Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis

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1. ABSTRACT

Impaired fibrinolysis may be associated with development of atherothrombotic cardiovascular disease (CVD) in metabolic syndrome or type 2 diabetes. Plasma plasminogen activator inhibitor (PAI)-1, a potent inhibitor of fibrinolysis, is elevated in a number of clinical situations that are associated with high incidence of CVD. Impaired fibrinolysis resulting from high plasma PAI-1 can lead to excessive fibrin accumulation within vessels, resulting in atherothrombosis. Increased expression of PAI-1 is found in atherosclerotic lesions in humans, especially atherosclerotic plaques in patients with type 2 diabetes. This increased vascular expression of PAI-1 promotes neointima formation via accumulation of fibrin or fibrinogen as a result of inhibited clearance of platelet-fibrin thrombi. PAI-1, an acute phase protein, also could be involved in vascular inflammation. PAI-1 may be associated not only systemically but also locally with development of CVD.

2. INTRODUCTION

Coronary heart disease (CHD) is the leading cause of morbidity and mortality among adults worldwide (1). Over the past two decades, obesity, a major risk factor for cardiovascular disease (CVD) (2), has increased greatly in most countries, reflecting high-fat diet and sedentary life styles. Approximately 60% of adults in developed countries are reported to be overweight or obese (3). The increase in obesity is associated with a global epidemic of metabolic syndrome and diabetes (4). Patients with type 2 diabetes have a high incidence of atherosclerotic CVD, leading to increased morbidity and mortality due to CHD, stroke, and peripheral arterial disease (5, 6). CVD is the major cause of death in patients with type 2 diabetes in Western societies (7). Several epidemiologic studies have shown a 2- to 4-fold increase in risk of CVD among patients with type 2 diabetes compared to subjects without diabetes (5, 6, 8). The Framingham Heart Study has shown a 2- to 4-fold excess in risk of CAD, stroke, heart failure, and death from
CVD among subjects with diabetes compared with non-diabetic subjects (5, 8). Metabolic syndrome, also known as insulin resistance syndrome, is defined by clustering of several cardiovascular risk factors in an individual patient, including impaired glucose tolerance (diabetes), hypertension, dyslipidemia, and visceral obesity (9, 10). Several studies have demonstrated that this syndrome strongly predicts CVD, especially CHD (11, 12), independently of low-density lipoprotein (LDL)-cholesterol.

Hypercoagulability and reduced fibrinolysis may be associated with development of atherothrombotic vascular disease in metabolic syndrome or type 2 diabetes (13, 14). Plasma concentrations of plasminogen activator inhibitor (PAI)-1, a potent inhibitor of fibrinolysis, are elevated in patients with metabolic syndrome and type 2 diabetes. Among a number of hemostatic abnormalities (15), an increase in PAI-1 is considered a core feature of the metabolic syndrome (16). Thus, because of high plasma PAI-1, these patients have a well-described tendency toward relative hypercoagulability arising from impaired fibrinolysis (13, 17). Reduced fibrinolysis reflecting elevated plasma PAI-1 may increase risk of atherothrombotic events in metabolic syndrome and type 2 diabetes. Furthermore, evidence has been accumulating recently that increased vascular PAI-1 itself may contribute directly to acceleration of atherothrombosis formation by favouring neointimal formation within plaques.

3. ROLE OF INFLAMMATION AND THE COAGULATION-FIBRINOLYSIS SYSTEMS IN Atherosclerotic plaques consist of monocyte-derive macrophages and T lymphocytes (18). In the initial stage of atherosclerosis, rolling and adherence of monocytes and T lymphocytes occur at the inflamed sites as a result of the upregulation of adhesion molecules on both the endothelium and leukocytes (18). Integrins then mediate their firmer attachment. Proinflammatory cytokines produced by atheroma provide a chemotactic stimulus to the adherent leukocytes, directing their migration into the intima (19). Vascular inflammation is also associated with the destabilization of the plaque. PAI-1 is the main physiological inhibitor of tissue-type and urokinase-type plasminogen activator and thereby regulates the fibrinolysis system, while PAI-1 can act as an acute phase protein. Several studies have demonstrated that PAI-1 is produced at the site of inflammation after tissue injury (24, 25), suggesting that PAI-1 plays a role in the regulation of local inflammatory process. The role of PAI-1 in vascular inflammation remains to be determined in humans. Renckens et al reported that PAI-1 deficiency mice showed a reduction in the early induction of IL-6, a main inflammatory cytokine, in plasma and tissues with subsequently lower acute phase protein levels (26). Thus, PAI-1 could have a proinflammatory property, thereby participating in vascular inflammation.

