite formation was calculated by the following multiple regression equation: 2117.91 + 15.907×CYP1A2 activity + 243.369×CYP2B6 activity + 12.397×CYP2C19 activity.

R^2: coefficient of correlation by simple linear regression.

Figure 8. Correlation between the activities of CYP2B6, CYP2C9, CYP2C19 and CYP3A4 and active metabolite formation rates from prasugrel in individual human liver microsomes (N = 20).

Figure 9 Correlation between estimated and observed prasugrel active metabolite in individual human liver microsomes (N = 20).

DMD Fast Forward. Published on August 24, 2009 as DOI: 10.1124/dmd.109.028498
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The estimated prasugrel active metabolite formation was calculated by the following multiple regression equation: 15.978 + 0.0091×CYP3A4 + 23.862×CYP2B6 + 1.121×CYP2C9 + 1.764×CYP2C19 activity.

R^2: coefficient of correlation by simple linear regression.

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Table 1 Activities of CYP isoforms and hCE1 in individual human liver microsomes (N = 20).

	Activity (pmol/mg/min)							
	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4	hCE1
HLM-009-1	1.02	0.37	4.00	9.00	35.00	23.0	52.0	7804.0
HLM-012-1	10.14	0.52	5.00	3.00	16.00	48.0	120.0	15830.0
HLM-014-3	85.27	5.95	190.00	40.00	73.00	31.0	2100.0	40300.5
HLM-056-1	34.00	0.27	40.47	4.39	0.00	2570.0	277.2	19595.0
HLM-057-1	8.97	0.36	11.55	0.00	40.28	555.8	1159.0	12769.0
HLM-059-1	98.02	1.45	89.47	50.70	21.36	1258.0	1491.0	32470.0
HLM-061-1	182.92	2.61	74.45	8.26	14.79	1537.7	4010.6	41414.5
HLM-062-1	7.42	0.70	13.54	0.93	28.72	773.9	1544.9	11473.5
HLM-087-1	5.32	0.48	18.00	0.00	31.01	2095.2	1469.7	9886.0
HLM-089-1	7.23	1.13	150.75	9.47	67.28	2404.0	3010.4	21509.0
HLM-090-1	15.91	0.39	76.26	1.52	2.13	2286.1	1672.5	17608.5
HLM-101-1	27.45	0.40	57.36	40.56	61.37	2241.9	2255.7	14409.5
HLM-104-1	25.79	1.75	52.13	2.17	2.62	4130.5	3823.6	17833.0
HLM-105-1	48.71	1.30	90.88	3.19	31.49	1879.7	3170.2	19704.0
HLM-116-1	35.25	1.79	65.79	8.23	24.30	2681.5	11727.0	15076.5
HLM-138-1	51.43	3.62	57.22	3.58	16.91	1606.0	866.5	24086.0
HLM-152-1	33.63	1.41	28.85	2.28	5.40	1036.0	278.1	20349.0
HLM-170-1	50.62	3.81	22.20	4.07	5.87	550.6	786.1	19032.5
HLM-188-1	42.23	11.13	0.64	8.07	56.38	1907.0	1238.0	23808.0
HLM-225-1	9.41	1.25	46.90	2.92	11.90	832.0	643.0	21034.5
Mean	39.0	2.0	54.8	10.1	27.3	1522.4	2084.8	20299.7
SD	42.8	2.6	49.2	14.9	22.4	1066.2	2557.8	8943.0
CV(%)	110	130	90	148	82	70	123	44

The CYP activities were determined by measuring the following activities: CYP1A2, phenacetin deethylation; CYP2B6, 7-benzyloxyresorufin debenzylation; CYP2C9, tolbutamide methylhydroxylation; CYP2C19, S-mephenytoin 4'-hydroxylation, CYP2D6, debrisoquine 4-hydroxylation; CYP2E1, chlorozoxazone 6-hydroxylation; CYP3A4, diazepam 3-hydroxylation. The hCE1 activity was determined by analyzing hydrolysis of temocapril.

