Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease

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Summary

Although plasminogen activator inhibitor 1 (PAI-1) is one of the primary regulators of the fibrinolytic system, it also has dramatic effects on cell adhesion, detachment and migration. PAI-1 also differs from other serine protease inhibitors (serpins) in that it is a trace protein in plasma, it has a short half-life in vivo, its synthesis is highly regulated, and it binds to the adhesive glycoprotein vitronectin (VN) with high affinity and specificity. These unique and diverse properties of PAI-1 probably account for the many observations in the literature that correlate abnormalities in PAI-1 gene expression with a variety of pathological conditions. In this review, we discuss the discovery, origin, properties and regulation of PAI-1, and then speculate about its potential role in vascular disease, fibrosis, obesity and the metabolic syndrome, and cancer.

Keywords

PAI-1, vascular disease, fibrosis, obesity, cancer

Background

Cells regularly invade and migrate through tissues during embryogenesis, angiogenesis, cancer, and a number of other normal and pathological processes. Much of this movement occurs through quite formidable tissue barriers, and is thus dependent upon the action of highly regulated and specific proteinases that can locally digest tissue proteins without the widespread damage associated with, for example, chronic inflammatory disease. This “targeted” proteolysis is accomplished in part by the plasminogen activation system, a versatile, temporally controlled enzymatic cascade that, when activated, generates locally expressed extracellular proteolytic activity (1–3). Activation of this system is initiated by the release of two plasminogen activators (tissue type, tPA; urokinase-like, uPA) from cells in response to signals (e.g., growth factors, cytokines, hormones) liberated during tissue remodeling, inflammation, and thrombosis, and it leads to the formation of the broadly acting, trypsin-like protease, plasmin. Remarkably, this same enzyme system in blood functions to maintain vessel patency by specifically removing fibrin deposits from the vasculature (fibrinolysis) (4). This diversity of function suggests that the plasminogen activating system is broadly used in human physiology, and that PA activity must be precisely regulated. This regulation is achieved primarily at the level of PA inhibitors (PAIs) which prevent the escape of this potentially destructive protease system. Although five molecules with PAI activity have been described, PAI-1 appears to be the primary inhibitor of plasminogen activation in vivo (5).

Discovery

The early days in the PAI field were at times difficult and confusing (6–8). For example, although numerous tissues and cells were reported to produce PAIs, available assays could not, in general, distinguish between these inhibitors and plasmin inhibitors, and it was not clear whether there was one or multiple PAIs. Another confounding problem in the literature at the time was the idea that PAIs were not even necessary since the formation of fibrin itself was thought to be sufficient to regulate tPA activity. The field began to solidify in the early 1980s with the description of assays specific for PAIs, and it grew explosively after the purification of PAI-1 and the development of specific antisera to it (9), and the cloning of PAI-1 cDNA (10–13). These successes led to the realization that there were actually four molecules that could inhibit PAs in vitro, including PAI-1, PAI-2, PAI-3, and protease nexin 1 (PN-1). Subsequent studies revealed that PAI-1 (previously referred to as the fast acting inhibitor, the endothelial cell inhibitor, and the β-migrating endothelial inhibitor) is the primary physiological inhibitor of tPA and uPA in vivo. Although PAI-2 (the placental inhibitor) also inhibits uPA and 2 chain tPA, it is a poor inhibitor of single chain tPA.
tPA and it is mainly an intracellular protease inhibitor. The primary substrates for PAI-3 and PN-1 are probably activated protein C and thrombin, respectively. Recently, a fifth molecule, neuroserpin, was shown to regulate tPA activity in the brain (14). These observations suggest that there are only two inhibitors that function to regulate plasminogen activation in vivo, PAI-1 and neuroserpin.

**Origin**

The origin of circulating PAI-1 under normal and pathological conditions remains the source of speculation. Human studies suggest that the liver may be a primary source (15, 16). However, PAI-1 is an acute phase response gene (17), and it is thus virtually impossible to obtain normal, non-stressed/diseased human tissues. In this regard, mouse studies indicate that the concentration of PAI-1 mRNA is relatively low in the liver, but is high in the heart, lung, aorta, muscle, and adipose tissue (18). In spite of the low concentration of PAI-1 mRNA in the liver, its relatively large size emphasizes that its total biosynthetic capacity may still be high. Moreover, PAI-1 gene expression in the liver is dramatically up-regulated by endotoxin and a variety of inflammatory mediators (18, 19). Thus, it seems likely that following major surgery/trauma, and in sepsis, stress and inflammatory disease, the liver may be an important source of circulating PAI-1. PAI-1 was also detected in adipocytes, and PAI-1 mRNA is up-regulated in the adipose tissues of obese mice and humans (20). In obesity, the adipose tissue may be the largest organ in the body, and thus the major source of circulating PAI-1. Although platelets store high amounts of PAI-1 (21, 22), they do not seem to contribute to plasma PAI-1 levels under normal conditions. However, during thrombosis, the release of PAI-1 from activated platelets may increase PAI-1 levels significantly, thus stabilizing newly formed thrombi against premature lysis and contributing to the known resistance of platelet-rich thrombi to thrombolytic agents (23). Another source of PAI-1 may be the vasculature (24). In this regard, the observation that PAI-1 can be detected in most tissues and in a variety of cultured endothelial cells, initially suggested that it was produced by the endothelium. However, in situ hybridization and immunohistochemistry experiments failed to detect PAI-1 in the endothelium of untreated control mice (24). These studies indicated that PAI-1 was expressed in relatively high levels in vascular and non-vascular smooth muscle cells present in multiple tissues. Interestingly, endotoxin-induced PAI-1 mRNA in endothelial cells at all levels of the vasculature, from larger arteries to veins and capillaries, including those vessels within different tissues (24, 25). These data suggest that the alterations in plasma PAI-1 levels observed during sepsis may result, at least in part, from increased biosynthesis of PAI-1 by endothelial cells. However, it should be noted that endotoxin (lipopolysaccharide; LPS) also induces PAI-1 mRNA in murine hepatocytes and adipocytes (25, 26).

