2.6.2.3 Secondary pharmacodynamics

The secondary pharmacodynamic studies with prasugrel are summarized in Section 2.6.2.4 and Section 2.6.3.

At high concentrations, prasugrel (0.26 mM) produced non-specific effects such as inhibition contractility of rabbit ileum *ex vivo*, and decreased amplitude and increased frequency of contractions of isolated rat uterus. Prasugrel HCl salt (10^{-5} g/ml) significantly inhibited acetylcholine-, histamine- and serotonin-induced contractions in isolated Hartley guinea pig ileum. These activities were elicited only at the high doses, and were considered to be non-specific.

Selective binding of prasugrel to $P2Y_{12}$ receptors

In CHO K-1 cells expressing human $P2Y_1$ and $P2Y_{12}$ receptors, R-138727, R-99224 and R-100364 inhibited the [^{3}H]-2-Methylthio-ADP binding to human $P2Y_{12}$ receptors in a concentration-dependent manner with IC_{50} values of 2.5, 1.3 and 45 μM, respectively (Figure 11). The [^{3}H]-2-MeS-ADP binding to human $P2Y_1$ receptors was potently inhibited by MRS2179, a selective $P2Y_1$ receptor antagonist, but not by R-138727 (100 μM), R-99224 (110 μM) or R-100364 (110 μM). These results indicated that R-138727 and R-99224 have potent and selective $P2Y_{12}$ antagonistic activity.

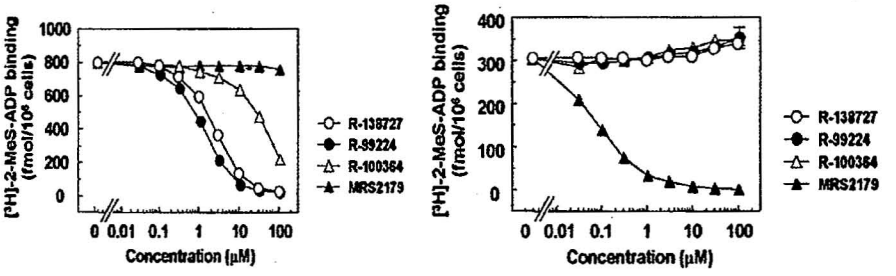

Figure 10. *In vitro* binding of R-138727, R-99224, R-100364 and MRS2179 on [^{3}H]-2-MeS-ADP binding to $P2Y_{12}$ (right panel) and $P2Y_1$ receptors (left panel) expressed on CHO K-1 cells.

Effects of metabolite R-99224 on platelet shape change in human platelets:

The active metabolite mixture, R-99224 (0.03 - 3 μM), inhibited ADP (10 μM)-induced platelet aggregation with an IC_{50} of 0.65 μM (Figure 8). Adenosine 3'-phosphate phosphosulfate (A3P5PS, 3 - 300 μM), a $P2Y_1$ receptor antagonist, also inhibited ADP-induced platelet aggregation (IC_{50} = 34 μM). Platelet shape change was inhibited by A3P5PS (IC_{50} = 260 μM), but not by R-99224 (IC_{50} = 3 μM). The active metabolite enantiomer, R-138727, potently inhibited [^{3}H]-2Me-S-ADP (stable ADP analog) binding to the human $P2Y_{12}$, but not the $P2Y_1$ receptor, expressed in CHO K-1 cells. These results support that R-99224 selectively blocks binding of ADP to the $P2Y_{12}$ receptor.

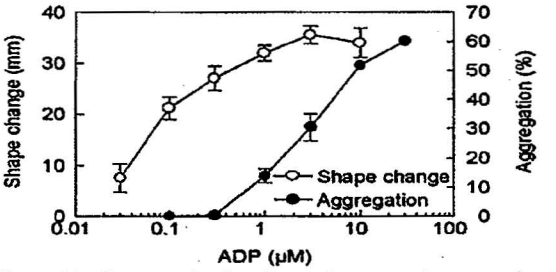

Figure 11. *In vitro* platelet shape change and aggregation induced by ADP in human platelets treated with R-99224 (n=5-6).

Cyclic AMP:

In vitro human platelets, ADP (10 μM)-induced attenuation of PGE_1 (10 μM) stimulated intracellular cAMP levels (via adenylate cyclase) were neutralized by pretreatment with R-99224 (0.22 - 2.2 μM) in a concentration-dependent manner indicating that R-99224, an active metabolite of prasugrel, selectively blocks $P2Y_{12}$ receptors.

