

Prasugrel on embryo-fetal development in the rabbit:

Key findings: Prasugrel does not have any teratogenic effects based on the absence of changes in the frequency of external, visceral or skeletal anomalies (30 times higher than human exposure at daily dose of 10 mg prasugrel) (Section 2.6.7. Tabulated Summary: Table 47).

Study no.:	APR-148-098	
Conducting laboratory:	_____	
Date of study initiation:	Oct 12, 2000	Report date: Aug 19, 2002
GLP compliance:	Yes,	QA reports: yes (√) no ()
Drug:	Prasugrel free base	
Doses:	30, 100 and 300 mg/kg/day, Vehicle: Tragacanth, 0.5%	
Species/strain:	Kbl:NZW rabbits	
Number/sex/group:	24/sex/dose	
Route:	Oral gavage	

b(4)

Study design: Prasugrel was administered by gavage to pregnant rabbits from Day 6 to 18 of pregnancy.

Parameters and endpoints evaluated: Effects on dams and development of embryos and fetuses were examined.

Results:

Mortality (dams): One dam died in the 100 mg/kg group.

Clinical signs (dams): Yellow urine was observed once to 5 times in 6 animals of the 300 mg/kg group for Days 14 to 19 of pregnancy. In the 30 mg/kg group no abnormal changes in clinical signs were observed.

Body weight (dams): A slight decrease in body weight gain was observed in the 300 mg/kg group.

Food consumption (dams): A significant decrease in food intake was observed in the 300 mg/kg group, and a slight decrease was observed in the 100 mg/kg group.

Toxicokinetics: R-106583: On day 6 of gestation at 30, 100 and 300 mg/kg, mean AUC_{0-24h} 2.4, 13.6 and 55 µg·h/mL. On day 18 of gestation, mean AUC_{0-24h} 2.5, 7.7 and 12.3 µg·h/mL.

Terminal and necroscopic evaluations: At autopsy of dams on Day 28 of pregnancy, no lethal effects on embryos or fetuses were observed but a decrease in live fetus body weights was observed in the 300 mg/kg group. There were no differences between the control and treated groups in the number of corpora lutea, number of embryos and fetuses, number of live fetuses or sex ratio. There were low values in the number of implantations in the 100 mg/kg group and of live fetal weights in the 300 mg/kg group, but there were no significant differences in any group. In the macroscopic observation of thoracic and intraperitoneal organs of dams, there were no abnormal changes in any of the animals.

Offspring: There was 1 fetus with absent eye bulge, misshapen nose and naris atresia in the 100 mg/kg group and 1 fetus with paw hyperflexion in the 300 mg/kg group. There were no external anomalies in the control or 30 mg/kg group. The

incidence rates of fetuses with visceral anomalies were 17% in the control group and 19% in the 300 mg/kg group. The incidence rates of fetuses with skeletal anomalies were 1.8 % in the control group and 3.4% in the 300 mg/kg group. The incidence rates of fetuses with skeletal variations were 76% in the control group and 85% in the 300 mg/kg group. There were no significant changes in the incidence rates of each variation or fetuses with variations in the 300 mg/kg group. The mean number of ossified sacrocaudal vertebrae was 19 in both the control and 300 mg/kg groups, and no significant differences between both groups.

Summary: Since there were no changes in the frequency of external, visceral or skeletal anomalies, prasugrel is considered not to be teratogenic at a dosage affording 30 times higher than the human major metabolite at daily dose of 10 mg prasugrel. The non-toxic dose in terms of the development of the next generation was considered to be 100 mg/kg. The non-toxic dose in terms of general toxicity in dams was considered to be 30 mg/kg (Section 2.6.7. Tabulated Summary: Table 47).

Prenatal and postnatal development study in CD rat:

Key findings: There was a decrease in offspring body weight on Day 1 and growth between Day 1 of age and weaning compared with control. This effect occurred only at 300 mg/kg, a dose that produced decreased maternal body weight and food consumption and which affords about 120-fold higher than the major metabolite levels at daily dose of 10 mg prasugrel. Pre- and post-natal development of the offspring was considered unaffected by maternal treatment with prasugrel at doses up to 110 mg/kg (100-fold higher than clinical exposure) (Section 2.6.7. Tabulated Summary: Table 48).

