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

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Abstract

Objective—Pepducins are membrane-tethered, cell-penetrating lipopeptides that target the cytoplasmic surface of their cognate receptor. Here, we report the first human use of a protease-activated receptor-1–based pepducin, which is intended as an antiplatelet agent to prevent ischemic complications of percutaneous coronary interventions.

Approach and Results—PZ-128 was administered by 1 to 2 hours continuous intravenous infusion (0.01–2 mg/kg) to 31 subjects with coronary artery disease or multiple coronary artery disease risk factors. Safety, antiplatelet efficacy, and pharmacokinetics were assessed at baseline and 0.5, 1, 2, 6, 24 hours, and 7 to 10 days postdosing. The inhibitory effects of PZ-128 on platelet aggregation stimulated by the protease-activated receptor-1 agonist SFLLRN (8 μmol/L) at 30 minutes to 6 hours were dose dependent with 20% to 40% inhibition at 0.3 mg/kg, 40% to 60% at 0.5 mg/kg, and ≥80% to 100% at 1 to 2 mg/kg. The subgroup receiving aspirin in the 0.5 and 1-mg/kg dose cohorts had 65% to 100% inhibition of final aggregation to SFLLRN at 30 minutes to 2 hours and 95% to 100% inhibition by 6 hours. The inhibitory effects of 0.5 mg/kg PZ-128 were reversible with 50% recovery of aggregation to SFLLRN by 24 hours. There were no significant effects of PZ-128 on aggregation induced by AYPGKF, ADP, or collagen, indicating that the observed effects were specific to protease-activated receptor-1. The plasma half-life was 1.3 to 1.8 hours, and PZ-128 was nondetectable in urine. There were no effects on bleeding, coagulation, clinical chemistry, or ECG parameters.

Conclusions—PZ-128 is a promising antiplatelet agent that provides rapid, specific, dose dependent, and reversible inhibition of platelet protease-activated receptor-1 through a novel intracellular mechanism.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01806077.

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Disclosures

The other authors report no conflicts.

Keywords

aspirin; collagen; coronary artery disease; lipopeptides; risk factors

Coronary thrombosis during acute coronary syndromes (ACS) and percutaneous coronary interventions (PCIs) is dependent on reactive platelets.[1,2] Antiplatelet therapy plays a central role in preventing stent thrombosis and recurrent myocardial infarction. Platelet activation involves multiple signaling pathways activated by thrombin, thromboxane A_2, ADP, and collagen.[3] Blockade of more than one of these pathways has proven superior in attenuating thrombotic event occurrence than mono-blockade. However, it remains unclear which pathway is central to the generation of thrombotic events and thrombin activation of the protease-activated receptors (PARs) and platelet-dependent thrombin generation may vary greatly from patient-to-patient.[4–6] Glycoprotein IIb–IIIa inhibitors (abciximab, eptifibatide, and tirofiban) that block the final common pathway of platelet clot formation exhibit potent antiplatelet activity in patients with PCI, but also significantly increase the risk of bleeding proportional to their potency.[7,8] Upstream inhibition of thrombin with bivalirudin provides significant protection from thrombin-induced platelet aggregation in the PCI setting, but by design also directly inhibits coagulation leading to a commensurate increase in bleeding risk.[5,9,10]

The antiplatelet effect of the most widely used $P2Y_{12}$ inhibitor, clopidogrel, is slow in onset, variable and irreversible. Newer, more potent oral $P2Y_{12}$ inhibitors, such as ticagrelor, have a faster peak onset of action than clopidogrel[11]; however, the pharmacodynamic effect of the new $P2Y_{12}$ inhibitors may be delayed in subjects with ACS.[12] A relation exists between fast and effective on-treatment platelet reactivity and ischemic event occurrence in patients with PCI.[12–14] In addition, the slow offset of $P2Y_{12}$ inhibition and residual bleeding risk by all currently approved oral agents may be problematic in patients requiring coronary artery bypass graft surgery.[15]

PAR1 or PAR4 inhibition is an emerging strategy to target thrombin-induced platelet activation.[4,16] Two orally active PAR1 inhibitors, vorapaxar[17] and atopaxar,[18] have been studied in phase 2 trials and have been associated with a reduction in ischemic event occurrence without an increase in major bleeding. However, acute and then chronic administration of vorapaxar in patients with non–ST elevation ACS in the Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome (TRACER) trial did not reduce the composite occurrence of cardiovascular death, myocardial infarction, stroke, hospitalization for ischemia, or urgent revascularization but increased major bleeding and intracranial hemorrhage.[19] The ability to reversibly inhibit PAR1 signaling by a parenteral strategy may reduce bleeding in the high-risk patient undergoing PCI. Whether rapid, short-term PAR1 blockade improves outcomes in patients undergoing PCI remains unknown.

Pepducins are lipidated peptides, which specifically target the cytoplasmic surface of their cognate receptor and can act as either allosteric agonists or antagonists.[20–22] PZ-128 is a cell-penetrating lipopeptide pepducin that selectively inhibits PAR1-G protein signaling on the inner leaflet of the lipid bilayer.[9,23,24] PZ-128 is being developed for prevention of acute

thrombotic complications of PCI. In this study, we assessed the pharmacokinetics, pharmacodynamics, safety, and tolerability associated with single ascending intravenous doses of PZ-128 in subjects with multiple risk factors for coronary artery disease (CAD).

