



Metabolism and Excretion of PRT060128, a New Competitive, Reversible P2Y₁₂ Inhibitor, in Healthy Human Subjects

Athiwat Hutchaleelaha¹, Christine Ye¹, Joe Lambing¹, Manik Desai², Zhe-ming Gu², C. James Kissling³, Kevin Romanko¹, Hsiao Dee Liu¹, Dan Gretler¹

¹Portola Pharmaceuticals Inc, ²Xenobiotic Laboratories, ³MDS Pharma Services

Abstract

PURPOSE. To determine mass balance and metabolite profile following single oral administration of ¹⁴C-PRT060128 in human

METHODS. ¹⁴C-PRT060128 was administered orally to five healthy subjects at 50 mg (100 μCi). Blood, urine and feces samples were collected and measured for total radioactivity by LSC. Metabolite profiling was performed by HPLC followed by radioactivity determination using a TopCount NXT™ Microplate counter. Characterization of metabolites was performed by LC/MS/MS.

RESULTS. Mean total radioactivity C_{max} and AUC(0-∞) were 3895 ± 952 ng eq/mL and 28985 ± 10963 ng eq*hr/mL, respectively. Approximately 56% of the total dose administered was recovered in urine and 48% in feces. The mean total radioactive recovery was 105 ± 0.58%. PRT060128 was moderately metabolized. Unchanged PRT060128 was the dominant circulating radioactivity in plasma and the major radioactive component in urine and feces, accounting for 66.2% of the total administered dose in 0-36 hr urine and 0-120 hr feces. The major metabolic route was demethylation to form PRT060301, which was determined to be the only prominent circulating metabolite in plasma (AUC approximately 10% of PRT060128) and the only major metabolite in urine and feces (22.4% of the dose).

CONCLUSIONS. Following a 50 mg oral dose, ¹⁴C-PRT060128 could be completely recovered. One major metabolite was found. PRT060128 was eliminated as an unchanged and demethylated metabolite in urine and feces.

Background

- Platelet activation and aggregation play a critical role in the pathogenesis of thrombotic diseases, such as acute coronary syndrome.
- ADP released from platelets is important in the stabilization of the thrombus infrastructure and propagation of the thrombotic process. The ADP receptor on platelets mediating this process is the P2Y₁₂ receptor, the target of clopidogrel.
- PRT060128 is an investigational drug being developed by Portola Pharmaceuticals, Inc. as both an oral and IV formulation. It is a competitive and reversible P2Y₁₂ receptor inhibitor.

Objective

- To determine mass balance and metabolite profile following single oral administration of ¹⁴C-PRT060128 in human

Method

Study Design

- Open-label, single oral administration of 50 mg (100 μCi) ¹⁴C-PRT060128 to six human subjects
- Subject age range from 24 – 48 years with mean weight of 170 lbs
- Blood samples for analysis of total radioactivity in plasma were taken at predose, and at 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, and 120 hr
- Urine samples were collected for analysis of total radioactivity at predose, 0-6, 6-12, 12-24, 24-36, 36-48, 48-60, 60-72, 72-96, 96-120, 120-144, 144-168, 168-192, and 192-216 hr after dosing
- Feces samples were collected for analysis of total radioactivity at predose, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, and 192-216 hr after dosing

Sample Analysis: Total Radioactivity

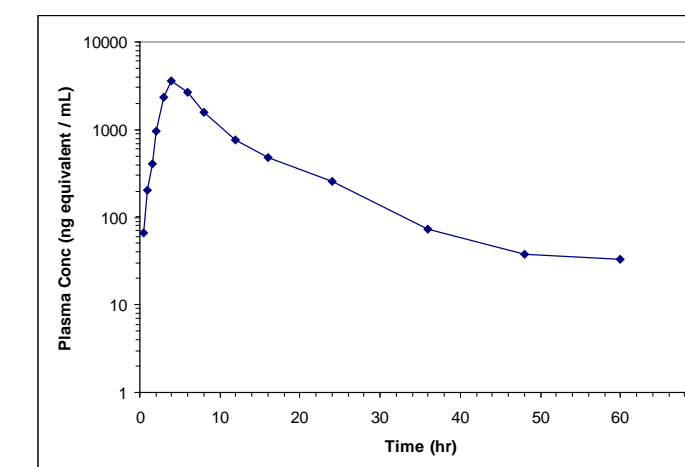
- Determination of radioactivity was conducted under GLP by MDS Pharma Services (Nebraska)
- Total [¹⁴C] radioactivity were determined in duplicate for dosing solution, plasma, blood, urine and feces using Tri-carb™ Liquid Scintillation Analyzer Model 1600 TR. All samples were counted in vials with liquid scintillation cocktail (Ultima Gold) for 5 min or until 2.8% error was reached.
- Blood and feces samples were dried and oxidized on a Packard Model 307 Oxidizer prior to counting

