

Plasmin Activation System in Restenosis: Role in Pathogenesis and Clinical Prediction?

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Abstract. During recent years it has become increasingly recognized that the plasmin activation system is involved in the development of atherosclerosis and restenosis. Responsible pathophysiologic mechanisms, however, remain elusive. This review focuses primarily on the clinician's point of view, suggesting that increases in plasminogen activator inhibitor type-1 (PAI-1) plasma levels after balloon angioplasty or permanently elevated lipoprotein (a) (Lp(a)) plasma levels might be helpful in the prediction of restenosis after coronary angioplasty. In contrast, tissue-type plasminogen activator (tPA) plasma levels appear unrelated to restenosis, and data regarding a possible role of urokinase-type plasminogen activator (uPA) in circulation are not available at present. Furthermore, a new hypothesis on the pathophysiological role of local PAI-1 overexpression as a beneficial negative feedback mechanism to limit excess cellular proliferation in atherogenesis and restenosis is presented.

Key Words. restenosis, plasmin activation system, plasminogen activator inhibitor type-1, lipoprotein (a)

Restenosis limits the long-term outcome of originally successful percutaneous transluminal coronary angioplasty (PTCA) or related coronary interventions. Up to 50% of the PTCA sites tend to narrow again over a period of 3–6 months due to a hyperplastic response of the vessel wall [1,2]. Restenosis after coronary angioplasty is a multifactorial process, initiated by vessel trauma, and involves mechanisms such as inflammation, platelet activation, thrombin activation, plasmin activation, and stimulation of growth factors [3–6] (Figure 1).

The plasmin activation system consists of a cascade of trypsin-like serine proteases, secreted as inactive single-chain zymogens, which are converted to the active two-chain proteases by cleavage of a peptide bond [7,8]. The major components are plasminogen, two plasminogen activators, and three serine protease inhibitors (serpins). Plasminogen consists of a noncatalytic A-chain containing five kringle domains, responsible for fibrin binding via high and low-affinity lysine-bind-

ing sites, and a catalytic B-chain containing the active site consisting of serine, histidine, and aspartic acid. Following activation of plasminogen to plasmin by cleavage of an arginine-valine bond, plasmin has a broad spectrum of substrates. Besides digestion of fibrin and most other matrix proteins, it activates metalloproteases and latent forms of certain growth factors.

The two plasminogen activators — tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) — differ mostly in their domain structures and function of their noncatalytic A-chain. While tPA possesses fibrin affinity via its finger domain and kringle 2, uPA binds to the specific plasma membrane receptor via its growth factor domain only. The single kringle domain of uPA has no affinity to fibrin. These interactions result in a fibrin-specific plasminogen activation by tPA, and a non-fibrin-specific plasminogen activation by uPA, suggesting a different biological role. Within the circulation, where the primary role of plasmin activation is the maintenance of patency and fluidity by dissolution of fibrin thrombi, tPA seems to be the main activator because it efficiently activates plasminogen only in the presence of fibrin. Interestingly, tPA in the single-chain form is almost as active as in the two-chain form after plasmin-mediated cleavage. In contrast, localized plasmin activation within the tissue is primarily triggered by uPA via receptor-directed proteolysis and extracellular matrix (ECM) turnover during cell migration, cell proliferation, and tissue remodeling. Plasmin activation is strongly regulated by three serpins with different target enzymes. Plasminogen activator inhibitor type-1 (PAI-1) inhibits both tPA (the single-chain as well as two-chain form) and uPA. It is secreted in an active form, but rapidly

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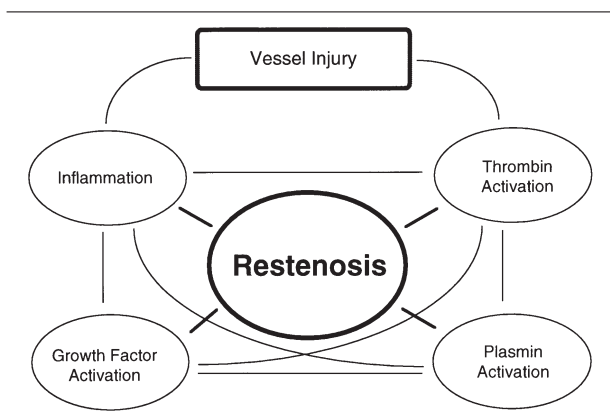