Study no.:	APS-151-155		
Conducting laboratory:	_____		
Date of study initiation:	1/11/2005	Report date:	8/21/2005
GLP compliance:	Yes	QA reports:	yes (✓) no ()
Drug:	Prasugrel HCl		
Doses:	33, 110 or 330 mg/kg/day (equivalent to 30, 100 and 300 mg/kg prasugrel free base).		
Species/strain:	~:CD (SD) IGS BR rat		
Number/sex/group:	F ₀ : 22/group (female rats); F ₁ : 20 M, 20 F/dose group		
Route:	Oral gavage, 0.5% w/v Gum Tragacanth		
Satellite groups for toxicokinetics:	n=9/group female rats.		

Study design: Prasugrel was administered from Day 6 after mating to Day 20 of lactation. Animals were allowed to litter and rear their offspring to weaning and were killed on Day 21 of lactation (Figure 19). F₁ females were killed on Day 14 after mating, and F₁ males were killed soon afterwards.

b(4)

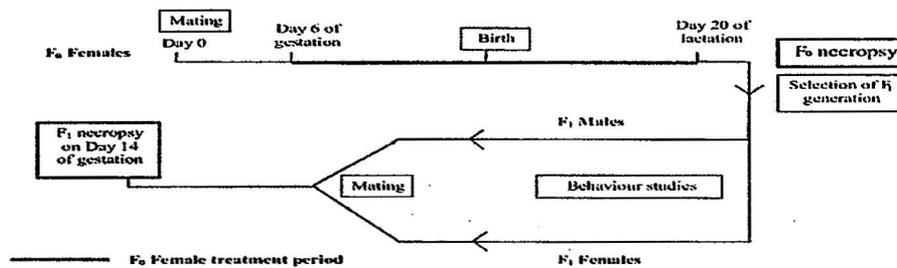


Figure 19. Study design of pre- and post-natal in mice

Parameters and endpoints evaluated: Reflex development in the F₁ offspring was assessed prior to weaning. F₁ offspring selected at weaning were subjected to functional and behavioral testing after weaning, and were reared to sexual maturity for subsequent assessment of reproductive capacity. F₁ females uterine contents were examined at necropsy.

Results (F₀ maternal responses):

Mortality: There were no unscheduled deaths during the course of the study.

Clinical Signs: Salivation and/or staining resulting from salivation was seen after dosing in most females treated at 110 or 330 mg/kg and salivation was seen in seven females treated at 33 mg/kg. Abnormal yellow staining of the cage tray paper was observed in females treated at 110 and 330 mg/kg. No cage tray paper staining was observed for females receiving 33 mg/kg.

Bodyweight: From commencement of treatment at 330 mg/kg, group mean bodyweight gain was lower than that in the controls until Day 14 of lactation. At 110 mg/kg, there was no effect of treatment on bodyweight gain during gestation. During lactation, mean cumulative weight gain during Days 1 - 14 was significantly lower than in controls. Bodyweights for females treated at 33 mg/kg/day showed no difference to control values.

Food consumption: At 330 mg/kg, food consumption during Days 6 - 9 of gestation were lower than controls by 18%. Food consumption was lower than control throughout lactation. At 110 mg/kg, group mean food consumption was considered unaffected during gestation and lactation. Food intake during Days 14 - 17 of lactation was significantly lower than in controls. Food consumption was unaffected by treatment at 33 mg/kg.

Gestation length, parturition and gestation index: At 330 mg/kg, a lower proportion of females had a gestation length of 22 days and a higher proportion had a gestation length of 22.5 or 23 days compared with the controls, however, all of the values were within the expected range of 22 to 23 days. Gestation length was unaffected by treatment at 33 or 110 mg/kg.

Macropathology: The type and incidence of macroscopic findings did not indicate any adverse effects of treatment with prasugrel.

Toxicokinetics: On Day 6 of gestation, the AUC_{0-24h} for 106583 were 19, 40 and 47 µg·h/mL, in the 33, 110 and 330 mg/kg groups, respectively. On Day 20 of lactation, the AUC_{0-24h} were 18, 43 and 53 µg·h/mL, in the 33, 110 and 330 mg/kg groups, respectively.

Results (F₁ Generation responses):

Mortality (F₁): There were no mortalities among the selected F₁ offspring.

Clinical signs: Treatment of the parental females did not affect the clinical condition of the offspring in adulthood.

Bodyweight: Group mean offspring bodyweight on Day 1 of age at 330 mg/kg was 9% lower than control for males and females. Offspring bodyweight performance was impaired to weaning, such that bodyweight gain between Days 1 - 21 of age was approximately 20% lower than control for males and females. Following removal of the parent female, bodyweight gain appeared to show some recovery, with weight gain between Days 21 - 25 of age only marginally lower than control. Offspring bodyweight increase was unaffected by maternal treatment at the 110 or 33 mg/kg dosages.

