

Short Communication

The Intestine As an Important Contributor to Prasugrel Active Metabolite Formation In Vivo

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ABSTRACT:

Prasugrel [2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine], a thienopyridine antiplatelet agent, undergoes rapid hydrolysis in vivo to a thiolactone intermediate, 2-[2-oxo-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl]-1-cyclopropyl-2-(2-fluorophenyl)ethanone (R-95913), which is further converted to a pharmacologically active metabolite, 2-[1-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-mercapto-3-piperidinylidene acetic acid (R-138727), by oxidation via cytochromes P450. In this study, we investigated how much the intestine and liver contribute to the formation of R-95913 and R-138727 after intraduodenal administration of prasugrel (1 mg/kg) to portal vein- and hepatic vein-cannulated

dogs. The areas under the plasma concentration-time curve up to 2 h of R-95913 in the portal, hepatic, and systemic veins were 525, 32, and 17 ng · h/ml, respectively, and those of R-138727 were 564, 529, and 495 ng · h/ml, respectively. The dose of prasugrel was absorbed and then converted to R-95913 and R-138727 by 93 and 13%, respectively, in the intestine. In the liver, 23% of the R-95913, which passed through the intestine, was converted to R-138727. In conclusion, this is the first report to directly demonstrate that the conversion of prasugrel to R-138727 in the intestine is comparable to that converted in the liver of dogs.

Introduction

Prasugrel [2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine; Effient in the United States and Effient in the European Union], clopidogrel (Plavix/Iscover), and ticlopidine (Ticlid) are thienopyridine antiplatelet agents. Prasugrel is indicated to reduce the rate of thrombotic cardiovascular events and stent thrombosis in patients with acute coronary syndrome who are undergoing percutaneous coronary intervention (Wiviott et al., 2007; Effient package insert, Eli Lilly and Company, Indianapolis, IN). The thienopyridines are prodrugs that are converted in vivo to their pharmacologically active metabolites possessing a thiol group via a corresponding thiolactone metabolite (Farid et al., 2010). In clinical trials, prasugrel achieved a greater and faster antiplatelet effect than clopidogrel (Payne et al., 2007; Wallentin et al., 2008). Such responses to prasugrel are attributed to higher and faster exposure to its active metabolite R-138727 (2-[1-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-mercapto-3-piperidinylidene acetic acid) than clopidogrel's response (Sugidachi et al., 2007; Ernest et al., 2008). Prasugrel is rapidly hydrolyzed to a thiolactone intermediate R-95913 (2-[2-oxo-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-

yl]-1-cyclopropyl-2-(2-fluorophenyl)ethanone), mainly by human carboxylesterase 2 during absorption through the gastrointestinal tract (Williams et al., 2008). R-95913 is metabolized to R-138727 by cytochrome P450 isoforms, and the main contributors are CYP3A4 and CYP2B6 (38–70 and 2–36%, respectively), with smaller contributions by CYP2C9 and CYP2C19 (14–19 and 8–11%, respectively) (Rehmel et al., 2006). CYP3A5 is as effective as CYP3A4 in converting R-95913 to R-138727 (Baker et al., 2008), indicating that CYP3A is a key isoform for R-138727 formation. Considering that CYP3A represents approximately 80% of the intestinal cytochrome P450 forms (Paine et al., 2006), a large proportion of R-138727 could be formed during first-pass metabolism in the intestine. We previously detected R-138727 in the portal vein after intraduodenal administration of prasugrel to rats (Hagihara et al., 2009), possibly indicating the formation of R-138727 in the intestine. However, that finding does not prove the intestinal contribution unequivocally because the appearance of R-138727 in the portal vein was slow and could have been derived from the circulation after formation in the liver (Hagihara et al., 2009).

In this study, we determined quantitatively the contribution ratio of the intestine and liver to the formation of the intermediate R-95913 and the active metabolite R-138727 from prasugrel in dogs.