Fig. 1. Mechanisms of restenosis. Restenosis after coronary angioplasty is a multifactorial process, which is initiated by vessel trauma and involves mechanisms such as inflammation, platelet activation, thrombin activation, plasmin activation, and stimulation of growth factors.

converts to an inactive, latent form. PAI-1 binding to plasma vitronectin or ECM vitronectin stabilizes it in the active conformation. Plasminogen activator inhibitor type-2 (PAI-2) inhibits uPA primarily and, to some extent, two-chain tPA, but less efficiently than PAI-1. The third serpin, α_2 -antiplasmin, is the primary inhibitor in plasma. Finally, lipoprotein(a) (Lp(a)), although not a genuine component of the plasmin activation system, possesses the ability to act as a fibrinolysis inhibitor by preventing the binding of plasminogen to cell surfaces due to its structural homology with plasminogen (Figure 2).

The overview summarizes the present literature about tPA, uPA, PAI-1, and Lp(a), with special atten-

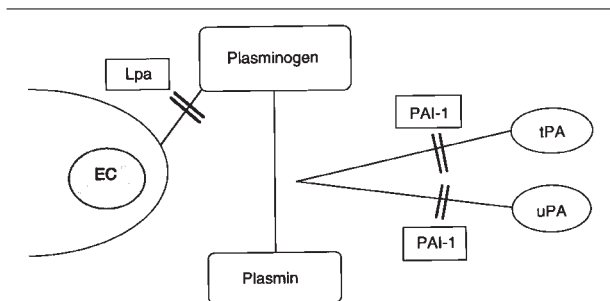


Fig. 2. Plasmin activation system. The most important components of the plasmin activation system are tissue type plasminogen activator (tPA), which is the most important plasminogen activator within the bloodstream, urokinase type plasminogen activator (uPA), which is the predominant activator in tissues, and plasminogen activator inhibitor type-1 (PAI-1), which is the main regulator of the plasmin activation system in circulation and tissue. In addition, lipoprotein (a) (Lp(a)) also acts as a fibrinolysis inhibitor by preventing the binding of plasminogen to cell surfaces due to its structural homology with plasminogen.

tion to pathophysiologic mechanisms in the development of restenosis after PTCA, and discusses a possible role of these parameters as clinical predictors of restenosis after initially successful PTCA. Furthermore, a new hypothesis on the pathophysiological role of local PAI-1 overexpression as a beneficial negative feedback mechanism to limit excess cellular proliferation in atherogenesis and restenosis is presented.

Tissue-Type Plasminogen Activator (tPA)

Tissue-type plasminogen activator is produced by endothelial cells and smooth muscle cells (SMCs). Compared with healthy controls, patients with atherosclerotic diseases, including coronary artery disease (CAD), have been shown to exhibit increased plasma levels of tPA antigen [9–13] as well as a reduced or absent increase in tPA plasma levels after certain stimuli, for example, venous occlusion of the forearm or physical exercise [14].

The paradoxical increase of tPA antigen levels in CAD is thought to be due to an increase in tPA/PAI-1 complexes secondary to the elevated PAI-1 plasma levels [11,13,15] and appears to reflect the high fibrinolytic inhibitory capacity in these patients. The reduced increase in plasma tPA levels to certain stimuli might reflect generally diseased endothelial cells, which have lost their ability to produce tPA in sufficient amounts to protect against intravascular thrombus formation.

In animal models, tPA is expressed by migrating arterial wall cells after injury and is capable of increasing smooth muscle cell migration, similar to uPA in in-vitro migration studies [16–18]. However, within the human atherosclerotic tissue, tPA appears not to play a major role as a plasminogen activator in vivo [19]. Accordingly, most previous publications have not shown a relationship between tPA plasma levels measured before or after PTCA for the development of restenosis (Table 1) [20–24]. Only one trial showed a significant positive correlation between elevated tPA levels and restenosis [25], which might be due to the very small number of patients included. The tPA response to venous occlusion with respect to the incidence of restenosis has been investigated retrospectively in two studies. Kirschstein et al. demonstrated a reduced tPA response in patients with restenosis compared with nonrestenotic patients [26]. We could show that only patients with a tendency towards recurrent restenoses exhibit a significantly decreased tPA response to venous occlusion, as compared with patients without or with only one restenotic event [27]. However, due to differences in the quality and duration of occlusion as well as coexisting environmental risk factors, the results of venous occlusion tests are mostly not sufficiently reproducible [28]. Therefore, venous occlusion has not become a clinical standard test and cannot be recommended for the detection of patients