Ca^{2+} mobilization:

In vitro human platelets, R-99224 (0.065 - 2.2 μM) did not show any significant effects on increase in intracellular Ca^{2+} concentration induced ADP indicating that R-99224 does interact with Ca^{2+} mobilization mediated by $P2X_1$ and $P2Y_1$ receptors.

2.6.2.4 Safety pharmacology

Neurological effects: Prasugrel at a dose of 300 mg/kg (*p.o.*) produced hypersensitivity to touch stimuli in rats, but caused no changes in other behavioral patterns in mice or Wistar rats. Prasugrel at a dose of 100 mg/kg decreased paradoxical sleep on spontaneous electroencephalogram in rats. Prasugrel (10, 30 and 100 mg/kg, *p.o.*) had no effects on normal body temperature in rats, and no effects on spontaneous locomotor activities, thiopental-induced sleeping times, acetic acid-induced writhing, and electroshock- and pentylenetetrazol-induced seizures in mice. The dose levels (10 to 100 mg/kg, *p.o.*) were 10- to 100-times higher than the doses demonstrated to exert pharmacological activities in rats (1 - 10 mg/kg, *p.o.*). Thus, prasugrel is considered to have no significant stimulative or depressive effects on the central nervous system at doses up to 100 mg/kg (600 mg/m^2), about 100-fold higher that the human therapeutic dose of 10 mg/day (6 mg/m^2).

Cardiovascular effects: Prasugrel (30 and 100 mg/kg, *i.d.*) had no effects on heart rate, blood pressure, carotid blood flow, or pressure response to acetylcholine, norepinephrine or bilateral carotid occlusion in anesthetized male beagle dogs. Prasugrel at the highest concentration of 10^{-4} g/ml had no effects on the contractile force and beat rate of the isolated right atrium of guinea pig.

Pulmonary effects: Prasugrel (30 and 100 mg/kg, *i.d.*) had no effects on respiration rate, in anesthetized male beagle dogs.

Renal effects: Prasugrel at oral doses of 10, 30 and 100 mg/kg had no effects on urinary volume, excretion of electrolytes or osmotic pressure in male SD rats.

Gastrointestinal effects: Prasugrel at oral doses of 10, 30 and 100 mg/kg did not inhibit intestinal propulsion or gastric emptying in mice. Prasugrel at a dose of 100 mg/kg decreased gastric acid content and gastric volume in male SD rats.

Effect of R-95913 on potassium currents (*h*ERG): R-95913 (de-esterified metabolite of prasugrel) at concentrations of 0.15, 1.5 and 15 μmol/L had no significant effect on the tail peak potassium currents of *h*ERG transfected CHO-K1 cells. R-138727 (active metabolite) and R-106583 (primary human metabolite) at 0.3, 3 and 30 μmol/L had no effects on tail peak currents of *h*ERG. E-4031, a selective blocker for the rapidly activating component of delayed rectifier potassium current (*IKr*), at 0.3 μmol/L significantly decreased tail peak currents. These results indicate that R-95913 up to 15 μmol/L had no effects on tail peak currents of *h*ERG.

2.6.2.5 Pharmacodynamic drug interactions

The additive or synergistic platelet inhibitory effects that result from co-administration of prasugrel and aspirin are demonstrated in several studies of platelet aggregation (*ex vivo*) in rats and dogs, thrombus formation *(in vivo*) in rats, and bleeding time in rats. Consistent with these findings, *in vitro* studies with blood from humans demonstrated that a combination of R-138727 and aspirin has additive effects on collagen-induced platelet aggregation.

Combined effects of prasugrel and aspirin on platelet aggregation in rats:
ADP (3 μM)-induced *ex-vivo* platelet aggregation was inhibited rats-treated orally with prasugrel, but not aspirin (Figure 12, panel A). The inhibitory effect of prasugrel on ADP-induced platelet aggregation was not potentiated by combination with aspirin (Figure 10, panel A). Prasugrel (0.6 and 1 mg/kg, *p.o.*) or aspirin (10 mg/kg, *p.o.*) alone did not show inhibition of collagen (10 μg/ml) induced platelet aggregation (Figure 12, panel B). In contrast, prasugrel in combination with aspirin showed a significant synergistic inhibition of collagen-induced platelet aggregation (Figure 10, panel B). These results suggest that prasugrel in combination with aspirin can produce a synergistic inhibitory effect on collagen-induced platelet aggregation.