Sexual maturation: The mean age at day of attainment of vaginal opening for the females in the 330 mg/kg group was significantly delayed compared with the control, which may be related to the lower bodyweight in this group.

Mating performance and fertility: F₁ mating performance and fertility was unaffected by treatment as assessed by the pre-coital interval, the percentage of animals mating, conception rate and fertility index.

Litter data on Day 14 after mating: The numbers of corpora lutea, uterine implantations, live embryos and resorptions and pre- and post-implantation losses were considered to be unaffected by treatment of the F₀ females.

Macropathology: Findings at necropsy of the F₁ generation were unremarkable and did not indicate any adverse effects of maternal treatment.

F₁ physical development: There were no clear treatment-related signs observed for the offspring. Offspring bodyweights on Day 1 of age were low in the 330 mg/kg group with impaired growth recorded between Day 1 of age and weaning. Gestation length also appeared to be slightly longer and this may be associated with the lower Day 1 offspring weights.

F₁ behavioral evaluation: Pre-weaning development, as assessed by the mean age of attainment for surface and air righting reflexes and the percentage success rate on Day 20 of age for auditory and visual response was similar in all groups. For selected F₁ offspring, there was no evidence of an adverse effect of maternal treatment on motor activity, neuromuscular function, Morris maze performance (learning and memory), attainment of sexual maturity or reproductive performance and fertility. There was no adverse effect on the growth rate of selected F₁ males, and overall bodyweight gains of females prior to pairing and during gestation were similar to controls.

F₁ reproduction: Prasugrel had no effect on the parturition process and most females gave birth to live offspring and successfully reared their litter to weaning. There was no evidence of an adverse effect of maternal treatment with prasugrel on litter size, survival (both pre-natal and post-natal). Attainment of sexual maturity was considered not to be adversely affected; vaginal opening was delayed in the group treated at 330 mg/kg when compared with the control group, which may be related to the lower bodyweights of the offspring.

Summary: Treatment of female rats with prasugrel from Day 6 after mating to Day 20 of lactation resulted in reductions in bodyweight gain and food consumption in females treated at 330 mg/kg. Although pre- and post- natal development was affected at 330 mg/kg, in terms of lower birth weight and impaired growth prior to weaning, subsequent development of the offspring to adulthood was unaffected. A delay in vaginal opening at 330 mg/kg was attributed to the lower bodyweights. Pre- and post-natal development of the offspring was considered unaffected by maternal treatment with prasugrel at dosages up to 110 mg/kg (i.e., 100-fold higher than human metabolite exposure). The NOAEL for maternal toxicity, and the growth of the F₁ offspring was considered to be 110 mg/kg (Section 2.6.7. Tabulated Summary: Table 48).

2.6.6.7 Local tolerance

This section is not applicable because prasugrel is administered orally, and therefore, local tolerance studies were not conducted.

2.6.6.8 Special toxicology studies

Antigenicity in mice:

In female A/J mice treated with single oral or intraperitoneal administration of prasugrel or prasugrel plus Alum (0.1 and 1 mg/animal), IgE titer by rat passive cutaneous anaphylaxis reaction was not detected, but the positive control TNBS (sodium 2,4,6-trinitrobenzenesulfonate dihydrate) plus Alum intraperitoneal administration (0.3 mg/body) produced antibody response suggesting that prasugrel may not be antigenic.

Antigenicity in Guinea pigs:

In female Hartley guinea pigs, single oral or subcutaneous administration of prasugrel or prasugrel plus FCA (Freund's complete adjuvant, 0.3 and 3 mg/animal) did not cause antibody titer as determined by passive cutaneous anaphylaxis reaction or systemic anaphylaxis. The positive control, TNBS plus FCA subcutaneous administration group (3 mg/animal) caused an increase in antibody titer in blood suggesting that prasugrel is not antigenic.

Acute dermal toxicity in rabbits:

In New Zealand White rabbits, single dermal administration of prasugrel at a dose of 1000 mg/kg (n=5/group) caused no dermal irritation at site of application indicating that prasugrel may be nonirritant to the skin.

Assessment of phototoxic potential:

The active and major circulating human metabolites of prasugrel have limited absorption in the UV-Vis range. There was little presence of drug in the skin of pigmented rats after 21 days of exposure. Similarly, there was limited prasugrel-

associated radioactivity in the eyeball after 21 days of exposure. There were no indications of skin or eye effects or effects on the immune system in the standard toxicity studies, and thus prasugrel was considered to have a low phototoxic potential.

