Plasminogen Activator Inhibitor Type 1 in Ischemic Cardiomyopathy

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Plasminogen activator inhibitor type 1 (PAI-1) is a protease inhibitor constituting the key regulator of the activity of the fibrinolytic system, an important protective mechanism against thrombosis. Because a reduced fibrinolytic activity, mainly caused by increased plasma levels of PAI-1, was a common finding in cross-sectional studies of patients with coronary artery disease (CAD), high PAI-1 levels have been regarded as a risk factor for recurrent episodes of myocardial infarction (MI). It has also been proposed that levels of PAI-1 have been regarded as a risk factor for recurrent episodes of MI. It has also been proposed that levels of PAI-1 have been regarded as a risk factor for recurrent episodes of MI. It has also been proposed that levels of PAI-1 have been regarded as a risk factor for recurrent episodes of MI. It has also been proposed that levels of PAI-1 have been regarded as a risk factor for recurrent episodes of MI.

Thus, we shall focus on recent advances in the knowledge of the role of PAI-1 and its gene in the development of ischemic cardiomyopathy.

Biochemical Features

Intravascular fibrinolytic activity results from a balance between plasminogen activators, such as the tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), and inhibitors, such as PAI-1 and a2-antiplasmin. PAI-1 is a glycoprotein with a molecular mass of ~54 kDa that belongs to the serine protease inhibitor superfamily (serpins). Its primary structure was deduced from the sequence of its cDNA. It is considered the major physiological inhibitor of t-PA and urokinase, because plasmin formation and fibrinolysis, as well as formation of other extracellular proteases, strongly depend on PAI-1 levels. Although the principal source of plasma PAI-1 is unknown, available evidence indicates that several cell types, including endothelial and vascular smooth muscle cells (VSMCs), platelets, hepatocytes, fibroblasts, and adipocytes, can all produce PAI-1. Furthermore, the finding of immunocytochemical localization of PAI-1 in human endothelial cells and VSMCs suggests an action of the peptide not only in the vessel lumen, but also in the vascular wall.

Plasma levels of PAI-1 can be measured either as activity or as immunoreactive (ir) PAI-1 (PAI-1 antigen). Both measurements require utmost care in blood collection and sample handling because PAI-1 is a labile molecule. In addition, precautions must be taken to avoid release of PAI-1 from the platelets, which contain a large amount of mostly the inactive form (see below). PAI-1 reacts rapidly with t-PA and u-PA, forming very stable stoichiometric 1:1 complexes, as do many serpins. A unique feature of PAI-1 among serpins is its spontaneous transition into an inactive "latent" form, which can be reactivated by treatment with denaturing agents. It has been established that denaturing agents lead to an exposure of the scissile bond at the surface of PAI-1, making it available for interaction with plasminogen activators. Crystalllographic studies have clarified the threedimensional structure of the latent form of PAI-1. Elegant, limited proteolysis studies have allowed detection of conformational differences between the different forms, ie, latent, active, and complexed PAI-1, as well as identification of some flexible regions that seem to be implicated in the conformational changes during the inhibitory reaction of PAI-1 on plasminogen activators. In plasma, vitronectin forms a relatively tight complex via its NH2-terminal domain with PAI-1 and this interaction may contribute to stabilize PAI-1 in its functional state. The differential proteolytic susceptibility of the aforementioned flexible joint region is likely to affect affinity to vitronectin. Although the half-life for the transformation into the inactive latent form is ~4 hours in vitro, at neutral pH and 37°C the half-life of PAI-1 activity is very short (~2 hours), and the in vivo half-life is even shorter (<10 minutes). Therefore, samples for PAI-1 activity measurements must be handled and plasma frozen as quickly as possible.