**Properties**

PAI-1 is a single-chain glycoprotein with a molecular weight of 50,000 Da, and it belongs to the serpin gene family (5, 27). The reactive centre of the inhibitor (Arg_{146}/Met_{347}) is contained within a strained loop region at the carboxy terminus of the molecule, and serves as a pseudosubstrate (“bait”) for the target serine protease. The inhibition of PAs by PAI-1 occurs in a rapid and stoichiometric manner, and the inhibitor is consumed in the process, giving rise to the term “suicide inhibitor”. The second-order rate constant for the interaction of PAI-1 with these PAs is approximately 3.5x10^{7} M^{-1}s^{-1} which is somewhat higher than those of PAI-2 and neuroserpin, and at least two orders of magnitude higher than that of PAI-3 and PN-1.

PAI-1 has been detected in two different activity states in vivo (active and latent), and conformational changes in the reactive centre loop (RCL) of the inhibitor seem to determine this state (28–30). In the active form, the RCL is exposed on the surface of the molecule and is able to interact with its target proteinases. This form is synthesized and secreted by cells but is unstable in solution and spontaneously converts into the inactive (latent) form (i.e., the RCL inserts into β sheet A) with a half-life of about 1–2 hours at 37°C (29, 31, 32). The latent form can be converted into the active form by treatment with denaturants (28) or negatively charged phospholipids (33). The fact that negatively charged phospholipids are expressed on the surface of activated platelets led to early speculation that the latent form of PAI-1 may be a “pro-inhibitor”. However, there is no data to support this idea, suggesting that the latent form simply reflects the normal biological instability of the inhibitor, perhaps because it lacks stabilizing disulfide bonds. Although the majority (approximately 80%) of PAI-1 in human platelets is latent, a sufficient amount of active PAI-1 is still released from stimulated platelets to inactivate tPA and prevent premature clot dissolution (23, 34). In this regard, all active PAI-1 in plasma seems to circulate complexed to VN (35, 36), and this interaction not only stabilizes the inhibitor (increasing its half-life by 2–10-fold), but also may alter its specificity (37). Thus, VN may be a cofactor for PAI-1. The high affinity binding site for PAI-1 in VN has been mapped to the somatomedin B domain (38). Although two additional forms of PAI-1 have been detected in vitro (i.e., the ‘cleaved’ form and the ‘substrate’ form (39), the biological significance of these forms, if any, remains to be demonstrated. Taken together, these considerations suggest that the active form of PAI-1 is the only biologically significant form in vivo. Interestingly, active PAI-1 resembles α_{1}-antitrypsin in that it is rapidly inactivated by oxidizing agents including oxygen radicals liberated by activated neutrophils or other cells (40). This sensitivity to oxidants provides a potential mechanism to regulate PAI-1 activity at sites of inflammation. Complexes between the PAs and PAI-1 are efficiently cleared by the low density lipoprotein receptor related protein (LRP) (41), and the primary site of clearance in vivo may be the liver.

**Gene expression**

Although physiological plasma levels of PAI-1 are low (6–80 ng/ml), its short half-life in blood (~10 min) and its ability to be rapidly induced in plasma suggest a high biosynthetic rate. In this regard, a variety of cultured cells (e.g., endothelial cells, adipocytes, smooth muscle cells, hepatocytes, granulosa cells, epithelial cells, megakaryocytes, fibroblasts and several tumour cell lines) can synthesize PAI-1, and the production of the inhibitor by these and other cells can be induced by a variety of growth factors, cytokines and hormones (24, 27, 42). These observations suggest that PAI-1 may be one of the most highly
regulated components of the fibrinolytic system, and that its expression by cells is under strict local control. As already mentioned, endotoxin, a component of the cell wall of gram-negative bacteria that causes sepsis, is a strong inducer of PAI-1 production both in vitro and in vivo in a variety of cultured cells (27, 42).