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Demographics

This phase 1 study of the antiplatelet efficacy, pharmacokinetics and safety of a pepducin was conducted in both male and female subjects with equal race distribution. The age range was 43 to 74 years. Twenty-two percent of subjects had documented CAD and all subjects had multiple risk factors for coronary disease, including smoking, hypertension, diabetes mellitus, and dyslipidemia (Table 1). Subjects were concurrently taking medications for the treatment of cardiovascular disease, including aspirin (63%), antihypertensives (66%), statins and other lipid-lowering drugs (56%), and agents to control blood glucose (22%). Sixty-nine subjects were screened and 31 were given a dose of PZ-128. One enrolled patient developed hypertension after pretreatment with an antihistamine and was not administered study drug. All enrolled patients completed 30-day follow-up (Figure 1).

Pharmacodynamics

PZ-128 (P1pal-7) is a cell-penetrating, membrane-tethered lipopeptide, which closely resembles the off-state of the corresponding juxtamembrane region of the PAR1 third-intracellular loop and TM6 region (Figure 2A). PZ-128 targets PAR1-G protein signaling, which can be assayed ex vivo in platelets as a pharmacodynamic marker of drug activity and specificity.[9] Accordingly, the ability of PZ-128 to block maximal and final platelet aggregation to the PAR1 agonist SFLLRN versus agonists for other platelet receptors (PAR4, ADP, and collagen) was determined at several time points (0, 30 minutes, 1, 2, 6, 24, and 192 hours). There were no significant effects of PZ-128 on platelet aggregation at the lowest doses (0.01–0.1 mg/kg) for all agonists. PZ-128 rapidly inhibited mean PAR1 platelet aggregation at the 0.5-mg/kg dose (2-hour infusion) with 40% to 50% inhibition of final aggregation to 8 μmol/L SFLLRN at 30 minutes to 2 hours (Figure 2B). By the 6-hour time point, PZ-128 significantly inhibited aggregation induced by 8 μmol/L SFLLRN in the 0.5-mg/kg dose cohort (Table 2). At 24 hours, aggregation induced by 8 μmol/L SFLLRN recovered by 50%, with near complete recovery by the 192-hour time point (Figure 2B). The inhibitory effects of 0.5-mg/kg PZ-128 were reversed by higher concentrations of PAR1 agonist (20 μmol/L SFLLRN) at 30 minutes to 24 hours with no significant inhibition observed (Table I in the online-only Data Supplement).

Analysis of the subgroup of subjects on concomitant aspirin (acetylsalicylic acid) revealed stronger apparent effects of PZ-128 on inhibition of maximal and final aggregation to 8 and 12 μmol/L SFLLRN in the 0.5-mg/kg dose cohort. The subjects also receiving acetylsalicylic acid in the 0.5-mg/kg dose cohort had 60% to 80% average inhibition of final aggregation to 8 μmol/L SFLLRN at 30 minutes to 2 hours and 98% inhibition by 6 hours

(Table 2; Figure 2C). There was 40% recovery of aggregation induced by 8-μmol/L PAR1 agonist by 24 hours and 60% recovery by 192 hours. Higher concentrations of PAR1 agonist (20 μmol/L SFLLRN) showed 80% recovery of aggregation by 24 hours and 95% by 192 hours in the 0.5-mg/kg dose cohort receiving concomitant acetylsalicylic acid. At the higher dose of 1 mg/kg, PZ-128 gave rapid and sustained ≈80% to 100% inhibition at 30 minutes to 6-hour time points regardless of whether the subject was taking concomitant acetylsalicylic acid (Figure 3). Similar 96% to 98% inhibitory effects on final aggregation to 8 μmol/L SFLLRN were also seen at the highest dose of 2 mg/kg at 1 to 2-hour time points, however, recovery of platelet aggregation was faster at the 6 to 192-hour time points at the highest dose with only 15% to 21% inhibition observed (Table 2).

The inhibitory effects of PZ-128 on PAR1 platelet aggregation (8 μmol/L SFLLRN, final aggregation) were dose dependent with 20% to 40% mean inhibition at 0.3 mg/kg, 40% to 60% at 0.5 mg/kg, and ≤80% to 100% at 1 to 2 mg/kg (Figure 4). PZ-128 was specific to PAR1 with no significant effects on aggregation induced by any other platelet agonists, including 160-μmol/L AYPGKF, 5-μmol/L ADP, 20-μmol/L ADP, 4-μg/mL collagen, or 20-μg/mL collagen in any dose cohort (Figure I in the online-only Data Supplement). As anticipated, there were nonsignificant inhibitory effects of PZ-128 on aggregation induced by collagen (≈10% to 20% inhibition at 0.5 to 2-mg/kg doses), consistent with suppressing a previously described collagen–MMP1 (matrix metalloprotease-1)–PAR1 mechanism.[28]

Pharmacokinetics

Plasma drug levels of the pepducin peaked (C_{max}) at the end of the 1- or 2-hour infusion in all dose cohorts with a terminal $t_{1/2}$ of elimination of 1.3 to 1.8 hours (Figure 5; Table II in the online-only Data Supplement). Drug was undetectable in plasma at 24 to 192 hours in all subjects. There was little or no PZ-128 detected in urine at any time point in all subjects. The mean volume of distribution of PZ-128 was 0.11 to 0.17 L/kg indicating that the palmitoylated peptide was not distributed in the water compartments (intracellular and extracellular) that constitute ≈60% of body volume (eg, volume of distribution at steady state-0.6 L/kg for a 100 kg subject).