Sample Analysis: Metabolite Profiling and Identification

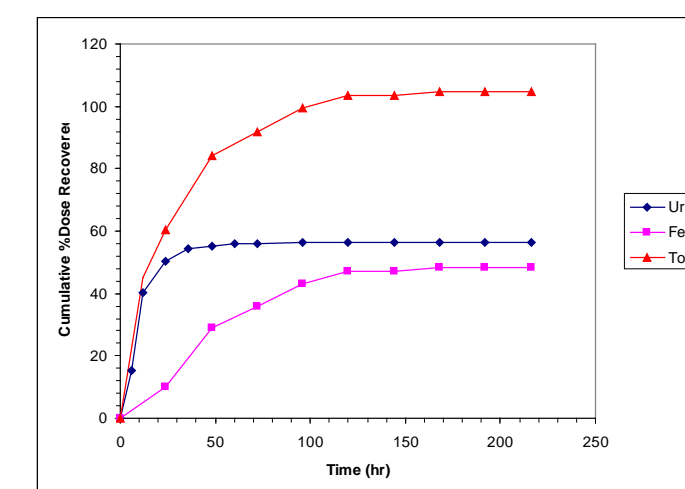
- Plasma samples were pooled by time point across subjects, i.e., 2, 4, 12 and 24 hr
- Urine samples were pooled by time interval across subjects, i.e., 0-12 and 12-36 hr
- Feces samples were pooled by time interval across subjects, i.e., 0-24 and 24-48, 48-72, 72-96, and 96-120 hr
- Samples were extracted and determined by HPLC radio-chromatography. Fraction of chromatography effluents were collected by time (15 sec/fraction) to Deepwell LumaPlate™-96 plates and were subsequently dried by a SpeedVac® concentrator. The radioactivity in each fraction was determined by Packard TopCount NXT™ Microplate Scintillation and Luminescence Counter technology. HPLC radio-chromatograms were reconstructed using ARC® Convert and Evaluation software. Radioactivity peaks were integrated to determine the percent distribution of individual radioactivity peaks or regions in each profile.
- PRT060128 and metabolite PRT060301 observed in the radioprofiles were confirmed by LC/MS and MS/MS analyses.

Results

Mean Plasma Total Radioactivity Concentration Equivalent (ng equivalent/mL) versus Time Following 50 mg (100 μCi) Oral Administration of ¹⁴C-PRT060128



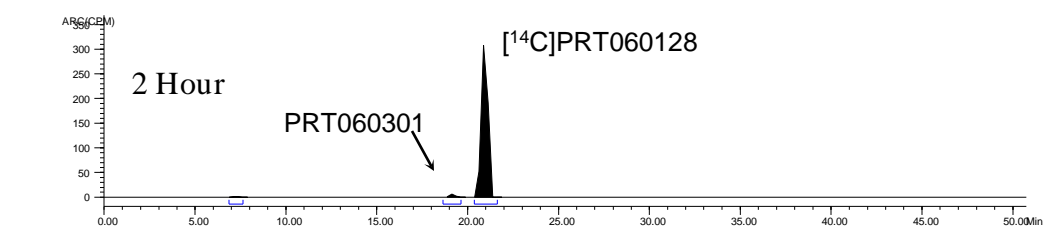
Cumulative % Dose Excretion Following 50 mg (100 μCi) Oral Administration of ¹⁴C-PRT060128



Mean SD Pharmacokinetic Parameters of Total Radioactivity Following 50 mg (100 μCi) Oral Administration of ¹⁴C-PRT060128

Parameters	Unit	
T _{max}	hr	4.4 ± 0.90
C _{max}	ng eq/mL	3895 ± 952
AUC(0-∞)	ng eq*hr/mL	28984 ± 10953
T _{1/2}	hr	9.62 ± 2.01

HPLC Radio-Chromatograms of Pooled 2, and 12 Hour Plasma Extracts from Male Human Subjects



12 Hour

HPLC Radio-Chromatograms of Pooled 0-12 Hour Urine Samples from Male Human Subjects

[¹⁴C]PRT060128

0-12 Hour

PRT060301

HPLC Radio-Chromatograms of Pooled 0-48 Hour Fecal Samples from Male Human Subjects

[¹⁴C]PRT060128

0-48 Hour

PRT060301

Summary

- Following a single 50 mg dose of ¹⁴C-PRT060128, the mean total radioactivity C_{max} and AUC(0-∞) were 3895.18 ng eq/mL, and 28984.51 ng eq*hr/mL, respectively.
- High radioactive recovery was achieved, with mean total radioactive recovery of 105% (range 104% to 106%), with approximately 56% of radioactive dose excreted in urine and approximately 48% excreted in feces.
- PRT060128 was moderately metabolized. Unchanged PRT060128 was the dominant circulating radioactivity in human plasma and the major radioactive component in urine and feces, accounting for a total of 66.2% of the administered dose in 0-36-hr urine and 0-120-hr feces. The major metabolic route of PRT060128 in humans was demethylation to form PRT060301, which was determined to be the only prominent circulating metabolite in human plasma and the only major metabolite in human urine and feces (22.4% of the dose).