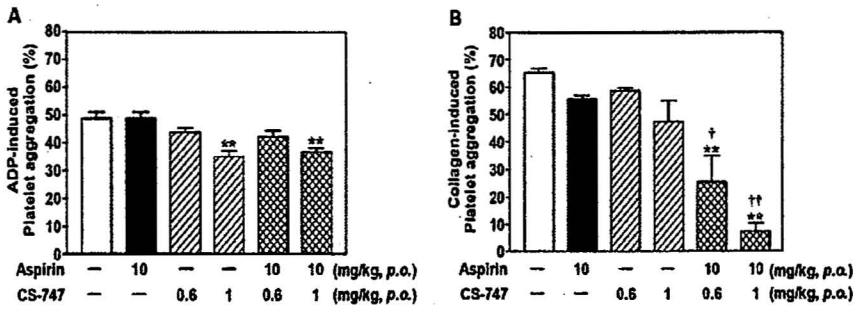

Figure 12. *Ex vivo* effects of CS-747 (prasugrel) and aspirin on ADP (panel A) or collagen (panel B) induced platelet aggregation in rats (n=5).

Ex vivo platelet aggregation in dogs treated with prasugrel and aspirin:

In *ex-vivo* platelets from the beagle dog, combined treatment with prasugrel and aspirin produced a more potent antiplatelet effect compared with prasugrel or aspirin alone (Figure 13).

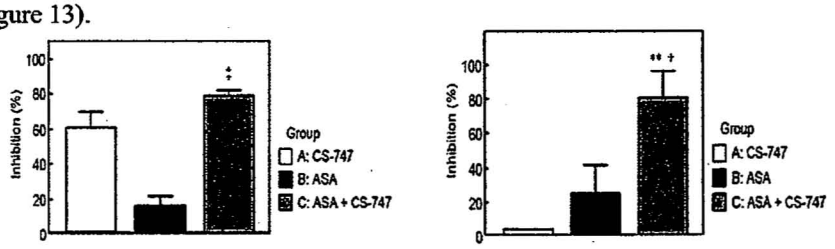

Figure 13. *Ex-vivo* effects of CS-747 (prasugrel, 0.1 mg/kg/day) and aspirin (ASA, 1 mg/kg/day) on ADP (left panel) or collagen (right panel) induced platelet aggregation in dogs (n=6).

Prasugrel and aspirin on thrombus formation and bleeding time in rats:

Prasugrel or aspirin alone, dose-dependently inhibited thrombus formation in the rat arterio-venous shunt thrombosis model (Figure 14). The combination of prasugrel with aspirin resulted in a more pronounced antithrombotic effect compared to that of each agent alone. Both prasugrel and aspirin alone also prolonged tail transection bleeding time in a dose-dependent manner in rats. In contrast, prasugrel combined with aspirin did not show any significant prolongation of the bleeding time. These results suggest that the combination of prasugrel with aspirin resulted in more potent antithrombotic effects compared to each agent alone in the rat arterio-venous shunt thrombosis model.

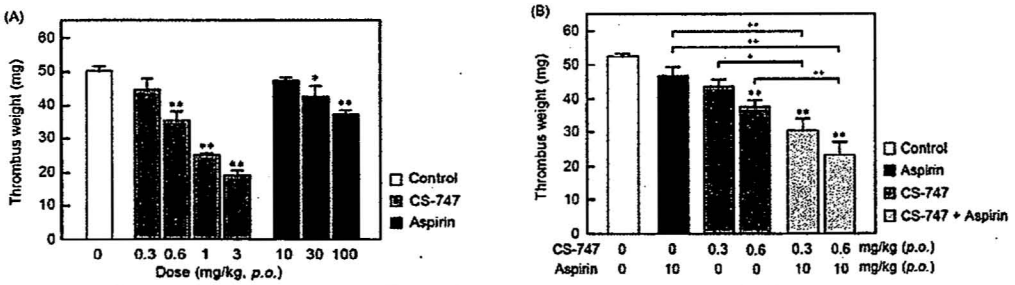

Figure 14. Antithrombotic effects of CS-747 (prasugrel) and aspirin alone (panel A), and in combination (panel B) on arterio-venous shunt rat model (n=6).