Drug concentrations were highly linearly correlated with increasing dose at 0.25 to 8-hour time points with R values of 0.980 to 0.998 (Figure II in the online-only Data Supplement). Likewise, area under the curve and C_{max} of PZ-128 in plasma were linearly correlated with dose (Figure III in the online-only Data Supplement). Supra-therapeutic drug concentrations were achieved at the 1- and 2-mg/kg dose levels with mean peak PZ-128 levels reaching 5 and 12 μmol/L, respectively (Figure 5). Accordingly, PZ-128 blocked 85% to 100% of aggregation to 8 μmol/L SFLLRN agonist at the 1 to 2-hour time points in the 1- to 2-mg/kg dose cohorts. At 0.3- to 0.5-mg/kg doses where anti-PAR1 effects on platelet aggregation were observed at 30 minutes to 2 hours, drug levels of PZ-128 achieved therapeutic concentrations of 1 to 3 μmol/L. In agreement with preclinical studies in nonhuman primates,[9] persistent antiplatelet effects of 60% to 100% inhibition were still observed at the 6-hour time point with the 0.5-mg/kg dose where plasma PZ-128 drug levels had fallen to <0.5 μmol/L. This is consistent with the mechanism of action of the pepducin, which flips

across the cell membrane where it remains associated with its cognate receptor on the inner leaflet of the lipid bilayer to give prolonged inhibition of PAR1 activity.[24,29,30]

Safety and Effects of PZ-128 on Hemostasis Parameters

PZ-128 was well tolerated in the 0.01-, 0.03-, 0.1-, and 0.3-mg/kg dose cohorts using a 1-hour intravenous infusion. At higher doses, adverse events were transient in nature and resolved by 24 hours after administration of the study drug (Table III in the online-only Data Supplement). Transient tingling or a numb sensation in the skin was commonly reported at the moderate to high dose cohorts (0.5–2 mg/kg). The most important adverse event was acute allergic reactions occurring in several subjects receiving the highest doses of PZ-128. The occurrence of drug allergic reactions at the high (1–2 mg/kg) doses using a 1-hour infusion time necessitated dropping the dose to 0.5 mg/kg and extending the infusion time to 2 hours. This strategy along with premedication mitigated the allergic reaction and resulted in a tolerated and efficacious dose of 0.5 mg/kg.

No significant effects on coagulation, hemostasis parameters, or bleeding were evident at any dose, despite the fact that 63% of the subjects (20/32) were also taking aspirin (Figure 6). There were no significant changes in heart rate or any ECG parameter including RR, PR, QRS, QT, QTc, or QTd intervals or pulmonary function with PZ-128 dose. Likewise, all other laboratory outcomes including clinical chemistry and hematology, and urinalysis were not significantly affected by PZ-128.

Discussion

This study describes the first-in-man effects of PZ-128, a cell-penetrating pepducin specific for the PAR1 receptor. We demonstrated that the platelet inhibitory effects of PZ-128 were: (1) dose dependent and rapid in onset with moderate to high levels of platelet inhibition stimulated by 8 μmol/L SFLLRN observed with doses ≥0.5 mg/kg, (2) sustained for ≥6 hours after the start of the infusion, (3) reversible, with recovery of platelet function by 24 hours at the 0.5-mg dose and 80% to 100% aggregation achieved by 20 μmol/L SFLLRN at all time points, and (4) specific for the PAR1 receptor with no significant effects on aggregation stimulated by a PAR4 agonist, ADP, or collagen. There were no effects on bleeding time, PTT, international normalized ratio, activated clotting time, pulmonary function, heart rate, or other laboratory or ECG parameters.

An apparent enhancement of the antiplatelet effects of PZ-128 by aspirin was observed in the 0.5-mg/kg group. Similar to thrombin, thromboxane A_2 also signals through $_{G12/13}$ to cause the platelet shape change reaction and trigger aggregation. In this regard, aspirin monotherapy has been shown to inhibit PAR1 response in platelets in subjects with PAD.[31] Similarly, based on a microchip-based flow chamber system, it was demonstrated that antithrombotic effects of another PAR1 inhibitor, SCH79797, was significantly enhanced in the presence of aspirin and a $P2Y_{12}$ receptor blocker.[32]

Analysis of the pharmacokinetic effects demonstrated peak plasma drug levels at the end of the infusion period in all dose cohorts with no detectable drug in plasma at 24 and 192 hours. The absence of PZ-128 in urine demonstrated that the pepducin is not renally cleared.

Plasma drug concentrations were highly linearly correlated with dose. At high-drug concentrations of PZ-128 achieved with 1 to 2-mg/kg dosing there was 85% to 100% inhibition of aggregation stimulated by 8 μmol/L SFLLRN regardless of whether the subject received concomitant aspirin. Prolonged antiplatelet efficacy was more prominent at 6 to 192 hours with the 1-mg/kg dose, despite 2-fold higher peak plasma drug levels in the 2-mg/kg dose cohort. The mechanistic basis for this is not clear and could be because of the small sample size; however, it is possible that the high concentrations of PZ-128 (7910–23 300 ng/mL) observed in the 2-mg/kg cohort resulted in more stable pepducin micelle formation with less overall adsorption as monodispersed molecules to the target tissue, namely platelets. At the 0.5 to 1-mg/kg dose cohorts, there was an additional late spike in inhibitory activity evident at 6 to 24 hours. Although speculative, the late spike in inhibition in the higher dose cohorts could also be because of the cell-penetrating pepducin moving from a fatty reservoir/vascularized-compartment (eg, liver, kidney, and bone marrow) into the continuously circulating platelets.