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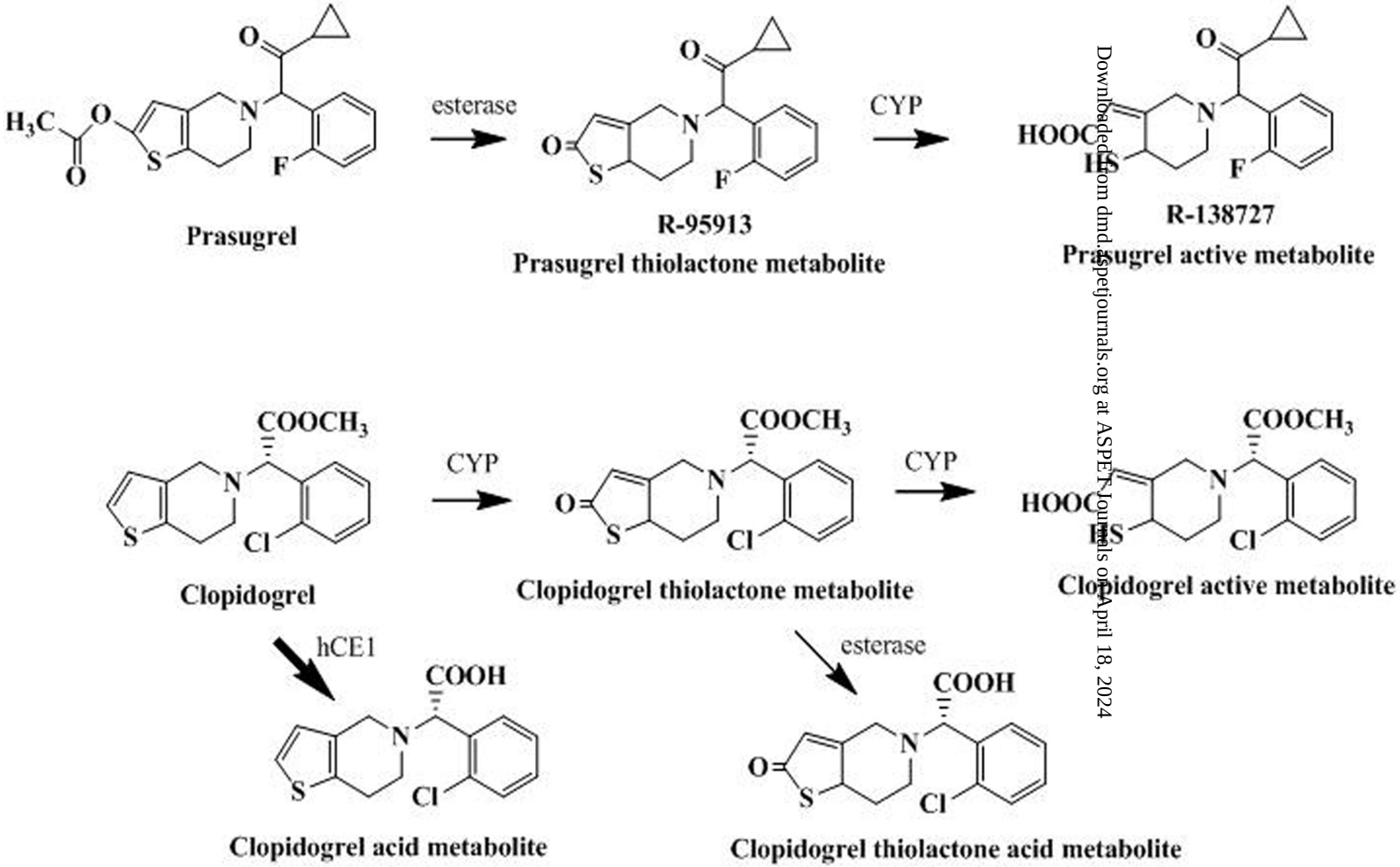
Table 2 Formation ratio of clopidogrel active metabolite and clopidogrel thiolactone acid from clopidogrel thiolactone (1 μM) in individual human liver microsomes (N = 20).

