

Plasminogen activator inhibitor-1 in kidney pathology (Review)

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Received September 17, 2012; Accepted November 9, 2012

DOI: 10.3892/ijmm.2013.1234

Abstract. Plasminogen activator inhibitor type-1 (PAI-1) inhibits tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which convert plasminogen to plasmin, a strong proteolytic enzyme. Thus, PAI-1 is a primary and negative regulator of plasmin-driven proteolysis. In addition to its main role as an inhibitor of fibrinolysis, PAI-1 has been implicated as a mediator in other processes, including fibrosis, rheumatoid arthritis, atherosclerosis, tumor angiogenesis and bacterial infections. It also significantly modulates cellular adhesion or migration, wound healing, angiogenesis and tumor cell metastasis. However, in the present study, we have reviewed the literature in relation to different kidney diseases where PAI-1 regulates fibrinolysis and acts independently of proteolysis. PAI-1 is normally produced in trace amounts in healthy kidneys but is synthesized in a wide variety of both acute and chronic diseased kidneys. We reviewed the role of PAI-1 in diabetic kidney nephropathy, chronic kidney disease, hemodialysis, peritoneal dialysis and in kidney transplantation. Increased PAI-1 expression results in accumulation of extracellular matrix (ECM) leading to numerous kidney diseases. Predisposition to some diseases is due to the genetic role of PAI-1 in their development. A number of studies demonstrated that the inhibition of PAI-1 activity or therapy with a mutant PAI-1 increases matrix turnover and reduces glomerulosclerosis by competing with endogenous PAI-1. This strongly suggests that PAI-1 is a valid target in the treatment of fibrotic renal disease. However, net proteolytic activity depends on the delicate balance between its negative regulation by PAI-1 and activation by uPA and tPA. Also, plasmin activated by its inhibitors upregulates activity of other enzymes. Thus, assessment of prognosis for the diseased kidney should include a variety of proteolysis regulators and enzymes.

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1. Introduction

The kidney plays a critical role in human health and failure in glomerular filtration leads to severe handicap and mortality unless the diseased kidney is replaced by transplantation. Kidney diseases often accompany diabetes and obesity. These two ailments are also interrelated with cardiovascular (CV) risk and deep vein thrombosis. The above suggest involvement of proteolytic enzymes of fibrinolysis. In this review, we have focused on plasminogen activator inhibitor type-1 (PAI-1), a potent regulator of fibrinolysis.

PAI-1 inhibits tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). These two convert plasminogen to plasmin, a strong proteolytic enzyme. Therefore, PAI-1 is a primary and negative regulator of plasmin-driven proteolysis (1-3). PAI-1 is involved in several biological processes, and, depending on the disease, its overexpression or deficiency may trigger unexpected outcomes. For example, patients with PAI-1 deficiency (defined as activity of PAI-1 in blood lower than 1 IU/ml) suffer from frequent bleeding episodes. This is a result of a premature clot lysis by overproduction of plasmin (4-6). Hereditary PAI-1 deficiency is caused by variants of PAI-1 Ala15Thr, Val17Ile resulting from polymorphisms at a signal peptide (7) and frame-shift mutation in exon 4 of the PAI-1 gene that resulted in complete PAI-1 deficiency due to nonfunctional protein (4). By contrast, hyperactivity of PAI-1 has been associated with an increased risk for coronary artery disease and myocardial infarction due to inhibition of fibrinolysis (8-10). A common functional deletion/insertion polymorphism (4G/5G) in the promoter region of the PAI-1 gene located at 675 bp was reported to result in the enhanced plasma expression of PAI-1 (11).

In addition to its major role as an inhibitor of fibrinolysis, PAI-1 has been implicated as a mediator in other processes,

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Key words: kidney, plasminogen activator inhibitor, kidney disease

including fibrosis, rheumatoid arthritis, atherosclerosis, tumor angiogenesis and bacterial infections. It also significantly modulates cellular adhesion or migration, wound healing, angiogenesis, and tumor cell metastasis (12-14). However, in the present study, we have reviewed literature in relation to different kidney diseases where PAI-1 regulates fibrinolysis and acts independently of proteolysis. PAI-1 is normally produced in trace amounts in healthy kidneys but is synthesized in a wide variety of both acute and chronic diseased kidneys (15,16).