Table 1. tPA plasma levels and restenosis

Author	[Reference no.]	Year	Patients	Restenosis (%)	Time of investigation related to PTCA	Correlation tPA and restenosis	P value
Shah	[20]	1992	68	41	Before	No	n.s.
Huber	[21]	1992	104	33	Before and after	No	n.s.
Benchimol	[25]	1993	23	26	Before	Positive	0.04
Brack	[22]	1994	68	41	Before	No	n.s.
Montalescot	[23]	1995	107	48	Before	No	n.s.
Sakata	[24]	1996	72	43	Before and after	No	n.s.
Kirschstein	[26]	1989	19 vs. 16		Reduced tPA response after VO in restenosis		
Huber	[27]	1994	72	42	Reduced tPA response after VO in restenosis		

PTCA = percutaneous transluminal coronary angioplasty; tPA = tissue type plasminogen activator; VO = venous occlusion.

with increased risk of restenosis formation after PTCA.

Urokinase-Type Plasminogen Activator (uPA)

Although uPA plasma levels have been shown to be useful as independent tumor markers in patients with certain malignant diseases [29–31], data on uPA plasma levels in patients with CAD are very limited [32]. Furthermore, uPA has not been measured in the circulation of patients with restenosis following coronary interventions. Despite the hypothesized pathophysiologic role of uPA in the development of restenosis as a primary plasminogen activator mediating SMC migration [16,17,33,34], no clinical support has been presented thus far. This lack of data is most likely due to the fact that uPA is responsible for plasmin activation in the vessel wall and does not enter the circulation in detectable amounts.

Lipoprotein (a) (Lp(a))

One of the most investigated parameters influencing the plasmin activation system is Lp(a), which was identified by K. Berg in 1963 [35]. It was not until the 1970s and 1980s that a strong relationship between high levels of Lp(a) and atherosclerosis was found in population-based studies [36–38]. Lp(a) can be distinguished from other lipoproteins in that it is a low-density lipoprotein particle containing an additional and unique apolipoprotein, apo(a), which is covalently linked to apo-B100 by a disulfide bridge. Protein and cDNA sequences of the apo(a) moiety show a remarkable similarity to plasminogen [39]. In addition to a plasminogen-like protease domain, apo(a) contains at least 10 repeats of the kringle 4 domain and one kringle 5 domain, which are highly homologous to plasminogen kringles 4 and 5. Due to these similarities, Lp(a) is a potential molecular link between atherosclerosis and thrombosis. As shown in several studies, Lp(a) com-

petes with plasminogen for binding to fibrinogen — ECM — endothelial cells and inflammatory cells [40–43]. This competition may lead to a local reduction in plasmin formation and thus create a prothrombotic and antifibrinolytic milieu.

With respect to the development of restenosis, several authors were able to demonstrate a significant correlation between elevated Lp(a) plasma levels and late restenosis [44–49] (Table 2). Although some variability exists, it could be shown that plasma Lp(a) levels of >19 mg/100 mL are associated with the incidence of restenosis. Studies without significant correlation of elevated Lp(a) plasma levels and late restenosis [20,25,50–53] either reported unusually high levels of Lp(a) before PTCA (restenosis group 6 ± 7.9 mg/mL, nonrestenosis group 7 ± 6.7 mg/mL) [20] or a positive correlation between different lipid fractions and restenosis (i.e., apolipoprotein E; apolipoprotein B) [50,51], or at least a trend towards an increased risk [53]. Furthermore, the reduction of Lp(a) plasma levels by low-density lipoprotein apheresis has been shown to reduce the rate of restenoses [54,55].