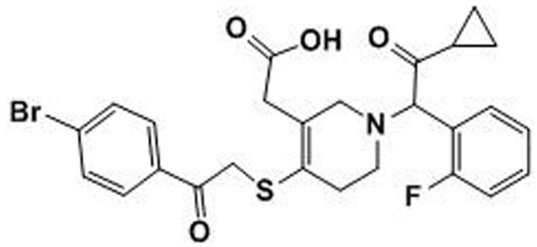
	Metabolite formation ratio (%)	
	Clopidogrel active metabolite	Clopidogrel thiolactone acid
Mean	52.21	47.79
SD	27.86	27.86

Figure 1

(a)

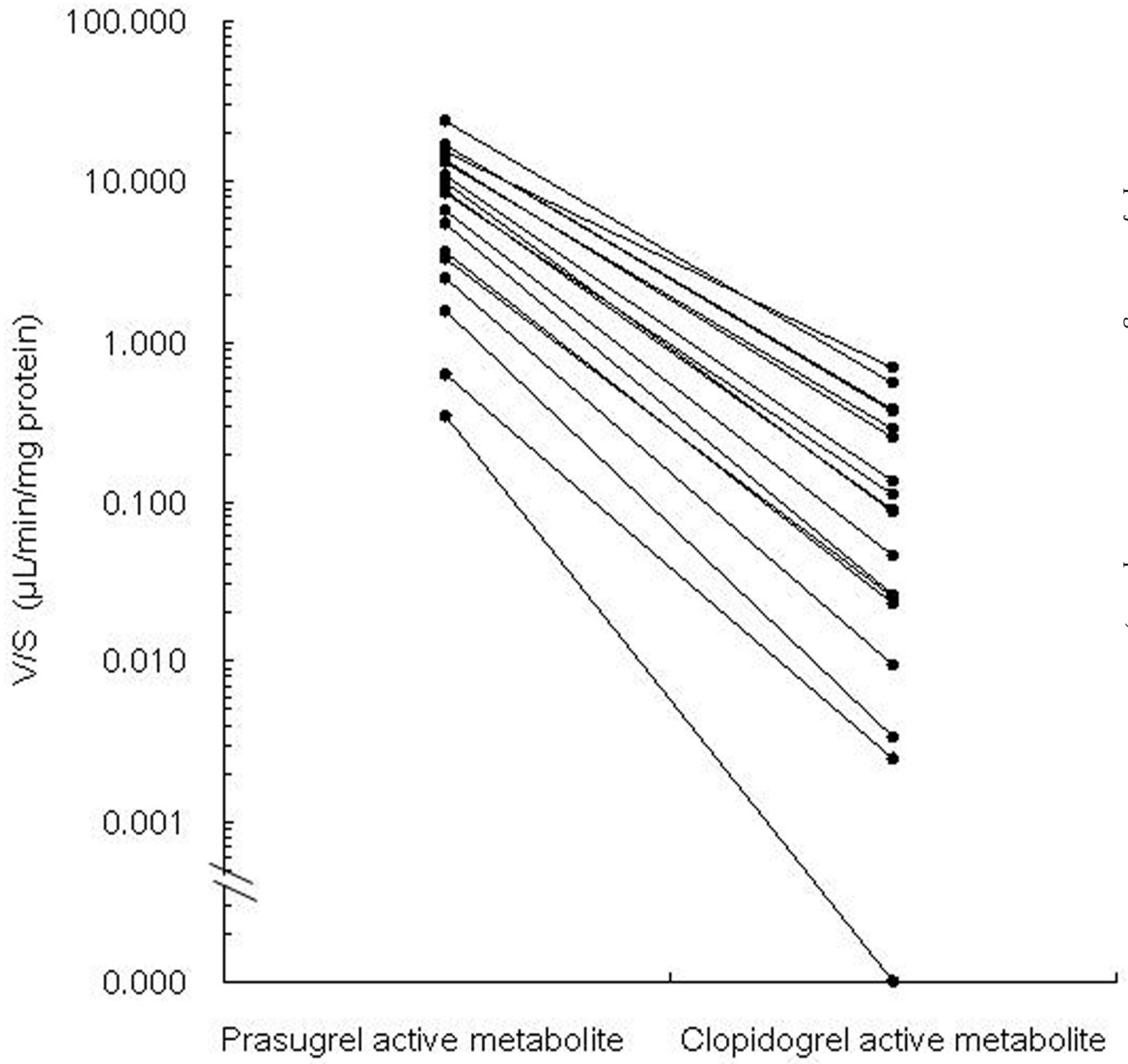


(b)



