Clinical Implications of the Contrasting Effects of In Vivo Thrombin Receptor Activation (Protease-Activated Receptor Type 1) on the Human Vasculature*

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Thrombin is the ultimate protease in the coagulation cascade whose pleiotropic actions can lead to thrombosis after tissue injury. Thrombin is the key effector of the coagulation cascade and converts fibrinogen to fibrin, which is essential for laying the meshwork for clot formation. In addition, thrombin displays a diverse range of effects in vascular cells that functionally connects tissue damage to both hemostatic and inflammatory responses (1,2). The majority of the cellular effects of thrombin are initiated via activation of a family of protease-activated receptors (PARs), which are coupled to heterotrimeric G proteins. The PARs are unique among G protein-coupled receptors (GPCRs) in that they are activated by proteases through proteolytic generation of a tethered ligand (3–5). Thrombin cleaves at the conserved arginine residue of the N-terminus of PAR-1, -3, and -4 to unleash the tethered ligand that activates the receptor. Specific activation of PARs can be achieved by synthesizing peptides that mimic the tethered ligand for each of the PARs.

Similar to the vascular effects of many other GPCRs, thrombin has been known to cause endothelium-dependent arterial vasodilatation since the early 1980s (6,7). Since PAR-1 was first cloned in 1991, multiple groups have established that thrombin activation of PAR-1 promotes arterial vasodilatation in mammals and activation of platelets ex vivo. However, the clinical applicability of many of these prior studies is questionable because the human studies examining arterial vasoreactivity were conducted in vitro using isolated coronary rings. Furthermore, the in vivo studies showing PAR-1 activation of arterial vasodilatation were conducted in dogs or rodents. Recently, Newby et al. (8) showed for the first time that infusion of a PAR-1 activating peptide in normal human brachial arteries produces arterial vasodilatation, vasoconstriction, activation of platelets, and release of tissue-type plasminogen activator (t-PA). This investigation established that in vivo activation of PAR-1 caused these diverse, powerful effects in the human forearm vasculature. Although these studies were quite provocative and convincing, the cautious interpretation of these findings requires consideration of the possibility that PAR-1 activation of platelets releases vasoactive substances that account for the observed alterations in arterial and venous vasoreactivity.

In this issue of the Journal, Gúnmundsdo´ttir et al. (9) confirm and extend their observations describing the selective effects PAR-1 activation in vivo in humans. The endothelium regulates the tone of vascular smooth muscle via production of endogenous vasodilators. The endothelium-derived relaxing factors (EDRF) include nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin. Gúnmundsdo´ttir et al. (9) show that PAR-1–dependent arterial vasodilatation is dependent on the endothelium, because inhibition of NO and EDHF nearly completely attenuated the vasodilator response (Fig. 1). Importantly, inhibition of prostacyclin production with aspirin did not alter PAR-1–mediated arterial vasodilatation. In contrast, denudation of the venous endothelium failed to influence PAR-1–mediated vasoconstriction. Interestingly, inhibition of NO production augmented PAR-1–mediated t-PA release. This intriguing finding is consistent with prior studies indicating that NO decreases von Willebrand factor (vWF) secretion in cultured endothelial cells (10). Endothelial cells contain elongated endothelial storage vesicles called Weibel-Palade bodies (WPB), which are involved in the regulated secretion of mature vWF (11,12). The contents of WPBs also include premature vWF, P–selectin, and possibly t-PA (13). The vWF facilitates platelet aggregation at sites of vascular injury (14), and P-selectin mediates adhesion of leukocytes to endothelial cells (15). Both of these molecules are linked to endothelial pathology. The vWF has been proposed to be a marker of endothelial cell dysfunction, and elevated levels of vWF predict adverse outcomes in patients with acute coronary syndromes (16,17). Intriguingly, the in vivo activation of PAR-1 in the human forearm model stimulated a rapid and robust release of t-PA, but not vWF (8). These functional differences may have a structural explanation, as it has been reported that t-PA is stored in small, dense vesicles distinct from WPBs (18). This observation is debatable because other studies...
have shown that t-PA is stored in WPBs in human endothelial umbilical vein cells (HUVEC) (19–21). However, we have shown previously that stimulation of PAR-1 in HUVEC cells causes robust release of vWF (21,22). Furthermore, other groups have reported that either thrombin or PAR-1 mediated the release of vWF in a variety of different endothelial cells, including arterial endothelial cells.

Initial studies using the administration of a specific antagonist to PAR-1, designated thrombin receptor antagonists (TRA), have shown promise in patients undergoing percutaneous coronary intervention (PCI). With increasing numbers of PCIs performed in patients with acute coronary syndrome (ACS) (23), bleeding complications are increasingly a major concern in this era of aggressive, multimodality antiplatelet and coagulation therapies. An agent that has the capacity to selectively block the inflammatory and thrombotic effects of thrombin by preventing PAR activation, without altering the protective activated protein C pathway or inhibiting fibrin generation, could potentially have a more desirable risk–benefit ratio. Indeed, the new oral PAR-1 TRA, SCH530348, has not been associated with an increase in minor or major bleeding in patients undergoing PCI when the agent was added to standard antiplatelet therapy (aspirin and clopidogrel). In addition, administration of this TRA has been reported to be associated with a trend (nonsignificant) toward reduced ischemic events. Based on this and other phase II data, the U.S. Food and Drug Administration has granted fast-track designation for a phase III clinical trial powered to test the effectiveness of SCH530348 for the reduction of ischemic events. Whether the inhibition of PAR-1–induced arterial vasodilatation and t-PA secretion has adverse consequences in comparison with the potential beneficial antiplatelet effects is unknown.

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