The PAI-1 activity (PAI-1act) assay detects free active PAI-1, whereas PAI-1 antigen (PAI-1ag) assay measures free active PAI-1, inactive latent PAI-1, and also inactive (complexed with t-PA or u-PA) PAI-1. Many studies, performed in healthy subjects or patients with CAD, showed a correlation between t-PA antigen (t-PAag) and PAI-1ag, with coefficients ranging between 0.36 and 0.86. These proteins are highly correlated with each other also in the circadian variation, but the real value of this...
TABLE 1. Environmental Factors Affecting PAI-1 Synthesis

<table>
<thead>
<tr>
<th>Factor</th>
<th>In Vitro</th>
<th>In Vivo</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>↑</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>Interleukin-1α</td>
<td>↑</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>TGF-β</td>
<td>↑</td>
<td>NA</td>
<td>18</td>
</tr>
<tr>
<td>PDGF</td>
<td>↑</td>
<td>NA</td>
<td>19</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>↑</td>
<td>NA</td>
<td>17</td>
</tr>
<tr>
<td>Insulin</td>
<td>↑</td>
<td>NE</td>
<td>38, 39</td>
</tr>
<tr>
<td>Glucose</td>
<td>↑</td>
<td>NA</td>
<td>39</td>
</tr>
<tr>
<td>LDL-Ox</td>
<td>↑</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>↑</td>
<td>NA</td>
<td>38, 39</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↑</td>
<td>↑</td>
<td>23, 24</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>NE or ↓</td>
<td>NA</td>
<td>25, 26</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>↑</td>
<td>NE</td>
<td>28</td>
</tr>
<tr>
<td>IL-GF-1</td>
<td>↑</td>
<td>NE</td>
<td>29</td>
</tr>
<tr>
<td>Endotoxemia</td>
<td>NA</td>
<td>↑</td>
<td>30, 31</td>
</tr>
<tr>
<td>Testosterone</td>
<td>NA</td>
<td>↓</td>
<td>34, 35</td>
</tr>
<tr>
<td>Estrogens</td>
<td>NA</td>
<td>↑</td>
<td>35–37</td>
</tr>
<tr>
<td>Ang II</td>
<td>↑</td>
<td>↑</td>
<td>20, 53</td>
</tr>
<tr>
<td>Smoking</td>
<td>NA or NE</td>
<td>↓</td>
<td>48–51</td>
</tr>
<tr>
<td>Physical training</td>
<td>NA</td>
<td>↓</td>
<td>32, 33</td>
</tr>
</tbody>
</table>

TGF indicates transforming growth factor; PDGF, platelet-derived growth factor; LDL-Ox, oxidized LDL; TNF, tumor necrosis factor; IL-GF-1, insulin-like growth factor-1; Ang II, angiotensin II; NA, not available; NE, no effect.

relation is still unclear. It has recently been proposed that t-PAag could accumulate in the presence of a high concentration of PAI-1 because of the delayed clearance of the t-PA/PAI-1 complex.16 It is our opinion that the aforementioned potential problems in sample handling and processing, as well as differences in the type of assay used, may contribute to explaining the discrepant results obtained in different studies, as discussed later in this review. We did our best to specify whether PAI-1ag or PAI-1act were measured in the studies that are reviewed in this article; however, the generic term PAI-1 levels was used whenever it was not possible to determine which type of assay was used.

Environmental Influences on Plasma PAI-1 Levels

Plasma PAI-1 levels show an intrinsic within-individual variability over time, and are affected by several environmental factors, which are summarized in Table 1. It has been shown that in vitro the expression of PAI-1 is regulated by glucocorticoids,17 thrombin,18 platelet-derived growth factor,19 angiotensin II (Ang II),20 and oxidized LDLs.21 In particular, various cytokines are known to affect PAI-1 expression. Interleukin-1α, transforming growth factor-β, and tumor necrosis factor-α were found to enhance the secretion of PAI-1 in vitro,18,22,23 and tumor necrosis factor-α also increases PAI-1 plasma levels when injected in healthy men.24 Interferon-γ has only a small direct effect on PAI-1ag expression in vitro, but may downregulate both basal and thrombin- or endotoxin-induced PAI-1 expression in cultured human endothelial cells.25,26 It is likely that the regulation occurs at the level of transcription, because sequence elements mediating the response to different regulators have been identified within the promoter region of the PAI-1 gene.27 With regard to other growth factors, in contrast to the results of in vitro experiments, endothelin-1 infusion does not appear to affect fibrinolysis in healthy men,28 and insulin-like growth factor-1 was not found to induce PAI-1 synthesis in vivo when infused in type II diabetic patients.29

