SUPPLEMENTAL MATERIAL

Cell-Penetrating Pepducin Therapy Targeting PAR1 in Subjects with Coronary Artery Disease

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Supplemental Methods

Study Population

Inclusion criteria included male and non-pregnant female volunteers between 18 and 75 years with documented vascular disease (peripheral vascular disease, carotid artery disease or coronary artery disease) or ≥2 of the following coronary artery disease risk factors: dyslipidemia (total cholesterol ≥200 mg/dL, triglycerides ≥200 mg/dL, or high-density lipoprotein (HDL) <40 mg/dL (males), <50 mg/dL (females)); diabetes (Type 1 or 2) or pre-diabetes (hemoglobin A1c ≥6%); hypertension (consistent elevation in systolic blood pressure ≥140 mm Hg or diastolic ≥90 mm Hg and/or on medication; diabetics with systolic blood pressure ≥130 mm Hg or diastolic ≥80 mm Hg; smoking (any smoking history; must be currently <2 pack/day); obesity (BMI ≥30 kg/m^2); family history of premature cardiovascular disease (coronary heart disease, peripheral arterial disease or ischemic stroke; first-degree male relative diagnosed before the age 55, or first-degree female relative diagnosed before the age of 65 based on subject's self-report or from documented medical records); or age (male >45 or female >55). Exclusion criteria included participation in an investigational drug study within 30 days of dosing; a history or presence of any clinically significant medical or surgical condition known to interfere with drug absorption or metabolism; consumption of anticoagulants, $P2Y_{12}$ inhibitors, non-steroidal anti-inflammatory drugs (more than three time per week) or other drug(s) with the potential to affect coagulation or PAR1 receptor inhibition within 2 weeks of dosing and for 2 weeks post dosing (aspirin was allowed); previous history of anaphylaxis to drugs or any environmental stimuli including foods or hymenoptera (e.g., ants, bees, wasps) stings; asthma requiring bronchodilator/inhaler therapy; current tobacco use ≥2 packs of cigarettes per day; consumption of herbal supplements within 1 week of dosing and for 24 hours post dosing; prior history or clinical suspicion of cerebral vascular malformations, intracranial tumor, transient ischemic attack, stroke, gastric ulcers and any form of bleeding disorder; prior history of myocardial infarction with the last 3 months or unstable angina; thrombocytopenia (platelet count <130,000/mm^3); hematocrit <30%; impaired renal function (serum creatinine >1.5 times upper limit of normal; those with an estimated creatinine clearance ≥60 mL/min using Cockcroft-Gault formula were eligible); liver enzymes ≥3 times upper limit of normal; alcohol consumption within 48 hours prior to dosing and for 24 hours post dosing; history of substance or alcohol abuse (positive baseline test for drugs with a high potential for abuse and/or positive alcohol breath test); uncontrolled hypertension defined as sustained systolic blood pressure >160 mmHg and/or diastolic blood pressure >90 mm Hg; uncontrolled hypotension defined as sustained systolic blood pressure <100 mm Hg and/or diastolic blood pressure <50 mmHg; international normalized ratio (INR) >1·5; insufficient venous access for drug delivery; those with a history of testing positive for human immunodeficiency virus (HIV) or hepatitis; those having an invasive surgical procedure within 3 months of dosing or planning to have one within 1 month post dosing; and any condition which could interfere with or for which the treatment might interfere with the conduct of the study, or which

would in the opinion of the investigator increase the risk to the subject (e.g., epilepsy, psychiatric disease). Subjects were allowed to continue their study-approved prescription and over the counter medications prior to drug administration. Subjects had normal or clinically acceptable physical exam, vitals, 12-lead electrocardiogram (ECG), clinical laboratory tests [complete blood count (CBC), blood chemistries, coagulation, urinalysis], bleeding time and pulmonary function tests.