The effects of LPS are mediated through the release of cytokines from inflammatory cells, and these molecules represent another group of important regulators of PAI-1 gene expression. For example, tumor necrosis factor alpha (TNF-α), which has been implicated in septic shock and hemorrhagic necrosis of certain tumors, induces PAI-1 expression in the same tissues of the mouse as does LPS (18). Interleukin-1 (IL-1) is another mediator of the immune response, and it can also stimulate PAI-1 gene expression in endothelial cells, adipocytes, hepatocytes, etc., both in vitro and in vivo (43, 44). Growth factors such as transforming growth factor-β (TGF-β) also play a role in the regulation of PAI-1 (18). In fact, the very high sensitivity of the PAI-1 gene to TGF-β was employed to develop a novel assay to detect low levels of TGF-β in complex biological samples (45). TGF-β is released from activated platelets and it induces PAI-1 in many cell types (18, 46). The release of TGF-β from platelets at sites of vascular injury, inflammation and thrombosis, may induce PAI-1 in surrounding endothelial cells and thus suppress the fibrinolytic system of the vessel wall. A number of other factors can induce PAI-1 expression in vitro including epidermal growth factor, platelet derived growth factor, basic fibroblast growth factor, insulin, angiotensin II, corticosteroids and others (20, 27, 42).

The regulation of PAI-1 is achieved primarily by alterations in the rate of gene expression (47). The human PAI-1 gene is approximately 12.2 kb in length, it is composed of 9 exons and 8 introns, and it is located on the long arm of chromosome 7. Two distinct PAI-1 mRNA species (approximately 2.3 and 3.2 kb in length) are expressed by human cells, and these differ in the length of their 3’ untranslated regions as a consequence of alternative polyadenylation. The 3’ end of the larger transcript contains an AT-rich sequence which may play a role in the regulation of PAI-1 mRNA stability. The 5’-flanking region of the human PAI-1 gene contains the transcription initiation site, a TATA box, and regulatory sequences that confer transcriptional responsiveness to a variety of mediators including glucocorticoids, TGFβ, VLDL, glucose, angiotensin II, TNFα, and insulin (47, 48).

Changes in plasma PAI-1 levels can be correlated with genetic variations in the PAI-1 promoter (49, 50). Several polymorphisms in this region of the gene have been described including a cytosine-adenine (CA)n dinucleotide repeat polymorphism, a HindIII-restriction-fragment-length polymorphism, and a single nucleotide insertion/deletion (4G/5G) polymorphism 675 bp upstream of the transcriptional start site in the promoter. The 4G allele in the 4G/5G polymorphism appears to promote an increased transcriptional rate compared to the 5G allele, and humans homozygous for the 4G allele present with approximately 25% higher PAI-1 plasma levels than in 5G/5G subjects. Although some studies suggest that the 4G allele is linked to an increased risk for myocardial infarction, other studies were unable to confirm this correlation, and two recent meta-analyses indicated only a weak association. Conflicting results were also reported for a possible link between PAI-1 polymorphisms and the risk for venous thromboembolism. The 4G/4G polymorphism was associated with a poor outcome in severely injured patients after trauma or in patients with meningococcal sepsis compared to the 4G/5G or 5G/5G genotype (51). Unexpectedly, and in contrast to these findings, several studies of PAI-1 polymorphisms and the risk for cerebrovascular events indicate a protective role for the 4G allele despite upregulated PAI-1 levels (52). The protective effect of PAI-1 in the cerebrovascular system might be due to increased plaque stability and neutralization of neurotoxic tPA (14, 53).

In conclusion, current evidence linking genetic variations in the PAI-1 promoter to human diseases are conflicting, possibly because of variations in unknown modifying factors and/or different study designs. Until it can be unequivocally demonstrated that high risk patients will profit from genotype specific therapy, there seems to be little clinical justification for screening thrombotic patients for PAI-1 polymorphisms.

**Cell motility**

Cell migration is a critical component of many normal and pathological processes including wound healing and cancer, and it is clear that regulated changes in the affinity state of adhesion receptors (e.g., integrins and uPAR) are essential for optimal motility (54). It is also clear that this process is influenced by PAI-1 (55–58) and other components of the plasminogen activation system. Although it was originally thought that the proteolytic activity of this system was required for optimal cell migration, recent in vitro studies have raised the possibility that the contribution of this system to cell migration may be far more complex than simple proteolysis. In fact, components of the system seem to influence several steps in the migratory process (e.g., cell attachment, cell detachment), and these effects do not depend upon the activation of plasminogen. These unexpected findings appear to result from direct interactions between components of this system (i.e., PAI-1, uPA, and uPAR) and both integrins and matrix proteins, including VN.