There have been no other previous reports of fast-acting parenteral PAR1 blockade in humans. The oral PAR1 inhibitor, vorapaxar was administered acutely as a loading dose and maintained at a lower dose for a median exposure of 386 days to patients with ACSs in a large phase 3 trial.[19] Vorapaxar therapy was associated with a reduction in recurrent myocardial infarction at the expense of more bleeding, including more intracranial bleeding. Vorapaxar gave no appreciable inhibition at 30 minutes, but reached ≥80% inhibition of SFLLRN-induced aggregation at 2 hours after a 40-mg loading dose (≈0.4 mg/kg) in 89% to 96% of PCI and non–ST-segment elevation–ACS patients.[17,33] Atopaxar, a second orally active PAR1 inhibitor, was investigated in several phase 2 studies including the Lessons From Antagonizing the Cellular Effect of Thrombin-Coronary Artery Disease Trial (LANCELOT)-ACS trial (n=603),[34] and studies in a Japanese ACS (unstable angina and non–ST-segment–elevation myocardial infarction) patient population (J-LANCELOT; n=241),[18] and in patients with CAD (LANCELOT-CAD; n=263) on top of standard antiplatelet therapy.[35] The LANCELOT-ACS subjects reached maximal platelet inhibition 6 hours after the loading dose of atopaxar. The LANCELOT-ACS trial results demonstrated that atopaxar significantly reduced Holter-detected ischemia without a clear increase in bleeding compared with placebo. Atopaxar resulted in more minor bleeding in the 200-mg daily dose group, and a trend toward fewer ischemic events in patients with CAD.[35] However, a transient rise in liver enzymes was observed in 3% to 6% of subjects and prolongation of the QTc interval was observed in some individuals at the higher dose levels, causing the development of atopaxar to be halted.[34,35]

Unlike the relation of ADP-induced platelet aggregation to clinical thrombotic event occurrence, little is known about whether the magnitude of SFLLRN-induced aggregation is related to or predictive of clinical event occurrence. In the Primary Prevention Parameters Evaluation (PREPARE) POST-STENTING trial, we demonstrated that high thrombin–induced platelet–fibrin clot strength was a marker for recurrent ischemic event occurrence in patients treated with PCI.[36] The effects of vorapaxar and PZ-128 on thrombin activation of platelets in patients are also unknown, whereas evidence exists that the direct thrombin inhibitor, bivalirudin inhibits thrombin-induced platelet activation.[5] At the 0.5 to 2-mg/kg doses, PZ-128 provided rapid and reversible inhibition of SFLLRN-induced aggregation.

Whether a fast-onset of reversible PAR1 blockade induced by PZ-128 provides a better safety profile with preservation of clinical efficacy remains to be determined.

An important adverse event was acute allergic reactions occurring at the highest doses of PZ-128. Anaphylactoid reactions occurred either during the infusion or within 40 minutes from the completion of the infusion with skin manifestations and hemodynamic effects ranging from 10 minutes to 12 hours. None of these acute events were deemed life threatening and all were effectively treated with either stopping the infusion or administering antihistamine(s) or intravenous fluids. The transient hypotension was asymptomatic and without compensatory tachycardia. Of the subjects with allergic reactions, the C_{max} levels of PZ-128 were 4.7 to 21.5 μmol/L. Conversely, 50% of the subjects receiving the 2-mg/kg dose over the 1-hour infusion had no hemodynamic effects. To achieve a well-tolerated dose and to mitigate any potential allergic reactions by lowering C_{max}, we added the last 0.5-mg/kg dose cohort using a longer 2-hour infusion instead of a 1-hour infusion, along with antiallergic premedication. Accordingly, none of these subjects had an allergic response. The C_{max} levels (1.8–2.7 μmol/L) in the 0.5-mg/kg group were all well below the levels reached in the 1 to 2-mg/kg dose cohorts. With the prolonged infusion time of 2 hours, area under the curve levels were still maintained with the 0.5-mg/kg dose, thereby achieving anti-PAR1 pharmacodynamic efficacy especially in the context of concomitant aspirin therapy. On the basis of the above efficacy and safety data, the 0.5-mg/kg dose of PZ-128 infused >2 hours will be used in an upcoming multicenter phase 2 study in PCI/ACS subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

ACS	acute coronary syndromes
CAD	coronary artery disease
PAR1	protease-activated receptor-1
PCI	percutaneous coronary intervention

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Significance

PZ-128 is a pepducin that modulates platelet function by inhibiting signaling at the receptor–G protein interface. We demonstrated that PZ-128 selectively inhibits the protease-activated receptor-1 receptor in subjects with coronary artery disease or risk factors and at a dose of 0.5 mg/kg appeared to be well tolerated. The property of a rapid pharmacodynamic onset targeting a key platelet activation pathway that is reversible makes PZ-128 an attractive adjunctive agent for percutaneous coronary intervention. Moreover, the reversibility lends promise for enhanced safety. A phase 2 percutaneous coronary intervention/acute coronary syndromes study to further investigate the efficacy and safety of PZ-128 to block protease-activated receptor-1 is underway.