2. PAI-1 in kidney transplantation

Steady improvements in patient first-year and graft survival rates have been reported by Rerolle *et al* (17). Nevertheless, chronic allograft dysfunction (CAD) remains the leading cause of the late renal allograft loss. Thus, numerous investigators have attempted to decipher the mechanism of renal kidney transplant failure.

Persistent fibrin accumulation has been observed in kidney chronic rejection. The process to remove fibrin in tissues is initiated by tPA and suppressed by PAI-1. For that reason, Wang *et al* (18) investigated their roles in chronic rejection and fibrin deposition in a Fisher 344 to Lewis rat renal transplant model. The authors examined the expression of tPA and PAI-1 in a chronic rejection model at 0, 2, 4, 6, 10, 12, 16 and 20 weeks post-transplantation. They found that tPA was over-expressed only in the acute phase of rejection, but PAI-1 was upregulated and persistently expressed during the progressive phase of chronic rejection, synchronously with fibrin deposition in the grafts. They propose that induction of PAI-1 may be responsible for the fibrin deposition, which leads to irreversible damage and chronic kidney loss (18).

The chemokine receptor (CCR1), is believed to play a crucial role in the migration of leukocytes to sites of inflammation and is expressed in T cells, dendritic cells, monocytes, and occasionally in neutrophils. The chemokines and their receptors have been proposed to damage the allograft. Thus, Bedke *et al* (19) investigated the effects of CCR1 antagonist BX 471 in Fischer to Lewis renal transplants at Days 10, 21 and 42 after transplantation. Therapies initiated on Day 21 after transplantation reduced glomerulosclerosis and tubulointerstitial fibrosis by Day 42 after transplantation. The PAI-1 gene was downregulated, as was the transforming growth factor (TGF)- β and others. Also, reduced collagen deposition was observed. The authors suggested that CCR1 antagonists may be a therapeutic option for fibrosis in renal grafts.

Inside the glomerulus, PAI-1 is mainly synthesized by mesangial cells. Delarue *et al* (20) hypothesized that thrombin, via its protease activated receptor type-1 (PAR-1) on the membrane of glomerular cells, is a mediator of PAI-1 overexpression in humans. They analyzed 23 kidney transplant patients and followed them up for up to five years. They examined mRNA expression of the PAI-1, the thrombin receptor PAR-1, among others, by reverse transcription-polymerase chain reaction. They found a significant upregulation of PAI-1 mRNA in acute rejection ($P<0.05$) in comparison with normal kidneys. A positive correlation exists between $\alpha 2$ IV collagen mRNA level and the degree of cellular infiltration. A negative correlation was found between the level of mRNA of PAR-1

and the degree of vascular thrombosis ($P=0.005$). However, a positive correlation was observed between the degradation of renal function and the mRNA level of PAI-1 ($P<0.05$). The authors concluded that glomerular PAI-1 mRNA could be predictive of long-term renal graft function.

Since PAI-1 plays an important role in renal fibrosis, Chang *et al* (21) examined whether serum PAI-1 has a role in predicting chronic allograft nephropathy (CAN). They examined 50 kidney transplant recipients to determine if there was a correlation between serum levels of PAI-1 and the chronic allograft damage index (CADI). They found that CADI score was associated with serum PAI-1 activity ($r=0.405$, $P=0.003$) and concluded that the serum PAI-1 level may be a potential marker to predict CADI score.

The plasma level of PAI-1 is genetically determined by a homozygous polymorphism in the 4G allele rather than other genotypes in the promoter region. Chow *et al* (3) investigated if such genetic variation in the fibrinolytic system affects the long-term renal transplant outcome. One hundred and thirty patients were assessed over a period of 75 months. Baseline clinical variables were comparable among three genotype groups. There was no association between primary event and PAI-1 genotype among the entire cohort. However, they found that among subjects with prior acute rejection episodes, those homozygous for 4G polymorphism had a significantly higher risk of kidney rejection (3).