Materials and Methods

Materials. Prasugrel, prasugrel thiolactone (R-95913), and prasugrel active metabolite (R-138727), shown in Fig. 1, were synthesized by Ube Industries, Ltd. (Ube, Japan). Phenacetin and *N,N*-dimethylacetamide were purchased from Sigma-Aldrich (St. Louis, MO). Polyethylene glycol 400 was obtained from Wako Pure Chemicals (Osaka, Japan). Methoxyphenacyl bromide was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Human intestinal

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ABBREVIATIONS: R-138727, 2-[1-2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4-mercapto-3-piperidinylidene acetic acid; R-95913, 2-[2-oxo-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl]-1-cyclopropyl-2-(2-fluorophenyl)ethanone; LC, liquid chromatography; MS, mass spectrometry; HPLC, high-performance liquid chromatography; AUC_{0–2 h}, area under the plasma concentration-time curve up to 2 h; MS/MS, tandem mass spectrometry.

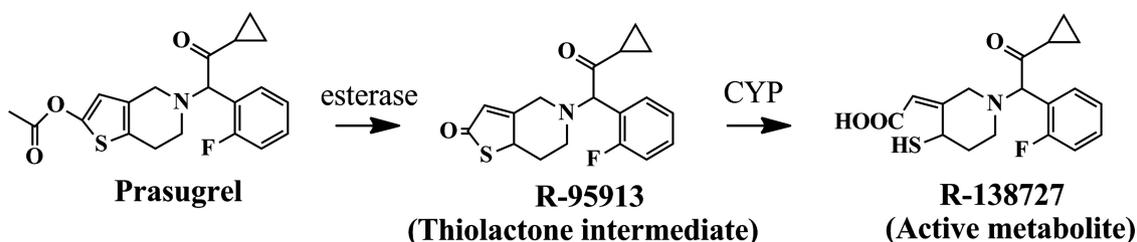


FIG. 1. Bioactivation pathway of prasugrel. CYP, cytochrome P450.

microsomes, dog intestinal microsomes, and dog liver microsomes were purchased from Tissue Transformation Technologies Inc. (Edison, NJ), Biopredic International (Rennes, France), and XenoTech, LLC (Lenexa, KS), respectively. All other chemicals and reagents were commercially available and of the highest grade.

Preparation of Dosing Formulations. Prasugrel was dissolved in 5% *N,N*-dimethylacetamide-95% polyethylene glycol 400 (v/v) solution at concentrations of 3 mg/ml. The solution was prepared immediately before use.

Experimental Animals. Male beagle dogs ($n = 6$) at age 2 to 3 years were obtained from Nosan Corporation (Yokohama, Japan) and were acclimatized in a controlled animal area set at $23 \pm 2^\circ\text{C}$ (acceptable range, $20\text{--}26^\circ\text{C}$) and relative humidity of $55 \pm 5\%$ (acceptable range, $30\text{--}70\%$) under a 12-h light/dark cycle of artificial lighting (lighting time, 7:00 AM–7:00 PM). Food (DS-A; Oriental Yeast Co., Ltd., Tokyo, Japan) and water were provided freely. Dogs, weighing 9 to 12 kg, that underwent the portal- and hepatic-vein cannulating surgery were used in the experiments and were fasted for approximately 19 h before prasugrel administration. All animal experiments were performed according to the guidelines provided by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

Portal Vein- and Hepatic Vein-Cannulated Dogs. Under pentobarbital anesthesia, the abdomen was incised on the median line. The portal vein was clamped onto the liver side and intestinal side of the cannulation point, into which the catheter (Medicut LCV-UK kit, single lumen, outer diameter 16 gauge, length 70 cm; Sherwood Medical Company, St. Louis, MO) was inserted. A purse-string ligature was applied, and the clamps were released. After the portal vein cannulation, the same catheter was also inserted into the hepatic vein and a purse-string ligature was applied. The catheters were conjugated with each injection port, which was embedded subcutaneously.

Animal Experiments. From the jugular vein, 2 ml of blood was extracted before dosing. The dosing solution of prasugrel was intraduodenally administered to the cannulated dogs ($n = 6$) at a dose level of 1 mg/kg. After approximately 1 ml of blood drainage from the portal and hepatic veins via the injection port, the syringe was changed to another one, and 1 ml of blood was collected with the heparinized syringe at 1, 5, 10, 15, 30, 60, and 120 min postdose. The blood in the cannula was pushed back by the injection of 2 ml

of heparin solution in saline (100 units/ml). At each time point, 1 ml of blood was also collected from the jugular vein. The blood samples were immediately centrifuged at 14,000 rpm for 3 min at 4°C (Hitachi Koki Co., Ltd., Tokyo, Japan) to collect plasma samples. A total of $50 \mu\text{l}$ of plasma was mixed with $100 \mu\text{l}$ of acetonitrile and $50 \mu\text{l}$ of the internal standard solution ($4 \mu\text{M}$ phenacetin in acetonitrile), and the mixture was centrifuged at 14,000 rpm for 3 min at 4°C (Hitachi Koki Co., Ltd.). A total of $10 \mu\text{l}$ of the supernatant was injected into the LC-MS system.

