Research Articles

Small molecule PAI-1 functional inhibitor attenuates vascular smooth muscle cell migration and survival: Implications for the therapy of vascular disease

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ARTICLE INFO

ABSTRACT

Focal points

- Bedside
  Targeted pharmacologic disruption of PAI-1 function with small molecule inhibitors may have general applicability for the treatment of fibroproliferative disorders, in general, and vascular disease, in particular. The development orally compatible drugs would likely simplify delivery and patient compliance.
- Benchside
  It is apparent that PAI-1 is a multi-functional SERPIN, affecting such diverse physiological and pathophysiological processes as smooth muscle cell growth, migration, survival and pericellular proteolysis activity. Clearly, PAI-1 functional blockade has widespread clinical implications that are not restricted to one aspect of the tissue response to injury.
- Industry
  The original small molecule panel of PAI-1 inhibitors derived from a collaboration between basic scientists and, largely, cardiovascular disease-oriented medical chemists as well as pharmaceutical investigators. This collegial relationship is expected to expand as it is evident that PAI-1 inhibition has generally utility in the treatment of cardiovascular diseases, tissue fibrosis (in particular, the renal and pulmonary systems) and cancer.

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1. Introduction: PAI-1 in vascular pathology

Vascular restenosis, the pathologic re-narrowing of a blood vessel after percutaneous coronary intervention, involves increased vascular smooth muscle cell (VSMC) migration, elevated proliferation and decreased VSMC apoptosis [1,2]. Few treatment options for vascular restenosis exist aside from re-catheterization. Among several factors implicated in the pathophysiologic vascular response to injury (balloon angioplasty is one type of trauma), studies in animal models and the available clinical evidence suggest that plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor (SERPIN) superfamily and the major physiologic regulator of the plasmin-based pericellular proteolytic cascade, is perhaps the most prominent. PAI-1 attenuates fibrinolysis and promotes tissue fibrosis by inhibition of the plasminogen→plasmin-generating system (Fig. 1).

Elevated PAI-1 expression is a significant causative factor in vascular disease and a major contributor to the pathophysiology of a number of significant human disorders including diabetes, pulmonary/renal fibrosis, metabolic syndrome, intravascular septic coagulopathy, atherosclerosis and restenosis, particularly in the setting of increased tissue TGF-β1 levels. PAI-1 exerts spatial and temporal control over the integrated processes of pericellular proteolysis and extracellular matrix (ECM) deposition/turnover that impact stromal remodeling, inflammation, cell migration, proliferation and apoptosis, each of which are critical determinants in tissue fibrosis and vascular disease (Fig. 2).

2. PAI-1 structure/function

During the interaction of PAI-1 with its target proteases, the sissile bond in the reactive center loop (RCL) is cleaved by the target protease to form a covalent ester bond between a serine hydroxyl group of the enzyme and a PAI-1 carboxyl group. Upon PAI-1 cleavage, the N-terminus of the RCL inserts into β-sheet A, while the RCL C-terminus forms strand s1C in β-sheet C producing a 70 Å separation of the P1 and P1′ residues, thereby deforming

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Tel.: +1 518 262 5168; fax: +1 518 262 5669.
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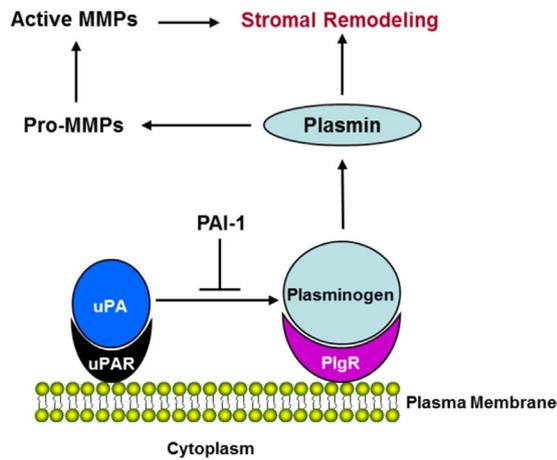**Fig. 1.** The plasmin/matrix metalloproteinsase (MMP) axis in pericellular proteolytic control. Urokinase plasminogen activator (uPA), tethered to its cell surface receptor (uPAR), converts plasminogen receptor (PlgR)-bound plasminogen to the broad-spectrum protease plasmin that, in turn, activates several MMP family members. Collectively, plasmin and MMPs regulate the extent, duration and locale of stromal remodeling.
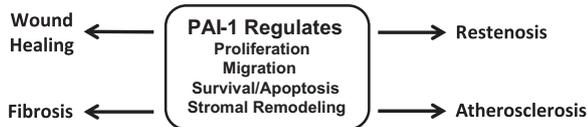
Fig. 2. The repertoire of PAI-1 functions in the context of normal and pathologic tissue injury repair. The findings in animal model systems as well as the available clinical evidence implicate PAI-1 as a critical element in normal and dysfunctional injury repair. Use of small molecule PAI-1 inhibitors has led to the realization that PAI-1 targeting may have therapeutic implications for patients with vascular disease and various fibrotic disorders.