In a different study, 82 renal allograft recipients were investigated for the PAI-1 polymorphism. Kidney recipients with CAD had significantly lower frequencies of the 5G/5G genotype and the 5G allele compared to those without CAD ($P<0.001$ and $P<0.05$). The authors concluded that determination of PAI-1 genotypes prior to transplantation may help identify patients who are at risk for chronic renal transplant dysfunction (22).

Furthermore, Rerolle *et al* (17) determined PAI-1 genotype in over 300 donors and kidney recipients in relation to the development of fibrosis, interstitial fibrosis and tubular atrophy (IFTA). They found that donor or recipient genotype did not influence the PAI-1 plasma level in recipients. However, kidney graft survival was statistically significantly reduced in the 4G/4G (higher PAI-1 activity) donor group (107 months vs. 147.5 months, $P=0.013$), but recipient PAI-1 genotype did not show any effects on kidney survival. The authors suggested that donor PAI-1 polymorphism impacts kidney graft survival and the donor 4G/4G genotype is an independent risk factor for kidney loss as well.

Characteristic for the CAN is deposition of extracellular matrix (ECM) in all renal compartments, thus PAI-1 plays a pivotal role in ECM turnover in this condition. Rapamycin (RAPA) has been shown to improve long-term graft survival in patients with CAN and Pontrelli *et al* (23) investigated whether that treatment has any effect on PAI-1 gene expression. They evaluated six patients on calcineurin inhibitors (CNI) and 12 patients who received RAPA. Patients underwent a renal biopsy at time 0 (T0) and after 24 months of treatment (T24) and PAI-1 expression was evaluated by immunohistochemistry. They reported that the RAPA group exhibited a significant regression of glomerulosclerotic lesions and a modest increase in interstitial fibrosis (26%) at T24. By contrast, CNI patients showed progress of glomerulosclerosis and a 112% increase in

fibrosis. Immunohistochemistry showed that glomerular and tubulointerstitial PAI-1 expression was reduced in the RAPA group, but remained unchanged in the CNI patients. They concluded that RAPA reduces ECM deposition in CAN by downregulating PAI-1 expression (23).

Ishikawa *et al* (24,25) treated kidney transplant patients with angiotensin II receptor blockers (ARB) as it is known that proteinuria from a renal graft is significantly decreased by administration of ARB. To examine the underlying mechanism, they conducted an additional clinical study to investigate changes in plasma PAI-1 levels among renal allograft recipients. Four kidney transplant patients were treated with 50 mg/day of losartan (LOS) and four others served as a control. One year after starting the treatment, PAI-1 levels in losartan-treated patients were $78.6 \pm 6.7\%$, while controls showed $110.4 \pm 9.2\%$ of pretreatment levels. The authors emphasized that PAI-1 reduction by ARBs was key for renal preservation (24). In follow-up studies, they reported that patients treated with LOS immediately after transplantation had PAI-1 levels at the end of two years of treatment $81.5 \pm 10.3\%$ of the initial PAI-1 level. A similar effect was observed in patients who started treatment two years after kidney transplantation and were observed for two additional years showing $90.1 \pm 12.5\%$ of initial PAI-1 levels. Furthermore, microscopic examination revealed less renal interstitial fibrosis among LOS-administered groups than control groups (25).