Supplemental Tables

Table I. Inhibitory Effects of PZ-128 on Light Transmission Aggregometry (LTA) to High Concentration of PAR1 Agonist (20 μM SFLLRN) in Subjects with CAD Risk Factors

		PAR1 Agonist			
		20 μM SFLLRN max		20 μM SFLLRN final	
Dose Level	Time (h)	All	+Aspirin	All	+Aspirin
0.5 mg/kg	0	0	0	0	0
All (n=6)	0.5	7	11	7	12
+ASA (n=4)	1	3	5	5	7
	2	3	4	4	6
	6	11	17	14	20
	24	12	18	16	24
	192	3	5	4	6
1 mg/kg	0	0	0	0	0
All (n=6)	0.5	5	5	11	11
+ASA (n=5)	1	12	12	18	18
	2	9	7	11	10
	6	8	10	11	14
	24	10	9	17	16
	192	13	16†	17	21
2 mg/kg	0	0	0	0	0
All (n=6)	0.5	8	7	13	16
+ASA (n=4)	1	8	7	8	8
	2	7	8	7	8
	6	3	≤0	3	≤0
	24	3	3	2	2
	192	11	4	12	4

Data are presented as mean platelet inhibition (%) measured by light transmission aggregometry (LTA). ANOVA analyses of the effects of PZ-128 on platelet inhibition over time for each agonist within each dose cohort was performed with the Dunnett's post-test correction using the predose (0) time point as the control. A subgroup analysis was done among subjects taking concomitant aspirin. Average standard error of mean (SEM) was 4. ASA indicates acetylsalicylic acid (aspirin).

†0·050<P<0·099

Table II. Pharmacokinetic Parameters of PZ-128 Following Single Ascending IV Doses Administered Over 1 to 2-hours

	Dose (mg/kg), Infusion time							
	0·01 1 h (n=3)	0·03 1 h (n=3)	0·1 1 h (n=3)	0·3 1 h (n=3)	0·5 2 h (n=6)	1 1 h (n=6)	1 2 h (n=1)	2 1 h (n=6)
AUC_{last}, ng·h/mL								
Mean	95	236	1,060	4,190	7,420	11,200	16,100	29,000
SD	85	43	47	692	1,150	2,910	-	6,930
Range	37-193	192-278	1,010-1,100	3,620-4,960	5,190-8,400	7,660-15,100	-	18,600-38,900
C_{max}, ng/mL								
Mean	113	193	578	2,150	2,670	5,290	5,400	13,300
SD	52	40	67	243	423	1,030	-	5,340
Range	62-166	148-225	530-654	1,890-2,370	1,910-3,010	4,050-6,520	-	7,910-23,300
$t_{1/2,}$ h								
Mean	NC	NC	NC	1·26	1·57	1·65	1·79	1·82
SD	-	-	-	0·33	0·22	0·17	-	0·15
Range	NC	NC	NC	0·97-1·62	1·38-1·95	1·39-1·92	-	1·59-1·99
Cl, mL/min								
Mean	NC	NC	NC	113·4	89·8	134·5	86·7	101·5
SD	-	-	-	35·9	18·3	35·2	-	36·3
Range	NC	NC	NC	86·2-154·1	71·3-117·5	96·2-184·8	-	57·6-147·9
V_{ss}, L/kg								
Mean	NC	NC	NC	0·107	0·116	0·167	0·111	0·144
SD	-	-	-	0·004	0·014	0·037	-	0·040
Range	NC	NC	NC	0·104-0·112	0·103-0·137	0·128-0·217	-	0·091-0·213

AUC_{last} indicates area under the plasma concentration-time curve from time of dosing to last observation greater than the lower limit of quantitation (LLOQ=50 ng/mL); Cl, clearance; C_{max}, maximum observed plasma concentration; NC, not calculable due to insufficient data points in the terminal phase of drug elimination; SD, standard deviation; $t_{1/2}$, terminal elimination half-life; and V_{ss}, volume of distribution at steady state.

Table III. Treatment-Related Adverse Events by Dose

	Dose Level (mg/kg)						
Adverse event	0·01 (n=3)	0·03 (n=3)	0·1 (n=3)	0·3 (n=3)	0·5 (n=6)	1 (n=7)	2 (n=6)
Allergic reaction	0	0	0	0	0	3	3
Anaphylaxis (Anaphylactoid reaction)	0	0	0	0	0	1	3
Bruising	0	0	0	0	0	0	1
Dizziness	0	0	0	0	0	0	1
Glucosuria	0	0	0	0	0	0	1
Headache	0	0	0	0	0	1	1
Hypertension	0	0	0	0	0	1	0
Hypotension	0	0	0	0	0	1	0
Injection site reaction	0	0	0	1	2	5	4
Nausea	0	0	0	0	0	1	0
Orthostatic changes in vital signs	0	0	0	0	0	0	3
Paresthesia	0	0	0	0	5	4	5
Peak expiratory flow decrease	0	0	0	0	0	0	1
Purpura	0	0	0	0	0	0	1
Vomiting	0	0	0	0	0	1	0

Data reflect treatment-emergent adverse events characterized as possibly, probably, or definitely related to PZ-128. Data are presented as the number of individual events.