The effects of PAI-1 on cell adhesion are related to the observation that both integrins and uPAR can bind to VN (59, 60), and that the binding of these adhesion receptors can be competed by PAI-1 (55, 61, 62). Interestingly, the somatomedin B domain of VN is the primary high affinity binding site for both PAI-1 and uPAR (63), and it is immediately adjacent to the only RGD sequence (i.e., integrin binding site) in VN. The close proximity of the binding sites for PAI-1 and the two adhesion receptors explains why PAI-1 can competitively inhibit uPAR- and/or integrin-mediated cell attachment to VN, and that it can do so independently of its inhibitory activity towards uPA. In this regard, PAI-blocks the integrin-mediated adhesion of MCF7 cells to VN, it inhibits the uPAR-mediated binding of U937 cells to VN, and it blocks the adhesion of cells which adhere to VN via both receptors (62).

Besides its effects on the adhesion of cells to VN, PAI-1 can also detach cells from a variety of extracellular matrix (ECM) proteins and this adhesive activity does not depend on its interaction with VN (64, 65). However, PAI-1-mediated cell detachment does have an absolute requirement for uPA and uPAR. The urokinase receptor is a glycosyl phosphatidyl inositol (GPI)-anchored protein which was shown to localize uPA on the cell sur-
face at the leading edge of migrating cells (2, 66). Recent studies demonstrate that the binding of uPA to uPAR on cell surfaces leads to conformational changes in the receptor, causing it to bind to matrix-engaged integrins (67–69). This interaction also induces the activation of signalling molecules that are important for cell migration (70, 71). The addition of PAI-1 to these cells leads to the transient formation of PAI-1/uPA/uPAR/integrin complexes which are rapidly internalized into early endosomes via receptors of the LDL-receptor family (64). The end result is the rapid disengagement of the two adhesion receptors from their association with the ECM, and cell detachment. The internalized PAI-1 and uPA are degraded in lysosomes whereas the receptors are recycled back to the surface. Thus, the deadhesion property of PAI-1 actually results from the PAI-1-mediated internalization of adhesion receptors. This model of cycled attachment-detachment-reattachment of integrins is an important feature of the cell migration process and occurs independently of the matrix composition.

Finally, it is clear that cell migration itself is also influenced by PAI-1. For example, the inhibitor impairs the migration of smooth muscle cells by competing with the integrin receptor $\alpha_\beta$, for binding to VN (56). PAI-1 also decreases the migration of other cell types, including endothelial cells, human amnion cells (WISH), epidermal carcinoma cells and human monocytes (57, 72). In contrast to these results, PAI-1 was found to stimulate the migration of rat smooth muscle cells and human fibrosarcoma cells (73). This effect did not depend on the binding of PAI-1 to either VN or uPA, and was associated with the LRP-induced activation of the Jak/Stat signalling pathway. A promigratory effect of PAI-1 was also seen in human breast cancer cells (58, 74) and melanoma cells (75). These apparently conflicting results on migration are reminiscent of the dose-dependent effects of PAI-1 on other cellular properties, with low concentrations being stimulatory and high dosages being inhibitory (27); see “Cancer” below.

In conclusion, PAI-1 can potentially influence cell motility at several levels, and in each case, it does so quite independently of its ability to inhibit plasminogen activation. Thus, it blocks cell adhesion to VN by competing with uPAR and integrins for binding to this adhesive glycoprotein. It detaches cells from a variety of matrix proteins by inducing the internalization of adhesion receptors. Finally, it seems to have a dual effect on cell migration, with low PAI-1 levels inducing migration and high levels inhibiting it. Based on these considerations, it would seem that the net effect of PAI-1 on cell motility will depend on the composition of ECM, the concentration of PAI-1, the levels of expression of adhesion receptors and LRP on the cell surface, and on the relative amounts of tPA and uPA. In the latter case, the PAs bind to PAI-1 and prevent it from binding to VN, and uPA is required for cell detachment.

Pathological consequences of PAI-1

Because regulation of the PA system is of such fundamental importance, workers in the field originally speculated that PAI-1 deficiency would be inconsistent with normal development and haemostasis. It is now clear that this hypothesis is incorrect. Thus, while PAI-1-deficient humans (76) and mice (77) develop a mild hyperfibrinolytic state and a bleeding tendency, they are fertile, develop normally, and lack gross or histological abnormalities. However, a number of recent observations do suggest that alterations in PAI-1 may contribute to the pathogenesis of a variety of disorders, including vascular disease, fibrosis, obesity and the metabolic syndrome, and cancer.

Vascular disease

Alterations in PAI-1 have been implicated in a variety of vascular disorders, from pathologic thrombosis in the blood to abnormal vascular remodeling in the vessel wall itself. We would suggest that these very different pathologies reflect the effects of PAI-1 on the plasma fibrinolytic system on the one hand, and its effects on cells in vascular lesions on the other.