Lahlou *et al* (26) investigated PAI-1 as a fibrogenic molecule whose secretion is regulated not only by genetic factors but also by several metabolic, inflammatory elements. They sought to verify if PAI-1 secretion in renal transplant patients is correlated with the decline in renal function following transplantation. One hundred and five renal transplant patients were included in the study. In addition to the routine clinical and biological data collected, the 4G/5G polymorphism of the kidney transplant patient PAI-1 gene was determined, as well as the PAI-1 plasma level. The multiple linear regression analysis indicated that the rate of decline in renal function was significantly correlated with the PAI-1 plasma level ($P=0.0051$). Also, the PAI-1 plasma level was significantly correlated with body mass index ($P=0.038$), insulin ($P<0.0001$), platelet count ($P<0.0001$), and fibrinogen ($P=0.024$). However, the PAI-1 gene polymorphism did not influence the rate of decline in renal function following transplantation. The study suggests that PAI-1, whose secretion is affected by metabolic and inflammatory factors, could be related to the rate of decline in renal function after transplantation.

Revelo *et al* (27) analyzed renal biopsy/nephrectomy files from 82 patients and scored all cases for severity of fibrosis in vasculature (0-3 scale), glomeruli and interstitial fibrosis. They immunostained for PAI-1 and assessed on a 0-3 scale in glomeruli, vessels and tubules. PAI-1 was increased in CAN compared with non-scarred native or transplant control kidneys. The authors speculated that modified matrix metabolism can be implicated in the development of CAN.

Knowing that CAN displays extensive interstitial fibrin deposition, Grandaliano *et al* (28) investigated if thrombin, present within the fibrin clots, can act together with PAR-1 and modulate a variety of pathways. They investigated 16 CAN biopsies and 10 normal human kidney grafts and found that fibrin deposits were observed in the interstitial space and arte-

rial wall of CAN but not in normal grafts. Notably, PAI-1 gene expression, scarcely detectable in control tissue, was markedly increased in CAN, and showed distribution similar to the arrangement of fibrin deposition. Fibrin deposits in CAN are a result of increased expression of PAI-1 and the subsequent inhibition of uPA activity that leads to a decrease of plasmin-driven fibrinolysis of ECM. Finally, thrombin preserved in the fibrin may upregulate PAR-1 and induce ECM by the TGF- β depended pathway. They also found that urine from CAN patients contained significantly higher levels of TGF- β (25). TGF- β stimulates expression of PAI-1 but PAI-1 can stimulate TGF- β expression creating positive feedback loop further promoting ECM presence (29-31).

Wang *et al* (32) studied PAI-1 gene expression by *in situ* hybridization in human renal tissue showing severe acute vascular rejection, clinically irreversible vascular rejection, mild vascular rejection, parenchymal rejection, non-rejecting kidneys and normal kidneys. They found that in 87.5% of cases showing severe acute vascular rejection and in 100% of chronic vascular rejection cases, PAI-1 mRNA was positive in endothelial cells of arterioles and arteries, and interstitial inflammatory cells but not in any other group. Notably, PAI-1 was detected frequently in areas of hemorrhage. However, it is not clear if PAI-1 caused thrombosis and ischaemia, the catastrophic consequence of severe vascular rejection or if it was produced as a response to hemorrhage in a potentially protective role.

Patrassi *et al* (33) used a more detailed approach in investigating fibrinolytic potential. They determined t-PA and PAI-1 activities and antigen as well as euglobulin lysis time. As measured by these determinants a hypofibrinolytic state was found in 68.4% of renal transplant patients. They concluded that an imbalance in the fibrinolytic system is a typical feature of renal transplantation patients in a long time following transplantation.

3. PAI-1 in diabetic kidney nephropathy

Obesity is a major risk factor for type 2 diabetes mellitus that is associated with chronic inflammation and consequential activation of the innate immune system (34). This activation results in the release of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and IL-6 (35-37). These cytokines initiate the production of serum amyloid-A, haptoglobin, C-reactive protein, and PAI-1 (35). Consequently, other proteins are activated, including JNK, IKK- β and PKC and protein tyrosine phosphatases such as PTP1B and PTEN, which impair insulin signaling at insulin receptor and insulin receptor substrate levels. Insulin resistance further stimulates the production of CRP and PAI-1 (38).