Supplemental Figures

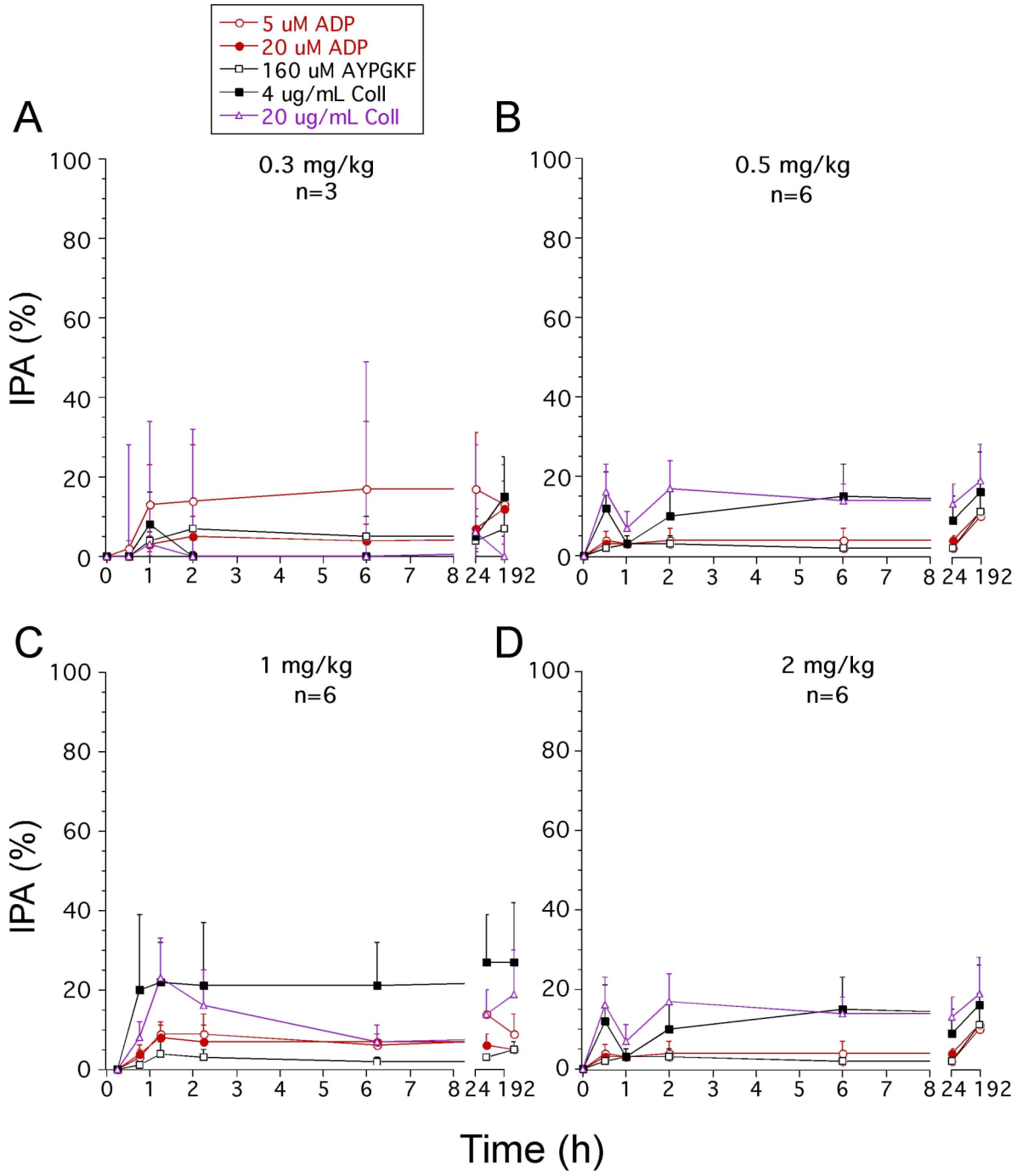

Figure I. Mean Inhibition of Platelet Aggregation (IPA) in the (A) 0.3 mg/kg, (B) 0.5 mg/kg, (C) 1 mg/kg, (D) 2 mg/kg PZ-128 dose cohorts to 5 µM ADP, 20 µM ADP, 160 µM AYPGKF, 4 µg/mL collagen, and 20 µg/mL collagen. Ex vivo aggregation was conducted in PRP by LTA and maximal aggregation (mean ± SD) normalized to baseline (t=0) for each agonist for all subjects in the dose cohort.