Bacteremia and endotoxemia were also found to affect PAI-1 in vivo. Both the infusion of endotoxin in animals and the intravenous injection of lipopolysaccharides in healthy men induce a rapid increase in PAI-1 plasma levels, suggesting a role for PAI-1 in the development of disseminated intravascular coagulation occurring during Gram-negative sepsis.30,31

Moderate physical activity lowers PAI-1ag after 30 and 60 minutes in normotensive and hypertensive men.32 Physical training also decreases PAI-1act, but not PAI-1ag, in men with and without a history of MI.33

With regard to sex, PAI-1 levels are higher in men than in women, but it is noteworthy that testosterone is inversely associated with plasma PAI-1 levels.34 Hypogonadism in males is associated with an increased synthesis of PAI-1, and androgen medication, with stanozolol and danazol, was found to reduce PAI-1 plasma levels,34 whereas estrogens seem to lower plasma PAI-1 levels.35 A significant non–dose-dependent decrease in PAI-1 activity in fertile women taking oral contraceptives, at estrogen doses ranging between 30 and 50 μg, compared with nonusers, was observed.36 Moreover, hormone replacement therapy in postmenopausal women with estrogen alone or estrogen plus progesterone lowers the morning values of PAI-1.37 These findings may explain both the low and high cardiovascular risk profile of premenopausal and postmenopausal women, respectively.

Insulin and triglycerides were found to stimulate PAI-1 production by human cultured endothelial cells or hepatocytes (for review, see Juhan-Vague et al.38), but acute infusions of either insulin or triglycerides in humans did not increase PAI-1 levels.39 Glucose can also increase PAI-1 release in the medium of cultured human endothelial cells; however, there is only a weak association between glycemia and PAI-1 levels in vivo and a short-term glucose infusion did not change PAI-1 concentration.39 Nonetheless, according to some reports, plasma PAI-1 levels would be higher in non–insulin-dependent diabetic (NIDDM) patients than in nondiabetic subjects.40,41 Collectively, available findings suggest that the increase in PAI-1 level in NIDDM patients is not related to hyperinsulinemia per se, but rather to insulin resistance, a contention also supported by the observation that the increase of insulin sensitivity because of weight loss or metformin treatment lowers PAI-1ag levels.41,42 Epidemiological studies have also shown a strong positive correlation between plasma PAI-1act and markers of insulin resistance, such as plasma insulin and proinsulin-like molecules (intact proinsulin and des 31.32 proinsulin) levels, body mass index, (VLDL) triglycerides, and ApoB, in healthy subjects and in patients with NIDDM and CAD.33,34 Therefore, it has been proposed that increased PAI-1 levels may be a component of the “insulin resistance syndrome,”37 a metabolic disorder characterized by upper body obesity, hypertriglyceridermia, and hyperinsulinemia, which may correspond to a prediabetic stage with an increased cardiovascular risk.

The effect of cigarette smoking on PAI-1 plasma levels is still debated. Increased PAI-1act has been observed in healthy
smokers and in both smokers and past smokers with CAD,48,49 and in cigarette smokers compared with pipe/cigar smokers.50 However, no influence of cigarette smoking was found in a series of monozygotic twins discordant for smoking, and in 228 healthy families of the Stanislas cohort.51,52 Therefore, it is not inconceivable that increased PAI-1 plasma levels are related to the presence of atherosclerosis in smokers and past smokers, rather to cigarette smoking itself.