R-135766

Figure 2



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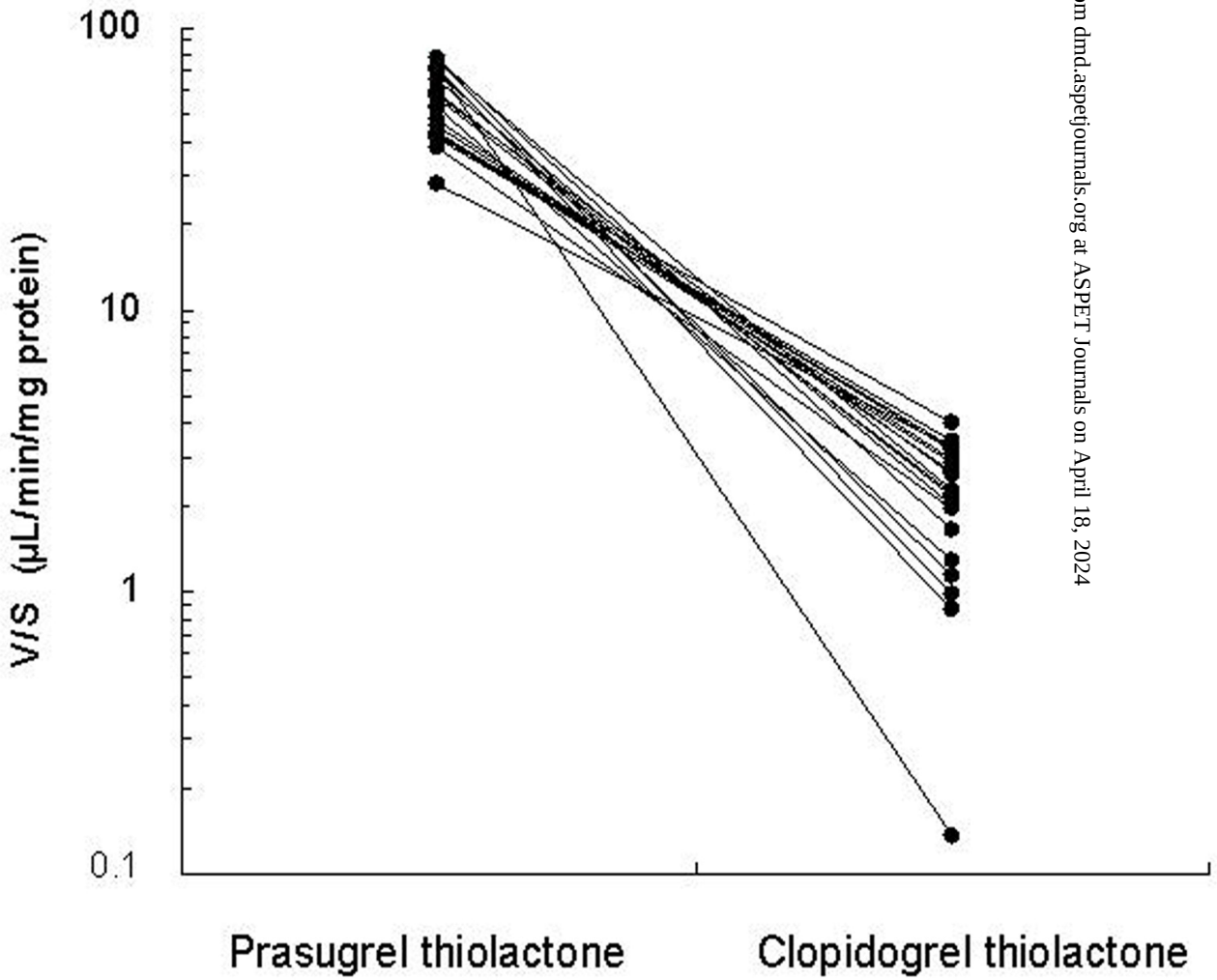