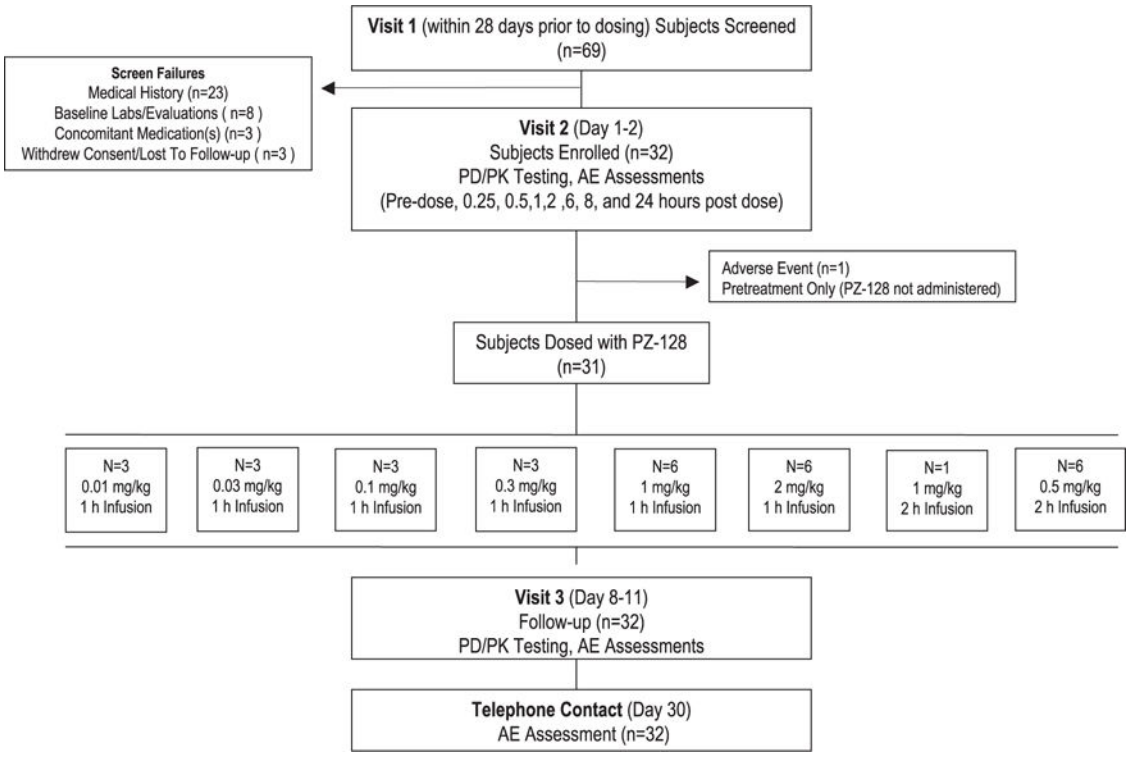

Figure 1.

Disposition of subjects. A total of 32 volunteers with vascular disease or coronary artery disease risk factors were prospectively assigned to 8 sequential dose levels and all subjects completed the study protocol. One subject became ineligible to receive the study drug after the administration of the pretreatment regimen in the predose setting. PD indicates pharmacodynamics; and PK, pharmacokinetics.