What possible mechanisms could be involved in Lp(a)-associated restenosis formation? Firstly, thrombus formation could be favored via competitive inhibition of plasminogen binding to its cell-surface receptors, an essential requirement for the conversion of plasminogen to plasmin. Secondly, increased lipid deposition and modulation of smooth muscle cell activity in the atherosclerotic plaque, as well as induction of endothelial cell dysfunction, may play important roles [56]. At present, the genetically determined elevation of Lp(a) plasma levels has to be regarded as a general risk factor for restenosis, although prospective trials with sufficient patient numbers are still to be performed.

Plasminogen Activator Inhibitor Type-1 (PAI-1)

Elevated plasma levels of PAI-1 have been correlated with the anatomical extent and clinical course of CAD

Table 2. Lp(a) plasma levels and restenosis

Author	[Ref. no.]	Year	Patients	Restenosis (%)	Time of investigation related to PTCA	Correlation Lp(a) and restenosis	P value
Hearn	[44]	1992	69	71	Before	Positive	0.018
Shah	[20]	1992	68	41	Before	No (but high values in both groups)	n.s.
Benchimol	[25]	1993	23	26	Before	No	n.s.
Tenda	[45]	1993	63	35	Before	Positive	<0.001
van Bockxmeer	[50]	1994	195	40	Before	No (apo E positive)	n.s.
Cooke	[51]	1994	62	46	Before	No (apo B positive)	n.s.
Desmarais	[46]	1995	240	40	Before	Positive	<0.0001
Yamamoto	[47]	1995	71	34	Before	Positive	<0.01
Ishikawa	[52]	1995	87	53	Before	No	n.s.
Miyata	[48]	1996	80	38	Before	Positive	<0.003
Bussiere	[53]	1996	103	49	Before	No (but trend >25 mg%)	n.s.
Kotamäki	[49]	1996	122	43	Before	No (but both groups <19 mg%)	0.075
					After (6 m)	Positive	0.048

Lp(a) = lipoprotein (a); PTCA = percutaneous transluminal coronary angioplasty; n.s. = not significant; apo E = apolipoprotein E; apo B = apolipoprotein B; m = months.

[15,57–63]. Data regarding a possible role of PAI-1 plasma levels in the development of restenosis are rare and controversial (Table 3). When PAI-1 plasma levels were investigated before coronary interventions, no association with restenosis formation was found [22,23,25]. Only one study showed a significant but paradox negative relation between PAI-1 levels and later development of restenosis [20]. We determined PAI-1 plasma levels before PTCA and serially after the intervention, and were able to show that PAI-1 plasma levels measured before and up to 3 days after PTCA are not related to the clinical outcome. However, during the follow-up period, a significant decrease of PAI-1 plasma levels to about 70% of the pre-PTCA values

was measured in patients who did not develop restenosis, whereas a stable PAI-1 level or a further increase was significantly associated with restenosis [21]. These findings have been confirmed by two other studies [24,64]. In addition, Ishiwata and coworkers found a reduced incidence of restenosis in patients whose PAI-1 plasma levels could be reduced in parallel to triglyceride levels due to bezafibrate therapy after PTCA [65]. We hypothesized that the upregulation of PAI-1 plasma levels might be related to a chronically active lesion, whereas the decrease in PAI-1 plasma levels in patients without later restenosis might indicate a healing of the traumatized vessel site without a hyperplastic response [21,66]. Interestingly, a recent

Table 3. PAI-1 plasma levels and restenosis

Author	[Ref. no.]	Year	Patients	Restenosis (%)	Time of investigation related to PTCA	Correlation of PAI-1 and restenosis	P value
Shah	[20]	1992	68	41	Before	Negative	0.04
Huber	[21]	1992	104	33	Before	No	n.s.
					After (1 w–6 m)	Positive	0.005
Benchimol	[25]	1993	23	26	Before	No	n.s.
Brack	[22]	1994	68	41	Before	No	n.s.
Montalescot	[23]	1995	107	48	Before	No	n.s.
Sakata	[24]	1996	72	43	Before	No	n.s.
					After (1 w–3 m)	Positive	0.01
Ishiwata	[64]	1997	73	37	Before	No	n.s.
					After (6 m)	Positive	<0.05
Lins	[67]	1997	35	23	Before	No	n.s.
					After (24 h)	Positive	<0.05
Ishiwata	[65]	1995	87	53	Decrease with bezafibrate therapy		

PAI-1 = plasminogen activator inhibitor-1; PTCA = percutaneous transluminal coronary angioplasty; w = week; m = months; h = hours.

study suggests that even an initial PAI-1 increase during the first 24 hours after directional coronary atherectomy might be associated with the development of late restenosis [67].