the protease and rendering it inactive. This cleavage also renders PAI-1 anti-proteolytically inactive, giving rise to its designation as a "suicide inhibitor". A substrate form of PAI-1 exists as well in which PAI-1 is cleaved by its target proteases without covalent formation of a PAI-1:protease complex [3,4–6].

Due to the complexity of PAI-1 structure and function, several low-molecular weight antagonists of PAI-1 have been developed to evaluate specific contributions of this SERPIN to disease pathologies (7). Tiplaxtinin (PAI-039), one of the most well-studied small-molecule inhibitors, attenuates asthmatic episodes, hyperlipidemia, hyperglycemia and angiogenesis [7–14]. The specific mechanism by which tiplaxtinin antagonizes the anti-fibrinolytic activity of PAI-1 involves promotion of a substrate-like conformation resulting in PAI-1 cleavage and impaired uPA and tPA inhibition [15,16]. The relative abundance of the different conformational states of PAI-1 within the immediate pericellular microenvironment likely dictates, in fact, whether VSMC migrate, proliferate or undergo apoptosis in response to injury. Indeed, in the context of a spectrum of cardiovascular diseases, it appears that PAI-1 is both pro- and anti-restenotic [17] depending on the nature of the wound model, level of both active or available PAI-1 and vessel TGF-β1 expression. Global PAI-1 deletion in knock-out mice, over-reliance on application of only the full-length, active form of PAI-1 to assess vascular remodeling, and uncertainties as to the role of PAI-1 conformation-dependent processes, however, are major confounders.

3. PAI-1: function beyond protease inhibition

It is increasingly evident that aside from its anti-proteolytic role, PAI-1 is also functions as a multifunctional signaling "ligand" where it impacts cellular responses at the site of injury. All three

forms of PAI-1 (full-length, latent and cleaved) interact with the low-density lipoprotein receptor-related protein 1 (LRP-1) and stimulate JAK/STAT1-mediated VSMC migration [18]. Outcomes, however, are clearly concentration-dependent. Low dose (2 nM), acute exposure (3 h) to cleaved PAI-1 stimulates VSMC migration [18]; chronic exposure (24 h) to high dose (40 nM) cleaved PAI-1 (via application of tiplaxtinin), in contrast, attenuated motility. Since both tiplaxtinin and cleaved PAI-1 stimulate apoptosis after 24 h, chronic exposure to cleaved PAI-1 appears to switch VSMC from the pro-migratory to a pro-apoptotic phenotype.

While full-length, active PAI-1 reduces both spontaneous and stimulated prostate cancer cell apoptosis, latent PAI-1 was unable to rescue neither response. The ability of active PAI-1 to inhibit apoptosis, furthermore, is not due to its urokinase PA (uPA) binding or uPA receptor (uPAR) signaling roles [19] suggesting that LRP-1 may be the more relevant "survival" receptor. Truncated PAI-1 (PAI-1$_{23}$), a mutant with deletions in much of the heparin-binding domain and the RCL, also stimulated endothelial cell apoptosis [20,21]. It appears that an intact RCL is required for the pro-survival function of PAI-1 and disruption of this structure promotes an apoptotic phenotype. This is consistent with findings that cleaved PAI-1 stimulates VSMC apoptosis. Indeed, unpublished recent data from this laboratory clearly indicate that application of an elastase-cleaved PAI-1 promotes VSMC apoptosis, both with and without additional death stimuli (e.g., chemotherapeutic drugs), while tiplaxtinin (10 μM), in the presence of recombinant PAI-1 (40 nM), promotes apoptosis to a greater extent than tiplaxtinin alone. Since 20 μM tiplaxtinin completely cleaves 400 nM PAI-1 in 30 min, it is likely under the latter conditions (24 h) tiplaxtinin effectively cleaves both exogenous recombinant and endogenous PAI-1 pools.