Ocular and dermal irritation:

In the hazard evaluation studies conducted in New Zealand white rabbits, prasugrel was a mild ocular irritant in that administration to the conjunctival sac of rabbits resulted in iritis and conjunctivitis. Prasugrel did not cause dermal irritation following a single application of 1000 mg/kg to the skin of rabbits.

Toxicological Qualification of Impurities:

The specifications levels of total impurities in prasugrel were considered justified from a toxicological perspective because they were present at sufficient levels in the lots of material used in the toxicology studies. Impurities found in the *in vivo* prasugrel

b(4)

Comparative toxicity of prasugrel and clopidogrel in rat:

Study no.: TR 143-040, 95-0067

Conducting laboratory: _____

Date of study initiation: Oct 27, 1995, Report date: Apr 2, 2000

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Key findings: The toxicity of prasugrel (R-91220) and clopidogrel (SR 25990C) at doses of 100 and 400 mg/kg was compared in F344/DuCrj rats administered orally for 28 days (n=10 M, 10 F). Decrease RBC, HCT and Hb, increase APTT, hepatocellular hypertrophy, proliferation of liver SER and thyroid follicular epithelium hypertrophy were observed at dosages of 400 mg/kg in both treatment groups. Pathological examination showed increase in liver weight, hypertrophy of liver cells, hypertrophy of follicular epithelium of thyroid and proliferation of smooth endoplasmic reticulum (SER) in both groups treated with ≥ 100 mg/kg/day. In summary, there was no difference in toxicity profile between prasugrel and clopidogrel in the rat.

Comparative toxicity of prasugrel and clopidogrel in dog:

Study no.: TR 143-093, 95-0072

Conducting laboratory: _____

Date of study initiation: Nov 13, 1995, Report date: March 24, 2000

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Key findings: In Beagle dogs treated with prasugrel (50 mg/kg/day) or clopidogrel (4, 20, 50 and 100 mg/kg/day) over a period of 28 days, vomiting was observed and pathological examination revealed stomach erosion, ulcer formation, and regeneration in the mucous membrane of the stomach in the clopidogrel group only (> 20 mg/kg/day), but not in the prasugrel group at doses up to 50 mg/kg. Treatment-related changes seen after administration of clopidogrel (> 20 mg/kg/day) or prasugrel (50 mg/kg/day) included reduced platelet aggregation, increased ALP activity, increased Cyt P₄₅₀ content, increased liver weight, hypertrophy and ground glass appearance in cytoplasm of the hepatocytes and proliferation of SER. There was no considerable difference between the degree or frequency of appearance of the two drugs.

2.6.6.9 Discussion and Conclusions

Prasugrel is a prodrug whose active metabolite irreversibly inhibits the P2Y₁₂ purinergic receptor, and consequently inhibits ADP-mediated platelet aggregation, thus prolonging the time required for normal blood clotting. The animal data substantiate the ability of prasugrel to be an effective inhibitor of thrombus formation. *In vitro* assays and *in vivo* animal models of thrombosis showed that prasugrel was 10- and 100-times more potent as an antithrombotic agent than clopidogrel and ticlopidine, respectively. The increased potency of prasugrel compared to clopidogrel in nonclinical pharmacology studies was consistent with the more efficient conversion of the prodrug to the active metabolite. There was no difference in the potency of the active metabolites of prasugrel and clopidogrel in the inhibition of platelet aggregation.

Following oral administration, prasugrel could not be detected in plasma because it is rapidly metabolized to an active sulfhydryl metabolite (R-138727) and several inactive metabolites. Among the hepatic metabolites, R-99224 is a mixture of two of the possible four stereoisomers of R-138727 and has an *in vitro* activity similar to the *ex vivo* activity of prasugrel. The AUC values of the metabolites increased with increasing the prasugrel dosage; however dose proportionality could not be established indicating induction of drug-metabolizing enzymes (Cyp2B and Cyp3A2). Pharmacokinetic studies showed adequate exposure was obtained in the toxicology studies, and all circulating metabolites in humans occurred in the circulation of the animal species including the pharmacologically active primary metabolite, R-138727, and the major human metabolite, R-106583. The elimination $t_{1/2}$ is 10 h and 33 h in the rat and dog, respectively. Biliary excretion appears to be the major route for elimination.