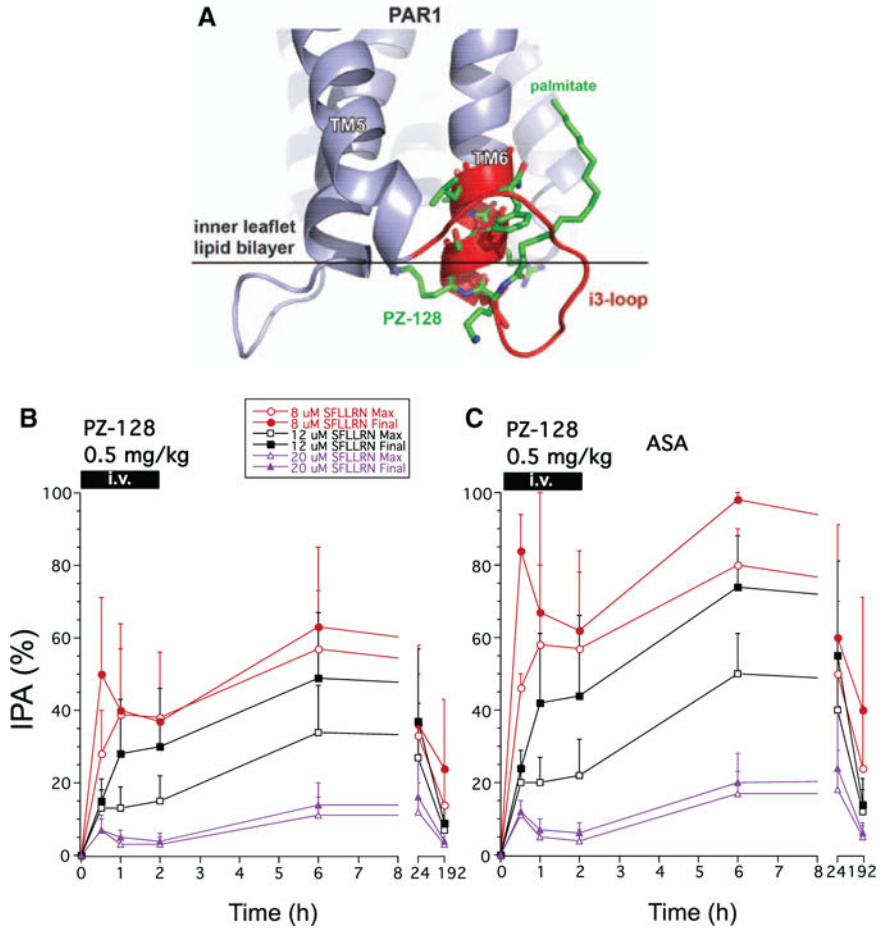

Figure 2.

Inhibition of platelet aggregation (IPA) in the 0.5 mg/kg PZ-128 dose cohort to 8, 12, and 20 μmol/L protease-activated receptor-1 (PAR1) agonist peptide, SFLLRN. **A**, The structure of the PZ-128 pepducin[9] (green) aligns closely (backbone root mean square deviation 1.4 Å) with the i3-loop/cytoplasmic α-helical extension of TM6 based on the rhodopsin[25,26] (red), and PAR1-vorapaxar[27] (blue) *x*-ray structures. Ex vivo aggregation was conducted in platelet-rich plasma by standard light transmission aggregometry and maximal and final (at 6–7 minutes) aggregation (mean±SD) normalized to baseline (t=0) for each agonist for (**B**) all subjects, and the subset receiving (**C**) aspirin (acetylsalicylic acid [ASA]).

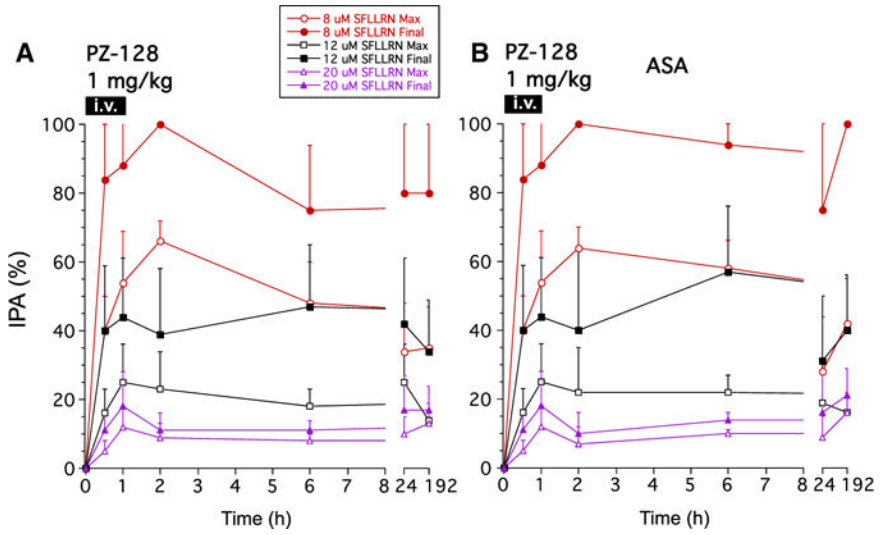

Figure 3.

Inhibition of platelet aggregation (IPA) in the 1 mg/kg PZ-128 dose cohort to 8, 12, and 20 μmol/L protease-activated receptor-1 agonist peptide, SFLLRN. Ex vivo aggregation was conducted in platelet-rich plasma by light transmission aggregometry and maximal and final (at 6 minutes) aggregation (mean±SD) normalized to baseline (t=0) for each agonist for (**A**) all subjects, and the subset receiving (**B**) aspirin (acetylsalicylic acid [ASA]).

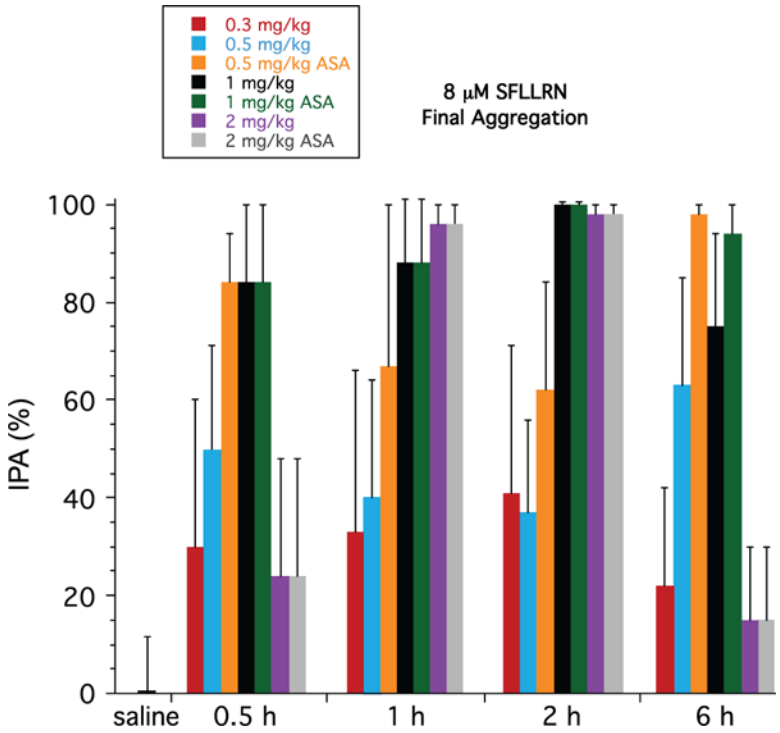

Figure 4.