Prasugrel or clopidogrel combined with aspirin on rat platelet aggregation:

Prasugrel inhibited platelet aggregation by about 22% (Figure 15, left panel). Aspirin showed a minimal effect on collagen-induced platelet aggregation (6.8%), but the combination with prasugrel produced marked inhibition (61%) indicating synergistic effects. Clopidogrel produced a significant but weak inhibition (15%), and in combination with aspirin showed moderate effect (22%), but this effect was not synergistic (Figure 15, right panel). These results demonstrate combination of prasugrel with aspirin is more efficacious antithrombotic agent than that of clopidogrel and aspirin.

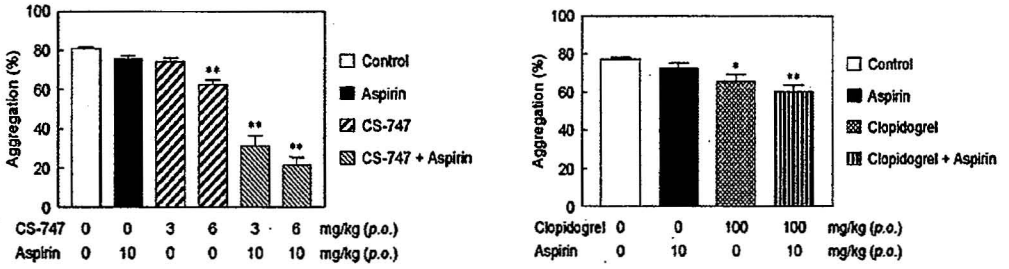

Figure 15. *Ex vivo* effects of CS-747 (prasugrel, left panel) and clopidogrel (right panel) in combination with aspirin on collagen-induced platelet aggregation in rat platelets (n=6).

In vitro human platelet aggregation of R-138727 and aspirin:

R-138727 is a mixture of four stereoisomers of the active metabolite of prasugrel. R-138727 moderately inhibited platelet aggregation induced by arachidonic acid (AA) (Figure 16, left panel). Aspirin completely inhibited AA-induced platelet aggregation. In the combination study of R-138727 and aspirin, no further effects were seen compared to aspirin alone because aspirin alone completely inhibited AA-induced aggregation. Aspirin alone produced weak effect on ADP-induced aggregation (Figure 16, right panel). The combination of R-138727 with aspirin significantly inhibited ADP-induced platelet aggregation compared to aspirin alone. Both R-138727 and aspirin alone showed moderate inhibition on collagen-induced platelet aggregation. The combination of R-138727 and aspirin showed marked combined effects on collagen-induced aggregation than aspirin alone. These *in vitro* studies show that combination of R-138727 and aspirin exhibit potent inhibition of platelet aggregation.

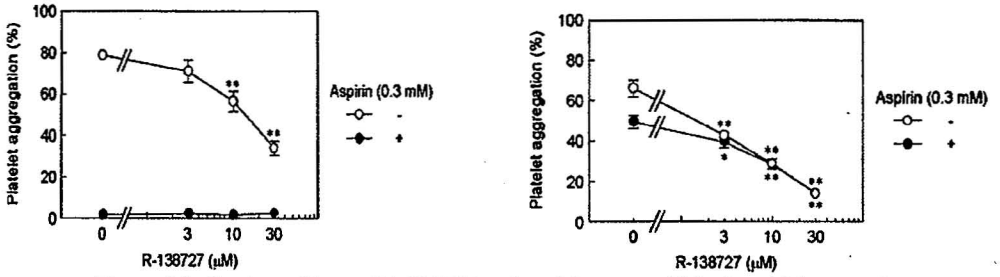

Figure 16. *In vitro* effects of R-138727 and aspirin on arachidonic acid (left panel) or ADP (right panel) induced human platelet aggregation (n=6).

Proton pump inhibitor on platelet aggregation in dogs treated with prasugrel:

In a cross over design to compare the antiplatelet effect of the free base form of prasugrel and its HCl salt, beagle dogs were pretreated with lansoprazole, a proton pump inhibitor. Oral administration of prasugrel free base or its HCl salt form caused similar inhibition of the platelet aggregation induced by ADP (Table 1). The plasma concentrations of prasugrel metabolites at 1 hour post-dosing were reduced in both formulation forms indicating that increasing the gastric pH with lansoprazole reduced the rate of generation of prasugrel's active metabolite. Both formulations of the free base and HCl salt of prasugrel have similar antiplatelet potency during lansoprazole treatment but dose adjustment of prasugrel may be warranted during treatment with proton pump inhibitors.