How could elevated PAI-1 plasma levels reflect the ongoing process of restenosis? High amounts of PAI-1 antigen and PAI-1 mRNA have been demonstrated in both atherosclerotic and restenotic lesions compared with normal vessels [19,68–70]. The increased PAI-1 content can be explained potentially by the fact that PAI-1 synthesis in the different cell types involved in the restenosis process (inflammatory cells, endothelial cells, and SMCs) is upregulated by the same factors that promote cellular proliferation and migration (e.g., growth factors, cytokines, thrombin) [4,5,69,71–73]. Production of PAI-1 commences very early and can be detected by 3 hours after vessel trauma. Interestingly, PAI-1 can be detected first in the adventitia, is present after 1 day in both the adventitia and media, and is later found to be highly expressed in the neointima [74]. PAI-1 thus seems to be primarily produced by cells during cell doubling and proliferation. We were able to show that SMCs after several cell doublings *in vitro* exhibit equivalent high levels of PAI-1 as primary cultures of SMCs obtained from atherosclerotic plaques, which are thought to have already undergone several cell doublings *in vivo* [75]. Furthermore, depending on their duplication status (expressed by the number of passages) and origin (normal vs. atherosclerotic vessels) SMCs are either able to downregulate [76] or to induce PAI-1 synthesis of endothelial cells, resulting in an overall increase of vascular wall PAI-1 expression [75] (Figure 3). The role of this PAI-1 overexpression at the site of atherosclerotic and restenotic lesions within the arterial wall is still a matter of debate. Recent data, however, suggest that PAI-1 exerts inhibitory effects on atherogenesis and restenosis by inhibiting SMC migration. Carmeliet et al. were able to demonstrate that in PAI-1-deficient mice excess neointima formation occurs in response to vascular injury due to impaired wound healing [77,78], whereas in uPA-deficient mice almost no neointima formation is visible [33]. Furthermore, Stefansson and Lawrence showed that PAI-1 inhibits SMC migration by blocking the binding of the $\alpha_v\beta_3$ integrin to its matrix receptor vitronectin [79].

In view of these findings, we propose a new hypothesis on the pathophysiological role of PAI-1 in atherogenesis and neointima formation. The local augmentation of vascular wall PAI-1 expression, triggered by proliferating SMCs, seems to reflect a self-regulatory feedback mechanism, attempting to terminate or limit excess cellular proliferation after vascular injury. Upon stimulation to proliferate, SMCs not only increase their own PAI-1 expression, but simultaneously upregulate endothelial PAI-1 synthesis through a presently unknown paracrine factor. As a result, one could speculate that local PAI-1 overexpression inhibits ex-