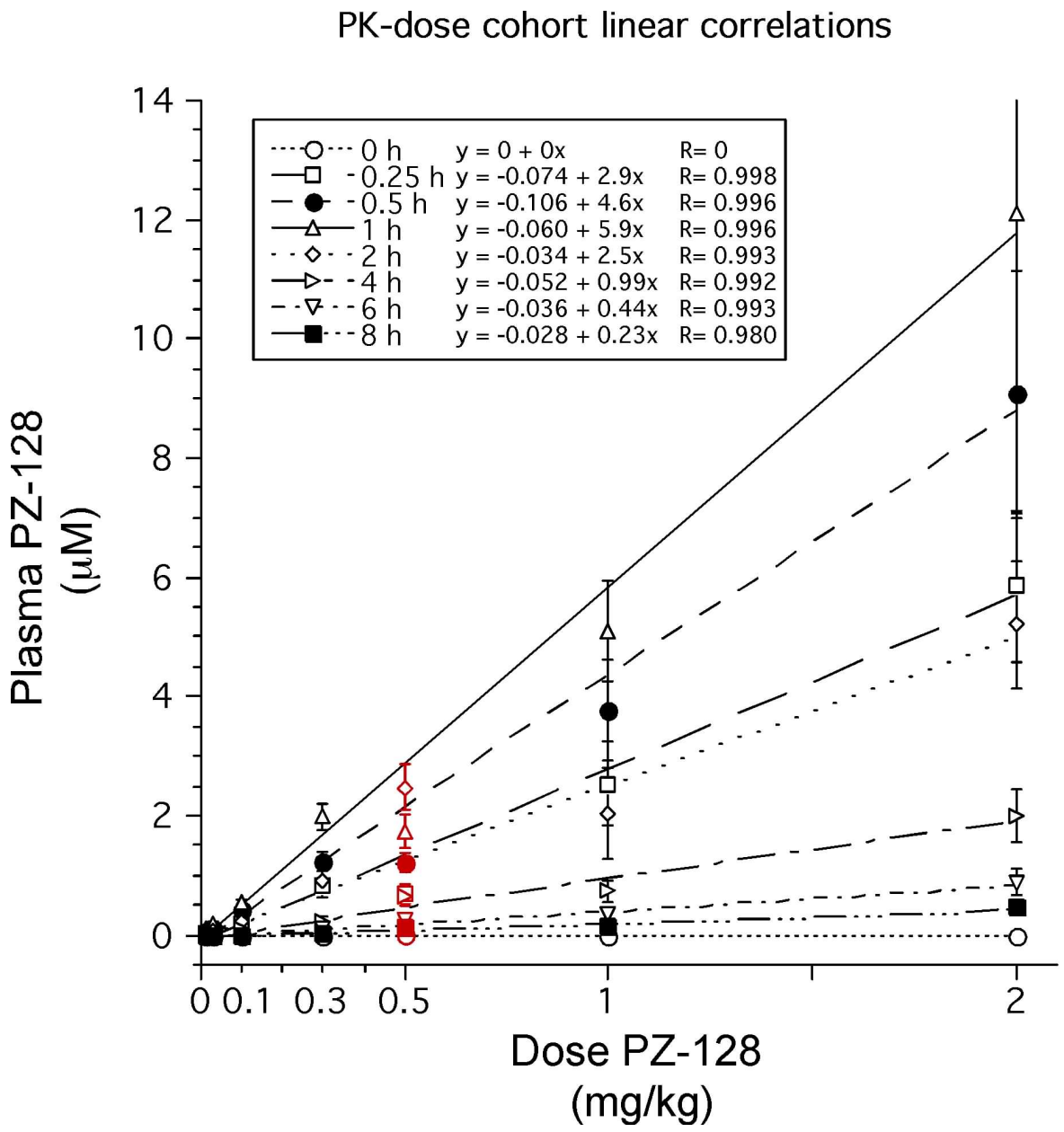

Figure II. Plasma concentrations of PZ-128 (μM) versus dose for 0·01-2 mg/kg PZ-128 cohorts. The data points shown are the means ± sd for 3-6 subjects per point at 6 different doses of PZ-128 (0·01, 0·03, 0·1, 0·3, 1, 2 mg/kg) measured at 0, 0·25, 0·5, 1, 2, 4, 6, 8 h time points for each subject, reflecting a total of 188 individual PK measurements (188 of 192 time points successfully collected), or approximately 24 data points per fitted linear regression line. The 0·5 mg/kg dose cohort (red) was not included in the linear regression analysis because the drug infusion duration was 2 hours, whereas the administration time was 1 hour in the remaining cohorts.