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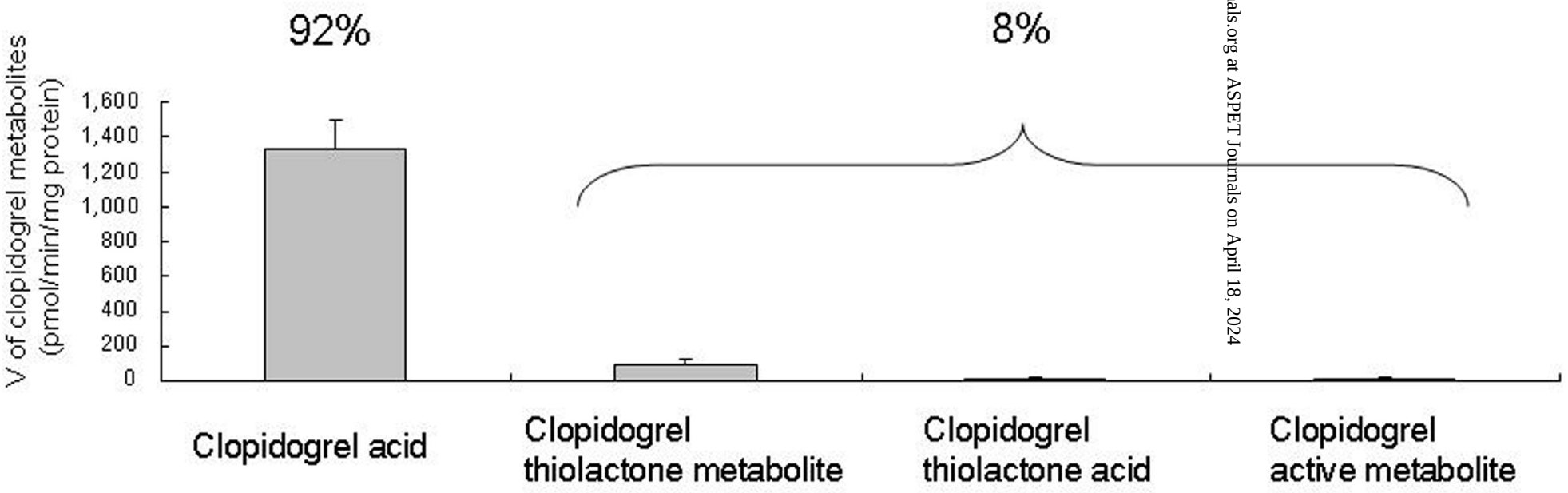

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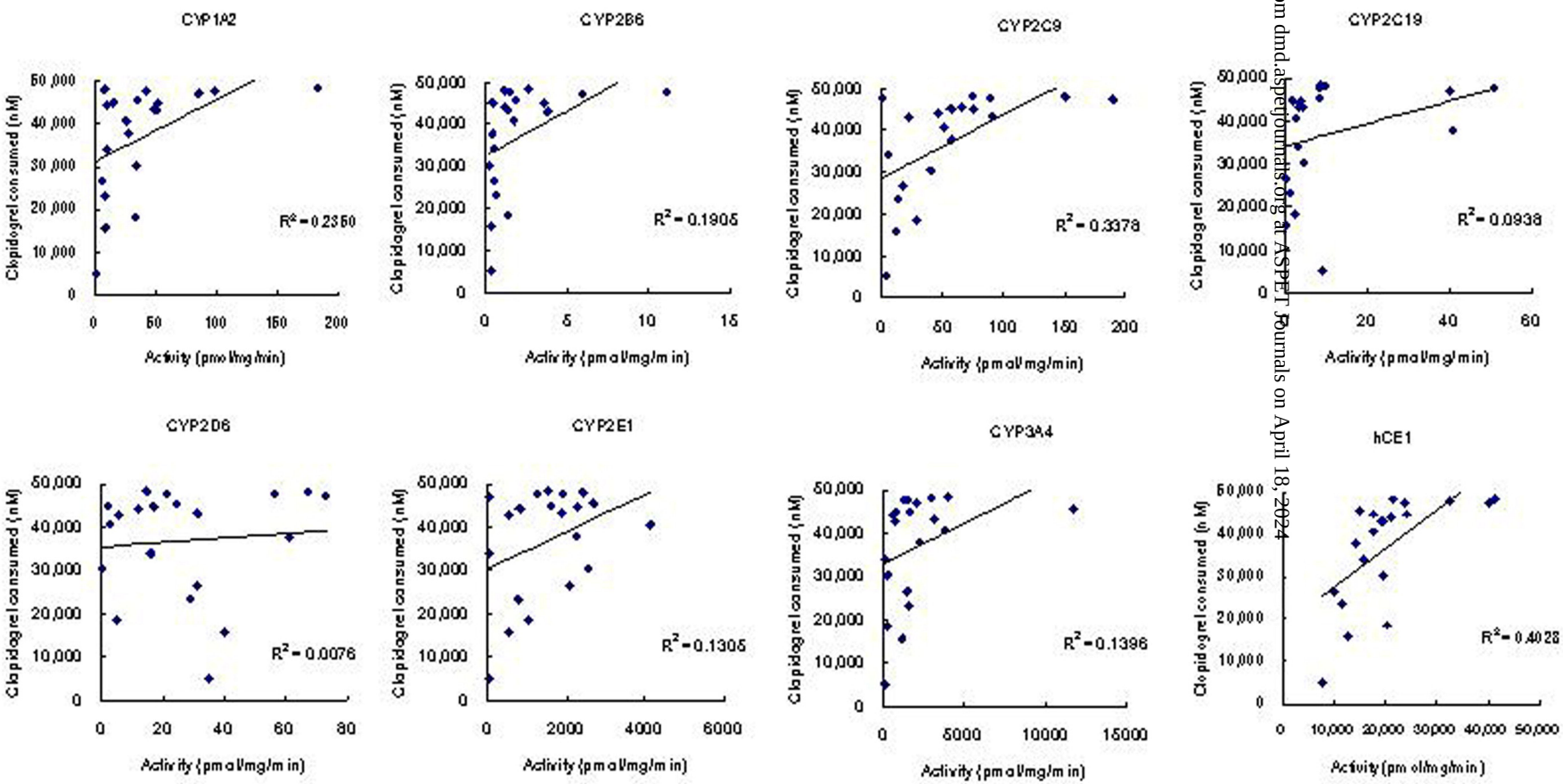

