The intestine as an important contributor to prasugrel active metabolite formation in vivo.

Katsunobu Hagihara, Miho Kazui, Hidenori Ikenaga, Toshihiko Nanba, Kiichi Fusegawa, Takashi Izumi, Toshihiko Ikeda and Atsushi Kurihara

*Drug Metabolism & Pharmacokinetics Research Laboratories (K.H., M.K., T.I. and A.K.) and Pharmacology Research Laboratories (H.I., T.N. and K.F.), Daiichi Sankyo Co., Ltd., Tokyo, Japan; Yokohama College of Pharmacy, Yokohama, Japan (T.I.)*
Running Title: Prasugrel active metabolite formation in intestine

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: 14
Tables: 3
Figures: 6
References: 21

Abstract: 168 words
Introduction: 334 words
Discussion: 807 words

Abbreviations: prasugrel,
2-acetoxy-5-(α-cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine; S9, 9,000g supernatant
Prasugrel, a thienopyridine antiplatelet agent, undergoes rapid hydrolysis in vivo to a thiolactone intermediate, R-95913, which is further converted to a pharmacologically active metabolite, 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 (AUC0-2h) 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 dosed 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 (Effient® in the US and Efient® in EU), 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 that are undergoing percutaneous coronary intervention (Wiviott et al., 2007; Effient package insert). 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 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, than clopidogrel’s (Sugidachi et al., 2007; Ernest et al., 2008). Prasugrel is rapidly hydrolyzed to a thiolactone intermediate, R-95913, 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 (CYP) 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 about 80% of the intestinal CYP 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 does not prove unequivocally the intestinal contribution 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 Figure 1 were synthesized by Ube Industries, Ltd (Ube, Japan). Phenacetine and N,N-dimethylacetamide were purchased from Sigma-Aldrich (St. Louis, MO). Polyethylene glycol 400 was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methoxyphenacetyl bromide was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Human intestinal microsomes, dog intestinal microsomes and dog liver microsomes were purchased from Tissue Transformation Technologies Inc. (Edison, NJ), Biopredic International (Rennes, France) and XenoTech (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. Solution was prepared immediately before use.

Experimental animals. Male Beagle dogs (n=6) at ages of 2-3 years were obtained from Nosan Corporation and were acclimatized in a controlled animal area set at 23±2°C (acceptable range: 20-26°C) and relative humidity of 55±5% (acceptable range:
Portal- and hepatic-vein cannulated dogs. Under pentobarbital anesthesia, the abdomen was incised on the median line. The portal vein was clamped onto the liver-leaning and intestine-leaning sites of the cannulation point, into which the catheter (Medicut LCV-UK kit, single lumen; outer diameter, 16G; 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 about 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 post-dose. 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 μL of plasma was mixed with 100 μL of acetonitrile and 50 μL of the IS solution (4 μM 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 μL of the supernatant was injected to LC-MS.

Quantitation of R-95913 and R-138727. The quantitation of R-95913 and R-138727 in dog plasma was carried out on an Alliance HPLC system consisting of a 2690 Separations Module (Waters Co., Milford, MA) coupled to a Quattro LC MS system (Micromass Ltd., Milford, MA) with the ESI 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 μL of each sample was injected onto a CAPCEL PAK C18 column (5 μm, 150 mm × 1.5 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.

The detection was performed in the multiple reaction monitoring (MRM) 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 (ver. 4.0.1, Pharsight Corp., Mountain View, CA) based on the non-compartmental method. The area under the plasma concentration-time curve up to 2 h (AUC<sub>0-2h</sub>) was calculated by the trapezoidal method and are expressed as the mean±SD.