Quantitation of R-95913 and R-138727. Quantitation of R-95913 and R-138727 in dog plasma was performed on an Alliance HPLC system consisting of a 2690 Separations Module (Waters, Milford, MA) coupled to a Quattro LC-MS system (Micromass Ltd., Milford, MA) with the electrospray ionization source in positive ion mode. The mobile phase containing acetonitrile, 5 mM ammonium acetate, and formic acid (32:68:0.2, v/v/v) was applied onto the column. A total of $10 \mu\text{l}$ of each sample was injected onto a CAPCELL PAK C18 column ($5 \mu\text{m}$, $150 \text{ mm} \times 1.5 \text{ mm}$; Shiseido Co., Ltd., Tokyo, Japan). The operating parameters of the MS detector were set as follows: capillary voltage, 3.5 kV; ion source temperature, 120°C ; and desolvation temperature, 350°C . Detection was performed in the multiple reaction monitoring mode. The concentrations of each analyte in the samples were calculated using the computer software MassLynx (version 3.4; Micromass Ltd.).

Pharmacokinetic Analysis. The pharmacokinetic parameters were calculated using the computer program WinNonlin Professional (version 4.0.1; Pharsight, Mountain View, CA) on the basis of the noncompartmental method. The area under the plasma concentration-time curve up to 2 h ($\text{AUC}_{0\text{--}2\text{h}}$) was calculated by the trapezoidal method, and values are expressed as means \pm S.D.

Calculation of Availability of R-95913. The availability of R-95913 was calculated using eqs. 1 and 2:

$$F_a \cdot F_{g \text{ pras} \rightarrow 913} = Q_p \cdot R_b \cdot (\text{AUC}_{\text{por}, 913} - \text{AUC}_{\text{sys}, 913}) / \text{dose} \quad (1)$$

$$F_{h 913} = \text{AUC}_{\text{hep}, 913} / \text{AUC}_{\text{por}, 913} \quad (2)$$

where F_a , $F_{g \text{ pras} \rightarrow 913}$, and $F_{h 913}$ are fraction of intestinal absorption of prasugrel, fraction of prasugrel hydrolysis to R-95913 in the intes-

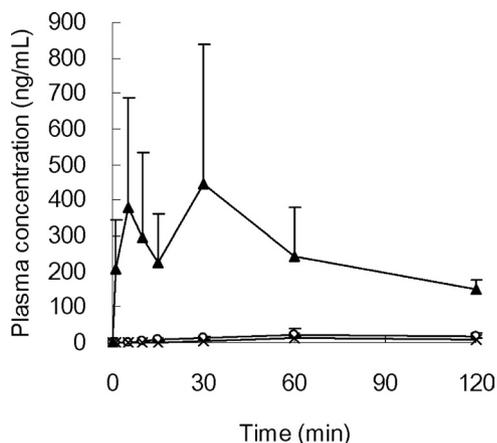


FIG. 2. Plasma concentrations of R-95913 after intraduodenal administration of prasugrel to dogs at a dose of 1 mg/kg. Concentrations of R-95913 in the portal vein (▲), hepatic vein (○), and systemic vein (×) were determined by LC-MS/MS. The data are expressed as the mean \pm S.D.

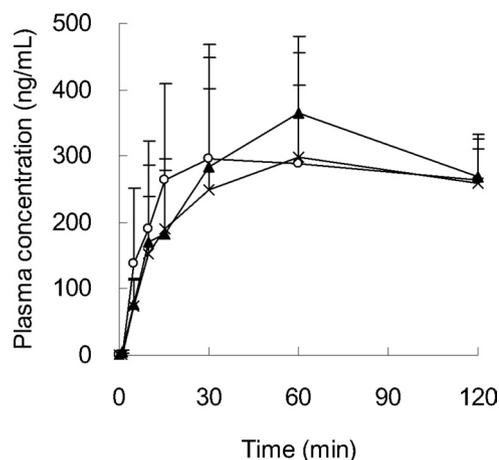


FIG. 3. Plasma concentrations of R-138727 after intraduodenal administration of prasugrel to dogs at a dose of 1 mg/kg. Concentrations of R-138727 in the portal vein (▲), hepatic vein (○), and systemic vein (×) were determined by LC-MS/MS. The data are expressed as the mean \pm S.D.