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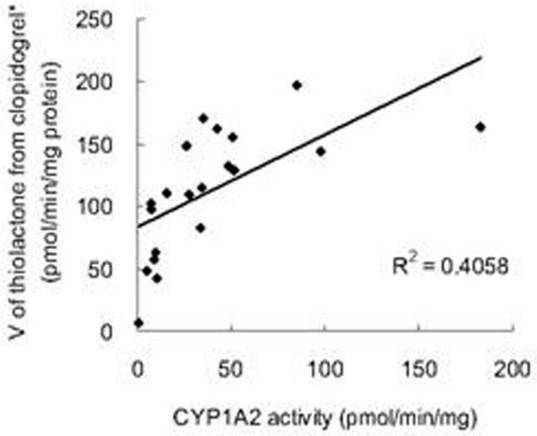
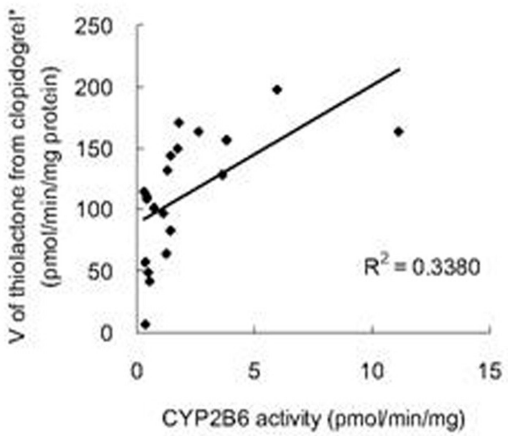




