Suppression of Arterial Thrombosis Without Affecting Hemostatic Parameters With a Cell-Penetrating PAR1 Pepducin

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Background—Thrombin-dependent platelet activation is heightened in the setting of percutaneous coronary intervention and may cause arterial thrombosis with consequent myocardial necrosis. Given the high incidence of adverse effects in patients with acute coronary syndromes, there remains an unmet need for the development of new therapeutics that target platelet activation without unduly affecting hemostasis. The thrombin receptor, PAR1, has recently emerged as a promising new target for therapeutic intervention in patients with acute coronary syndromes.

Methods and Results—We report the development of a first-in-class intracellular PAR1 inhibitor with optimized pharmacokinetic properties for use during percutaneous coronary intervention in patients with acute coronary syndromes. PZ-128 is a cell-penetrating pepducin inhibitor of PAR1 that targets the receptor–G-protein interface on the inside surface of platelets. The structure of PZ-128 closely resembles the predicted off-state of the corresponding juxtamembrane region of the third intracellular loop of PAR1. The onset of action of PZ-128 was rapid and suppressed PAR1 aggregation and arterial thrombosis in guinea pigs and baboons and strongly synergized with oral clopidogrel. There was full recovery of platelet function by 24 hours. Importantly, PZ-128 had no effect on bleeding or coagulation parameters in primates or in blood from patients undergoing percutaneous coronary intervention.

Conclusions—Based on the efficacy data in nonhuman primates with no noted adverse effects on hemostasis, we anticipate that the rapid onset of platelet inhibition and reversible properties of PZ-128 are well suited to the acute interventional setting of percutaneous coronary intervention and may provide an alternative to long-acting small-molecule inhibitors of PAR1. (Circulation. 2012;126:83-91.)

Key Words: antiplatelet therapy | drug delivery system | platelets | thrombosis | PAR1
the PAR4 thrombin receptor and fibrinogen-dependent hemostasis.\textsuperscript{19} Currently, there are no approved PAR1 inhibitors for the treatment of ACS or other cardiovascular indications. Two PAR1 small-molecule inhibitors, vorapaxar (SCH530348)\textsuperscript{20,21} and atopaxar (E5555),\textsuperscript{22} have been evaluated in phase II trials and have been associated with a reduction in ischemic event occurrence. In the Thrombin Receptor Antagonist Percutaneous Coronary Intervention (TRA-PCI) trial with nonurgent PCI patients\textsuperscript{20} and an accompanying study in patients with non–ST-elevation ACS,\textsuperscript{21} vorapaxar reduced the occurrence of periprocedural myocardial infarction when added to dual-antiplatelet therapy. Similarly, atopaxar significantly reduced early ischemia on Holter monitoring in the Lessons From Antagonizing the Cellular Effects of Thrombin–Acute Coronary Syndromes (LANCELOT-ACS) phase II study.\textsuperscript{21} In the recently completed Thrombin Receptor Antagonist for Clinical Event Reduction in Acute Coronary Syndrome (TRACER) and Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA-2P) phase III trials, vorapaxar was found to significantly reduce the composite end point of death from cardiovascular causes, myocardial infarction, or stroke in ACS patients\textsuperscript{12} and in patients treated chronically for secondary prevention of atherothrombotic events.\textsuperscript{13} However, the limitations of vorapaxar include an extremely long pharmacodynamic half-life of up to 3 weeks and oral administration leading to a slower onset of pharmacodynamic effects during PCI, and an elevated risk of bleeding.\textsuperscript{12,13,20,24} The ability to rapidly and reversibly inhibit PAR1 signaling by a parenteral strategy would be an attractive option in high-risk patients undergoing PCI.

To block thrombin activation of platelets without interfering with the normal hemostatic functions of thrombin, we report a first-in-class intracellular inhibitor of PAR1. PZ-128 is a lipiodated pepducin that targets the cytoplasmic surface of PAR1 and interrupts signaling to internally located G proteins.\textsuperscript{25–29} The structure of PZ-128 was found to mimic the off-state of the corresponding intracellular region of PAR1 that is critical for coupling to G proteins. PZ-128 rapidly and reversibly inhibits PAR1 platelet activation and arterial thrombosis in guinea pigs and primates without affecting bleeding or other coagulation parameters. These data provide support for the further development of PZ-128 as a novel intervention of PAR1-driven arterial thrombosis in patients undergoing PCI.