In the case of thrombosis, inhibition of the fibrinolytic system by PAI-1 is expected to promote a procoagulant state because of reduced fibrin degradation and thus increased intravascular fibrin deposition. This hypothesis is supported by both animal and human studies. For example, transgenic mice overexpressing PAI-1 spontaneously developed thrombi in their extremities (78) and coronary arteries (79). Moreover, stress induces both PAI-1 expression and thrombosis in adipose and renal tissues of mice (80), and the lack of PAI-1 seems to protect mice from venous thrombosis induced by endotoxin (77). Finally, PAI-1 (and VN) were shown to be essential for the formation of stable arterial thrombi in a mouse model of carotid artery injury (81, 82). In humans, low fibrinolytic activity is associated with the development of coronary artery disease (83), and individuals with elevated PAI-1 in their blood have an increased risk for thrombosis, including myocardial infarction (17, 84). High circulating PAI-1 levels may precede a first acute myocardial infarction in these patients (49). This observation may be clinically important since PAI-1 levels are subject to circadian regulation and are higher at night than during the day (85). Thus, the PAI-1 mediated decrease in fibrinolytic activity could contribute to the higher incidence of acute cardiovascular complications in early morning hours (86).

A number of other conditions are known to be associated with high PAI-1 levels and an increased risk for thrombosis. For instance, the so-called “postoperative fibrinolytic shutdown” generally observed within 24 hours after major surgery has been attributed to increased PAI-1 activity, with concomitant decreases in t-PA levels (87). Plasma PAI-1 levels also rise after severe trauma (17), during pregnancy (88), and in disseminated intravascular coagulation (DIC) (89), a serious haemostatic complication in critically ill patients that contributes to multiple organ failure. In patients with endotoxemia and sepsis, very high plasma PAI-1 levels were associated with the highest risk for death (90). These studies suggest that PAI-1 may be a useful clinical marker to identify patients who are at particularly high risk for poor outcome in DIC and/or septic shock.

The role of PAI-1 in cerebral ischemia is less well characterized, possibly because the inhibitor may exert some neuroprotective effects in this setting (14, 91). For example, in an animal model of cerebral ischemia, the lack of PAI-1 resulted in a twofold increase in infarct volume. This neuroprotective effect of PAI-1 may be related to the observation that tPA has deleterious effects on ischemia-induced neuronal cell death (92).
In addition to thrombosis, elevated PAI-1 may contribute to the pathogenesis of diseases of the vessel wall itself. The development of vessel disease frequently involves hyperplasia of arterial smooth muscle cells (SMC), the formation of fatty streaks, the development of atheromas, and finally, plaque rupture which in turn causes acute thrombosis and vessel occlusion (93). The involvement of PAI-1 in these processes can be inferred from several observations. First of all, PAI-1 can be expressed by most of the cells that play an active role in the atherosclerotic process (e.g., endothelial cells, smooth muscle cells and macrophages). Secondly, the expression of PAI-1 by these cells can be induced by factors that have been implicated in the progression of atherosclerotic lesions such as TNF-a (94) and oxidized LDL (95). Expression of PAI-1 also was rapidly induced in SMC and EC in the vessel wall following vascular injury in mice and rabbits (81, 96). Finally, levels of PAI-1 mRNA were significantly increased in severely atherosclerotic arteries compared to relatively normal human arteries (97, 98), and in arteries from hypercholesterolemic mice prone to atherosclerosis (99). The amount of PAI-1 seemed to correlate with the progression of atherosclerosis from normal vessels to fatty streaks and plaques (100).

The exact contribution of PAI-1 to vascular remodeling remains speculative. On the one hand, it is known that the extent of neointima formation correlates with the degree of thrombosis (101), and as already mentioned, PAI-1 seems to promote the formation of stable thrombi (81, 82). Thus, PAI-1 may contribute to vascular remodeling through its effects on fibrin deposition/thrombosis. The ability of PAI-1 to regulate cell motility in vitro (see “Cell motility”) raises the additional possibility that the presence of elevated PAI-1 within injured and/or diseased segments of the vessel wall may promote cell migration and neointima formation as well as deposition of extracellular matrix. In this regard, PAI-1 was found to induce neointima formation in different artery injury models (cooper-induced injury, oxidative vascular injury, carotid ligation, balloon injury) (102). In particular, hypercholesterolemic mice prone to atherosclerosis developed enhanced neointimal growth and luminal stenosis after injury and this effect was reduced by the absence of PAI-1 (99, 103). Thus, in these models, neointima formation correlated with the presence of PAI-1, and one recent study showed that PAI-1 can enhance SMC migration in vitro under certain experimental circumstances (104). However, in less severe injury models without a strong thrombotic reaction, the opposite was observed. In this case, the absence of PAI-1 (i.e., PAI-1-/- mice) was associated with enhanced cell migration in the vessel wall (105). Thus, endogenous PAI-1 may inhibit migration under some conditions.

In conclusion, there is evidence from clinical trials to support a role for PAI-1 in thrombotic diseases. Moreover, in vitro cell culture experiments together with in vivo animal studies implicate the inhibitor in remodeling processes of the vessel wall, and suggest that it may be a pathogenic factor in the development of atherosclerosis. However, opposite results regarding the effect of PAI-1 on neointima growth make it difficult to define the exact cellular mechanism by which PAI-1 regulates intramural processes. As discussed in the “Cell motility” section, PAI-1 can regulate cell migration by several mechanisms and the net effect may depend on the composition of the local environment, the presence of receptors and other factors involved in the migratory process, and whether high or low levels of PAI-1 are expressed. All these factors may also play a role in the effect of PAI-1 on neoointimal growth and, in particular, on migration of SMC. Despite numerous clinical and experimental studies on the role of PAI-1 in vascular diseases, it is still too early to conclude that alterations in systemic or local PAI-1 levels represent a useful strategy to treat or prevent vascular diseases.