Prasugrel did not exhibit secondary pharmacology activity related to the CNS, respiratory, renal, or cardiovascular function. Toxicology studies identified the liver as a target organ as shown by the induction of hepatic enzymes and elevations of ALP activity. In mice repeated oral administration of prasugrel for 3-months at doses ≥ 300 mg/kg caused increase in liver weight, hypertrophy of the centrilobular hepatocytes and diffuse hepatocyte vacuolation. In rats repeated oral administration of prasugrel for 3- and 6-months at doses ≥ 100 mg/kg was associated with increased liver weight, hepatocellular hypertrophy, hypertrophy of thyroid follicular epithelium, and proliferation of smooth endoplasmic reticulum, changes consistent with microsomal enzyme induction. In dogs repeated oral administration of prasugrel for 3- and 9-months at doses > 4 mg/kg was associated with increase in ALP activity, hypertrophy of hepatocytes accompanied by ground glass appearance of the liver, proliferation of smooth endoplasmic reticulum which may be due to an activation of drug metabolism enzymes. In the repeat dose study in the rat and dog, the NOAEL of prasugrel were approximately 30 and 4 mg/kg, respectively, which is about 18- and 1.5-fold higher than the exposure of major metabolite in human therapeutic daily dose of 10 mg prasugrel.

Comparative 1-month toxicity studies with prasugrel and clopidogrel (400 mg/kg) demonstrated similar toxicity profiles including increase in liver weight, ALP and Cyt P₄₅₀, proliferation of liver SER and hypertrophy of follicular epithelium in thyroid. There was no difference in toxicity profile between prasugrel and clopidogrel in the rat.

No genetic toxicity was observed for the active metabolite prasugrel in standard tests that included *in vitro* bacterial mutation test and Chinese hamster lung chromosomal aberration assay and *in vivo* mouse micronucleus test. There was no evidence of

treatment-related tumors in a 2 year rat study with prasugrel exposures ranging to about 50 times the recommended therapeutic exposures in humans. There was an increased incidence of tumors (hepatocellular adenomas) in mice exposed for 2 years which was observed at high doses prasugrel (i.e., 190 times human major metabolite exposure). The sponsor considers the hepatocellular adenoma findings to be secondary to prasugrel-induced hepatic metabolizing enzyme-induction. However, this is unlikely because there was induction of hepatic metabolizing enzymes in the rat but no adenomas were observed in the two year study.

In the rat and mouse, there were high incidence of spontaneous carcinomas (liver, lung, thyroid, adrenal,) but these were not enhanced by treatment with prasugrel, which rules out possibility of tumor promoter effects. A non-neoplastic finding of a tendency for an increase in the incidence of eosinophilic altered cell foci was observed in the mid and high dose groups in the rat and mouse studies. Thus, in the liver lesions, the tendency for an increase in the incidence of eosinophilic altered cell foci may be consequences of hepatic drug-metabolizing enzyme induction. It is considered that altered cell foci are progenitor lesions from which hepatocellular neoplasia might arise and some of the benign hepatocellular neoplasia may progress to malignant hepatocellular neoplasia. However, there was no evidence of malignant tumors in the 2-year lifetime rat and mouse studies of prasugrel. FDA computational quantitative structure activity relations by MC4PC or MDL-QSAR showed that prasugrel is not predicted to be carcinogenic, which is consistent with the negative carcinogenicity data of the *in vivo* rodent studies. Another member of the thienopyridine class, clopidogrel (plavix[®]), administered to rats for 104 weeks and mice for 78 weeks at plasma exposures >25 times than that in humans showed no evidence of tumorigenicity.

There was no significant effect of prasugrel on male or female fertility or on early embryonic development at oral doses up to 100 mg/kg (30 times human exposure). At doses \geq 100 mg/kg, decrease adrenal gland, seminal vesicle/prostate gland, and epididymal weights and reduction in mean fetal weight was observed. Dose associated maternal toxicity and decrease in fetal weight were observed; however, there were no adverse effects on *in utero* survival or morphological development of the conceptus at 100 mg/kg dose (100 times human exposure). Prasugrel does not have any teratogenic effects based on the absence of changes in the frequency of external, visceral or skeletal anomalies (100 times human exposure). Placental transfer of prasugrel metabolites to the fetus of pregnant rats was low. However, ¹⁴C-prasugrel was excreted in the milk of lactating rats.

In conclusion, the toxicological documentation are appropriate to support the intended clinical use of prasugrel at 10 mg/day. The proposed labeling of the animal carcinogenicity studies, developmental and reproductive studies, and advice on breast feeding are provided in Section 2.6.7 (Overall Conclusions and Recommendations).