**Calculation of availability of R-95913.** The availability of R-95913 was calculated using the following equations.

\[ F_a * F_{g pras-913} = Q_p * R_h * (AUC_{por,913} - AUC_{sys,913}) / Dose \]  (1)

\[ F_{h 913} = AUC_{hep,913} / AUC_{por,913} \]  (2)

where \( F_a \), \( F_{g pras-913} \) and \( F_{h 913} \) are fraction of intestinal absorption of prasugrel, fraction of prasugrel hydrolysis to R-95913 in the intestine and availability of R-95913 in the liver, respectively. \( AUC_{por,913} \), \( AUC_{hep,913} \) and \( AUC_{sys,913} \) indicate the AUC<sub>0-2h</sub> 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 the following equations.

\[
F_a \cdot F_{g \text{ pras-.727}} = Q_p \cdot R_b \cdot (AUC_{\text{por,727}} - AUC_{\text{sys,727}}) / \text{Dose} \quad (3)
\]

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

\[
F_a \cdot F_{g \text{ pras-.727}} \cdot F_{h \text{ 727}} + F_a \cdot F_{g \text{ pras-.913}} \cdot F_{m \text{ 913-.727}} = \text{Relative BA} \quad (5)
\]

where \(F_{g \text{ pras-.727}}, F_{m \text{ 913-.727}}\) 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 \(AUC_{0-2h}\) values of R-138727 in the portal, hepatic and systemic veins, respectively. Relative BA means 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 \(\mu\)L) in triplicate contained potassium phosphate buffer (7 mM, pH 7.4), an NADPH generating system containing 2.5 mM \(\beta\)-NADP, 25 mM G-6-P, 0.5 units/mL G-6-PDH and 10 mM MgCl\(_2\), 5 mM glutathione and dog or human intestinal S9 (10 mg
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. Ninety μL of ethanol was added to 30 μL of the reaction mixture to stop the reaction at each time point and the mixture was centrifuged (15,000g, 3 min, 4°C). Twenty five μL of the supernatant was injected into the HPLC system. HPLC was performed using YMC-ODS A-302 (4.6 mm i.d. ×150 mm) pumped at flow rate of 0.5 mL/min with a mobile phase consisting of acetonitrile, isopropyl alcohol, distilled water, 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. 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 MgCl₂), 5 mM glutathione and human or dog intestinal or dog liver microsomes (1 mg 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 IS solution (100 ng/mL R-135766) in acetonitrile were added to 100 μL of the reaction 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 extract column (Captiva; Varian, Inc., Palo Alto, CA). The assay of R-138727 was performed following the methods previously reported (Hagihara et al., 2009). 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. A lower limit of quantification was set at 1.6 nM. Data acquisition and analyses were performed using MassLynx software (Version 4.0, Micromass Ltd.).

**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. 1 using WinNonlin Professional (version 4.0.1, Pharsight Corp.).

\[
V = \frac{V_{\text{max}} \times S}{(K_m + S)}
\]

where \(S\), \(K_m\) and \(V_{\text{max}}\) is the substrate concentration, Michaelis-Menten constant and maximal formation rate, respectively. The intrinsic clearance (\(\text{CL}_{\text{int}}\)) was calculated as

12
a ratio of $V_{\text{max}}$ to $K_m$. These parameters are expressed as mean±SD.
Results

