PAI-1 Inhibition – Another Therapeutic Option for Cardiovascular Protection

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ABSTRACT

Current research suggest that plasminogen activator inhibitor type 1 (PAI-1) is an important contributor to a number of disease processes. The aim of the current paper is to emphasize the deleterious effects of PAI-1 on cardiovascular diseases development and progression. The plasminogen system is known by its role in hemostasis and thrombosis regulation. Lifestyle changes and pharmacological treatment can regulate PAI-1 levels and functions.

Some pharmacologic agents currently used in the management of atherosclerosis and its complications can counteract the deleterious effects of PAI-1.

Keywords: plasminogen activator inhibitor type-1, cardiovascular protection, PAI-1 antagonists

INTRODUCTION

Plasminogen activator inhibitor 1 (PAI-1) was discovered a quarter of century ago as an important component of the coagulation system. Over the time, many researchers tried to understand the PAI-1 contribution to different disease states. An association between elevated PAI-1 levels and cardiovascular risk factors such as hypertension, obesity, insulin resistance, and diabetes, was observed (1,2).

In this paper, we provide an overview of the current role and function of PAI-1 and we look at the potential therapeutic benefits of different agents that may influence the clinical outcomes.

Potential PAI-1 role in cardiovascular pathology

Research studies suggest that reduced blood fibrinolytic activity and elevated plasma PAI-1 activity, PAI-1 antigen, and TPA antigen are predictive for coronary artery disease (CAD) and related acute events (2,3). A recent meta-analysis proved that PAI-1 polymorphism (4G/5G) is associated with myocardial infarction (4).

PAI-1 is linked closely to renin-angiotensin system (RAS), an important contributor to vascular disease initiation and progression. However, the RAS blockade slows the progression of processes related to vascular disorders without stopping them. This can probably be explained by involvement of additional pathways, such as...
decreased fibrinolysis. Conversely, the fibrinolytic balance is modulated by RAS.

The main risk factors of atherosclerosis are closely linked to fibrinolysis components.

Hypertension and pre-hypertension are associated with an increased risk of thrombosis suggesting an interaction between the RAS and hemostasis (5-7).

Insulin increases the expression of PAI-1 through several molecular mechanisms (8). Type-2 diabetes together with CAD may be associated with increased production and circulating levels of PAI-1(8). Some researchers find that PAI-1 4G/5G polymorphism can be associated with type 2 diabetes risk (9).

Patients with metabolic syndrome present significantly higher levels of PAI-1 and t-PA antigen (10). Obese subjects have increased production and circulating levels of PAI-1 (10).

In healthy young adults, PAI-1 may be independently associated with cardiovascular diseases (CVD) risk factors, but it was not demonstrated as a biomarker for early atherosclerosis (1).

**PAI-1 functions**

PAI-1 acts by inhibition of tissue-type (tPA) and urokinase-type plasminogen (uPA) activators. T-PA is the predominant plasminogen activator PA in blood, and u-PA, the predominant one in tissues. Elevated PAI-1 plasma levels are associated with vascular thrombosis, whereas PAI-1 deficiencies may result in increased fibrinolysis and a bleeding diathesis (3).

Fibrinolysis in the vascular system is initiated by tPA. The inhibition of PA activity in the blood by PAI-1 is necessary for maintenance of homeostasis and bleeding prevention.

PAI-1 is synthesized under the control of diverse promoters in multiple tissues such as adipose tissue, liver, endothelial cells and platelets.

Recently, increased PAI-1 synthesis in adipose tissue due to acute systemic inflammation was demonstrated in vivo, in humans (10).

Its regulation is complex and incompletely understood. PAI-1 is stabilized by binding to vitronectin, which is present in plasma and extracellular matrix (3).

PAI-1 levels may have a diurnal variations pattern, vary with exercise and rest (11).

Elevated levels of plasminogen activator inhibitor-1 are implicated in the pathogenesis of fibrosis in kidney, skin, lung, heart, and liver (12).

There are conflicting data about the role of overexpressed PAI-1 in the development of intimal hyperplasia after vascular injury. Some studies found neointima formation after vascular injury, others suggesting inhibition of neointima formation. Neointima formation is supported by the fact that PAI-1 promotes vascular smooth muscle cells (VSMC) proliferation and inhibits apoptosis (13). Meanwhile, in some studies reduction in neointima formation were explained by: (a) inhibition of u-PA and related plasmin formation; (b) binding to extracellular matrix vitronectin that have overlapping binding domain for PAI-1 and for receptors located on VSMC; and (c) recombinant PAI-1 inhibits intimal hyperplasia by inhibiting proteases and binding vitronectin (13).

A more recent paper challenges again the concept that PAI-1 determines nonthrombotic obstructive vascular disease. In vein grafts, PAI-1 overexpression did not increase intimal hyperplasia, although it promotes VSMC migration in vitro. This can be explained by its antiproteolytic function, including its antithrombin activity (3).