**Fibrosis**

Fibrosis is characterized by excessive accumulation of extracellular matrix in basement membranes and interstitial tissues. The two main degrading proteases that limit abnormal deposition of matrix are plasmin and the matrix metalloproteinases (MMP). Plasmin itself has been reported to activate some MMPs, and both systems degrade matrix proteins. PAI-1 reduces plasmin formation, and thus may decrease MMP activation as well, thus augmenting the formation of the matrix. This concept is supported by studies of animal models of fibrotic disease which show that PAI-1 is upregulated in glomerulosclerosis induced by hypertension or x-irradiation, in liver fibrosis induced by carbon tetrachloride or spontaneously occurring, and in bleomycin-induced pulmonary fibrosis (106). Perhaps most convincingly, mice deficient for PAI-1 show an attenuated fibrogenic response to ureteral obstruction (107), and they accumulate less fibrin in their lungs and develop less severe fibrosis after administration of bleomycin than normal mice or transgenic mice overexpressing PAI-1 (108). A recombinant, non-inhibitory variant of human PAI-1 that competes with native PAI-1 for binding to VN, was shown to decrease matrix accumulation in a rat model of glomerulonephritis (109). This decrease in the matrix may result from enhanced plasmin formation because of the presence of the non-inhibitory PAI-1, or from the ability of this PAI-1 variant to decrease the migration of inflammatory cells into the region. These cells may stimulate the production of collagen and other ECM proteins. The observations that PAI-1 is upregulated in kidney diseases such as glomerulonephritis and diabetic nephropathy (110) and in bronchoalveolar lavage fluids from patients with adult respiratory distress syndrome (111), suggests that the inhibitor also plays a role in the pathogenesis of human fibrotic disease.

**Obesity and the metabolic syndrome**

The metabolic syndrome defines a cluster of abnormalities including obesity, insulin resistance, hyperinsulinemia and glucose intolerance, and hypertension and hypertriglyceridemia/dyslipidemia, and is associated with an increased risk for the development of cardiovascular disease (112). Interestingly, PAI-1 gene expression is induced by many components of the metabolic syndrome, thus providing a potential link between the elevated PAI-1, the decreased fibrinolytic response, and the increased risk for cardiovascular disease in this metabolic disorder (113). For example, in obesity, the adipose tissue has a dramatically increased capacity for PAI-1 production, and may be the major source of plasma PAI-1 in this condition. As mentioned earlier (see “Origin”, above), cultured murine (25, 114, 115) and human adipocytes and stromal-vascular cells (116), and human adipose tissue explants (114, 117) all express PAI-1, and PAI-1 expression is increased in adipose tissues from obese ob/ob mice.
(118) and in human visceral fat (117). In this regard, circulating PAI-1 plasma levels are approximately five-fold higher in obese mice and humans than in their lean controls (20, 113).

The relationship between obesity and high PAI-1 levels is further strengthened by the observation that there is a significant correlation between the amount of visceral fat and the plasma levels of PAI-1 in humans, and by the fact that weight loss due to surgical treatment, diet, etc. significantly reduces plasma PAI-1 levels in obese individuals (113). Thus, elevated PAI-1 expression in obesity may be the result of an increased capacity of the adipose tissue to produce more PAI-1 and/or the consequence of direct stimulation of adipocytes by hormones and cytokines that are themselves upregulated in obesity (e.g., TNF-α, TGF-β and insulin; (20). Unexpectedly, PAI-1 itself may also influence the development of obesity and the metabolic syndrome. This possibility is supported by the observation that obese ob/ob mice crossed into a PAI-1 deficient background had significantly reduced body weight and improved metabolic profiles compared to control mice (119). Moreover, in one recent study it was demonstrated that the lack of PAI-1 in normal weight mice attenuated nutritionally-induced obesity and insulin resistance (120). It should be noted, however, that PAI-1 deficiency did not attenuate obesity and insulin-resistance in another study (121). The different results (120, 121) may reflect differences in genetic background, age, and/or the severity of the diabetes that developed.

Hyperinsulinemia and insulin-resistance are two other components of the metabolic syndrome. Insulin induces PAI-1 production in adipocytes in the mouse (118), and administration of insulin to rabbits (122), mice (118, 123) and humans (124) increases PAI-1 plasma levels. Finally, hyperinsulinemic individuals have elevated PAI-1 levels in their plasma (125). The observation that insulin continues to induce PAI-1 gene expression in insulin-resistant mice and adipocytes (123), may account for the correlation between high PAI-1 and insulin-resistance/hyperinsulinemia. In this regard, improvement of insulin resistance by weight loss or troglitazone treatment, is associated with reduction in PAI-1 levels (126, 127).