Unexpectedly, a previous study reported that PAI-1 inhibits the attachment of cells to the extracellular matrix protein by blocking the adhesive sites of vitronectin (27). PAI-1 may act as a de-adhesive molecule rather than adhesive one. Furthermore, a previous study demonstrated that active PAI-1 inhibits SMC migration by blocking integrin the binding of vitronectin to the integrin receptor αvβ3 (28). However, further study needs to be done to determine whether PAI-1 is associated with the attachment or detachment of cells to the extracellular matrix of arterial wall in humans.

The coagulation system is comprised of a complex cascade of coagulation molecules. Formation of stable, cross-linked fibrin, a main step in the coagulation cascade, occurs as a result of thrombin-induced cleavage of fibrinogen with concurrent activation of cross-linked fibrin polymers (29, 30). Thus, thrombin has a pivotal role in the
The fibrinolytic system is activated after formation of fibrin, when both plasminogen and t-PA bind to the fibrin surface (Figure 1). Spontaneous dissolution of thrombus is regulated mainly by fibrinolytic activity, which ultimately is dependent on generation of plasmin (29, 30). During fibrinolysis, insoluble fibrin is digested by plasmin, which is converted from its inactive precursor plasminogen by the action of urokinase-plasminogen activator (u-PA) or tissue-PA (t-PA) on the surface of the fibrin clot. Thus, plasmin, the enzyme responsible for degradation of fibrin, plays a pivotal role in the fibrinolytic system. Hypercoagulability, reduced fibrinolysis, or both may contribute to the development of CVD via formation of occlusive thrombi within coronary and cerebral arteries. The two main inhibitors of fibrinolysis in the circulation are PAI-1, a rapidly acting inhibitor of t-PA and u-PA (31), and α2-antiplasmin (32), a specific plasmin inhibitor that is covalently bound to polymerizing fibrin by activated factor XIII.

PAI-1, a single-chain glycoprotein with a molecular weight of 50 kDa, is a member of the serpin (serine proteinase inhibitor) family (17). PAI-1 binds to t-PA or u-PA, forming an inactive complex, thus negatively regulating fibrinolysis in the blood by inhibiting t-PA (31). This important function of PAI-1 is facilitated by vitronectin, a 75-kDa extracellular matrix glycoprotein (33) (Figure 1). An increase in the PAI-1 concentration in the circulation impedes fibrinolysis by impairing the action of t-PA, so thrombi cannot be removed from the vascular wall (31). Immunohistochemical studies have demonstrated that macrophage infiltration and thrombus formation are more prominent in advanced coronary plaques from unstable angina patients with diabetes than plaques from those without diabetes (34, 35). Another study has shown that coronary artery thrombi from MI patients with diabetes contain more fibrin than thrombi from those without diabetes (36). Several studies have demonstrated increased PAI-1 expression in human atherosclerotic lesions. Thus, imbalance between coagulation and fibrinolysis may contribute to excessive fibrin deposition in the vascular wall. In this manner, impaired fibrinolysis due to high plasma PAI-1 may contribute to the pathogenesis of atherothrombosis, leading to CAD, stroke, and peripheral arterial disease in type 2 diabetes.