Chen *et al* (39) treated diabetes-induced (streptozotocin, 25 mg/kg; STZ) male Wistar rats with fenofibrate, a fibrate class drug that is mainly used to reduce cholesterol levels but that also has protective effects on the diabetic kidney. The cohort included control untreated nondiabetic, untreated diabetic, and fenofibrate-treated (32 mg/kg/day, for 8 weeks) diabetic rats. Kidney nephropathies were associated with the overexpression of PAI-1 mRNA and its protein activity in the renal cortex, and a substantial raise in TGF- β . Treatment with fenofibrate lowered the expression of PAI-1 mRNA as well as

protein activity, and inhibited TGF- β . Furthermore, partially reversed pathophysiologic changes associated with diabetic nephropathy in the treatment group were also observed. The authors emphasized that renoprotective effects were achieved through suppression of PAI-1 and TGF- β in the renal cortex, which reduced ECM accumulation.

Hagiwara *et al* (40) investigated the gene expression of fibrinolytic factors in diabetic nephropathy in the kidneys of diabetic rats. As a model of type one diabetes, they used male Sprague-Dawley rats divided into three groups: control, STZ-induced diabetic, and insulin-treated diabetic. As a model of type two diabetes, they used Otsuka Long-Evans Tokushima Fatty (OLETF) rats and as the control they used Long-Evans Tokushima Otsuka (LETO) rats. The study examined uPA, tPA and PAI-1 genes by real-time PCR. Spatial distribution of these genes was determined by *in situ* hybridization. In STZ-induced diabetic rats (type one diabetic model) mRNA levels were increased by 60-80% and insulin treatment reduced expression to the control level. In OLETF rats, the renal PAI-1 mRNA level was 250% of that in age-matched LETO rats. Conversely, tPA and uPA mRNA levels were lower than those in LETO rats. They found PAI-1 mRNA in intraglomerular cells and tubular epithelial cells of both models. They concluded that the PAI-1 gene was upregulated in type one and type two diabetic rats, and they suggested that modulation of gene expressions of fibrinolytic factors played a central role in the development of diabetic nephropathy.

Lee and Ha (41) used PAI-1^{-/-} mice and found that PAI-1 induces ECM deposition in diabetic kidneys through increased ECM synthesis by TGF- β upregulation and through reduced ECM degradation by suppression of proteolytic activity of plasmin and MMP-2 activity. Lassila *et al* (42) reported that disruption of the PAI-1 gene protects mice against diabetic nephropathy in PAI-1^{-/-} mice. Effects of PAI-1 deficiency on the renal disease in experimental diabetes in mice were also determined in another study. Diabetes was induced by injection of STZ in 6-week-old PAI-1-deficient mice, and transgenic mice overproducing PAI-1 in comparison with wild-type mice. The authors detected that the PAI-1 message was higher in kidneys from normal mice with diabetes and in nondiabetic PAI-1 transgenic mice. Diabetes-associated glomerular injury, albuminuria and, renal α -smooth muscle actin production, were enhanced in diabetic mice in comparison with wild-type mice. Thus, they concluded that endogenous PAI-1 deficiency protects mice from glomerular injury.

A PAI-1 inhibitor may be a therapeutic agent for fibrotic diseases (43,44). However, only a few PAI-1 inhibitors have been identified so far and their clinical potential has yet to be evaluated (43). Huang *et al* (45) overcame the shortage of PAI-1 inhibitors by short-term administration of inactive PAI-1 mutant but otherwise in active form conformation (PAI-IR). This protein slowed down the progression of glomerulosclerosis in the db/db mice (46). They observed that PAI-IR increases glomerular plasmin generation, reverses PAI-1 inhibition of matrix degradation, and consequently reduces symptoms of disease in experimental animals. Non-treated db/db mice used as a control developed progressive albuminuria and mesangial matrix expansion. PAI-1 in plasma binds to vitronectin (Vn) that extends PAI-1 half-life. The authors suggested that PAI-IR interferes with WT-PAI-1: Vn binding by saturating vitro-

nectin with PAI-IR. Consequently, WT-PAI-1 not complexed with vitronectin converts quickly to latent form and the anti-protease activity of PAI-1 is diminished (45,47).