Besides obesity and insulin, other components of the metabolic syndrome can induce PAI-1 in vitro and/or in vivo, including glucose itself (128). In fact, improved glycemic control decreased plasma PAI-1 activity in patients with NIDDM treated with insulin (129). The metabolic syndrome is frequently associated with hypertension, and the renin-angiotensin system plays an important role in the control of blood pressure. Angiotensin II is another hormone that can stimulate PAI-1 expression in cultured adipocytes (130), and inhibition of angiotensin converting enzyme reduced PAI-1 levels in humans (131). Thus, there is also a link between hypertension and elevated PAI-1. Finally, elevated PAI-1 levels are associated with hypertriglyceridemia (132), and PAI-1 secretion by cultured endothelial cells can be induced by VLDL and unsaturated free fatty acids (133).

In conclusion, elevated PAI-1 is an important feature of the metabolic syndrome and may contribute to the prothrombotic state and increased cardiovascular risk associated with this disorder. In fact, patients with coronary artery disease frequently present with both abnormalities (134). This correlation between high plasma PAI-1 and coronary events disappeared after adjustment for BMI, triglyceride, and HDL cholesterol (i.e., markers of the metabolic syndrome; [113]).

Cancer

The idea that metastatic disease is associated with increased protease activity has been in the literature for quite some time (1), and elevated uPA, uPAR and tPA have been detected in various tumours and may be indicative of a poor prognosis for survival (135, 136). Thus, the observation that high PAI-1 is also associated with a poor prognosis for survival in breast cancer (137) was unexpected. However, it has now been convincingly demonstrated that high PAI-1 levels also indicate a poor prognosis for survival in a number of human cancers (3, 136, 138). In fact, the measurement of PAI-1 in tumour tissues is becoming an important prognostic parameter for a variety of malignant diseases. For example, together with uPA and next to nodal status, PAI-1 is the strongest prognostic marker in breast cancer. Moreover, in gastric cancer, PAI-1 levels, nodal status, and WHO classification were the only independent prognostic factors. PAI-1 was also an independent indicator for poor prognosis in pulmonary adenocarcinoma, and when considered together with uPA, formed a strong prognostic parameter in ovarian cancer after radical surgery. In urinary tract cancer, plasma PAI-1 levels were higher in patients with metastatic disease than in patients without this life-threatening complication.

The exact mechanism by which PAI-1 influences tumour growth and/or dissemination remains speculative. The simplest scenario is that the induction of PAI-1 is actually a response of the host to the high protease activity of the tumor since the increased PAI-1 is frequently detected in the surrounding stromal cells (139, 140). The presence of high PAI-1 at invasive foci may protect the extracellular matrix against plasmin-mediated degradation, thus preserving the matrix for cell migration. It should be noted however, that increased PAI-1 is also detected in the malignant cells themselves (141, 142). This observation together with the observation that uPA, uPAR and PAI-1 all correlate with a poor prognosis in cancer, raises the possibility that the PAI-1 effects may reflect the activation of the cell detachment system (see “Cell motility”). In this regard, the elevation of PAI-1 may promote cell metastasis by influencing cell attachment, detachment, and migration (as discussed under “Cell motility”). Recent studies demonstrate that the addition of low concentrations of PAI-1 to cells leads to the rapid rearrangement of the actin cytoskeleton, the loss of focal adhesions, and the assumption of the migratory phenotype (73).

Another possible mechanism by which PAI-1 may influence tumour growth and/or dissemination is related to its effects on angiogenesis (27). The formation of new blood vessels is fundamental to tumour growth. Like its effect on cell migration, PAI-1 seems to possess both pro- and anti-angiogenic effects depending on the PAI-1 concentration and specific experimental conditions employed. For example, in one study, no effect of PAI-1 on angiogenesis was observed (143), and in another, mice lacking the PAI-1 gene show impaired vessel formation when malignant keratinocytes were transplanted into the skin (144). A ‘bell-shaped’ dose-dependent effect of PAI-1 was found in a Matrigel implant assay (145) and in aortic ring explants in PAI-1-deficient mice (146) in which low doses enhanced angiogenesis but high...
dosages were inhibitory. The observations that PAI-1 is expressed in endothelial cells of small vessels in human colon adenocarcinomas and in proliferative vessels in high-grade gliomas and metastatic tumors, suggest that PAI-1 may play a role in angiogenesis in human cancers as well.

In conclusion, several clinical studies provide strong support for the unexpected correlation between high PAI-1 levels in tumors and poor prognosis for survival. In this regard, PAI-1 may emerge as an important prognostic parameter to identify patients with high risk for recurrence. The exact mechanism by which PAI-1 influences tumor growth and dissemination is still not fully understood, but may be related to recent observations indicating that PAI-1 exerts a broad spectrum of effects in tumor biology, from the inhibition of uPA-induced proteolysis and destruction of the extracellular matrix, to its effects on cell migration and angiogenesis.