5. HOW ENHANCED LOCAL PAI-1 EXPRESSION IN THE VASCULAR WALL MAY INFLUENCE ATHEROSCLEROTIC VASCULAR EVENTS

Several studies have demonstrated increased expression of PAI-1 in human atherosclerotic lesions (37-39). Immunohistochemical study demonstrated enhanced expression of PAI-1 in the macrophages and endothelial cells of atherosclerotic plaques (39). Patients with type 2 diabetes show higher expression of PAI-1 in the atherosclerotic vascular wall than those without diabetes (40, 41). Thus, increased PAI-1 in the arterial wall may contribute directly to development of CVD in type 2 diabetes. However, the causal role of arterial PAI-1 expression in atherothrombosis is controversial. Migration of vascular smooth muscle cells (VSMCs) from the media into the intima participates significantly in neointimal formation in atherosclerotic plaques or restenosis (18). Plasmin generated from plasminogen in the extracellular matrix of arterial wall activates MMPs, resulting in degradation of this matrix (42). By disrupting the arterial wall matrix, plasmin facilitates VSMC migration into the intima, which may contribute to neointima formation (42). Considering this, PAI-1 actually could attenuate plasmin-dependent proteolysis of the matrix by inhibiting t-PA or u-PA. In fact, in mouse studies, the VSMC migration was increased by the disruption of PAI-1 gene, and VSMC accumulated in the neointima after intraluminal electrical injury (43, 44). Furthermore, PAI-1 overexpression attenuated migration of VSMCs in response to injury (44). Considering these results together, increased PAI-1...
expression in the arterial wall unexpectedly could inhibit migration of VSMCs into the intima, thus diminishing luminal obstruction.

In contrast, several studies demonstrated that increased expression of PAI-1 in the arterial wall augmented neointimal formation in mice (45-47). As mentioned previously, PAI-1 promotes fibrin accumulation in the vascular lumen by inhibiting activation of t-PA or u-PA. Increased expression of PAI-1 may augment neointimal formation by favoring fibrin deposition within vessels (42). In fact, in mice PAI-1 overexpressing, PAI-1 promoted neointimal formation in association with fibrin or fibrinogen accumulation by inhibiting clearance of platelet-fibrin thrombi (45). Furthermore, in atherosclerosis-prone mice, increased vascular expression of PAI-1 is associated with enhanced thrombosis (48). Increased PAI-1 expression in the arterial wall could decrease local fibrinolysis, and also promote thrombus formation and unfavorable change in atherosclerotic plaques. Thus, results in animal models of neointimal formation concerning PAI-1 show disagreement. In the absence of obvious reasons for this discrepancies, Konstantinides et al. speculated that in the early stage of vascular remodeling in the absence of thrombi and fibrin, PAI-1 limits migration and proliferation of VSMCs, stabilizing the extracellular matrix; while in an advanced stage, where thrombus is present, PAI-1 correlates consistently with neointimal formation, which narrows the lumen (49).

6. ASSOCIATIONS OF HIGH PLASMA PAI-1 CONCENTRATIONS IN TYPE 2 DIABETES AND IN METABOLIC SYNDROME

High plasma PAI-1 concentrations have been associated with development of CHD. Many investigators have reported that plasma PAI-1 concentrations are elevated in type 2 diabetic patients (50-53). Elevated plasma PAI-1 now is considered a main component of metabolic syndrome, a pre-diabetic state (16, 17). Plasma PAI-1 also is strongly correlated in subjects with metabolic syndrome and type 2 diabetes with plasma concentrations of plasmin-α2-antiplasmin complex (PAP), a measure of fibrinolytic activity in blood, in type 2 diabetes (51). Elevated plasma PAI-1 may play a role in development of atherothrombosis in obese patients with type 2 diabetes, independently of other known cardiovascular risk factors (58).

7. REGULATION OF PAI-1 GENE EXPRESSION

The PAI-1 gene, located on chromosome 7 (bands q21.3 to q22), has an extent of approximately 12.2 kb and consists of 8 or 9 exons (17). Two distinct species PAI-1 mRNA, 2.3 kb and 3.3 kb, are expressed on human cells, resulting from alternative polyadenylation to yield an additional 3’ untranslated region (17). The 5’-flanking region (promoter) of the human PAI-1 gene contains the transcription initiation site, a TATA box, and a regulatory sequence that confer transcriptional responsiveness to a number of mediators, including a glucocorticoid response element (59) that can mediate aldosterone responsiveness, a VLDL response site (60), a glucose response site (61), as well as sites for TGF-β and TNFα (62).