PAI-1 levels are elevated in type two diabetes, and this elevation correlates with complications of diabetes, although the link between insulin and upregulation of PAI-1 remains unclear (48). Festa *et al* (49) reported the correlation between prevalence of diabetes and changes of fibrinogen and PAI-1. The duration of this study was more than 5 years and diabetes development was observed in 140 of 843 individuals. The increase of PAI-1 levels was associated with the incidence of diabetes. Furthermore, progression of PAI-1 levels over time was correlated with the development of type two diabetes as well as the rising glucose levels (49). Significant findings supporting the causative role of PAI-1 in diabetes complication were described by Nagi *et al* (50). An ethnic group of Pima Indians with a very high rate of obesity, insulin-resistant and hyperinsulinemic, have high frequencies of diabetes as well as risk of ischemic heart disease and other diabetes complications. In contrast to other ethnic groups, PAI-1 activity is similar between nondiabetic and diabetic Pima Indians and low PAI-1 activity protects this population from side-effects.

As was observed in complications of kidney transplantation, an elevated plasma level of PAI-1 expedites diabetic vascular complications and suggests diabetic nephropathy to be the major implication of high levels of PAI-1 (51,52). The PAI-1 4G polymorphism is the cause of high plasma PAI-1 levels in 4G allele carriers suggesting that this PAI-1 polymorphism is a genetic risk factor for diabetes. Thus, Meigs *et al* (52) tested this hypothesis among 2,169 participants. They concluded that elevated PAI-1 levels may indeed be associated with an increased risk for diabetes and endothelial dysfunction in these patients.

4. PAI-1 in chronic kidney disease

Defined as estimated glomerular filtration rate (eGFR) <60ml/min/1.73 m², chronic kidney disease (CKD) is an increasing medical problem. In the US, approximately 26 million people have CKD (53). Of the diseases leading to CKD, chronic glomerulonephritis, diabetes mellitus, hypertension, ischemia and urological obstruction contribute to renal fibrosis. A reduced glomerular filtration rate may lead to endothelial dysfunction and inflammatory activity, which can be detected by measurement of the biochemical markers which have been shown to predict CV disease. An impaired renal function is associated with the markers of endothelial dysfunction and increased inflammatory activity (54). The endothelial dysfunction links the processes of immune inflammation, hemorheology and fibrinolysis/proteolysis in the kidney (55). Also, recent epidemiological studies linked CKD with a risk of venous thrombosis directly related to increased levels of PAI-1 (56).

Several possible mechanisms explain the association of low eGFR and higher levels of the hemostatic factors. Decreased renal clearance may cause an increase in the levels of smaller molecular weight hemostatic markers. On the other hand, kidney dysfunction may generate a thrombotic milieu indirectly through the electrolyte or acid-base abnormalities,

which may alter activities of enzymes involved in coagulation (57). CKD stage 3 to 5 is an established risk factor for CV morbidity and mortality although the mechanisms underlying the association are not fully understood (58). This association begins in the preclinical stages of kidney disease (57). Several studies indicated that atherosclerosis and the increased risk of CV disease are associated with inflammation and coagulation (59). The relation between chronic inflammation and CKD are better documented, but deregulation of hemostasis contributing to the association of CKD and CVD has yet to be fully examined. Results of a study by Dubin *et al* (57) indicated that patients with eGFR <60 ml/min/1.73m² presented PAI-1 at 6.5% higher levels compared to subjects with eGFR >90. The study suggested that the deregulation of hemostasis may play an important pathologic role in CKD.

5. Hemodialysis (HD) and PAI-1

CKD (stage 5) patients face a more than 3-fold higher risk of CV events compared with the general population, and the chronic hemodialysis (CHD) patients face a 100-fold higher risk of CV mortality compared with the general population of patients aged ≤45 years. Traditional risk factors for atherosclerosis do not account for the increased incidence of the CV events. On the other hand, biomarkers of the oxidative stress and inflammation can predict CV events in CHD patients (60).