The renin–angiotensin–aldosterone system (RAAS) has recently been found to exert important effects on PAI-1. Ang II has been shown to induce PAI-1 mRNA expression in cultured rat astrocytes and VSMCs20 with a time-dependent increase in PAI-1 act in the medium. When infused in healthy human volunteers at doses capable to attain physiological levels of Ang II, caused a dose-dependent increase in plasma PAI-1 levels (but not in t-PA levels), thereby suggesting a role of Ang II in regulating basal PAI-1 expression in healthy tissues.53 The increased PAI-1 expression in response to Ang II was specific20 and not prevented in vitro by inhibitors of both AT1 and AT2 receptors, suggesting a role for additional receptor subtypes. Elegant in vitro experiments with different inhibitors of angiotensin biosynthesis pointed to Ang IV (Ang 3 to 8) and the recently identified AT4 receptors as the most likely mediators of the effect of the RAAS on PAI-1.54,55 Of interest, treatment with angiotensin-converting enzyme inhibitors was found either to lower or to unaffect plasma levels of PAI-1 act or PAI-1ag (for review, see Lottermoser et al56). The effect of the AT1 receptor antagonist losartan on plasma levels of PAI-1 act and PAI-1ag (for review, see Lottermoser et al56) has been investigated only in vitro studies demonstrated enhanced cytokine-stimulated secretion from vascular endothelium. Because this effect occurred only after angiotensin-converting enzyme inhibition,58 a role of angiotensin-converting enzyme in maintaining the physiological balance between PAI-1 and t-PA is suggested.59

Bradykinin administration in human hypertensives dose-dependently increases plasma t-PA levels by stimulating t-PA secretion from vascular endothelium. Because this effect occurred only after angiotensin-converting enzyme inhibition,58 a role of angiotensin-converting enzyme in maintaining the physiological balance between PAI-1 and t-PA is suggested.59

Of the other hormones, plasma aldosterone levels and renin, but not catecholamines atrial natriuretic factor and arginine-vasopressin, significantly correlated with plasma PAI-1 levels in a subset of patients enrolled in the SAVE (Survival and Ventricular Enlargement) study.59 This finding further supports the contention of a link between the RAAS and the fibrinolytic cascade.

**Genetic Influence on Plasma PAI-1 Levels**

The human PAI-1 gene has been mapped on chromosome 7 (q21.3-q22) and contains 9 exons and 8 introns.27,60 At present, 8 different polymorphisms of the PAI-1 gene are known (Table 2).61 The 4G/5G polymorphism in the promoter region was found to affect plasma PAI-1 antigen and activity levels in some, but not all, studies.45,61–66 Furthermore, in vitro studies demonstrated enhanced cytokine-stimulated gene transcription associated with the 4G, compared with the 5G, allele.62 According to the results of a transfection assay, the 5G allele would bind factor B, a nuclear protein acting as a transcriptional repressor, present in the human hepatocellular carcinoma cell line HepG2, human umbilical vein endothelial cells, and VSMCs.65 Both healthy subjects and patients with CAD or NIDDM, who were 4G homozygotes, were found to have the highest mean plasma PAI-1 antigen or activity levels.45,62–65 Thus, these data would suggest a direct relation between the plasma levels of PAI-1 and the number of 4G alleles, but discordant results are also available. In fact, no significant association was found between PAI-1 act levels and the 4G/5G polymorphism or the HindIII restriction fragment length polymorphism [in strong linkage disequilibrium with both 4G/5G and (CA)n repeat polymorphisms] in 189 patients with NIDDM, and between PAI-1 act levels and the 4G/5G polymorphism and other recently identified 4 polymorphisms in 256 healthy men, aged 50 to 59 years old.61,66

There is also preliminary evidence that the genotype at the PAI-1 gene may affect the relation between PAI-1 antigen and activity levels and serum triglycerides, the association being stronger in 4G/4G than 5G/5G in patients with high triglycerides levels, NIDDM, or CAD.45,62,63,66 Because this gene-environment interaction was not confirmed in a large population of the ECTIM (Etude Cas Temoins de l’Infarctus du Myocardie) study,64 nor in healthy families52 and men,61 this issue remains controversial.

Two recent studies investigated the influence of environmental and genetic background on the total variability of PAI-1 plasma levels.52,67 In Swedish middle-aged and elderly twins, a genetic effect on PAI-1 levels, comprising 42% of PAI-1 act variability, was reported.67 At variance, in 228 healthy French nuclear families, a low (3%) influence of the genotype was found only in women, and a greater importance of the environmental factors, namely, of markers of the insulin resistance syndrome, comprising the 49% in fathers and 29% in mothers of the total PAI-1 variability, was described.52 It is