PAI-1 is produced by several tissues including endothelial cells (63), adipose tissue (64), and liver (65). Human studies suggest that the liver ordinarily may be a main source. In subjects with obesity, adipose tissue is the main source of circulating PAI-1, since the mRNA for PAI-1 is up-regulated in adipose tissues of obese persons. A previous study reported that intensity of adipose tissue PAI-1 expression is related to plasma PAI-1 concentrations in human subjects (66). Platelets also synthesize and store PAI-1, which is released after platelet activation (67). A platelet-rich clot therefore is resistant to fibrinolysis.

In vitro studies have demonstrated that gene expression and synthesis of PAI-1 can be induced in cultured cells by a number of factors. Glucose induces PAI-1 synthesis and release by human endothelial (68) and smooth muscle (61). Both insulin and proinsulin can increase expression of PAI-1 in hepatocytes (69). Very low-density lipoprotein (VLDL) and free fatty acid stimulate secretion of PAI-1 by endothelial cells (70, 71). A number of cytokines have been found to induce the synthesis and release of PAI-1 in vitro. Tumor necrosis factor (TNF)-α and interleukin (IL)-1, both inflammatory cytokines, can produce PAI-1 in adipocytes (72). Growth factors, including transforming growth factor (TGF)-β, are known to stimulate synthesis of PAI-1 in endothelial cells (73). Components of the renin-angiotensin-aldosterone system can contribute to synthesis of PAI-1 in vitro. Angiotensin (Ang) II and Ang IV, a hexapeptide metabolite of Ang II, increase PAI-1 mRNA expression in vascular tissue (74). Aldosterone enhances the effects of Ang II on PAI-1 expression in vascular smooth muscle cells, as shown by transfection with a luciferase reporter construct containing variable lengths of the human PAI-1 promoter (75).
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8. REGULATION AND DETERMINANTS OF PLASMA PAI-1 CONCENTRATIONS

PAI-1 is present in blood as three different molecular forms: latent (inactive), active, and complexed to t- or u-PA (29). PAI-1 binds rapidly to t-PA or u-PA at a ratio of 1:1, forming a stable complex that is cleared from the circulation by liver (29, 76). PAI-1 extensively binds t-PA, acting to limit fibrinolysis. PAI-1 plasma concentrations exceed t-PA concentrations by a 4:1 ratio (29, 62). The half-life of PAI-1 in the circulation is about 10 min. PAI-1 circulates at a plasma concentration of 10 to 50 ng/ml (62), showing circadian variation; plasma PAI-1 concentrations peak in the early morning, corresponding to a nadir in fibrinolytic activity, while plasma PAI-1 concentrations fall in the afternoon (75).

In clinical studies, plasma concentrations of PAI-1 have shown relationships with components of the metabolic syndrome, especially the fasting plasma insulin concentration and central obesity (76, 77). Chronic hyperinsulinemia secondary to insulin resistance is believed to be the primary determinant of elevated plasma PAI-1 in individuals with metabolic syndrome and type 2 diabetes; on the other hand, hyperglycemic state generally is not considered to be a main regulator of plasma PAI-1 (52, 78). An insulin infusion in rats increases activity of PAI-1 in plasma, resulting in decreased fibrinolytic function (79). In contrast, a human study demonstrated that short-term insulin administration reduced plasma PAI-1 by inhibiting intranuclear nuclear factor κB (80). Most likely, synergistic actions of hyperglycemia, dyslipidemia, and hyperinsulinemia drive elevated plasma PAI-1 concentrations in type 2 diabetes (81). Several recent studies have demonstrated that plasma PAI-1 correlated negatively with serum adiponectin (82, 83), an adipocyte-derived protein that has antidiabetic and anti-atherosclerotic effects, suggesting a potential relationship between PAI-1 and adiponectin in adipocytes.