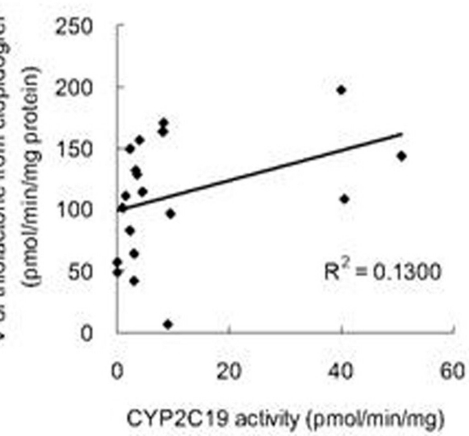


Figure 7

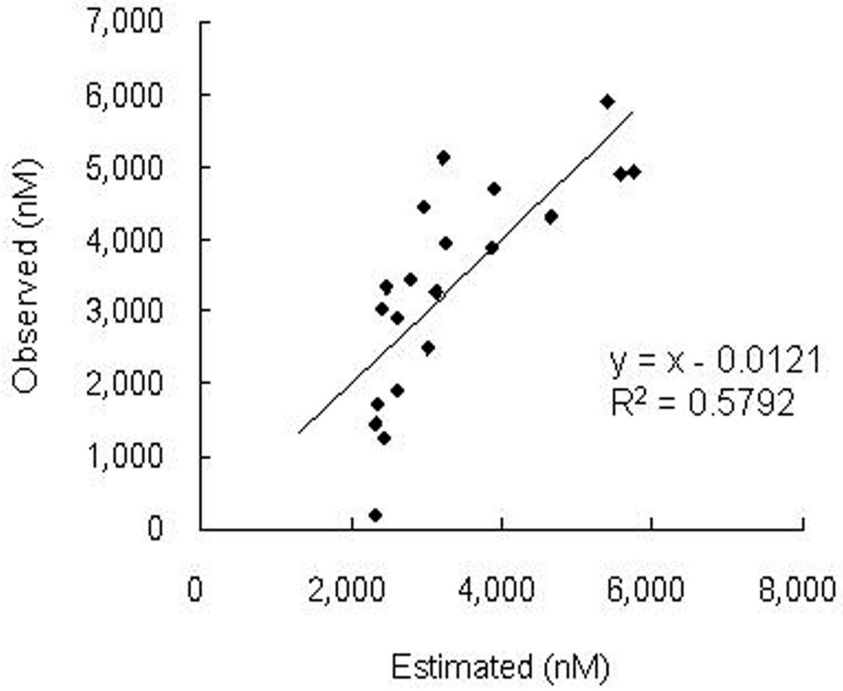
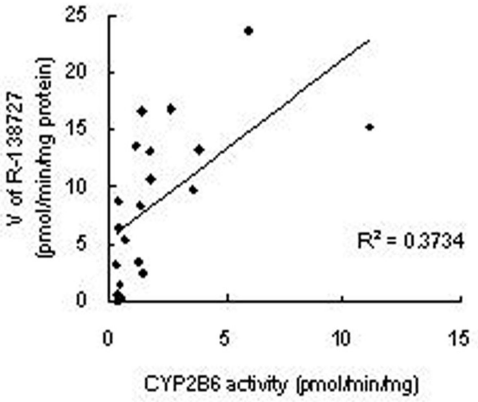
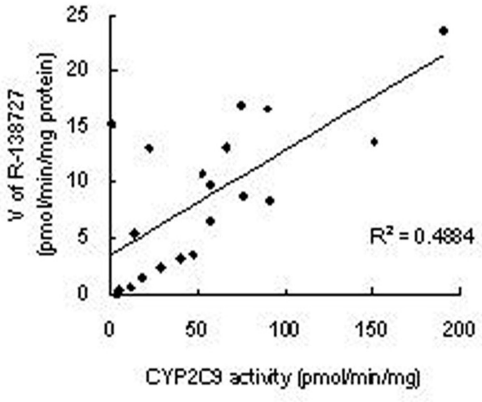
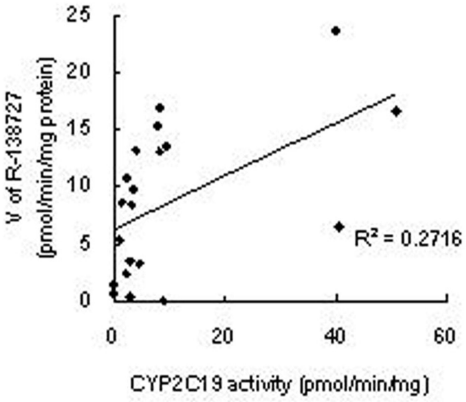


Figure 8







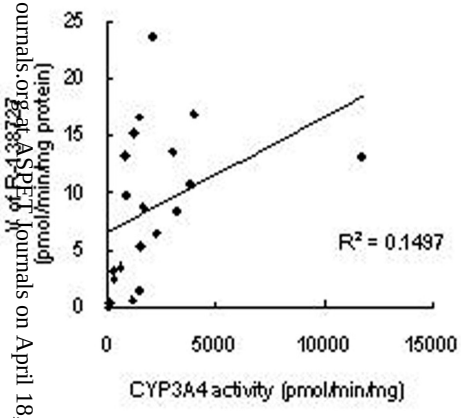


Figure 9