During HD, contact of the blood with the dialyzer and dialysate activates the kallikrein-kinin system (KKS) and induces a systemic inflammatory response characterized by the leukocyte activation, and generation of cytokines. For example, HD increases leukocyte expression of IL-1 β , IL-8 and TNF- α , and the circulating concentrations of IL-6 (61). Increased inflammation may, in turn, contribute to imbalances in the fibrinolytic system in CHD patients. Cytokines such as TNF- α , IL-1 β and IL-6 stimulate expression of PAI-1, the major physiologic inhibitor of fibrinolysis *in vivo*. t-PA concentrations and activity increase transiently during HD, whereas circulating PAI-1 concentrations are increased in the CHD population (62). It has been shown that dialysis patients treated by HD or peritoneal dialysis (PD) have increased plasma levels of PAI-1 which makes them vulnerable to CV risk (63). CHD patients exhibit abnormalities in the platelet function, fibrinolysis and coagulation (60). Fibrinolytic activity increases acutely during HD, largely related to the increase in t-PA, but decreases markedly after HD (64). Some investigators have reported no change in PAI-1 antigen or activity during HD, but have not measured PAI-1 in the postdialysis period. Marney *et al* (60) observed increased PAI-1 after dialysis. In their study, they showed that bradykinin B₂ receptor blockade abolished the increase in PAI-1 following HD, suggesting that the CHD patients exhibit endothelial dysfunction and that the pro-inflammatory effects of bradykinin predominates in regulating PAI-1, at least during HD. PAI-1 antigen is both an acute-phase reactant and an inhibitor of fibrinolysis. During HD, increased t-PA antigen may complex with PAI-1 antigen causing a decrease in its activity. The data from Marney *et al* (60) suggested that endogenous bradykinin receptor blockade attenuates fibrinolysis.

In agreement with previous observations (65), Stefoni *et al* (66) reported that PAI-1 values in hemodialyzed patients proved higher than among controls. The increase was more noticeable in cardiovascular HD patients. These results may be related to the high plasma cytokine levels found in hemodialyzed patients and related to the degree of endothelium dysfunction. In the study by Stefoni *et al* (66), PAI-1 levels showed a moderate, inverse correlation with TGF-1 levels. This effect may combine with the similar inhibition by Lp(a) (lipoprotein) and partly explain the low TGF-1 values.

The study of Segarra *et al* (62) consisted of 200 HD patients and investigated the relationship between the circulating levels of the endothelial cell glycoproteins, PAI-1, tPA, thrombomodulin and the major vascular risk factors described in dialysis patients, and determined the role of these endothelial cell products as independent predictors of atheromatous cardiovascular disease in a large group of nondiabetic dialysis patients. This study concluded that circulating PAI-1 was an independent predictor of complications in coronary artery stenosis after adjusting for the major vascular risk factors and CRP. Collectively, our data suggest that increased circulating PAI-1 could indicate a chronic endothelium activated state and could be used as an additional tool in identifying dialysis patients who are at risk for developing atheromatous CV disease.

6. Peritoneal dialysis (PD) and PAI-1

Kim *et al* (67) reported that PD patients with atherosclerosis had significantly higher tPA levels than those without atherosclerosis and the normal controls. An elevated tPA antigen level in patients with atherosclerotic vascular disease reflects primarily an increase in circulating complexes of tPA and PAI-1, which can explain a positive correlation between tPA and PAI-1 levels. Several longitudinal cohort studies have also provided evidence that impaired fibrinolysis due to increased PAI-1 activity is implicated in the pathogenesis of atherosclerotic disease. PD patients with atherosclerosis had significantly higher PAI-1 levels than those without atherosclerosis and the normal controls. Kim *et al* (67) observed correlations between PAI-1 and triglycerides, which were also reported by Gray *et al* (68). These findings suggest that fibrinolytic activity is correlated with lipid disturbances.