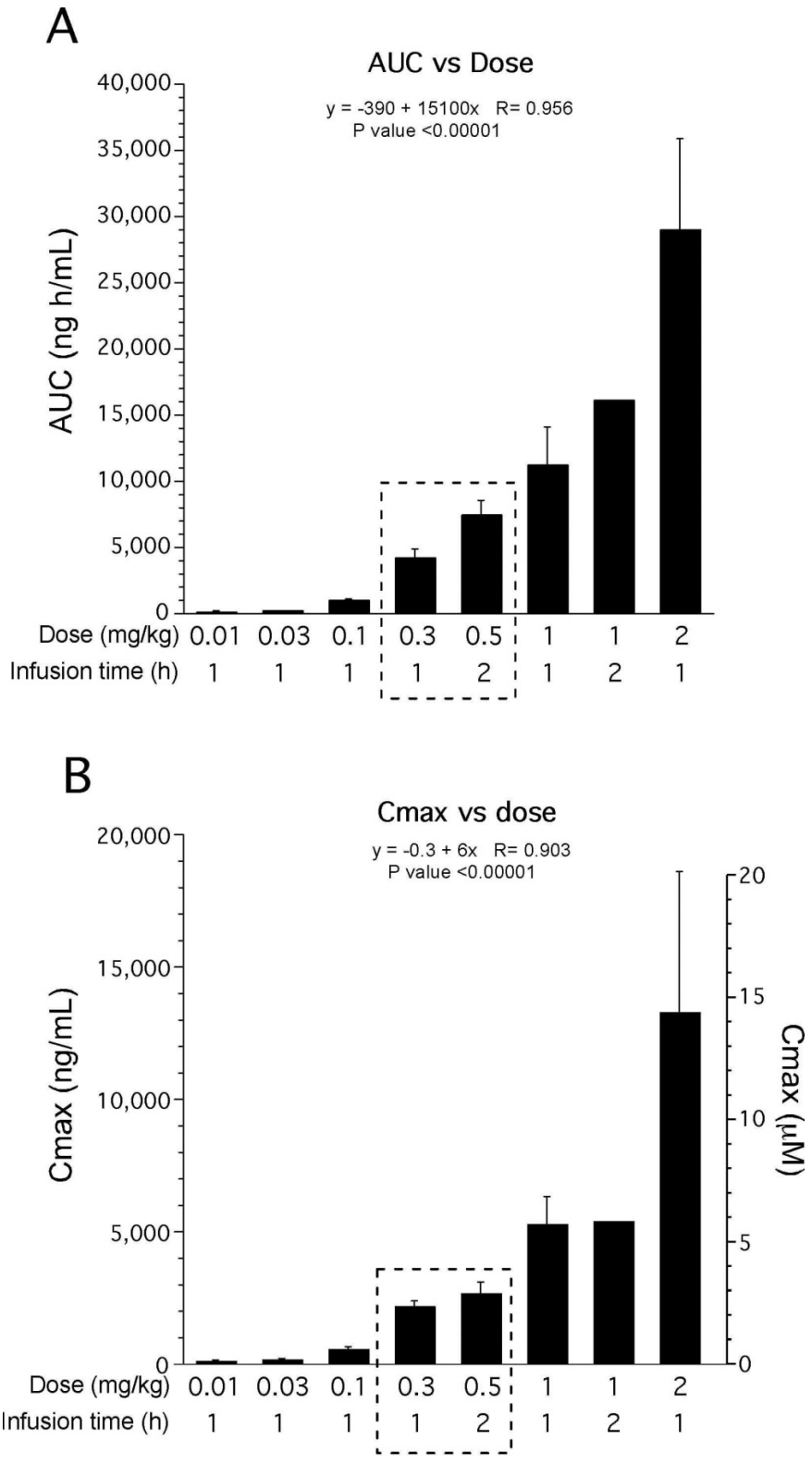

Figure III. Mean AUC (A) and mean C_{max} (B) versus dose. Dotted boxes indicate predicted therapeutic dose levels with an adequate safety margin.