**Plasma concentrations and AUC\(_{0-2h}\) after intraduodenal administration of prasugrel to cannulated dogs.** The plasma concentrations of R-95913 and R-138727 were determined by LC-MS after intraduodenal administration of prasugrel to the portal and hepatic vein cannulated dogs at a dose of 1 mg/kg. Plasma concentrations of prasugrel were not analyzed as 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 Figures 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 ng·h/mL, 529±136 ng·h/mL and 495±233 ng·h/mL, respectively. The bimodal peaks were detected in the plasma concentration-time profiles of R-95913 (Figure 2). This 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 \(F_a \cdot F_g\) and \(F_h\) values were 0.80±0.23 and 0.08±0.06, respectively (Table 1). The
F_a * F_g pras→727, F_m 913→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 dosed prasugrel was absorbed and converted to R-95913 by 93% ($F_a * F_g$ pras→727 + $F_a * F_g$ pras→913) and to R-138727 by 13% in the intestine. R-95913 which passed through the intestine was converted to R-138727 by 23% in the liver (Figure 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 (Figure 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. Each concentration - R-138727 formation curve was well fitted to equation 6 (Figure 6). Dog intestinal and liver microsomes and human intestinal microsomes exhibited apparent $K_m$ values of 42.7, 18.5 and 80.4 $\mu$M, respectively. The corresponding $CL_{int}$ values in these microsomes were 1.2, 20.6 and 1.1 $\mu$L/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 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. Also, AUC of R-95913 doubled and $C_{\text{max}}$ increased by 71% to 93%, while $t_{\text{max}}$ or $t_{1/2}$ of R-95913 did not change (Farid et al., 2007a). Considering that the AUC and $C_{\text{max}}$ 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. reported a clinical observation in patients with chronic liver disease, where 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 healthy controls (Small et al., 2009). Based on this information, we considered the intestine as an important contributor to R-138727 formation in vivo. Generally, it is difficult to perform quantitative kinetic analyses of a prodrug and its metabolites in vivo, which require complex models to describe respective concentration profiles (Tsukamoto et al., 2001). Therefore, we evaluated each metabolite’s availability using a simple calculation method without any differential equations. The results in the present study
demonstrated quantitatively important contribution rates of the intestine to R-138727 formation in dogs, where 13% of dosed prasugrel was converted to R-138727 in the intestine. Since availability of R-138727 in the liver was 75%, about 10% (13% * 75%) of dosed 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 likely attributed to intestinal bioactivation.

In this study, dosed 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 non-clinical observations. In human plasma, prasugrel was not detected unmodified due to rapid hydrolysis by carboxylesterases (Farid et al., 2007b; Williams et al., 2008). Also, 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 (Figure 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. Since the existence of 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 the 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 comparable CL_{int} values 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→727}/F_{g pras→727} = 1.8). Such discrepancy between in vitro and in vivo might be explained by the difference of physiological conditions in intestine and liver (i.e. more absolute exposure to the substrate in the intestine during absorption) or possibly 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 (Figure 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 similar fraction of R-138727 formed in the intestine in dogs could be expected in humans as well.

Clopidogrel seems not to be converted to either thiolactone intermediate or active metabolite in the intestine (Hagihara et al., 2009; Kazui et al., 2010). This could be the reason for slower onset of inhibitory effect on platelet aggregation by clopidogrel than 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.
Acknowledgements

The authors thank Drs. Takashi Ito and Daisuke Nakai of Daiichi Sankyo Co., Ltd. and Drs. Mary Pat Knadler and Steven A. Wrighton of Eli Lilly and Company for their helpful comments and discussion on this study.
Authorship Contributions

Participated in research design: Hagihara, Kazui, Ikenaga, Nanba, Fusegawa, 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.
REFERENCES


FOOTNOTES

A portion of this work was presented at the scientific session of International Society on Thrombosis and Haemostasis, Boston, MA, USA, in 2009.
FIGURE LEGENDS

Figure 1. Bioactivation pathway of prasugrel.

Figure 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 (closed triangle), hepatic vein (open circle) and systemic vein (cross) were determined by LC-MS/MS. The data are expressed as the mean±SD.

Figure 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 (closed triangle), hepatic vein (open circle) and systemic vein (cross) were determined by LC-MS/MS. The data are expressed as the mean±SD.