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Relation With PAI-1 Levels</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CA)n repeat polymorphism in the promoter</td>
<td>NA</td>
<td>61</td>
</tr>
<tr>
<td>(CA)n repeat polymorphism in intron 4</td>
<td>Yes</td>
<td>62</td>
</tr>
<tr>
<td>HindIII restriction fragment length polymorphism</td>
<td>Yes* or no</td>
<td>45, 62–65/61, 66</td>
</tr>
<tr>
<td>4G/5G polymorphism in the promoter</td>
<td>Yes* or no</td>
<td>45, 62–65/61, 66</td>
</tr>
<tr>
<td>6→A substitution at position −844 in the promoter</td>
<td>No</td>
<td>61</td>
</tr>
<tr>
<td>6→A substitution at position +9785 in the coding region</td>
<td>No</td>
<td>61</td>
</tr>
<tr>
<td>6→T substitution at position +11053 in the 3' untranslated region</td>
<td>No</td>
<td>61</td>
</tr>
<tr>
<td>Insertion/deletion of 9 nucleotides in the 3' untranslated region</td>
<td>No</td>
<td>61</td>
</tr>
</tbody>
</table>

*In patients with CAD45,64,65 or NIDDM.62,63
likely, therefore, that in subjects with more pronounced features of the insulin resistance syndrome, the proportion of variance of PAI-1 plasma levels caused by genetic factors is minimized. In agreement with this contention, we recently found that in young, healthy, normotensive twins with no features of insulin resistance syndrome, a predominant additive genetic component accounts for the largest proportion (70%) of variance of plasma levels of PAI-1.68

**PAI-1 Plasma Levels and Coronary Heart Disease**

Several pieces of evidence suggest that high local levels of PAI-1 might play a role in development of CAD. Increased PAI-1 mRNA expression was found in VSMCs and macrophages of human atherosclerotic lesions.69,70 This is not surprising because cytokines and thrombin can increase local synthesis of PAI-1,28,71 with ensuing increased fibrin deposition, incorporation into the intima, and subsequent plaque growth. Local PAI-1 can also affect VSMC migration or proliferation after vascular injury, as suggested by experiments in PAI-1-deficient mice, where vascular wound healing was found to be improved.72–74 Because adenoviral PAI-1 gene transfer was also shown to suppress luminal stenosis after vascular injury, it was concluded that PAI-1 plays an inhibitory role in arterial neointima formation after injury.72,73

In atherosclerotic plaque, VSMC migration is accompanied by production and accumulation of matrix molecules, such as collagen and glycoproteins, and it is known that matrix deposition depends on the balance between protease and antiprotease activity. Therefore, it is conceivable that an increase in PAI-1 expression in the thickened media of atherosclerotic arteries can reduce local plasmin activity and therefore the activation of matrix metalloproteinases, protecting the vessel wall after damage.69,74 Thus, although after plaque rupture an increased local PAI-1 expression may facilitate thrombosis, it is possible that local PAI-1 generation contributes to ensure plaque stabilization.

With regard to the relation between atherosclerosis and plasma levels of PAI-1, some evidence that an impaired fibrinolysis could play a role in the early stage of atherosclerosis has emerged from the ARIC study (Atherosclerosis Risk in Communities), a biracial prospective multicenter study in asymptomatic subjects with intima-media thickening of carotid arteries.75 A cross-sectional case–control study in 455 pairs from this cohort showed a relation between PAI-1ag plasma levels and the intima-media thickness of carotid arteries, although only in white subjects.76 Cross-sectional studies in coronary atherosclerosis have also shown a decreased fibrinolytic activity, associated with elevated PAI-1ag and t-PAag plasma levels in patients with angina pectoris, compared with healthy controls.76–80 In the European Concerted Action on Thrombosis and Disabilities (ECAT) prospective study, where the relation of increased circulating PAI-1ag and t-PAag plasma levels with coronary stenosis was investigated in 2578 patients undergoing coronary angiography, although slightly but significantly higher PAI-1ag (P = 0.0004) and PAI-1act (P = 0.0008) levels were found in patients with 1 to 4 stenosed or occluded vessels, compared with the subjects without stenoses >50%, no significant relation between PAI-1 plasma level and the extent of coronary disease, measured in term of involved vessels, was detected.50 This was consistent with the results of a more recent study on 453 Yorkshire patients, classified at angiography as having normal vessels or single-vessel or multivessel coronary disease, that showed only a borderline significant (P = 0.06) relation between PAI-1ag level and coronary artery stenoses.45 Thus, at the present time there is no conclusive evidence of a relation between PAI-1ag plasma level and the extent of coronary atherosclerosis, and further studies are needed.