TABLE 1

Availability of R-95913 in the intestine and the liver after intraduodenal administration of prasugrel to dogs at a dose of 1 mg/kg

	$F_a \cdot F_{g \text{ pras} \rightarrow 913}$	$F_{h \text{ 913}}$	$F_{g \text{ pras} \rightarrow 913}$
Mean	0.80	0.08	0.83
S.D.	0.23	0.06	0.23

tine, and availability of R-95913 in the liver, respectively. $AUC_{\text{por}, 913}$, $AUC_{\text{hep}, 913}$, and $AUC_{\text{sys}, 913}$ indicate the $AUC_{0-2 \text{ h}}$ values of R-95913 in the portal, hepatic, and systemic veins, respectively. Q_p is portal blood flow, and R_b is the blood/plasma concentration ratio. F_a , Q_p , and R_b are set as 0.968 (Hagihara et al., 2007), 521 ml/min (Hoshino et al., 1986), and 0.597 (in-house data, Daiichi Sankyo Co., Ltd.), respectively.

Calculation of Availability of R-138727. The availability of R-138727 was calculated using eqs. 3 to 5:

$$F_a \cdot F_{g \text{ pras} \rightarrow 727} = Q_p \cdot R_b \cdot (AUC_{\text{por}, 727} - AUC_{\text{sys}, 727}) / \text{dose} \quad (3)$$

$$F_{m \text{ 913} \rightarrow 727} = (AUC_{\text{hep}, 727} - AUC_{\text{por}, 727} \cdot F_{h \text{ 727}}) / AUC_{\text{por}, 913} \quad (4)$$

$$F_a \cdot F_{g \text{ pras} \rightarrow 727} \cdot F_{h \text{ 727}} + F_a \cdot F_{g \text{ pras} \rightarrow 913} \cdot F_{m \text{ 913} \rightarrow 727} = \text{relative BA} \quad (5)$$

where $F_{g \text{ pras} \rightarrow 727}$, $F_{m \text{ 913} \rightarrow 727}$, and $F_{h \text{ 727}}$ are fraction of prasugrel conversion to R-138727 in the intestine, fraction of R-95913 conversion to R-138727 in the liver, and availability of R-138727 in the liver, respectively. $AUC_{\text{por}, 727}$, $AUC_{\text{hep}, 727}$, and $AUC_{\text{sys}, 727}$ indicate the $AUC_{0-2 \text{ h}}$ values of R-138727 in the portal, hepatic, and systemic veins, respectively. Relative BA represents the relative bioavailability of R-138727 after oral dosing of prasugrel to dogs and was set as 24.8% (Hagihara et al., 2009).

Formation of R-95913 in Dog and Human Intestinal S9. The mixture (total volume, 247.5 μl) in triplicate contained potassium phosphate buffer (7 mM, pH 7.4); an NADPH-generating system containing 2.5 mM β -NADP, 25 mM glucose 6-phosphate, 0.5 units/ml glucose-6-phosphate dehydrogenase, and 10 mM MgCl_2 ; 5 mM glutathione; and dog or human intestinal S9 (10 mg of protein/ml each). The mixture was preincubated at 37°C for 5 min, and 2.5 μl of prasugrel (final concentration: 250 μM) was added to the mixture, which was incubated at 37°C for 0, 5, 15, 30, and 60 min. Then 90 μl of ethanol was added to 30 μl of the reaction mixture to stop the reaction at each time point, the mixture was centrifuged (15,000g, 3 min, 4°C), and 25 μl of the supernatant was injected into the HPLC system. HPLC was performed using a YMC-ODS A-302 (4.6 mm i.d. \times 150 mm) column pumped at flow rate of 0.5 ml/min with a mobile phase consisting of acetonitrile, isopropyl alcohol, distilled water, and trifluoroacetic acid (5:12:83:0.01, v/v/v/v). Absorption of the column effluent was monitored at 220 nm to detect the metabolites. The lower limit of quantification for R-95913 was 1 μM .