**PAI-1,ATHEROSCLEROSIS AND RELATED COMPLICATIONS**

In the arterial wall, both thrombosis and lipid deposition appear to contribute to atherosclerosis and its complications. Increased PAI-1 expression in vessel wall appears to precede formation of atherosclerotic plaques. The effects of PAI-1 on plaque infiltration, proliferation, migration- and apoptosis of VSMCs represent important ways to favor atherosclerosis (14). PAI-1 stimulates migration of VSMC by binding to low-density lipoprotein (LDL) receptor, present on VSMC. Oxidized-LDL stimulates the production of PAI-1 in endothelial and VSMC and plays an important role in the pathophysiology of atherosclerosis and atherothrombosis (3,10).

When the balance between thrombosis and thrombolysis is shifted toward thrombosis, elevated levels of PAI-1 increase the exposure of vessel walls to intermittent platelet activation. PAI-1 overproduction potentiates development of VSMC- poor plaques with thin fibrous caps, necrotic cores, inflammatory and phagocytic cells and favor plaque rupture. By this mecha-
THERAPEUTIC STRATEGIES FOR PAI-1 INHIBITION

Statins

Elevated serum lipids increase thrombogenicity. Statins, in addition to lowering cholesterol, appear to have a number of pleiotropic effects, lipid and nonlipid related.

Most effects are related to inhibition of HMG-CoA reductase that blocks the synthesis of cholesterol and a number of isoprenoid intermediate molecules involved in intracellular signaling (Rho, Ras, and Rac). As a result, statins improve endothelial function; have antiinflammatory effects, and antiatherogenic ones by plaque stabilization and regression (16). Statins also induce downregulation of prothrombotic factors (eg, PAI-1) (16).

Fibrinolysis seems to be influenced by statins through a mechanism that involves geranylgeranyl-modified intermediates (16,17). In vitro studies have shown that statins increase tPA and decrease PAI-1 levels. This effect involves geranylgeranyl transferase inhibition. The mechanism by which statins treatment reduces PAI-1 is different from those that increase t-PA (16).

Statins can modify PAI-1 expression also by influencing the inflammatory process (16). Those drugs influence thrombogenic responses of both vessel wall and blood. Statins decrease platelet aggregation, inhibit tissue factor and PAI-1 expression and increase tPA leading to decrease susceptibility for coagulation and thrombosis (16).

In vitro studies agree about the beneficial role of statins on promoting fibrinolysis, while clinical trials have shown discrepancies. The DALI study reported significant lowering of PAI-1 levels in patients with type 2 diabetes mellitus treated with atorvastatin (18). Only one out of 14 studies from an old meta-analysis reported a change in PAI levels with statin therapy (16). A recent work shows that pitavastatin may exert an anti-atherothrombotic adiponectin-dependent effect in hyperlipidemic patients with significant decreases in plasma PAI-1(16).

In summary, there are data that statins may increase tPA and diminish PAI-1 expressions. The reduction of morbidity and mortality with this class of drugs is mainly due to LDL lowering but the effects on fibrinolysis may contribute to that.

Renin-Angiotensin-Aldosterone System (RAAS) inhibitors

The ACE released by endothelium represents the link between RAAS and fibrinolysis. Two processes mediated by RAAS positively influence fibrinolysis by ACE inhibition: (a) increased t-PA release by a process intermediated by bradykinin (BK); (b) decreased PAI-1 release probably mediated by angiotensin II (AII) (19). Ang II or its metabolite, Ang IV, and aldosterone increase PAI-1 expression in a diversity of cell and the mechanisms is cell-type specific (5). ACE inhibitors impair the breakdown of kinins. Increased kinin-stimulated NO synthesis may contribute to the decreases in PAI-1 expression. Activated RAAS promotes abnormal secretion of PAI-1 released from adipose cells and endothelium (10). At his turn PAI-1 amplify endothelial dysfunction and impair fibrinolysis continuing the vicious circle that lead to atherosclerosis and raise the thrombotic risk (3).

Therapeutic strategies can be targeted also to Angiotensin II inhibition in order to decrease the synthesis of PAI-1. Inhibition of the synthesis process may facilitate migration of VSMCs and stabilization of atherosclerotic plaques.

While ACE inhibitors’ effects to decrease PAI-1 plasma level have been demonstrated in different experimental models, for ARBs there are conflicting data. Some papers report a neutral effect while other suggest that PAI-1 may increase through stimulation of the angiotensin II type 4 receptor, because ARBs induce a feedback increase in angiotensin II concentration. In HEART Study ramipril influenced plasma fibrinolytic balance in patients with acute anterior myocardial infarction (20). However, TRAIN study found no significant effect of fosinopril versus placebo on PAI-1 in subjects with a high cardiovascular risk profile (21).