Dose dependence of mean inhibition of platelet aggregation (IPA) in the 0.3 to 2 mg/kg PZ-128 dose cohorts to 8 μmol/L protease-activated receptor-1 agonist peptide, SFLLRN. Ex vivo aggregation was conducted in platelet-rich plasma by light transmission aggregometry and maximal and final (at 6–7 minutes) aggregation (mean±SD) normalized to baseline (t=0) for each agonist for either all subjects or the subset receiving aspirin (acetylsalicylic acid) as indicated. Saline was the IPA response at 30 minutes for subject TMC-27 who received a 1-hour infusion of saline only.

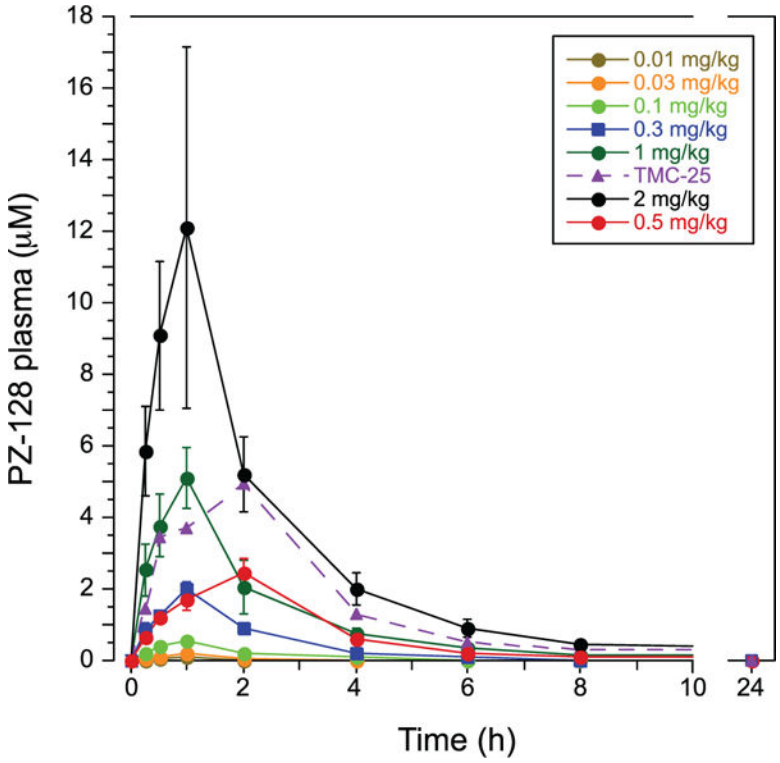

Figure 5.
Pharmacokinetics of PZ-128 (μmol/L) in plasma for 0.01 to 2 mg/kg dose cohorts.

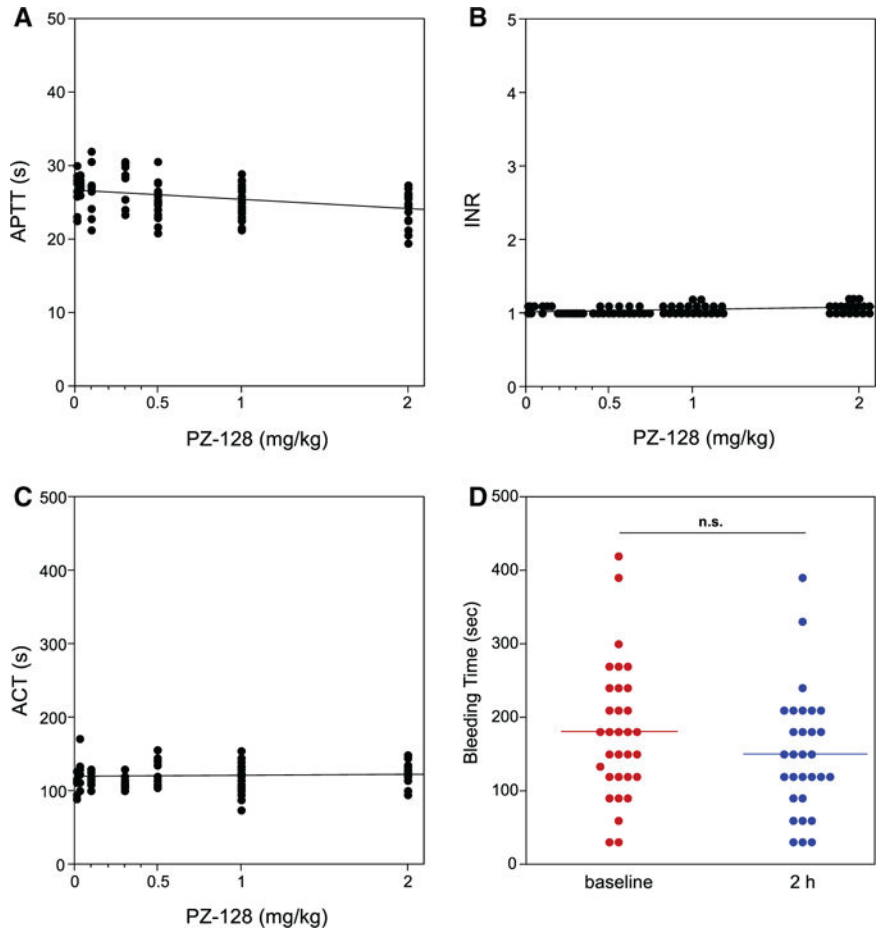

Figure 6.