**Methods**

**NMR Structural Determination of PZ-128**

PZ-128 (palmitate-KKSRALF-NH$_2$) pepducin was synthesized by standard f constructions and purified to 99.1% by reverse-phase high-performance liquid chromatography. NMR samples were prepared by dissolving lyophilized PZ-128 in a buffer comprising 5% glucose-d$_6$, 6.8 mmol/L PZ-128 (final concentration), pH 7.1 with 10% D$_2$O. Samples at acidic pH were prepared by adding perdeuterated acetic acid to 10 mmol/L and adjusting the pH to 4.9. Spectra were collected at 25°C onBruker Avance-600 and AMX-500 spectrometers. Two-dimensional nuclear Overhauser effect spectroscopy and total correlation spectroscopy spectra were collected by using mixing times of 100 ms and 31 ms, respectively. Spectra were assigned with the use of standard methodology, and the distances corresponding to 210 nuclear Overhauser effect measurements were calculated as previously described using Crystallography and NMR System.\textsuperscript{30}

**Human Platelet Aggregation**

In accordance with informed consent procedures approved by the Tufts Medical Center Institutional Review Board, whole blood from healthy donors was collected into a 30-mL syringe containing sodium citrate (0.4% vol/vol final). Platelets were isolated from platelet rich plasma (PRP) by use of Sepharose 2B columns in modified PIPES buffer as described previously.\textsuperscript{17}

**Human ACT Evaluation**

Adult outpatients with angina referred for coronary angiography or PCI were enrolled in the Tufts Medical Center Adult Cardiac Catheterization Laboratory. All patients provided written informed consent before the initiation of the study. The study protocol was approved by the Tufts Medical Center Institutional Review Board. Blood was collected before PCI or angiography, and PZ-128 was spiked into 1-mL samples of whole blood at a range of final concentrations (0–150 μmol/L). Activated clotting time (ACT) was measured immediately in duplicate. To serve as a positive control for elevated ACT, blood was also collected at the end of the PCI procedure from patients who received a weight-adjusted dosage of bivalirudin administered intravenously as a 0.75 mg/kg bolus followed by continuous infusion of 1.75 mg/kg per h during the procedure. Bivalirudin concentrations in plasma were measured by liquid chromatography and tandem mass spectrometry as previously described.\textsuperscript{18}

**Guinea Pig Arterial Thrombosis and Platelet Aggregation**

All guinea pig experiments were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of Tufts University School of Medicine. Male Hartley guinea pigs (150–220 g) were purchased from Charles River Laboratories. A 0.61-mm-diameter catheter was inserted into the left jugular vein of anesthetized animals for administration of infusions of 5% USP dextrose vehicle or PZ-128. A 0.5-V Doppler probe (Transonic Systems, Ithaca, NY) was placed around the right carotid artery to record blood flow. A range of doses of PZ-128 from 0.05 to 1.6 mg/kg in 0.9-mL volumes was delivered at an injection rate of 0.09 mL/min by a Harvard syringe pump. Five minutes after the infusion ended, arterial thrombosis was induced by placing a 5×5 mm$^2$ piece of filter paper soaked in freshly made 20% FeCl$_3$ solution on the right carotid artery 5 mm distal to the probe for 20 minutes. If vessel occlusion did not occur within 60 minutes of injury, the experiment was stopped, and time to occlusion was assigned a value of 60 minutes. To examine possible synergistic effects of PZ-128 and PY212-ADP receptor inhibition, 1 mg/kg clopidogrel was administered by oral gavage 4 hours before FeCl$_3$ injury. In these synergy experiments, the maximum end point was set at 90 minutes for occlusion time. Guinea pigs weighing 600 to 650 g were used for platelet aggregation experiments. PZ-128 (3 or 6 mg/kg) was administered by a 10-minutes intravenous infusion, and blood was collected by cardiac puncture into sodium citrate (0.4% vol/vol final) 5 minutes after cessation of the infusion. PRP was prepared, and Phe(d)-Pro-Arg-chloromethylketone was added to a final concentration of 100 μg/mL. PRP was calcified with 2.5 mmol/L CaCl$_2$ and aggregation was performed as described above.