A recent study, Kagoshima Collaborate Trial in Metabolic Syndrome (KACT-MetS), reports that Valsartan reduced plasma PAI-1 levels compared to non-RAS inhibition in hypertensive patients with metabolic syndrome. Although there are some study limitations, KACT-MetS suggests that Valsartan may be useful for improving fibrinolytic function (22). Another recent paper supports that the suppression of PAI-1 expression by azilsartan medoxomil...
(TAK-491) may attenuate the evolution of atherosclerotic plaques vulnerable to rupture (23).

The reduction in coronary thrombotic events by ACEIs and ARBs is not entirely understood and contemporary evidences are controversial and not fully satisfactory.

However, PAI-1 inhibition may be part of the mechanisms by which ACE inhibition could reduce cardiovascular mortality rates independent of BP lowering.

Current research papers suggest that aldosterone may play a role, dependent and independent of A II, not only in myocardial fibrosis but in vascular disease too.

Aldosterone augments the effect of All on PAI-1 expression in vitro (24). Aldosterone increase PAI-1 expression in VSMC and endothelial cells in vitro through a mineralocorticoid receptor (MR). Brown`s works found a relation between plasma levels of PAI-1 antigen and serum aldosterone concentrations in salt-depleted normal and hypertensive subjects (25). In hypertensive individuals, aldosterone receptor antagonism eliminates the relationship between aldosterone and plasma PAI-1 concentrations (25).

The hypothesis that MR antagonism potentially reduces the risk of vascular thrombotic events by decreasing PAI-1 remains interesting.

**Fibrates**

Fibrates attenuate PAI-1 expression in human arterial SMC in some clinical studies (26). Lowering plasma triglycerides with fibrates decreases PAI-1 levels in vivo by an unknown mechanism. Fibrates can downregulate the PAI-1 production in vitro also independently of triglyceride lowering effect. Different fibrates showed different potencies in suppressing PAI-1 production (26). Beneficial effects of fibrates on PAI-1 inhibition remains not enough and not consistently proven.

**Other therapies**

**Estrogen replacement**, particularly 17β-estradiol, may increase t-PA release and enhance fibrinolysis in postmenopausal women (27). However, estrogen therapy for reduction of cardiovascular risk was abandoned due to increased risk of breast and endometrial cancer.

**TNF α- inhibitors.** TNF-α is a significant stimulator of the PAI-1 expression in the adipose tissue and seems to be involved in his regulation. Incubation of human fat biopsies with TNF-α inhibitors determine the reduction of PAI-1 mRNA (28). Pentoxifylline, a TNF α-inhibitor, may lower PAI-1 levels in overweight individuals (29).

**Specific PAI-1 antagonists**

Small drug molecules have been developed over the time for specific PAI-inhibition. Those were designed first to treat thrombotic disorders (30).

Tiplaxtinin, (PAI-039), and piperazine-chemotype molecules were studied and their chemical structure was modified using hybridization and conformational restriction to obtain two new products of 5-nitro-2-phenoxybenzoic acid derivatives with PAI-1 inhibition properties (31). Small molecules anti-PAI-1 orally bioavailable as TM5001, TM5007, TM5275, were tested in animal models, with some in vitro good results, but they did not achieve enough data to be used (32).

Potential benefits of PAI1 inhibition were studied regarding hypertension (7,33).

Recombinant PAI-1 was also found to inhibit vascular restenosis without promoting thrombosis (13).

**Nondrug PAI-1 lowering conditions**

Cesari and colleagues recently reported than physical activity and weight loss are correlated with decreases in PAI-1 levels while alcohol does not influence PAI-1 (28).

Physical activity has an inhibitory effect on thrombogenic factors and enhances blood fibrinolytic potential by increasing t-PA activity and lowering PAI-1 levels (34).

Weight loss provokes PAI-1 levels reductions. Look AHEAD Study showed after 1 year that moderate weight loss in obese patients with type 2 diabetes determined significant reductions in PAI-1 levels even if the study does not support the physiological relationships between PAI-1, inflammation and coagulation balance (35).

**INSTEAD OF CONCLUSIONS**

Atherosclerosis and related complications remain the main cause of death in 21st century. The imbalance between t-PA and PAI-1 can generate intravascular thrombosis.
In the current clinical practice, only thrombolytic agents are able to increase fibrinolytic activity. Many cardiovascular drugs related to atherosclerosis may indirectly increase fibrinolytic activity by reducing plasma levels of PAI-1. RAAS inhibition, particularly by ACE inhibitors, was proven to exert a convergent action by t-PA release enhancement.

The development of PAI-1 antagonists may provide an interesting approach for modulating PAI-1 activity within the vessel wall, with the purpose to counteract its deleterious effects and improve clinical event outcomes. Few orally active PAI-1 antagonists have been tested with some positive results mostly in animal models.

PAI-1 inhibitors remain a future therapeutic option for chronic fibrinolytic balance improvement. Whether these strategies may alter the course of atherosclerosis remain to be established. Until then, we need to obtain the final answer to the following question: is PAI-1 a causal risk factor or a surrogate marker of vascular damage?

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REFERENCES