No relation between coronary atherosclerosis and the 4G/5G polymorphism was found,49 despite the contention that the 4G/4G homozygotes would have the highest PAI-1 plasma levels. This would also challenge the hypothesis of a role for PAI-1 plasma levels in the development of coronary atherosclerotic lesions, and might suggest that the increased PAI-1 plasma levels in patients with CAD may simply be a consequence of the presence of atheroma.45 However, it must be emphasized that it is not known whether the 4G/5G polymorphism has any relation with the local (arterial wall) levels of PAI-1 and whether the increased plasma levels of PAI-1 reflect an enhanced release of the inhibitor from endothelial cells. Thus, although it has been hypothesized that an increase in local levels of PAI-1 might contribute to the pathogenesis of atherosclerosis, it is still unclear whether the increase in PAI-1 plasma levels observed in this pathological condition represents cause, effect, or both. In our view, the cause–effect relation must be demonstrated in prospective studies of healthy populations before PAI-1 can be considered a risk factor for coronary atherosclerosis, because it is evident that PAI-1 can play an ambivalent role, either by contributing plaque stabilization or by enhancing the risk of thrombosis after plaque rupture.

**PAI-1 Plasma Levels and Complications of Coronary Heart Disease (CHD)**

In animals, disruption of the PAI-1 gene induces a mild hyperfibrinolytic state and a greater resistance to venous thrombosis,73 and conversely transgenic mice engineered to overexpress PAI-1 were found to develop spontaneous venous, but not arterial, thrombosis.81 In a canine model of coronary artery thrombosis, PAI-1act was shown to enhance both thrombosis and thrombus growth.52 Most PAI-1 found in occlusive platelet-rich clots comes from platelets and approximately one-third of PAI-1 in porcine coronary artery thrombi is active.83 As already mentioned, tumor necrosis factor-α can induce PAI-1 secretion from endothelial cells in vitro and in vivo23,24 and other cytokines produced in the atherosclerotic plaque can induce adjacent endothelial cells in vivo to secrete PAI-1, with subsequent clot stabilization.84 Thus, experimental studies suggest an independent pathogenetic role of PAI-1 in the development of complications of CHD, such as MI and stable or unstable angina.

It has been shown that predisposition to formation of an abnormal fibrin gel structure in vitro is associated with premature MI in humans.85 This could be the result of an increase in PAI-1act, because a strong inverse correlation between plasma PAI-1act and fibrin gel porosity, or fiber mass–length ratio, was seen in men with premature MI.85 Over the past few years, cross-sectional studies in patients
with stable and unstable angina and MI showed a decreased fibrinolytic activity, with increased PAI-1ag and t-PAag levels, to be related with CHD, increased PAI-1act being more strongly associated with MI than with angina or CAD. The first evidence of a link between increased plasma PAI-1 levels and CHD was provided by a study in young survivors of MI. Elevated PAI-1act levels were also observed in diabetic and nondiabetic MI patients, for up to 1 to 3 months after MI, despite normalization of other acute-phase proteins. In 62 nondiabetic patients with premature MI, plasma levels of triglycerides, cholesterol, fasting insulin, proinsulin-like molecules, and PAI-1act were higher than in age-matched healthy men. In the already mentioned ECAT study, slightly but significantly (P<0.0001) higher plasma PAI-1ag and PAI-1act levels were found in patients with a history of MI and diabetes.