Plasma PAI-1 concentrations can be affected by several polymorphisms in the promoter region of the PAI-1 gene, including a common single-base polymorphism (4 or 5 guanine) in the promoter region of the gene, 675 base pairs upstream of the transcriptional start site (84). Subjects homozygous for the 4G allele have plasma PAI-1 concentrations approximately 25% higher than those of subjects who are homozygous for the 5G allele (85). PAI-1 also has a genotype-specific interaction with plasma triglyceride (86).

Several interventional studies demonstrated a decrease in plasma PAI-1 concentration in obese subjects after weight reduction with low-calorie diet (87, 88). In a previous study we also found that in patients with type 2 diabetes, weight reduction by intensive metabolic control decreased in plasma PAI-1, resulting in a reciprocal increase in plasma plasmin-α2-antiplasmin complex (PAP) (89). Indeed, PAI-1 is released directly from adipose tissues, especially visceral fat, in subjects with obesity (64). Insulin sensitizing drugs such as metformin and thiazolidinedione (TZD) decrease plasma PAI-1 concentrations in type 2 diabetes. Metformin, which acts by decreasing hepatic glucose production and improving insulin sensitivity through peripheral glucose utilization, can reduce triglyceride and body weight. Grant et al. showed that plasma PAI-1 concentrations and activity fell significantly in diabetic patients treated with metformin compared with placebo-treated controls (90). TZDs, which are peroxisome proliferator-activated receptor-γ agonists, promote adipocyte differentiation, converting insulin-resistant adipocytes to insulin-sensitive adipocytes (91). Several studies reported that troglitazone, a TZD, decreased plasma PAI-1 concentrations and activity in patients with type 2 diabetes (92, 93).

Inhibition of the renin-angiotensin-aldosterone system by an angiotensin-converting enzyme (ACE) inhibitor or an Ang II receptor blocker (ARB) may decrease plasma PAI-1 concentrations. Short-term treatment with fosinopril, an ACE inhibitor, significantly reduced PAI-1 compared with amlodipine, a calcium antagonist, in a dose-dependent fashion in type 2 diabetic patients with hypertension (94). Short-term interruption of the renin-angiotensin-aldosterone system by either ACE inhibition or ARB decreases PAI-1 antigen, but the duration of this effect is greater for ACE inhibition than for Ang II receptor (AT1) receptor antagonism (95). Aldosterone blockade by spironolactone also decreases plasma PAI-1 in hypertensive patients (96). Unexpectedly, fenofibrate, a fibric acid derivative that lowers serum triglyceride, slightly increases PAI-1 activity in hyperlipidemic patients (97).

Our group and other investigators have shown that in poorly controlled patients with type 2 diabetes, insulin therapy decreased plasma PAI-1 concentrations with concurrent improvement in insulin sensitivity (89, 98), suggesting that exogenous insulin replacement may have no deleterious effect on fibrinolysis. In a crossover study in poorly controlled patients with type 2 diabetes, sulfonylurea therapy increased PAI-1 activity and antigen compared with insulin therapy (99).

9. SUMMARY AND PERSPECTIVE

Mechanisms by which PAI-1 may induce atherothrombotic events are summarized in Figure 2. Systemically, elevated plasma concentrations of PAI-1 induce hypofibrinolyis by impairing the action of t-PA, to prevent removal of thrombi from the vascular wall. Thus, elevated plasma PAI-1 may contribute to an excess of atherothrombotic events in metabolic syndrome or type 2 diabetes. Locally, PAI-1 promotes fibrin accumulation in the vascular lumen through its capacity to inhibit activation of t-PA or u-PA. Increased expression of PAI-1 may augment neointimal formation by promoting fibrin deposition within vessels. Enhanced local PAI-1 expression within the arterial wall may induce luminal obstruction via neointimal formation, leading to an atherothrombotic event. Medications that counteract impaired fibrinolysis by decreasing circulating concentrations of this inhibitor may
reduce risk of cardiovascular disease in metabolic syndrome or type 2 diabetes. Excessive intravascular PAI-1 expression may represent an ideal target for therapeutic interventions to prevent atherothrombotic events. Accordingly, a long-term clinical study should be undertaken to investigate the effects of pharmacologic inhibition of PAI-1 on atherothrombotic events.

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