Formation of R-138727 in Human and Dog Intestinal and Dog Liver Microsomes. The mixture (total volume, 110 μl) in triplicate contained potassium phosphate buffer (43 mM, pH 7.4), an NADPH-generating system (1.3 mM NADP^+ , 3.3 mM glucose 6-phosphate, 0.4 units/ml glucose-6-phosphate dehydrogenase, and 3.3 mM MgCl_2), 5 mM glutathione, and human or dog intestinal or dog liver microsomes (1 mg of protein/ml each). The mixture was preincubated at 37°C for 5 min, and 1.1 μl of R-95913 (final concentrations ranging from 1.6 to 200 μM) was added to the mixture. After incubation at 37°C for 15 min, 200 μl of 5 mM methoxyphenacyl bromide solution and 100 μl of the internal standard solution (100 ng/ml R-135766) in acetonitrile were added to 100 μl of the reaction

TABLE 2

Availability of R-138727 in the intestine and the liver after intraduodenal administration of prasugrel to dogs at a dose of 1 mg/kg

	$F_a \cdot F_{g \text{ pras} \rightarrow 727}$	$F_{g \text{ pras} \rightarrow 727}$	$F_{m \text{ 913} \rightarrow 727}$	$F_{h \text{ 727}}$
Mean	0.13	0.13	0.23	0.75
S.D.	0.13	0.13	0.06	0.21

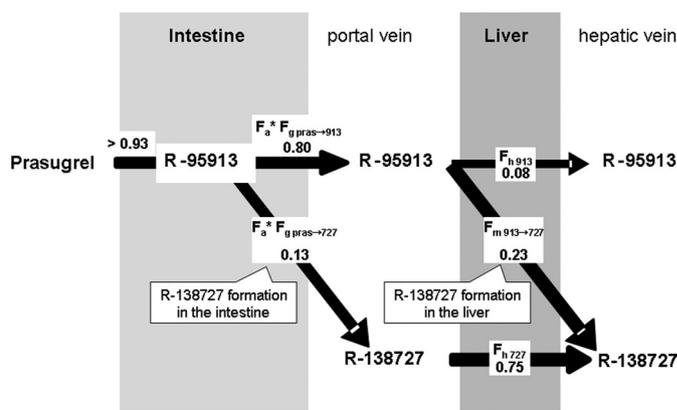


FIG. 4. Availability of R-95913 and R-138727 in the intestine and the liver in dogs.

mixture to stop the reaction, and the mixture was left for 10 min at room temperature to derivatize a thiol moiety of R-138727. The mixture was extracted by a solid-phase extraction column (Captiva; Varian, Inc., Palo Alto, CA). The assay of R-138727 was performed following the methods reported previously (Hagihara et al., 2009). Separation of the analytes by HPLC was conducted using an Alliance 2690 Separations Module (Waters). Mass spectra were determined using a Quattro LC-MS/MS system (Micromass Ltd.) in the positive ion detection mode using an electrospray ionization interface. A lower limit of quantification was set at 1.6 nM. Data acquisition and analyses were performed using MassLynx software (version 4.0).

Data Handling. The formation pattern of R-138727 in human or dog intestinal or dog liver microsomes appeared monophasic in each Eadie-Hofstee plot (data not shown). Therefore, the data were fitted to eq. 6 using WinNonlin Professional (version 4.0.1).

$$V = V_{\text{max}} \times S / (K_m + S) \quad (6)$$

where S , K_m , and V_{max} are the substrate concentration, Michaelis-Menten constant, and maximal formation rate, respectively. The intrinsic clearance (CL_{int}) was calculated as a ratio of V_{max} to K_m . These parameters are expressed as means \pm S.D.