**Baboon Arterial-Venous Shunt Thrombosis and Platelet Aggregation**

Nonterminal thrombosis and platelet aggregation studies were performed on 12 healthy male baboons (<i>Papio anubis</i>) weighing 9 to 12 kg at the Oregon National Primate Research Center. Protocols were approved by the Institutional Animal Care and Use Committee at Oregon Health and Sciences University. All animals had a chronic exteriorized silicone rubber shunt (arterio-venous shunt) placed between the femoral artery and vein, and arterial thrombosis on Dacron grafts (4-mm diameter) quantified as previously described.\textsuperscript{19} Whole blood (10 mL) was collected into Phe(d)-Pro-Arg-chloromethylketone at a final concentration of 100 μg/mL just before infusion (baseline) and...
15 minutes to 24 hours after the PZ-128 infusion was terminated. Platelet counts and hematocrit were measured immediately. PRP was prepared from whole blood, and platelet aggregation was performed as described above. Bleeding time measurements were performed on the shaved volar surface of the forearm by use of the standard template method as previously described.

Quantification of PZ-128 in Baboon Plasma
Various doses of PZ-128 were infused intravenously for 45 minutes to baboons. At sequential time points, whole blood was drawn into 3.2% citrate buffer and immediately centrifuged at 3000 rpm for 10 minutes. Platelet-poor plasma samples were harvested and stored at -80°C. PZ-128 drug levels in platelet-poor plasma samples were determined with use of an API 4000 LC/MS/MS system (Agilux Laboratories, Worcester, MA).

Prothrombin Time and Activated Partial Thromboplastin Time Measurements in Cynomolgus Monkeys
PZ-128 (0, 3, 10, or 30 mg/kg) was administered intravenously to 2.5- to 4.5-kg male and female cynomolgus monkeys by infusion over 1 hour at MPI Laboratories (Mattawan, MI). Peripheral venous blood was collected from cynomolgus monkeys into K3EDTA anticoagulant at baseline (day -8) and at 2 time points (day 1 and day 5) after daily 1-hour intravenous PZ-128 infusions on days 1 to 4. Prothrombin time and activated partial thromboplastin time were analyzed immediately on a MLA-800 coagulation analyzer.

Data Analysis
Statistical analyses were performed with the use of GraphPad Prism Software, STATA or SAS. Significance of antiplatelet effects in guinea pigs and baboons were assessed by using the nonparametric Kruskal-Wallis test with the Dunn multiple pairwise comparisons or by ANOVA with Bonferroni post test correction. Baboon arterial thrombosis experiments were analyzed by use of a repeated-measures mixed-effects model. Coagulation and hemostasis parameters in baboons and cynomolgus monkeys were compared by Wilcoxon matched-pairs signed rank test or by linear mixed-effects modeling. The null hypothesis was rejected at P<0.05.

Results
Structure and Antiplatelet Activity of PZ-128
From a screen of 57 pepducins derived from the i1, i2, i3, and i4 intracellular loops of PAR1, we identified PZ-128 as a highly efficacious inhibitor of PAR1-dependent platelet aggregation. PZ-128 is a cell-penetrating lipopeptide derived from the juxtamembrane region of the i3 loop and N terminus of transmembrane domain 6 of PAR1 (Figure 1A). This region has been shown to be essential for coupling of PAR1 with associated G proteins. Incorporation of the N-terminal palmitate lipid facilitates rapid and highly efficient translocation of the pepducin across the plasma membrane to the inner leaflet of the lipid bilayer.
of PZ-128 was determined by NMR (Figure 1B), and the peptide was found to form a well-defined α-helix extending from the palmitate lipid. We generated structural models of full-length PAR1 in the off- and on-states by use of the refined x-ray structures of rhodopsin (1HZX) and opsin (1BSS)34 as templates, respectively, for comparison with the NMR-derived structure of PZ-128. PZ-128 was found to form a well-defined β-sheet extending 0.5 α-helix from the palmitate lipid. We generated structural models of full-length PAR1 in the off- and on-states by use of the refined x-ray structures of rhodopsin (1HZX) and opsin (1BSS)34 as templates, respectively, for comparison with the NMR-derived structure of PZ-128. PZ-128 was found to form a well-defined β-sheet extending 0.5 α-helix from the palmitate lipid. We generated structural models of full-length PAR1 in the off- and on-states by use of the refined x-ray structures of rhodopsin (1HZX) and opsin (1BSS)34 as templates, respectively, for comparison with the NMR-derived structure of PZ-128. PZ-128 was found to form a well-defined β-sheet extending 0.5 α-helix from the palmitate lipid. 