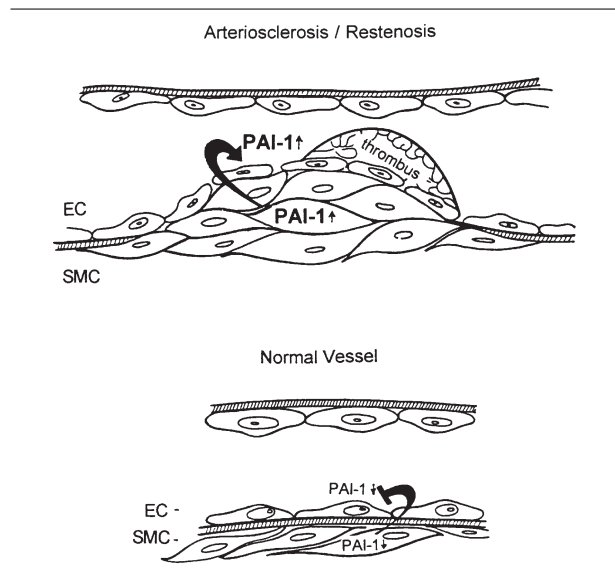


Fig. 3. Increase of plasminogen activator inhibitor type-1 (PAI-1) in arteriosclerosis/restenosis. The overall increase of vascular wall PAI-1 expression in arteriosclerotic and restenotic lesions seems to be mediated primarily through proliferating smooth muscle cells (SMC) by expressing higher PAI-1 synthesis levels as well as paracrine upregulation of endothelial cell (EC) PAI-1 synthesis. Under normal conditions, resting SMC seem to suppress overall vascular wall PAI-1 synthesis by expressing lower levels of PAI-1 synthesis as well as through paracrine downregulation of endothelial PAI-1 synthesis. (Slightly modified figure published by permission of Christ et al. [75].)

cess cell accumulation by several mechanisms, as illustrated in Figure 4:

PAI-1 expression reduces local *proteolysis* by inhibiting uPA-mediated plasmin generation. Plasmin appears to be a key enzyme in controlling ECM turnover because it is able to cleave most matrix proteins and also activates metalloprotease precursors [80]. Additionally, uPA and plasmin appear to control vascular remodeling by activation of latent growth factors [81–84]. Attenuation of local proteolysis might also affect cell migration. Receptor–ligand interactions at the leading edge of a migrating cell and simultaneous uPA-mediated loosening of such interactions at the trailing edge of the cell seem important for transmitting motor gene-induced movements of the cytoskeleton [17,85].

PAI-1 expression inhibits cell *migration* by preventing binding between the cellular integrin receptor ($\alpha_v\beta_3$) and the extracellular matrix–ligand (vitronectin) due to the high affinity for vitronectin [79].

PAI-1 expression inhibits cell *proliferation* by blocking uPA-mediated mitogenic activity. This growth factor–like activity of uPA has been proposed by several groups [81,82,86–89]. Furthermore, thrombin-mediated growth factor–like activities might also be reduced, because PAI-1 efficiently inhibits thrombin