Table 1. Inhibitory effects of prasugrel on platelet aggregation in lansoprazole-treated dogs

		Inhibition of Platelet Aggregation (% relative to predose)[a]			
		Time after Dosing (hr)			
Agonist	Test article[b]	Predose	0.5	2	4
ADP (5 µM)	Prasugrel base	0 ± 0	19.8 ± 7.2	54.6 ± 2.5	58.9 ± 2.2
	Prasugrel HCl	0 ± 0	16.3 ± 8.3	47.8 ± 10.3	51.7 ± 11.3
ADP (10 µM)	Prasugrel base	0 ± 0	13.6 ± 7.4	54.6 ± 5.5	58.1 ± 4.5
	Prasugrel HCl	0 ± 0	13.8 ± 8.6	51.8 ± 5.1	55.1 ± 4.3
ADP (20 µM)	Prasugrel base	0 ± 0	7.0 ± 5.2	45.4 ± 10.9	53.4 ± 8.9
	Prasugrel HCl	0 ± 0	15.6 ± 7.7	48.4 ± 6.4	53.9 ± 4.7

[a] n=6, [b] orally administered at 2.5 mg/dog

Effects of gastric pH on bioavailability of prasugrel in dogs:

Prasugrel metabolites were comparable for the two formulations (free base and HCl salt forms, 5 mg tablet) in dogs treated with tetragastrin (6 µg/kg, intramuscularly). (Table 2). However, in dogs treated with the H_2 blocker ranitidine (2.5 mg/kg, intramuscularly), the AUC_{0-2} for each metabolite of prasugrel decreased to about 24% (prasugrel base) and 53% (prasugrel HCl) of the comparable value in the tetragastrin-treated dogs. The AUC_{0-8} values were 32% and 74% in the prasugrel base and HCl groups, respectively. The oral bioavailability of prasugrel base decreased by more than 65% when the dogs were pretreated with the H_2 blocker ranitidine intramuscularly, which increased gastric pH to ≥6. Ranitidine-mediated increase of stomach pH to ≥6 decreased the amount of prasugrel absorbed, and that this effect was more pronounced with the prasugrel free base formulation. Thus, prasugrel HCl was selected for further development and additional studies were conducted with the HCl salt.

Table 2. Levels of plasma metabolites of single oral dose of prasugrel after intramuscular administration of rantidine in dogs

Test article	Prasugrel Base			Prasugrel HCl		
Analyte	R-95913	R-100932	R-106583	R-95913	R-100932	R-106583
Mean PK parameters						
Neutral gastric pH[a]						
C_{max} (ng/mL)	16.666	6.142	9.262	34.354	13.071	20.948
AUC_{0-last} (ng•hr/mL)	29.86	17.60	20.50	62.66	38.88	45.50
T_{max} (hr)	0.5	0.5	0.5	1.1	1.1	1.1
N	5	5	5	5	5	5
Acidic gastric pH[b]						
C_{max} (ng/mL)	85.653	26.452	49.710	73.075	24.580	50.268
AUC_{0-last} (ng•hr/mL)	108.26	51.49	85.15	94.43	48.04	80.69
T_{max} (hr)	0.6	0.7	0.7	0.7	0.7	0.7
N	6	6	6	6	6	6