PZ-128 Inhibits Platelet Aggregation and Arterial Thrombosis in Guinea Pigs

Aside from humans and other primates, the only other animal species known to harbor PAR1 on their platelets are guinea pigs.14 The PAR1 agonist, SFLLRN, was confirmed to activate guinea pig platelets with an EC50 value of 2.5 μmol/L (Figure 2A). PZ-128 was delivered by internal jugular vein infusions over 10 minutes. At the 15 minutes time point, 3 and 6 mg/kg PZ-128 provided significant, dose-dependent inhibition of ex vivo platelet aggregation to ADP or the thromboxane mimetic, U46119. Individ-ual data points (n=3) are overlaid on bar graphs depicting mean±SD. PZ-128 was delivered by 10 minutes infusion, 5 minutes before initiation of FeCl3 injury. The time at which the blood flow decreased to <0.01 V was recorded as occlusion time of vessels. E, Observed synergistic effect of coadministration of low dose of PZ-128 (0.05 mg/kg) and clopidogrel (1 mg/kg PO 4 hours before start of infusion) on the mean increase of occlusion time over a 90-minute period (n=5). Data in B to D were analyzed by the nonparametric Kruskal-Wallis test with the Dunn multiple pairwise comparison post test. Data in E were analyzed by 2-way ANOVA. *P<0.05, **P<0.01. 

Global probability values were 0.044 for B, 0.33 for C, 0.018 for D, and 0.047 for E.
PZ-128 Inhibits Platelet Aggregation in Baboons

The antiplatelet effects of PZ-128 were next examined in baboons at various time points after receiving different doses of intravenous infusions of PZ-128. Data from baboons showed excellent pharmacodynamic correlations with dose- and time-dependent inhibition of PAR1-induced ex vivo platelet aggregation (Figure 3). At the lowest dose tested, 1 mg/kg PZ-128 (30 minutes infusion), PAR1-dependent aggregation (5 μmol/L SFLLRN) was inhibited by only 5% to 10% at the 1- to 2-hour time points (Figure 3A). At the 3 mg/kg dose (30 minutes infusion), PAR1-dependent aggregation was inhibited by 85% at the 1- and 2-hour time points, but was not appreciably inhibited at the 24-hour time point (Figure 3B). At the 6 mg/kg dose (45 minutes infusion), PAR1-dependent aggregation was inhibited by 100% at 1- to 2-hour time points, 90% at the 6-hour time point, but was completely recovered by 24 hours (Figure 3A). Inhibition of PAR1 by PZ-128 was reversible, as evidenced by loss of inhibition with higher concentrations of SFLLRN agonist (10 μmol/L) at both the 3 mg/kg and 6 mg/kg doses (Figure 3B and 3C). As a further assessment of in vivo specificity, PZ-128 gave no inhibition at any dose of either the ADP or AYPGKF (PAR4) responses at any time point.

Effect of PZ-128 on Baboon Arterial Thrombosis

Baboon arterial thrombosis experiments were conducted to determine whether the PZ-128 pepducin had the potential to inhibit arterial thrombosis in primates. An arterial-venous shunt equipped with a Dacron vascular graft with an internal lumen diameter of 4 mm at a high flow rate of 100 mL/min was used. Thrombogenesis was assessed by measuring platelet-thrombus deposition in the baboon (data not shown). As shown in Figure 4B, the 6 mg/kg IV infusion dose of PZ-128 gave a significant protective effect against arterial thrombus formation in comparison with vehicle (P<0.0028). The effects of the 3 mg/kg dose were not significant but showed a tendency to be protective against arterial thrombosis. These data indicate that PZ-128 can inhibit platelet-dependent thrombus formation in nonhuman primates under conditions of high arterial flow.