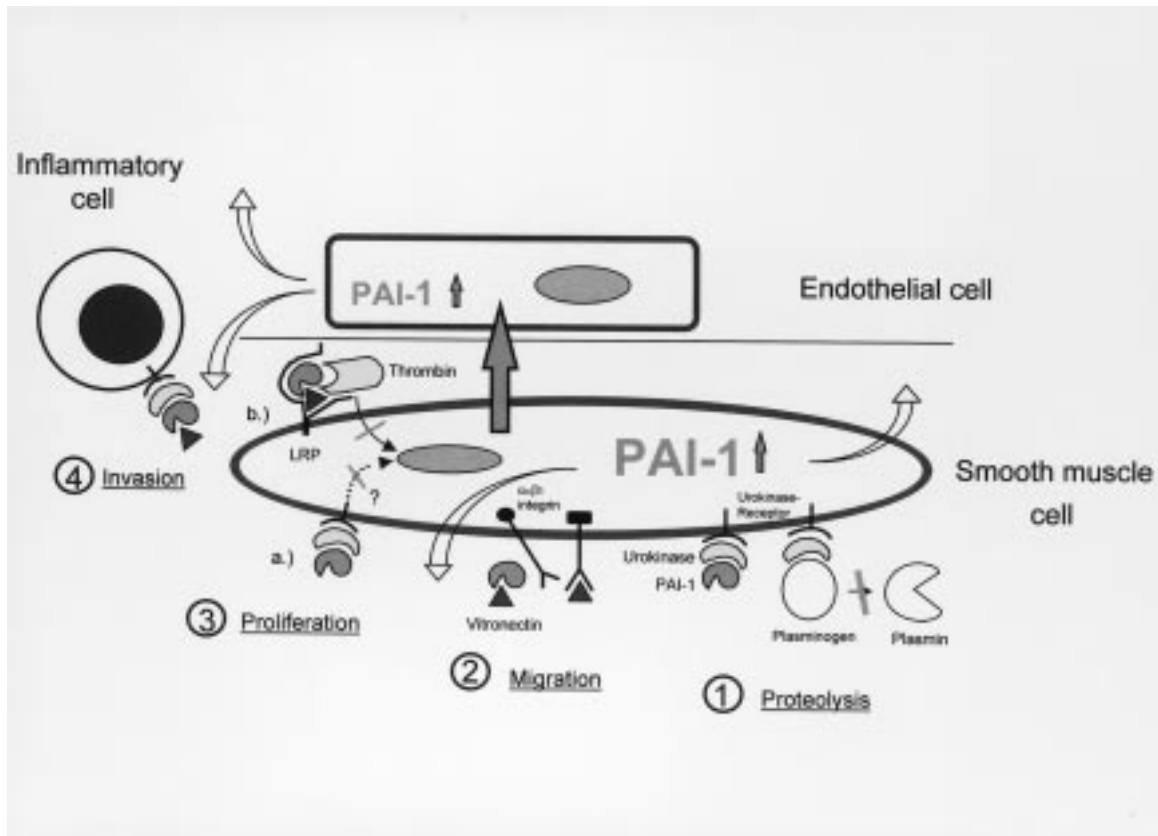


Fig. 4. Hypothesis on the role of plasminogen activator inhibitor type-1 (PAI-1) in arteriosclerosis/restenosis. The augmentation of vascular wall PAI-1 expression seems to reflect a self-regulatory feedback mechanism of proliferating smooth muscle cells (SMC), attempting to terminate or limit excess cellular proliferation after vascular injury. Upon stimulation to proliferate, SMC not only increase their own PAI-1 expression but simultaneously upregulate endothelial PAI-1. This local PAI-1 overexpression inhibits excess cell accumulation by several mechanisms: (1) Inhibition of urokinase-mediated proteolysis, with concomitant inhibition of extracellular matrix degradation, inhibition of activation of latent growth factors, and inhibition of cell migration because as urokinase-mediated loosening of receptor–ligand interactions between cells and the extracellular matrix are important in transmitting motor gene-induced movements of the cytoskeleton. (2) Inhibition of cell migration by prevention of binding between the cellular integrin receptor ($\alpha_5\beta_1$) and the extracellular matrix ligand (vitronectin) due to the high affinity of PAI-1 for vitronectin. (3) Inhibition of cell proliferation by blocking (a) urokinase-mediated mitogenic activity and (b) thrombin-mediated growth factor-like activities because PAI-1 efficiently inhibits thrombin in the presence of vitronectin, followed by clearance through the low-density lipoprotein receptor-related protein (LRP). (4) Inhibition of urokinase-mediated invasion of inflammatory cells.

in the presence of vitronectin, followed by clearance through the low-density lipoprotein receptor-related protein [90].

Finally, PAI-1 expression inhibits uPA-mediated invasion of inflammatory cells [91–94], attempting to attenuate the inflammatory process in atherosclerosis and restenosis.

In conclusion, the increase of local intravascular PAI-1 expression after vascular injury might reflect a primary physiological mechanism, triggered by proliferating SMCs in an attempt to control wound healing and to prevent the development of atherosclerotic and restenotic lesions. The increase of PAI-1 plasma levels after PTCA may reflect the ongoing attempts to counterbalance a pathologically increased stimulation of proliferation. Therefore, determination of PAI-1 in

plasma samples before and serially after PTCA could prove a useful method to clinically predict restenosis. Indeed, we recently demonstrated that an increase of PAI-1 plasma levels may enhance the predictive value of ^{201}Tl scintigraphy performed 3 months after angioplasty, thus improving non-invasive detection of restenosis in patients [95].

Conclusions

Lp(a) and PAI-1 have been shown to be involved in the pathophysiological mechanisms leading to restenosis following primarily successful PTCA. In contrast, tPA has not been linked to restenosis, and data regarding a possible role of uPA in circulation are presently un-

available. While Lp(a) plasma levels are mainly genetically determined and stable over a long period in the individual patient, PAI-1 plasma levels seem to be linked to the ongoing process of restenosis. We propose a novel hypothesis on the pathophysiological role of this increased local PAI-1 expression. It might reflect a physiological feedback mechanism of proliferating SMCs attempting to limit cell proliferation and migration, thus controlling wound healing. The determination of PAI-1 plasma levels before and after PTCA might be an interesting non-invasive clinical tool that allows early detection of those patients who have an increased tendency for the development of restenosis.

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