Plasma PAI-1act, but not t-PAag, was significantly higher in offspring of men with premature MI than in controls; however, it is unclear whether PAI-1act is a heritable risk factor for CAD in males, because in the European Atherosclerosis Research Study (EARS) PAI-1act did not differ between 682 offspring of men with premature MI and 1312 controls. In 165 patients with previous MI studied with coronary angiography, only a trend toward higher levels of PAI-1ag (22.2 versus 18.6 ng/mL; P=0.1), after adjustment for age, sex, body mass index, and triglycerides level, was found. (It must be acknowledged that with multivariate analyses, adjustments of particular variable values for all related parameters may cause a highly significant difference found in a univariate analysis to lose its significance. This may lead to underestimation of the importance of risk factors identified as significant in univariate analyses. Although these factors cannot be regarded as “independent” statistically speaking, they may well retain their relevance as markers of increased cardiovascular risk.) In the ECTIM study, a large 4-center case–control study of MI in patients aged 25 to 64 years, PAI-1act levels were higher in cases than in controls only in the North Irish cohort, whereas in the 3 French cohorts the opposite was found, a difference that cannot be explained by assay variability or blood handling.

Collectively, available data suggest that in men with a high metabolic risk because of insulin resistance, increase of ApoB, fasting insulin, triglycerides, body mass index, and reduced HDL cholesterol, who suffer premature MI, increased PAI-1 levels are likely to be present. Whether this is simply a consequence of the increase of insulin and triglycerides, which stimulate in vitro the synthesis of the peptide, remains to be clarified.

Prognostic Value of PAI-1
Reduced fibrinolytic activity has been linked to the risk of CHD-related events and mortality in several studies of patients with unstable and stable angina pectoris, and in healthy, middle-aged men, but the prognostic value of fibrinolytic variables is still controversial, because prospective cohort studies have given conflicting results. In a longitudinal study of 109 men with premature MI, evidence of a cause and effect relation between PAI-1act and risk of recurrent MI was found. In keeping with this finding, in the prospective trial Angina Prognosis Study in Stockholm (AP-SIS), PAI-1act was found to be an independent predictor for death or nonfatal MI, albeit only in male patients with angina.

Elevation of PAI-1act was also found to be related to cardiac death within 6 to 9 years in a prospective study in 108 nondiabetic men with premature MI, and to development of a thrombotic event after 1-year follow-up in a smaller case–control study of patients with atherosclerotic disease in the PLAT study.

However, other studies have given opposite results, possibly because, as fibrinolytic parameters are strongly related to other risk factors such as insulin resistance and inflammation markers, the adjustment for these variables could reduce the prognostic value of PAI-1. This explanation is also supported by the results of the previously mentioned ECAT study. In this study, 10 fibrinolytic variables were measured in >3000 patients (men and women) with angina pectoris, followed up for 2 years. Of these variables, 3 (t-PAag, P=0.0002; PAI-1act, P=0.02; and PAI-1ag, P=0.001) were found to be associated with an increased incidence of subsequent coronary events. However, after adjustment for markers of insulin resistance, PAI-1 antigen and activity could no longer be considered independent risk factors. [See note about multivariate analyses under section “PAI-1 Plasma Levels and Complications of Coronary Heart Disease (CHD).”]

With regard to the influence of PAI-1 on reperfusion after thrombolytic therapy, it had been established that the level of PAI-1 decreases slightly immediately after thrombolytic therapy, increases again several hours after therapy, and returns to normal 4 to 7 days after thrombolysis, and that raised PAI-1act in MI patients on admission would be associated with reduced likelihood of reperfusion. It has therefore been proposed that increased PAI-1act on day 3 may predict an increased risk of reinfarction, because an elevated PAI-1 level contributes to a prothrombotic state, increasing the likelihood of coronary thrombosis.

PAI-1 Gene Polymorphisms and Ischemic Cardiomyopathy
As already mentioned, 8 polymorphisms were found in the PAI-1 gene and there is preliminary evidence of a direct association of genotypes, particularly of the 4G allele, with plasma PAI-1ag and PAI-1act levels. This led to hypothesize that the 4G/5G polymorphism may be related to the occurrence of ischemic heart disease. This hypothesis has been investigated mainly in small and cross-sectional studies that seem to support the content of a significant association of the 4G allele with MI, although this relation was not confirmed in other studies. In an earlier study, no difference in frequency of 4G or 5G allele between long-term survivors of MI and controls was found. However, because patients were studied 5 to 7 years after MI, a selection bias caused by the early mortality of 4G/4G patients, who might be at highest risk, might have occurred. In the previously mentioned ECTIM study, no association was found between PAI-1 genotypes and MI in 476 cases compared with 601 controls. In the 374 middle-aged men of the American Physicians Health Study (APHS) who developed MI during the 8 years of follow-up, the distribution of the three 4G/5G genotypes was identical to those who remained free of cardiovascular disease. Thus, although this issue is
still controversial, a bulk of evidence supports the contention that the 4G/5G polymorphism in the promoter of the PAI-1 gene is not a major risk factor for MI. In keeping with this view is also the finding that in healthy ultracentenarians the 4G/4G genotype was significantly more frequent than in younger healthy individuals, indicating that the 4G homozygosity is compatible with successful aging.105