Effect of PZ-128 on Hemostatic Parameters in Primates and Blood From PCI Patients

We evaluated whether PZ-128 had any adverse effects on hemostasis or coagulation indices in baboons and monkeys. At all doses tested (1–6 mg/kg), PZ-128 had no effect on...
bleeding time, platelet counts, or hematocrit in baboons (Table 1). PZ-128 was also administered daily for 4 days to adult male and female cynomolgus monkeys with 1 hour intravenous infusions of 3 mg/kg, 10 mg/kg, and 30 mg/kg PZ-128. Coagulation parameters prothrombin time and activated partial thromboplastin time were unaffected in all monkeys at 3 to 30 mg/kg PZ-128 at both day 1 and day 5 in comparison with baseline or vehicle-treated animals (Table 2). No spontaneous, venous access or retinal bleeding was observed in any monkey (n=38) even at PZ-128 plasma levels (Cmax) exceeding 200 μM.

Table 1. PZ-128 Does Not Enhance Bleeding Time in Baboons

<table>
<thead>
<tr>
<th>PZ-128 Dose</th>
<th>Baseline</th>
<th>1–2 h</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets, k/μL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg, n=3</td>
<td>270±39</td>
<td>258±44</td>
<td>0.75</td>
</tr>
<tr>
<td>3 mg/kg, n=5</td>
<td>339±74</td>
<td>334±99</td>
<td>0.88</td>
</tr>
<tr>
<td>6 mg/kg, n=4</td>
<td>286±96</td>
<td>294±80</td>
<td>0.63</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg, n=3</td>
<td>39±3</td>
<td>41±3</td>
<td>0.25</td>
</tr>
<tr>
<td>3 mg/kg, n=4</td>
<td>36±1</td>
<td>39±2</td>
<td>0.13</td>
</tr>
<tr>
<td>6 mg/kg, n=4</td>
<td>36±4</td>
<td>40±4</td>
<td>0.13</td>
</tr>
<tr>
<td>Bleeding time, min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASA+clopidogrel, n=1</td>
<td>5.5</td>
<td>&gt;20</td>
<td>...</td>
</tr>
<tr>
<td>1 mg/kg, n=3</td>
<td>2.8±1.3</td>
<td>3.3±1.2</td>
<td>0.50</td>
</tr>
<tr>
<td>3 mg/kg, n=5</td>
<td>4.4±1.9</td>
<td>4.6±1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>6 mg/kg, n=3</td>
<td>4.0±2.3</td>
<td>3.7±1.6</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Venous blood was collected from male baboons into PPACK anticoagulant (final concentration 100 μg/mL) at baseline and at 1 to 2 hours after the start of the intravenous PZ-128 infusion (1 mg/kg over 15 minutes, 3 mg/kg over 30 minutes, or 6 mg/kg over 45 minutes). Platelet counts and hematocrit were measured by use of a micro-60 automated cell counter (Horiba ABX, Diagnostics, Montpellier, France). Template bleeding time (Surgicutt; ITC, Edison, NJ) was measured at baseline at either 1 or 2 hours after the start of the PZ-128 infusion. Data are reported as mean±SD and P values were determined by Wilcoxon matched-pairs signed rank test. ASA indicates acetylsalicylic acid.

Discussion

Disruption of atherosclerotic plaques and formation of occlusive platelet thrombi remains a leading cause of morbidity and mortality in the United States.7 Antiplatelet therapy thus plays a critical role in preventing arterial thrombosis and myocardial infarction in high-risk patients with ACS and atherothrombotic disease and in patients who have undergone PCI.3–5 PZ-128 is a first-in-class cell-penetrating pepducin inhibitor targeted against the PAR1 receptor–G-protein interface being developed as an antiplatelet agent to be used during coronary interventions. We demonstrated that PZ-128 is a rapid-acting and specific inhibitor of PAR1-dependent platelet aggregation and does not suppress ADP, thromboxane, or PAR4 responses. PZ-128 attenuated PAR1 aggregation and arterial thrombosis in guinea pigs within 15 minutes, and effectively inhibited PAR1 platelet activity and arterial thrombosis in baboons with full recovery of platelet function by 24 hours. The inhibitory effects of the pepducin were fully reversible and overcome by high concentrations of PAR1 agonist even at early time points. PZ-128 had no effect on bleeding or coagulation parameters in baboons and monkeys, or in blood samples from PCI patients.