Perspectives

Although PAI-1 is the primary inhibitor of plasminogen activation in blood, recent studies suggest that its biological functions may extend far beyond the regulation of plasmin formation and fibrinolysis. In this regard, the PAI-1 field has grown tremendously since the discovery of this inhibitor in the early 1980s, and it has evolved into an exciting discipline, one that seems to be relevant to a large and unusually diverse number of normal and pathological processes. Unfortunately, the field is also associated with a number of apparently conflicting observations, sometimes from what appear to be identical model systems. Because of this, simple cause and effect relationships are frequently difficult to develop and/or accept. We would suggest that the diversity of pathways affected by this inhibitor are, in fact, consistent with some of its unusual properties, and that problems of reproducibility may reflect differences in the levels of other molecules in tissues that can modify the activity and/or function of the inhibitor.

Unusual properties

In general, most plasma serpins are long-lived (days), they are present at high concentrations in plasma (mM), they are not highly regulated except perhaps during the acute phase response, and they are synthesized in the liver. In contrast, PAI-1 is short-lived (min), it is a trace protein in plasma (nM), it is highly regulated, and it is synthesized or can be induced in a variety of cells in a variety of tissues. Unexpectedly, PAI-1 is also an immediate early gene (148). These properties of PAI-1 not only set it aside from other serpins, but also argue against the notion that its only function is to regulate the fibrinolytic system. These unusual properties may also offer clues about the mechanisms by which this inhibitor can influence such a wide range of biological processes. In this regard, it was suggested (41, 55) that these features of PAI-1 (i.e., trace protein, short half-life, rapid induction by a diverse collection of molecules and/or changes in physiological state (e.g., thrombosis, inflammation, stress, wound healing, etc.), may be the properties of a molecular switch (i.e., a molecule that can rapidly “turn-on” and “turn-off” biological pathways). If this concept is true, then it is tempting to speculate that the molecular switch function of PAI-1 may represent the common mechanism by which it can contribute to diverse pathologies. We further speculate that in these instances, the cell migration system and not the fibrinolytic system, is the target. Obviously, the primary goal of future studies in this area should be the development of direct experimental proof that the postulated molecular switch function of PAI-1 is not only real, but is also important in vivo.

Modifying factors

Another unique property of PAI-1 is that it binds specifically and with high affinity to VN, a molecule present in blood and the extracellular matrix. Thus, an additional future goal is to develop a better understanding of the importance of VN for PAI-1 function in vivo. For example, the binding of PAI-1 to VN seems to be critical for the control of fibrinolysis in clots formed in vitro (149) and perhaps in vivo (81, 82). However, experimental proof is lacking for a role of VN in the regulation of PAI-1 function in tissues (e.g., in tissues undergoing physiological/pathological changes). Based upon available in vitro data, we would predict that the function of PAI-1 in tissue pathology may be different in the presence vs. the absence of VN.

Besides VN, a large number of other factors are expected to modify PAI-1 activity and/or function. For example, as pointed out by Dan Lawrence and his collaborators, the effects of PAI-1 on cell migration and angiogenesis are frequently dose-dependent, with low concentrations being stimulatory and high concentrations inhibitory. There is also some evidence to suggest that this ‘bell-shaped’ dose-dependent effect of PAI-1 may be relevant in other biological processes in vivo, including injury-induced neointima formation. In this regard, PAI-1 was reported to promote neointima formation in animal models in which arterial injury induces fibrin deposition and a strong thrombotic response. However, in other models with less severe injury, PAI-1 was inhibitory. Besides this dose-dependent effect, the local activity and function of PAI-1 may be modified by the composition of the extracellular matrix (e.g., the presence of fibrin), and by the presence of varying amounts of uPA, tPA, uPAR, LRP, and other PAIs. Such factors are not usually monitored in parallel with PAI-1, and differences in their concentrations in the tissues may explain the sometimes conflicting results observed in similar models. Future studies to more completely quantify PAI-1 and the relevant modifying factors may resolve some of these issues.

Finally, in spite of the potential problems employing animal models to study the role of PAI-1 in vessel wall physiology/pathology, clinical studies rather consistently correlate high levels of PAI-1 with the occurrence of thrombotic diseases, including myocardial infarction and DIC. Thus, one must wonder why the inhibitor has not been employed as a marker to predict or to monitor coronary artery events. The answer is probably related to the fact that so many different physiological and pathological mediators seem to induce PAI-1, that it is difficult to use it as a prognostic indicator of any specific thrombotic event. High PAI-1 in tumor extracts is also predictive for poor outcome in several types of cancer, and in this case, PAI-1 has emerged as a useful prognostic marker for many cancers. Future studies to identify cancer patients at high risk for recurrence as judged by elevated PAI-1 (and uPA) levels, may ultimately lead to risk-adapted individualized therapy.
References


40. Stefansson S, Lawrence DA. The serpin PAI-1 inhibits cell migration by blocking integrin alpha
Dellas, Loskutoff: Historical analysis of PAI-I