[a] Ranitidine, [b] Tetragastrin

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Organ Systems Evaluated	Species, Strain	Route	Test article Doses[a]	Sex #/Group	Noteworthy Findings	GLP
Autonomic and Somatic Nervous System; Cardiac, Smooth, and Diaphragmatic Muscle	Guinea pig, Hartley (atria, ileum); rabbit, Japanese white (ileum) Rat, Sprague Dawley (uterus, diaphragm)	In vitro	Prasugrel HCl: 1×10^{-6} – 1×10^{-4} g/mL	Male guinea pig and rabbit, female rat (uterus), male rat (diaphragm) 5 - 9 tissues	No effects on atria. 1×10^{-5} g/mL: inhibition of acetylcholine-, histamine-, and serotonin-induced contractions of guinea pig ileum 1×10^{-4} g/mL: ↓ spontaneous contraction of rabbit ileum, ↓ amplitude and ↑ frequency of spontaneous uterine contractions, ↑ twitch response in phrenic nerve-diaphragm preparation	No
Cardiac Ion Channel	hERG transfected CHO-K1 cells	In vitro	R-95913 0, 0.15, 1.5, and 15 µM	5 cells	No effect NOEL = 15 µM	Yes
Cardiac Ion Channel	hERG transfected CHO-K1 cells	In vitro	R-106583 0, 0.3, 3, and 30 µM	5 cells	No effect NOEL = 30 µM	Yes
Cardiac Ion Channel	hERG transfected CHO-K1 cells	In vitro	R-138727 0, 0.3, 3, and 30 µM	5 cells	No effect NOEL = 30 µM	Yes
Central Nervous System and Behavioral Effects	Rat, Wistar	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 5	No effects on wakefulness time or slow wave sleep time. 100 mg/kg: ↓ paradoxical sleep time	No
Nervous System and Behavioral Effects	Mouse, ddY Rat, Sprague-Dawley	Oral	Prasugrel 0, 10, 30, 100, or 300 mg/kg	Male 5	No effects on gross behavior in mice. 300 mg/kg: ↑ sensitivity to touch in rats	No
Nervous System and Behavioral Effects	Mouse, ddY Rat, Sprague-Dawley	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 10 - 12	No effects on spontaneous locomotor activity, thiopental-induced sleeping time, analgesic activity, convulsant activity, body temperature, or muscle traction. NOEL = 100 mg/kg	No
Local Anesthesia	Guinea pig, Hartley	Ocular	Prasugrel 1% or 5% solution	Male 5	No effect on corneal reflex. NOEL = 5% solution	No
Cardiovascular and Respiratory Effects	Dog, beagle (anesthetized)	Intraduodenal	Prasugrel 30 or 100 mg/kg	Male 5	No effect on respiratory rate, heart rate, blood pressure, carotid blood flow or ECG. NOEL = 100 mg/kg	No
Renal Effects	Rat, Sprague-Dawley	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 9 - 12	No effect on urinary volume, excretion of electrolytes, or osmotic pressure. NOEL = 100 mg/kg	No
Gastrointestinal Effects	Mouse, ddY Rat, Sprague Dawley	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 11	No effect on gastrointestinal motility or gastric emptying. 100 mg/kg: ↓ gastric acid content and gastric volume in rats NOEL = 30 mg/kg	No
Gastrointestinal Effects	Mouse, ddY Rat, Sprague Dawley	Oral	Prasugrel 0, 30, 100, or 300 mg/kg Single dose and daily for 3 days	Male 8 – 12	300 mg/kg : ↓ gastric emptying in mice given 3 daily doses NOEL = 100 mg/kg	No
Effect on Blood Glucose	Rat, Sprague-Dawley	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 10	No significant effect on blood glucose. NOEL = 100 mg/kg	No
Hemolytic Effects	Rabbit, Japanese White	In vitro	Prasugrel HCl 1×10^{-6} – 1×10^{-4} g/mL	5 samples	No hemolytic effect NOEL = 10^{-4} g/mL	No
Effect on Coagulation	Rat, Sprague-Dawley	Oral	Prasugrel 0, 10, 30, or 100 mg/kg	Male 5	No effect on prothrombin time or activated partial thromboplastin time. NOEL = 100 mg/kg	No

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Prasugrel is a prodrug that is metabolized by de-esterification in the gastrointestinal tract to form a thiolactone, R-95913, an inactive metabolite (Figure 17). Prasugrel was not detected in plasma because of rapid de-esterification to R-95913. The thiolactone ring of R-95913 is opened to form sulfhydryl compounds R-104434 (inactive) and R-138727 (active metabolite). The sulfhydryl compounds are further metabolized by S-methylation, producing R-106583 and R-100932, and by conjugation with cysteine, forming R-119251 and R-118443 (all inactive metabolites). Of the sulfhydryl compounds, only the enantiomers of R-138727 are pharmacologically active. The other metabolites, including R-106583 (major metabolite in human), are inactive. Thus, the pharmacokinetic parameters were determined for inactive metabolites found in plasma as indicator of exposure to prasugrel (R-106583 major metabolite, R-138727 active metabolite). Prasugrel metabolites identified in human plasma, urine, and feces were also identified in mice, rats, and dogs (Section 2.6.5). The AUC of the metabolites decreased after multiple dosing of prasugrel compared with the values obtained after the first dose in mice at ≥ 100 mg/kg, rats at 100 and 300 mg/kg, and in dogs at 20 mg/kg (Section 2.6.4.10.: Tables 6 and 7).