Current antiplatelet therapy for secondary prevention of vascular events mainly consists of oral administration of aspirin and blockade of the P2Y12 ADP receptor with thienopyridines.8 Patients with a higher risk of thrombosis while undergoing coronary interventions are also often treated with intravenous GP IIb/IIIa antagonists in addition to...
Table 2. PZ-128 Does Not Affect Coagulation Parameters in Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>PZ-128 Dose (mg/kg)</th>
<th>Day −8</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25.9±1.4</td>
<td>25±1.7</td>
<td>23.5±1.1</td>
</tr>
<tr>
<td>3</td>
<td>30.1±2.0</td>
<td>30.5±1.1</td>
<td>28.1±1.4</td>
</tr>
<tr>
<td>10</td>
<td>30.6±2.4</td>
<td>21.3±2.3</td>
<td>29.6±2.9</td>
</tr>
<tr>
<td>30</td>
<td>32.2±2.9</td>
<td>30.9±5.7</td>
<td>33.7±6.5</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26.9±2.8</td>
<td>27.1±2.7</td>
<td>25.5±2.8</td>
</tr>
<tr>
<td>3</td>
<td>27.2±2.2</td>
<td>27.2±2.7</td>
<td>26.8±2.6</td>
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</tr>
<tr>
<td>30</td>
<td>26.5±2.8</td>
<td>26.2±2.8</td>
<td>27.1±1.8</td>
</tr>
</tbody>
</table>

Venous blood was collected from cynomolgus monkeys into K$_3$EDTA anticoagulant at baseline (day −8) and at 2 time points (day 1 and day 5) after daily 1-hour intravenous PZ-128 infusions on days 1 to 4. PT and aPTT were measured by using a MLA-800 coagulation analyzer. Data are reported as mean±SD. *P* values were not significant as determined by repeated-measures linear mixed-effects modeling. aPTT indicates activated partial thromboplastin time; PT, prothrombin time.

aspirin, thienopyridine, and heparin. Although dual-antiplatelet therapy has been shown to attenuate ischemic event occurrence during ACS and PCI, drug response variability, the persistent occurrence of ischemic events, and the increased risk of bleeding events remain major concerns. Notably, treatment with the most potent P2Y$_{12}$ receptor blockers are associated with only a 16% to 19% relative risk reduction in comparison with clopidogrel, and ~10% of patients still experience recurrent ischemic events within 1 year of treatment. The latter observations indicate that there may be a ceiling effect in a strategy solely using aspirin and P2Y$_{12}$ receptor inhibition in the attenuation of platelet-mediated ischemic event occurrence. A current hypothesis is that persistent ischemic events in the presence of P2Y$_{12}$ blockade and aspirin are due to thrombin and collagen-mediated platelet activation under high arterial shear that is unchecked by currently available agents. PZ-128 was found to be effective at inhibiting both thrombin-induced PAR1 activation and collagen-initiated arterial thrombosis in guinea pigs triggered by FeCl$_3$ injury. Moreover, the PAR1 pepducin PZ-128 acts in synergy with the P2Y$_{12}$ antagonist, clopidogrel, to significantly inhibit arterial thrombosis in guinea pigs.

Figure 5. PZ-128 does not affect activated clotting time of blood from PCI patients. PZ-128 (○) was spiked at various concentrations (0–150 μmol/L) into fresh whole blood obtained from patients just before PCI. By comparison, blood was obtained at the 30-minute time point from PCI patients (n = 22) after a weight-adjusted dosage of bivalirudin (●) administered as a 0.75 mg/kg IV bolus followed by continuous infusion of 1.75 mg/kg per h during the procedure. ACT assays were performed immediately by use of a Hemochron 801 with FTCAS10–4 ACT cartridges containing silica, phospholipids, and diatomaceous earth (kaolin). ○, the mean (±SD) ACT and mean bivalirudin concentration at the 30-minutes-time point in the 22 PCI patients.

