Preventing Platelet Thrombosis with a PAR1 Pepducin

Running title: Abdel-Latif et al.; PAR1 pepducin as anti-platelet therapy

Ahmed Abdel-Latif, MD and Susan S. Smyth, MD, PhD

Division of Cardiovascular Medicine, Gill Heart Institute, the Lexington VA Medical Center, University of Kentucky, Lexington, KY

Address for Correspondence:
Susan S. Smyth, MD, PhD
Professor of Medicine
Division of Cardiovascular Medicine
The Gill Heart Institute
University of Kentucky
900 S. Limestone Street, 321 CTW
Lexington, KY 40536-0200
Tel: 859-218-0121
Fax: 859-257-6060
Email: SusanSmyth@uky.edu


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Platelet-mediated arterial thrombosis, such as occurs following atherosclerotic plaque rupture, erosion, or percutaneous coronary interventions (PCI), is the underlying cause for most myocardial infarctions and many ischemic strokes. Platelets adhere to damaged blood vessels, aggregate with one another, and facilitate the generation of thrombin, which in turn makes fibrin. Thrombin is also a highly potent stimulator of platelets, and shear stress in settings such as PCI may lead to peri-procedural complications due to thrombin-dependent platelet activation.

Human platelets possess two main thrombin G-protein coupled receptors (GPCR), protease activated receptor (PAR) 1 and 4 (Figure 1), which when cleaved by thrombin, trigger a host of intracellular signaling events resulting in secretion of granule contents including ADP, production of thromboxane A2 (TXA2), and activation of the platelet fibrinogen receptor integrin αIIbβ3 (GPIIb/IIIa). In the setting of acute coronary syndrome and PCI, antiplatelet therapy for secondary prevention of vascular events consists of aspirin, to reduce TXA2 production, P2Y12 antagonists to block the effects of ADP, and GPIIb/IIIa inhibitors. However, despite the use of these therapies, the rate of ischemic events remains high. Furthermore, this approach is estimated to prevent only ~15-17% of lethal cardiovascular events with a ceiling effect. The ability of thrombin to activate platelets in the presence of aspirin and P2Y12 antagonists may explain some of the residual risk. Thus, strategies that target thrombin signaling in platelets have been the focus of considerable attention.

On human platelets, PAR1 serves as a high-affinity thrombin receptor. In multiple animal studies and pre-clinical trials, PAR1 has emerged as a viable therapeutic target for inhibiting platelet activity. In the current issue of Circulation, Zhang and colleagues present data on a selective intracellular, reversible PAR1 receptor inhibitor, PZ-128. PZ-128, a “pepducin”, is a cell-permeant peptide fragment homologous to a region in PAR1 that couples to
G proteins. By anchoring in the cell membrane and disrupting the interactions of PAR1 and its G-proteins, PZ-128 prevents thrombin-induced platelet activation. In the current work, Zhang et al. demonstrate the clinical utility of the pepducin approach in reducing arterial thrombosis by targeting PAR1. Administration of PZ-128 to guinea pigs, which also possess platelet PAR1 receptors, effectively blocks ex vivo thrombin-mediated platelet aggregation without affecting responses to ADP or TXA₂. PZ-128 delays arterial thrombosis in a ferric chloride model, and sub-therapeutic doses of PZ-128 and clopidogrel work synergistically. In non-human primates, PZ-128 dose dependently inhibits platelet aggregation rapidly and reversibly, with recovery of platelet function by 24 hour of discontinuation of PZ-128. As might be expected based on its mechanism of action, PZ-128 does not alter coagulation parameters in non-human primates or in blood samples from humans undergoing elective PCI, nor does it prolong bleeding time when administered to animals. Taken together, these findings validate PZ-128 and intracellular blockade of PAR1 as an effective approach for preventing atherothrombosis that may come without the additive bleeding risk associated with targeting thrombin’s coagulant actions.