**PAI-1 and Restenosis After Percutaneous Transluminal Coronary Angioplasty (PTCA)**

Impaired fibrinolysis, by influencing proteolysis and neointima formation in the arterial wall, has been suspected to participate in the mechanism of restenosis occurring in as many as 25% to 40% of patients within the first 6 months after PTCA. Studies investigating whether PAI-1 levels before and after PTCA can predict the development of restenosis have shown increased PAI-1 levels after PTCA in patients who develop late restenosis. Three and 6 months after PTCA, plasma PAI-1 lact was significantly higher (P<0.005) in 34 patients who develop coronary restenosis than in 70 who did not, although PAI-1 lact was similar before the procedure.106 At variance, t-PAg levels did not differ in these patients at any stage during the whole observation period. In a subsequent clinical trial, fibrinolytic factors of 73 patients were studied before and within 6 months after elective PTCA.107 PAI-1 levels after PTCA were higher in the 27 patients with restenosis than in those without restenosis, despite similar levels before PTCA.107 PAI-1 levels were also assessed in 35 patients before and after directional coronary atherectomy. In 8 patients with late (within 6 months) restenosis, PAI-1 levels increased from 2.4 to 4.9 U/mL (P<0.05) 24 hours after directional coronary atherectomy, whereas there were no changes in patients without restenosis.108

These findings are probably because immediately after the vascular injury there is an inflammatory reaction with recruitment of leukocytes, activation of the coagulation and fibrinolytic cascade, and thrombin production and formation of a platelet-rich thrombus. The increase in PAI-1 plasma levels may therefore be regarded as a reactive phenomenon.

Early recollection caused by this increased thrombogenic reaction after endothelial damage and plaque rupture, which occurs within days after PTCA, does not seem to be influenced by PAI-1 levels. At variance, late restenosis, which is mainly caused by cytokine production, fibroblast and VSMC migration and proliferation, and synthesis of the extracellular matrix with subsequent intimal hyperplasia, is likely to be affected by PAI-1 levels. The increase in plasma PAI-1 levels 3 to 6 months after PTCA, or during the first 48 hours after directional coronary atherectomy in patients who developed late restenosis, may be a consequence of the local inflammatory process with an increase of mediators such as interleukin-1, platelet-derived growth factor, transforming growth factor, and tissue RAAS, which are known to stimulate PAI-1 synthesis. Thus, PAI-1 has been proposed to be an indirect marker of late restenosis.

**Conclusions**

The advancement of our knowledge on the biology of the clotting and fibrinolytic cascade, and of its role in cardiovascular disease, is the result of studies performed, to a large extent, in the past decade. Although several factors, some of which are known cardiovascular risk factors, have been found to affect PAI-1 synthesis, whether PAI-1 carries, per se, an increased risk of cardiovascular disease remains to be determined, perhaps with the exception of late restenosis after PTCA. The fact that increased plasma levels of PAI-1 are likely to be part of the insulin resistance syndrome (“metabolic” syndrome X) has contributed to complicate many studies aimed at clarifying the role of PAI-1. In addition, as for other “players” acting locally as autocrine/paracrine factors, the relation between circulating (plasma) and local (tissue) levels of PAI-1 must be taken into consideration, and it has been neglected in most available studies. Conflicting results are available concerning the genetic determinants of plasma levels of PAI-1 antigen and activity, despite intensive investigative efforts. It can be anticipated that a better understanding of the role of PAI-1 in the different cardiovascular diseases, which might be relevant for the development of novel therapeutic strategies, is likely to be attained in the next decade with the use of molecular techniques, which are becoming increasingly available to most clinical research laboratories.

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