Importantly, elastase levels increase immediately after balloon angioplasty, peak at one week and then decline [22] suggesting that lowered elastase activity levels, might contribute to a decrease in cleaved PAI-1 and, therefore, reduced apoptosis and increased VSMC persistence. As elastase cleaves PAI-1 at the peptide bond between Val^{343}–Ser^{344} (P4–P3) and, whereas, tiplaxtinin promotes substrate behavior of PAI-1, allowing for uPA/tPA protease attack at Arg^{346}–Met^{347} (P1–P1′) [23], cleavage at either of these sites may be sufficient for promotion of apoptosis. Tiplaxtinin could be a useful therapeutic option in the context of elevated vessel uPA and PAI-1, as it promotes a substrate-like form of PAI-1, a uPA target [24–30].

4. Conclusions

In the absence of PAI-1, the increase in active plasmin releases a pro-apoptotic Fas ligand that stimulates endothelial cell death [31]. Unlike endothelial cells, however, VSMC are relatively resistant to Fas ligand-stimulated apoptosis [32] suggesting that, when cleaved, PAI-1-stimulated VSMC apoptosis, is likely to be independent of Fas. Interestingly, PAI-1 expression is stimulated by both TNF-α [33,34] and tumor necrosis factor-like weak inducer of apoptosis (TWEAK) [35]. Furthermore, full-length PAI-1 binds to, and inhibits, caspase-3 in TNF-α-treated cells [36], implicating PAI-1 as a VSMC pro-survival factor in response to and dependent on members of the TNF-α-superfamily. Importantly, both tiplaxtinin and elastase-cleaved PAI-1 down-regulate TWEAK, NF-κB, and TNFSR1b, (a TNF-α-binding receptor) transcripts in VSMC. Cleaved PAI-1 might potentiate VSMC apoptosis by deregulating these pro-survival signaling pathways [37–40] inducing death receptor-initiated apoptosis and the executioner caspase cascade. Collectively, these data suggest that PAI-1 over-expression is both a causative factor in vascular and non-vascular fibrosis as well as a biomarker of cardiovascular disease mortality. The adaptation of PAI-1 function-disrupting strategies may have

eventual translational utility for the treatment of the pathophysiology of vascular disorders, thrombosis and tissue fibrosis. Targeted manipulation of the underlying PAI-1-dependent pro-survival pathways, with function-blocking PAI-1 mutants or small molecule pharmacologic inhibitors, is likely to have significant clinical implications in the context of PAI-1-dependent vascular pathologies.

Acknowledgments

Executive summary

- Among factors implicated in the tissue response to injury, plasminogen activator inhibitor-1 (PAI-1), the major negative regulator of the plasminogen→plasmin-generating cascade, is perhaps the most pathophysiologically-relevant.
- PAI-1 exerts spatial and temporal control over the integrated processes of pericellular proteolysis and matrix turnover that, collectively, impact stromal remodeling, the inflammatory response, cellular migration, proliferation and apoptosis; each of which are critical determinants in tissue fibrosis and vascular disease.
- Targeted blockade of PAI-1 function with small molecule inhibitors effectively inhibits the initiation and progression of pulmonary, renal, vascular and cutaneous fibrotic disease.
- Tiplaxtinin is one of the most well-studied small-molecule PAI-1 inhibitors. Tiplaxtinin attenuates asthmatic episodes, hyperlipidemia, hyperglycemia and angiogenesis.
- The specific mechanism by which tiplaxtinin antagonizes the anti-fibrinolytic activity of PAI-1 involves promotion of a substrate-like conformation resulting in PAI-1 cleavage and impaired uPA and tPA inhibition, and increased vascular smooth muscle cell apoptosis.
- Development of PAI-1 function-disrupting strategies may have clinical utility for the treatment of the pathophysiology of vascular disorders, thrombosis and tissue fibrosis.

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