The results by Zhang et al are important for two reasons. First, they suggest that the pepducin approach to inhibiting GPCR works in animal models of disease, which opens the door for directed targeting of a wide variety of receptor – G protein signaling pathways. Second, given the high incidence of ischemic events in patients with acute coronary syndromes and following PCI, PZ-128 may be an attractive candidate drug to inhibit thrombin-induced platelet activation. Studies in animals and humans have demonstrated a protective effect of adding PAR1 receptor inhibitors to standard-of-care antiplatelet agents in reducing the rates of atherothrombotic events. Two novel PAR1 receptor antagonists, vorapaxar (SCH530348) and atopaxar (E5555), have been studied in phase II clinical trials in patients undergoing PCI and
those with ACS\textsuperscript{9,11}; results of phase III trials of vorapaxar have recently been published. The Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA-2P)-Thrombolysis in Myocardial Infarction (TIMI) 50\textsuperscript{12} trial examined the ability of vorapaxar to reduce major cardiac events in patients with a history of myocardial infarction, ischemic stroke or peripheral vascular disease. Vorapaxar reduced the composite primary and secondary endpoints of death from cardiovascular causes, myocardial infarction, recurrent ischemia requiring revascularization, and stroke at 3 years follow up. However, the risk of bleeding, particularly intracranial hemorrhage, was significantly higher in the vorapaxar group, leading the data safety and monitoring board to recommend that patients with a history of stroke be removed from the study. In the Thrombin receptor Antagonist for Clinical Event reduction in Acute Coronary Syndrome (TRACER) trial, the addition of vorapaxar was compared to standard of care therapy in 12,944 patients with acute coronary syndrome failed to significantly alter the primary endpoint (a composite of death from cardiovascular causes, myocardial infarction, stroke, recurrent ischemia with rehospitalization, or urgent coronary revascularization), but did lower the composite of death from cardiovascular causes, myocardial infarction, and stroke. Use of vorapaxar came at a cost of increase in moderate and severe bleeding and intracranial hemorrhage, resulting in no net clinical benefit with efficacy and safety data combined\textsuperscript{12,13}. With the growing arsenal of antiplatelet therapies, an improved understanding of the mechanisms responsible for platelet activation and impact of genetics and environmental factors may be necessary to provide adequate protection in high-risk clinical scenarios such as acute coronary syndromes and PCI. A combination of therapies that provide effective and complimentary platelet inhibition without increased bleeding risk need to be identified. The preclinical data suggest a strategy of low dose PZ-128 in combination with P2Y12 antagonism
could possibly meet this need. While the data regarding PZ-128 is promising, the clinical trials with vorapaxar call for careful examination of the clinical utility of PAR1 inhibitors. Additionally, it is still not clear if the pepducin approach will have off-target effects, for example by altering G-protein coupling with other receptors. Future in vivo and clinical studies examining PZ-128 in at-risk individuals will undoubtedly shed light on its potential therapeutic role and its interaction with the current anti-platelet and anti-coagulant drugs. Regardless of its clinical fate, PZ-128 may ultimately stand out as a landmark drug that opened the door for pepducin-targeted therapy in human health and disease.

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**References:**


**Figure Legend:**

**Figure 1.** Platelet activation pathways, the role of thrombin receptors. In humans, protease-activated receptors (PAR)-1 and PAR-4 are coupled to intracellular signaling pathways through molecular switches from the Gi, G12, and Gi protein families. When thrombin (scissors) cleaves the amino-terminal of PAR-1 and PAR-4, several signaling pathways are activated, which result in ADP secretion. By binding to its receptor, P2Y12, ADP activates additional Gi-mediated pathways. Ca2+ = calcium; CalDAG-GEF1 = calcium and diacylglycerol-regulated guanine-nucleotide exchange factor 1; GP = glycoprotein; IP = prostacyclin; PKC = protein kinase C; PLC = phospholipase C; RIAM = Rap1-GTP interacting adapter molecule.
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