Hemostasis parameters: Effect of PZ-128 dose on (**A**) activated partial thromboplastin time (APTT), (**B**) international normalized ratio (INR), (**C**) activated clotting time (ACT), and (**D**) bleeding time for all subjects. Hemostasis parameter (at all time points after drug infusion was initiated) versus dose for all subjects TMC1-32 was analyzed with PZ-128 dose as a continuous variable and correlations and *P* values for each slope were not significant ($P>0.01$) using repeated measures, mixed effects models, based on a Bonferroni post hoc test correction. Changes in bleeding time between baseline and 2 hours for all individuals in **D** was also analyzed for significance by 2-tailed, paired *t* test, with the mean bleeding times shown as horizontal lines.

Table 1

Baseline Demographics and Medical History

	All Subjects (n=32)
Age, y	57±8
Range	43–74
Male, n (%)	19 (59)
Race, n (%)	
White	16 (50)
Black	15 (47)
Other	1 (3)
Weight, kg	91±17
Range	63–127
Body mass index, kg/m^2	31±6
Range	20–43
Vascular disease, n (%)	7 (22)
Coronary artery disease	6 (19)
Previous myocardial infarction	5 (16)
Previous percutaneous coronary intervention	4 (13)
Previous coronary artery bypass-grafting	4 (13)
Coronary artery disease risk factors, n (%)	
Dyslipidemia	26 (81)
Hypertension	22 (69)
Obesity *	17 (53)
Smoking history	13 (41)
Diabetes mellitus or prediabetes	11 (34)
Age †	25 (78)
Baseline medications, n (%)	
Blood pressure	21 (66)
ACE inhibitor, ARB	14 (44)
β-Blocker	7 (22)
Calcium channel blocker	7 (22)
Diuretic	5 (16)
Aspirin	20 (63)
Lipid lowering	18 (56)
HMG-CoA reductase inhibitor	18 (56)
Other (fibrate, absorption inhibitor)	3 (9)
Diabetes mellitus (insulin, oral hypoglycemic, and incretin mimetic)	7 (22)

Data represent mean±SD where indicated. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; and HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

*Defined as body mass index ≥30 kg/m^2.

†Males >45 years of age, females >55 years of age.

Table 2

Inhibitory Effects of PZ-128 on PAR1 Light Transmission Aggregometry (Maximal and Final) in Subjects With CAD Risk Factors

		% Inhibition of PAR1 Platelet Aggregation							
		8 µmol/L SFLLRN Max		8 µmol/L SFLLRN Final		12 µmol/L SFLLRN Max		12 µmol/L SFLLRN Final	
Dose Level	**Time, h**	**All**	**+ASA**	**All**	**+ASA**	**All**	**+ASA**	**All**	**+ASA**
0.5 mg/kg	0	0	0	0	0	0	0	0	0
All (n=6)	0.5	28	46	50	84†	13	20	15	24
+ASA (n=4)	1	39	58†	40	67	13	20	28	42
	2	38	57†	37	62	15	22	30	44
	6	57*	80**	63	98*	34*	50*	49†	74*
	24	33	50	36	60	27	40*	37	55
	192	14	24	24	40	7	12	9	14
1 mg/kg	0	0	0	0	0	0	0	0	0
All (n=6)	0.5	40†	40†	84**	84***	16	16	40	40
+ASA (n=5)	1	54**	54**	88**	88***	25	25	44	44
	2	66***	64**	100***	100***	23	22	39	40
	6	48*	58**	75**	94***	18	22	47	57
	24	34	28	80**	75**	25	19	42	31
	192	35	42*	80**	100***	14	16	34	40
2 mg/kg	0	0	0	0	0	0	0	0	0
All (n=6)	0.5	36	21	24	24	10	7	19	19
+ASA (n=4)	1	54**	45†	96*	96*	19	21*	50*	60*
	2	47*	50*	98*	98*	12	13	21	23
	6	29	20	15	15	9	≤0	21	2
	24	≤0	≤0	21	21	≤0	≤0	≤0	≤0
	192	18	5	19	19	12	3	16	4

Data are presented as mean platelet inhibition (%) of maximal and final (6 min) light transmission aggregometry (LTA) in response to 8–20 µmol/L PAR1 agonist SFLLRN. ANOVA analyses of the effects of PZ-128 on platelet inhibition over time for each agonist concentration within each dose cohort was performed with the Dunnett post-test correction using the predose (0) time point as the control. A subgroup analysis was done among subjects taking concomitant aspirin. Average SEM was 11. ASA indicates acetylsalicylic acid (aspirin); CAD, coronary artery disease; LTA, light transmission aggregometry; and PAR1, protease-activated receptor-1.

$^{\dagger}0.050<P<0.099$,

$^{*}P\leq 0.05$,

$^{**}P\leq 0.01$,

$^{***}P\leq 0.001$.