Results

Plasma Concentrations and $AUC_{0-2 \text{ h}}$ Values after Intraduodenal Administration of Prasugrel to Cannulated Dogs. The plasma concentrations of R-95913 and R-138727 were determined by LC-MS

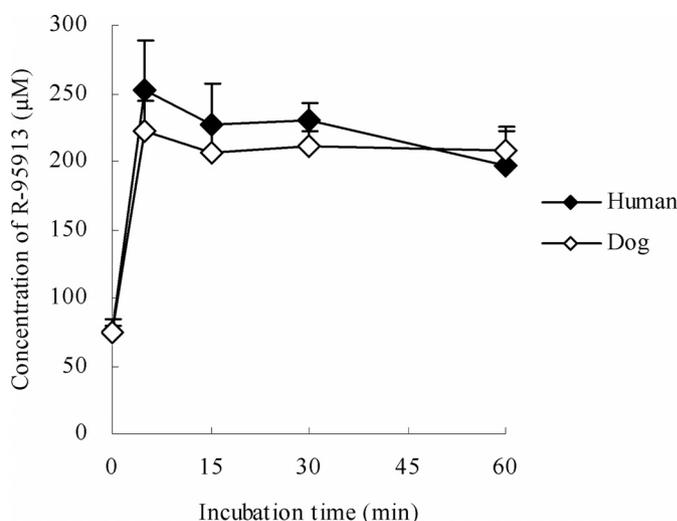


FIG. 5. Formation of R-95913 from prasugrel in dog and human intestinal S9. Prasugrel (250 μM) was incubated in dog and human intestinal S9 and the concentrations of R-95913 were determined.

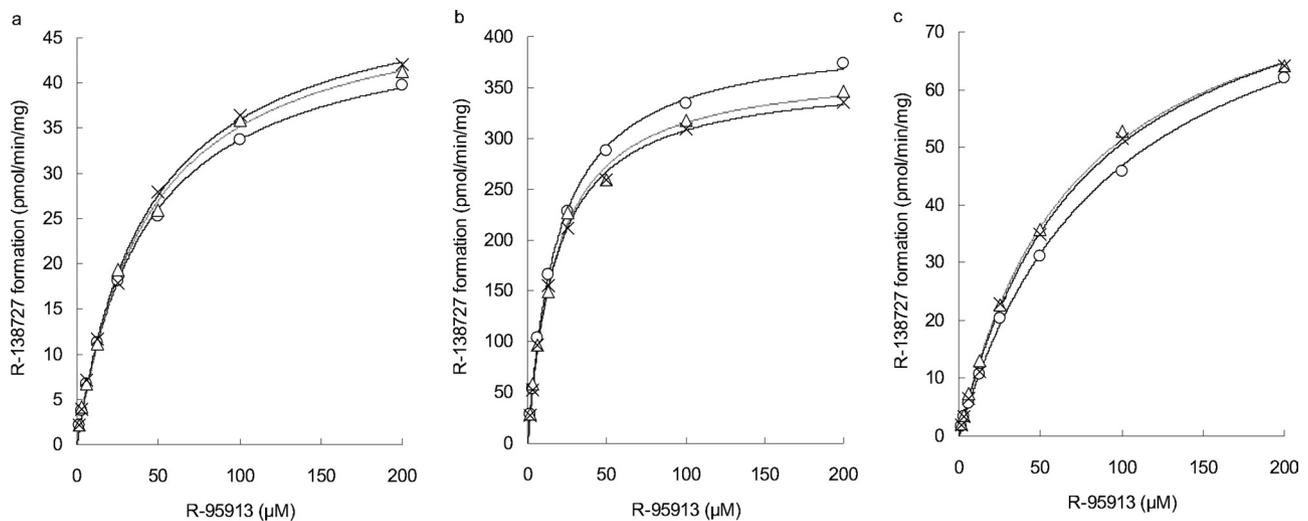


FIG. 6. Kinetic analyses of the formation of R-138727 from R-95913 in dog and human intestinal and dog liver microsomes. The formation rates of R-138727 from R-95913 in triplicate were determined in dog intestinal (a) and liver (b) microsomes and human intestinal microsomes (c).

after intraduodenal administration of prasugrel to the portal vein- and hepatic vein-cannulated dogs at a dose of 1 mg/kg. Plasma concentrations of prasugrel were not analyzed because prasugrel was not detected unmodified even in the portal vein in a previous study (Hagihara et al., 2009). The plasma concentration-time profiles of R-95913 and R-138727 are shown in Figs. 2 and 3, respectively. The AUC_{0-2h} value of R-95913 in the portal vein (525 ± 234 ng · h/ml) was much higher than those in hepatic and systemic veins (32 ± 17 and 17 ± 8 ng · h/ml, respectively). The AUC_{0-2h} values of R-138727 in the portal, hepatic, and systemic veins were 564 ± 187 , 529 ± 136 , and 495 ± 233 ng · h/ml, respectively. The bimodal peaks were detected in the plasma concentration-time profiles of R-95913 (Fig. 2). This finding may indicate reabsorption of R-95913 from the intestine as in the case of rats, which showed enterohepatic circulation of [^{14}C]prasugrel (Hagihara et al., 2007).