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

Fₐ : fraction of intestinal absorption of prasugrel.
F_{g \text{ pras} \rightarrow 913} : \text{fraction of prasugrel hydrolysis to R-95913 in the intestine.}

F_{h \text{ 913}} : \text{availability of R-95913 in the liver.}

F_{g \text{ pras} \rightarrow 727} : \text{fraction of prasugrel conversion to R-138727 in the intestine.}

F_{m \text{ 913} \rightarrow 727} : \text{fraction of R-95913 conversion to R-138727 in the liver.}

F_{h \text{ 727}} : \text{availability of R-138727 in the liver.}

**Figure 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.

**Figure 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).
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

<table>
<thead>
<tr>
<th></th>
<th>$F_a$</th>
<th>$F_{g\text{ pras},\ 913}$</th>
<th>$F_{h913}$</th>
<th>$F_{g\text{ pras},\ 913}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.80</td>
<td>0.08</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.23</td>
<td>0.06</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

$F_a$ : fraction of intestinal absorption of prasugrel.

$F_{g\text{ pras},\ 913}$ : fraction of prasugrel hydrolysis to R-95913 in the intestine.

$F_{h913}$ : availability of R-95913 in the liver.
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

<table>
<thead>
<tr>
<th></th>
<th>$F_a$</th>
<th>$F_{g \text{ pras}\to 727}$</th>
<th>$F_{g \text{ pras}\to 727}$</th>
<th>$F_{m 913\to 727}$</th>
<th>$F_{h 727}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.13</td>
<td>0.13</td>
<td>0.23</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.13</td>
<td>0.13</td>
<td>0.06</td>
<td>0.21</td>
<td></td>
</tr>
</tbody>
</table>

$F_a$ : fraction of intestinal absorption of prasugrel.

$F_{g \text{ pras}\to 727}$ : fraction of prasugrel conversion to R-138727 in the intestine.

$F_{m 913\to 727}$ : fraction of R-95913 conversion to R-138727 in the liver.

$F_{h 727}$ : availability of R-138727 in the liver.
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± SD.

<table>
<thead>
<tr>
<th></th>
<th>Incubation time</th>
<th>$K_m$ $\mu$M</th>
<th>$V_{max}$ pmol/min/mg</th>
<th>$CL_{int}$ $\mu$L/min/mg</th>
<th>$CL_{int}$ ratio (liver/intestine)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal microsome</td>
<td>15</td>
<td>42.7 ± 1.0</td>
<td>49.8 ± 2.0</td>
<td>1.2 ± 0.0</td>
<td>17.2</td>
</tr>
<tr>
<td>Liver microsome</td>
<td>15</td>
<td>18.5 ± 0.6</td>
<td>379.9 ± 20.7</td>
<td>20.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal microsome</td>
<td>15</td>
<td>80.4 ± 11.5</td>
<td>89.2 ± 1.3</td>
<td>1.1 ± 0.1</td>
<td>8.7</td>
</tr>
<tr>
<td>Liver microsome*</td>
<td>15</td>
<td>26</td>
<td>247</td>
<td>9.6</td>
<td></td>
</tr>
</tbody>
</table>

* Rehmel et al. (2006)
Figure 1

Prasugrel → esterase → R-95913 (Thiolactone intermediate) → CYP → R-138727 (Active metabolite)
Figure 2

A graph showing plasma concentration (ng/mL) over time (min) for different conditions, with error bars indicating variability.
Figure 4

Intestine

Portal vein

Liver

Hepatic vein

Prasugrel

R-95913

R-95913

R-138727

R-138727

F_a * F_g pras→913

0.80

F_h 913→727

0.08

F_m 913→727

0.23

F_h 727

0.75

R-138727 formation in the intestine

R-138727 formation in the liver

Prasugrel > 0.93

R-95913
Figure 5

The graph shows the concentration of R-95913 (μM) over time (min) for human and dog samples. The concentration decreases with increasing incubation time. The error bars indicate the variability in the measurements.
Figure 6

(a) Graph showing R-138727 formation vs. R-95913 (µM) with different markers.

(b) Graph showing R-138727 formation vs. R-95913 (µM) with different markers.

(c) Graph showing R-138727 formation vs. R-95913 (µM) with different markers.