Contrary to potent thrombin (eg, bivalirudin, hirudin, argatroban, dabigatran), or factor Xa inhibitors (rivaroxaban, apixaban), fully reversible PAR1 inhibitors do not directly affect coagulation, and increased bleeding should not accompany their use, an observation that is consistent with our studies in nonhuman primates. Because thrombin-dependent fibrin generation is unaffected by inhibition of PAR1 and reversible PAR1 antagonists can be overcome by robust hemostatic thrombin generation, a thrombin-receptor antagonist may provide a safer therapeutic index than a thrombin or Xa inhibitor in preventing arterial thrombosis. Likewise, the PZ-128 pepducin had no adverse effects on bleeding, coagulation, or clotting time in nonhuman primates and human blood samples. In recent studies, PZ-128 did not impact initial platelet adhesion to exposed collagen surfaces, but prevented large occlusive thrombi from forming. Taken together, these findings support the notion that PAR1 inhibitors such as PZ-128 can permit the formation of an initial platelet-fibrin monolayer necessary for control of hemostasis, but still block pathological thrombus propagation that occurs at the site of endothelial denudation.

It was notable that the highly potent PAR1 small-molecule inhibitor, vorapaxar, was recently shown to significantly increase the rate of moderate and severe bleeding in both ACS patients and in patients being treated for secondary prevention of atherothrombotic events. Two possible explanations for the elevated bleeding include: (1) the extremely long pharmacodynamic effect of vorapaxar that significantly inhibits platelet function for up to 3 weeks (plasma half-life of 5–11 days) with a single loading dose; and (2) vorapaxar was administered daily for a median time of 1 to 2.5 years in combination with both aspirin and a P2Y$_{12}$
inhibitor. 12, 13 In a subgroup analysis of TRACER, it was found that vorapaxar did not increase the hazard of Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) moderate or severe bleeding in the patients who did not receive a thienopyridine. 12 Therefore, it is likely that concomitant blockade of P2Y12 and thromboxane receptors along with PAR1 may also contribute to the observed bleeding risk in the ACS patients. A much shorter-acting and reversible PAR1 antagonist such as PAR-128 (plasma half-life of 50–80 minutes) is expected to help mitigate any untoward periprocedural bleeding in the context of dual-antiplatelet therapy. Moreover, small-molecule inhibitors such as vorapaxar and atopaxar interact with the ligand binding site on the extracellular surface of the receptor. By comparison, PAR-128 works by an entirely different mechanism of action on the inner surface of the lipid bilayer where it modulates the interactions of PAR1 with intracellular G proteins. 44, 45 The structure of PAR-128 was found to closely resemble the predicted off-state of the corresponding juxtamembrane region of the third intracellular loop and helix 6 region of PAR1, consistent with a mechanism whereby PAR-128 may stabilize or mimic the off-state of PAR1. Intervention of PAR1-dependent platelet activation with the PAR-128 pepducin thus represents an entirely new therapeutic strategy for suppressing arterial thrombosis that could potentially benefit PCI patients being treated for severe atherothrombotic heart disease.

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Disclosures
Drs Kuliopulos and Covic are scientific founders of Anchor Therapeutics.

References


**CLINICAL PERSPECTIVE**

Antiplatelet therapy is of paramount importance in the effective treatment of patients with acute coronary syndrome and those undergoing percutaneous coronary intervention. Thrombin is the most potent platelet activator. The thrombin receptor PAR1 has emerged as an important new therapeutic target to inhibit platelet function in patients with acute coronary syndrome undergoing percutaneous coronary intervention. We describe the development of PZ-128, a first-in-class PAR1 inhibitor that targets the cytoplasmic loops of the receptor. PZ-128 rapidly suppressed PAR1-induced platelet aggregation and arterial thrombosis in guinea pigs and baboons and was synergistic to oral clopidogrel. PZ-128 did not affect bleeding or coagulation in nonhuman primates or in blood from patients undergoing percutaneous coronary intervention. Platelet function returned to baseline 24 hours after PZ-128 infusion. PAR1 inhibition by PZ-128 appears to be a novel promising therapy for patients with acute coronary syndrome. Planned clinical trials will establish where this novel class of medication fits into our therapeutic armamentarium.

Zhang et al Pepducin-Based Suppression of Arterial Thrombosis 91
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