Availability of R-95913 and R-138727 in the Intestine and the Liver. The $F_a \cdot F_{g\text{ pras} \rightarrow 913}$, and $F_{h\ 913}$ values were 0.80 ± 0.23 and 0.08 ± 0.06 , respectively (Table 1). The $F_a \cdot F_{g\text{ pras} \rightarrow 727}$, $F_{m\ 913 \rightarrow 727}$, and $F_{h\ 727}$ values of R-138727 were 0.13 ± 0.13 , 0.23 ± 0.06 , and 0.75 ± 0.21 , respectively (Table 2). The dose of prasugrel was absorbed and converted to R-95913 by 93% ($F_a \cdot F_{g\text{ pras} \rightarrow 727} + F_a \cdot F_{g\text{ pras} \rightarrow 913}$) and to R-138727 by 13% in the intestine. R-95913 that passed through the intestine was converted to R-138727 by 23% in the liver (Fig. 4).

Formation of R-95913 in Dog and Human Intestinal S9. Prasugrel was almost completely hydrolyzed to R-95913 at 5 min in dog and human intestinal S9 (Fig. 5). The results indicated comparable hydrolytic activities of prasugrel by dog and human intestinal enzymes.

Formation of R-138727 in Dog and Human Intestinal and Liver Microsomes. The rates of R-138727 formation over a range of R-95913 concentrations were determined in dog intestinal and liver microsomes and human intestinal microsomes. The formation curve for each concentration of R-138727 was well fitted to eq. 6 (Fig. 6). Dog intestinal and liver microsomes and human intestinal microsomes exhibited apparent K_m values of 42.7, 18.5, and 80.4 μM , respectively. The corresponding CL_{int} values in these microsomes were 1.2, 20.6, and 1.1 μl per min/mg, respectively. The CL_{int} ratios (liver/intestine) were 17.2 and 8.7 in dogs and humans, respectively (Table 3).

Discussion

There have been several reports indicating that the active metabolite of prasugrel is formed in the intestine of humans. Farid et al. (2007a) showed that concomitant administration of a potent CYP3A4/5 inhibitor, ketoconazole, with prasugrel resulted in delayed appearance of R-138727 in plasma. In addition, the AUC of R-95913 doubled and maximum concentration (C_{max}) increased by 71 to 93%, whereas the t_{max} or $t_{1/2}$ of R-95913 did not change (Farid et al., 2007a). In consideration of the fact that the AUC and C_{max} reflect bioavailability, whereas $t_{1/2}$ depends directly on hepatic clearance, these differential pharmacokinetic effects are indicative of R-95913 as a substrate for intestinal CYP3A in humans. Small et al. (2009) reported a clinical observation in patients with chronic liver disease, in whom moderate hepatic impairment appeared to have no effect on exposure to prasugrel's active metabolite R-138727 and little or no effect on platelet aggregation relative to that in healthy control subjects. On the basis of this information, we considered the intestine to

TABLE 3

Kinetic parameters for the formation of R-138727 in dog and human intestinal and liver microsomes

Values for K_m and V_{max} are reported as the mean of parameter estimates \pm S.D.