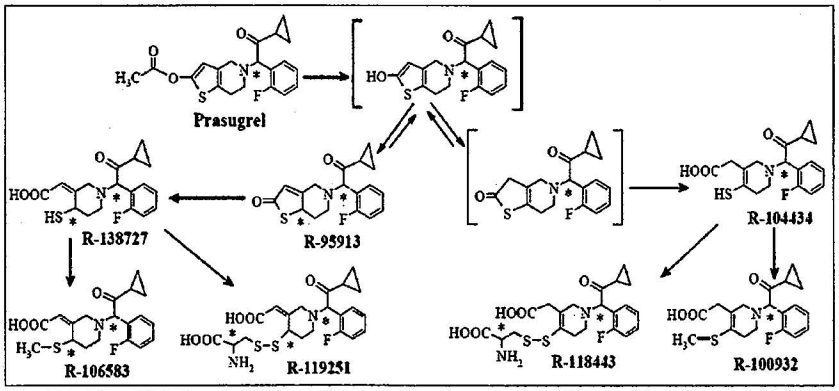

Figure 17. Simplified metabolic pathway of prasugrel. * indicate chiral centers, R-138727 is active compound, Compounds in brackets are proposed.

2.6.4.2 Methods of analysis

Plasma samples collected in the nonclinical studies were analyzed for prasugrel metabolites using validated liquid chromatography with tandem mass spectrometric detection (LC/MS/MS) methods. Radioactivity in plasma, tissues and excreta was measured following the administration of [^{14}C]-prasugrel by liquid scintillation counting. Metabolites in bile and urine were detected and identified using LC/MS/MS.

2.6.4.3 Absorption

After a single oral administration of ^{14}C-prasugrel (2 and 5 mg/kg) in the dog and rat, the radioactivity concentration in the plasma reached maximum at 0.7 h, and then declined with a half-life of 2.8 days and 23 h after 24 h, respectively. In rats administered single oral doses of [^{14}C]-prasugrel, oral bioavailability was approximately 77%. The absolute bioavailability of R-138727 (active metabolite) after single oral administration of prasugrel, (calculated by dividing the $AUC_{0\text{-}6h}$ of R-138727 after oral administration of prasugrel by that after intravenous administration of R-138727), was 25% in rats, indicating substantial intestinal absorption of prasugrel, and efficient biotransformation to active metabolite. The plasma exposure levels of metabolites after oral administration of prasugrel HCl salt was slightly higher (about 20-30%) compared with the prasugrel free base formulation (Table 3).

Table 3. Pharmacokinetic parameters of inactive metabolites and R-138727 after oral administration of 5 mg of CS-747 (prasugrel, free form) and CS-747S (HCl salt form) to rats

$AUC_{0\text{-}8h}$

Metabolite	CS-747			CS-747S		
R-119251	4650	±	960	5710	±	1860
R-118443	176	±	29	313	±	122
R-106583	1470	±	210	1390	±	530
R-100932	2330	±	310	2330	±	580
R-95913	27.6	±	21.9	25.0	±	31.9
R-138727	2630	±	530	3390	±	1110

($AUC_{0\text{-}8h}$: ng.h/ml)

2.6.4.4 Distribution

A single 5 mg/kg oral dose of ^{14}C-prasugrel to rats was distributed rapidly within 1 hr mainly to the liver, with some levels in the kidney, lungs, blood, adrenal glands and heart. The radioactivity in other tissues and organs were lower than that in the blood. The radioactivity in the central nervous system or testis was negligible. At 24 hours, most of the radioactivity was eliminated from the body, with some levels in the liver, renal cortex, and intestinal contents. The highest radioactivity was observed in the gastrointestinal tract indicating biliary excretion. The $t_{1/2}$ for plasma radioactivity was 1.6 days after the single dose and 2.3 days after the 21st dose.

Binding to plasma proteins:

Protein binding of prasugrel metabolites R-95913, R-100932, and R-106583 was similarly high (> 80%) in rat, dog and human. Binding of R-119251 was similar in rat and human plasma (71 to 77%), but much lower in dog plasma (26 to 36%). Binding of the active metabolite, R-138727, was 98% in human serum albumin. The active metabolite, R-138727 formed a covalent disulfide bond with the platelets; a high level in platelets was maintained for relatively long periods compared to the fast elimination of this metabolite from the plasma. Inactive metabolites (R-100932 and R-106583) were not bound to platelets, consistent with the lack of activity of these metabolites.