Arikan *et al* (63) investigated whether PAI-1 could independently predict CV outcome in PD patients. They studied 72 PD patients and in a multivariable Cox regression analysis showed that plasma PAI-1 at 41 ng/ml was independently predictive of higher CV mortality (P=0.021) and CV events (CVEs) (P=0.001). The only other independent predictor of CV mortality was CRP (5 mg/l; P=0.008). The authors suggested that plasma levels of PAI-1 >41 ng/ml are a significant predictor of CV mortality and CVEs in PD patients.

Pawlak *et al* (69) showed that PD patients had high concentrations of PAI-1 and that tissue factor (TF), its inhibitor (TFPI), prothrombin fragment 1+2 [F(1+2)], uPA, its soluble receptor (suPAR), plasmin/antiplasmin (PAP) complexes, KYN, kynurenic (KYNA) and quinolinic (QA) acid levels were significantly higher as well. Tissue-type plasminogen activator and PAI-1 were higher in patients with CVD compared to patients without CVD and normal controls.

Malyszko *et al* (70) indicated that adipose tissue secretes various bioactive substances including leptin, TNF, adiponectin and PAI-1 and may contribute to the CVD in the PD patients. PAI-1 is closely involved in the development of atherosclerosis. In PD patients prone to atherosclerosis and CV complications, adiponectin was inversely related to PAI-1 in both diabetic and nondiabetic peritoneally dialyzed subjects. Maruyoshi *et al* (71) reported a similar correlation in patients with a stable angina. Moreover, in a multiple regression analysis, in addition to gender and angina pectoris, PAI-1 was an independent determinant of a hyperadiponectinemia. In the study by Malyszko *et al* (70), PAI-1 was higher and adiponectin was lower in the PD patients with CVD, compared to the patients without CVD. However, in the patients with CVD, correlation between adiponectin and PAI-1 did not reach statistical significance ($r=-0.51$, $P=0.084$). Correlation between PAI-1 and adiponectin in the PD patients (both diabetic and nondiabetic) may support the hypothesis that adiponectin acts as a protective factor for the CV system.

An additional role of PAI-1 was reported by Lin *et al* (72), who described that PAI-1 measured during PD may reflect the intraperitoneal fibrinolytic balance. Elevated PAI-1 levels observed during PD may impede peritoneal solute and water transport by its maintenance and enhanced deposition of basement membranes and peritoneal interstitial ECM. They suggested that intraperitoneal plasminogen activators may be of therapeutic clinical value in such PD patients with elevated PAI-1 levels and altered peritoneal transport. Lin *et al* (72) observed a correlation between dialysate PAI-1 levels and the episodes of peritonitis, and concluded that the elevated PAI-1 levels in dialysate were likely from the local production and release of PAI-1. Also, repeated inflammation of the peritoneum was associated with an increased production and release of PAI-1 into the peritoneum.

7. Conclusions

A delicate balance exists between ECM synthesis and degradation that is regulated largely by PAI-1. Increased PAI-1 expression results in accumulation of ECM leading to numerous kidney diseases. Predisposition to some diseases is due to the genetic role of PAI-1 in their development (73).

Several studies have demonstrated that inhibition of PAI-1 activity or therapy with a mutant PAI-1 increases matrix turnover and reduces glomerulosclerosis by competing with endogenous PAI-1, strongly suggesting that PAI-1 is a valid target in the treatment of fibrotic renal disease. However, net proteolytic activity depends on the delicate balance between its negative regulation by PAI-1 and activation by uPA and tPA. Also, plasmin activated by its inhibitors upregulates the activity of other proteolytic enzymes such as collagenases and metalloproteinases (13,74,75). Thus, assessment of prognosis for the diseased kidney should include a variety of proteolysis regulators and enzymes.

Other than its protease inhibitory activity, the de-adhesive action of PAI-1 is less understood and plays an unknown role in kidney disease (76,77). Additional not proteolytic activity of PAI-1 includes upregulating TGF- β which in positive feedback promotes synthesis of PAI-1 (44,78-83).

Thus, TGF- β may be the therapeutic target in nephropathy moderating ECM production through the PAI-1 pathway (44,84-87).

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