	Incubation Time	K_m	V_{max}	CL_{int}	CL_{int} Ratio (Liver/Intestine)
	min	μM	pmol per min/mg	μl per min/mg	
Dog					
Intestinal microsomes	15	42.7 ± 1.0	49.8 ± 2.0	1.2 ± 0.0	17.2
Liver microsomes	15	18.5 ± 0.6	379.9 ± 20.7	20.6 ± 0.5	
Human					
Intestinal microsomes	15	80.4 ± 11.5	89.2 ± 1.3	1.1 ± 0.1	8.7
Liver microsomes	15	26	247	9.6	

Data from Rehmel et al. (2006).

be an important contributor to R-138727 formation in vivo. In general, it is difficult to perform quantitative kinetic analyses of a prodrug and its metabolites in vivo, because complex models are required to describe respective concentration profiles (Tsukamoto et al., 2001). Therefore, we evaluated the availability of each metabolite using a simple calculation method without any differential equations. The results in the present study demonstrated a quantitatively important contribution of the intestine to R-138727 formation in dogs, where 13% of the dose of prasugrel was converted to R-138727. Because the availability of R-138727 in the liver was 75%, approximately 10% ($13 \times 75\%$) of the dose of prasugrel is thought to reach the circulation as R-138727 via bioactivation in the intestine. The relative bioavailability of R-138727 after oral administration of prasugrel to dogs is 25% (Hagihara et al., 2009), and therefore approximately 40% (10:25%) of exposure to R-138727 in the circulation is probably attributable to intestinal bioactivation.

In this study, the dose of prasugrel was calculated to be substantially converted to R-95913 (by 93%) during the absorption process through the intestine, which is consistent with the previous clinical and nonclinical observations. In human plasma, prasugrel was not detected unmodified because of rapid hydrolysis by carboxylesterases (Farid et al., 2007b; Williams et al., 2008). In addition, a Caco-2 cell study showed the complete conversion of prasugrel to R-95913 during absorption (Williams et al., 2008). In the current study, carboxylesterase inhibitors were not added during blood sampling, indicating the possibility of hydrolysis of prasugrel in the extracted blood samples. However, prasugrel is rapidly hydrolyzed in dog intestinal S9 (Fig. 5), and therefore prasugrel administered to dogs is thought to be hydrolyzed in the intestine in vivo.

We used dogs to evaluate respective fractions of prasugrel bioactivation in the intestine and liver. Because CYP3A activities have been reported in the intestine and liver in dogs (Komura et al., 2002; Sahi et al., 2002), it is considered reasonable to use dogs for assessment of active metabolite formation of prasugrel, which is mediated mainly by CYP3A. Indeed, prasugrel was metabolized to R-138727 in dog intestinal and liver microsomes with CL_{int} values comparable to those in human intestinal and liver microsomes, respectively (Table 3). The ratios of CL_{int} (liver/intestine) were 17.2 and 8.7 in dogs and humans, respectively, and these were relatively higher than that of the fraction of R-138727 formation in dogs ($F_{m\ 913 \rightarrow 727}/F_{g\ pras \rightarrow 727} = 1.8$). This discrepancy between in vitro and in vivo might be explained by the difference in physiological conditions in intestine and liver (i.e., more absolute exposure to the substrate in the intestine during absorption) or possibly a higher unbound fraction of the substrate in the intestine. In dog small intestine, no carboxylesterases were found in the previous report (Taketani et al., 2007). However, prasugrel was hydrolyzed in dog intestinal S9 to the same degree as in human intestinal S9 (Fig. 5), indicating the existence of complementary esterases in dog intestine. This concept is also supported by the previous observation that prasugrel was not detected unmodified in the portal vein after oral administration of prasugrel to dogs (Hagihara et al., 2009). Thus, a fraction of R-138727 similar to that formed in the intestine in dogs could be expected in humans.

Clopidogrel seems not to be converted to either a thiolactone intermediate or an active metabolite in the intestine (Kazui et al., 2005; Hagihara et al., 2009). This could be the reason for the slower onset of an inhibitory effect on platelet aggregation by clopidogrel than by prasugrel in the clinical studies (Payne et al., 2007; Wallentin et al., 2008).

In conclusion, a significant portion of R-95913 was oxidized to R-138727 during intestinal absorption in dogs. This is the first report to quantitatively evaluate the contribution of the intestine and liver to

the formation of R-95913 and R-138727, and the calculation method used in this study may provide a useful tool for evaluation of the bioactivation of other prodrugs in the liver and intestine.

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Participated in research design: Hagihara, Kazui, Ikenaga, Izumi, Ikeda, and Kurihara.

Conducted experiments: Hagihara, Kazui, Ikenaga, Nanba, and Fusegawa.
Performed data analysis: Hagihara and Kazui.

Wrote or contributed to the writing of the manuscript: Hagihara, Kazui, Ikenaga, Izumi, Ikeda, and Kurihara.

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