Vascular Functions of the Plasminogen Activation System

William P. Fay, Nadish Garg, Madhavi Sunkar

Abstract—The plasminogen activator (PA) system, which controls the formation and activity of plasmin, plays a key role in modulating hemostasis, thrombosis, and several other biological processes. While a great deal is known about the function of the PA system, it remains a focus of intensive investigation, and the list of biological pathways and human diseases that are modulated by normal and pathologic function of its components continues to lengthen. Because of remarkable advances in molecular genetics, the laboratory mouse has become the most useful animal system to study the normal and pathologic functions of the PA system. The purpose of this review is to summarize studies that have used genetically modified mice to examine the functions of the PA system in hemostasis and thrombosis, intimal hyperplasia after vascular injury, and atherosclerosis. Particular emphasis is placed on the vascular functions of PA inhibitor-1, a key regulator of the PA system, and the multiple variables that appear to account for the complex role of PA inhibitor-1 in regulating vascular remodeling. Lastly, the strengths and limitations of using mice to model human vascular disease processes are discussed. (Arterioscler Thromb Vasc Biol. 2007;27:1231-1237.)

Key Words: atherosclerosis ▪ fibrinolysis ▪ mouse ▪ plasminogen ▪ vascular remodeling

The central reaction of the plasminogen activator (PA) system is the conversion of plasminogen to plasmin by PAs (Figure 1). Plasmin, a serine protease, degrades fibrin to fibrin degradation products. However, plasmin has several substrates other than fibrin, including blood coagulation factors, cell surface receptors, metalloproteinasises, and structural components of the extracellular matrix.1,2 Therefore, plasminogen activation is a key reaction not only for fibrinolysis but also for a variety of biological processes, particularly those involving cell adhesion and migration.3,4 While plasminogen resides primarily within the plasma, with the liver representing the primary site of plasminogen synthesis, plasminogen mRNA is present in several mouse tissues, including adrenal, kidney, brain, testis, heart, lung, uterus, spleen, thymus, and gut, supporting a broadly distributed functional role of the PA system.5 Tissue-type PA (t-PA) and urinary-type PA (u-PA), the 2 main mammalian PAs, activate plasminogen by cleaving a specific Arg-Val peptide bond located within the protease domain. The activation of plasminogen by t-PA is highly dependent on the presence of cofactors, such as fibrin, that bind and alter the conformation of plasminogen.6 Plasmin formation is intensely regulated by PA inhibitors, which inhibit t-PA and u-PA, most notably PA inhibitor-1 (PAI-1).7 Plasmin is directly inhibited by α2-antiplasmin, which circulates in plasma. Several bacterial species secrete PAs, such as streptokinase and staphylokinase, that promote bacterial cell migration and invasion of host tissues by supporting bacterial–cell-associated plasmin formation.8–10 In contrast to mammalian PAs, streptokinase and staphylokinase are not enzymes. Rather, these factors bind plasminogen or plasmin to form activator complexes that convert substrate plasminogen molecules to plasmin.11,12

Clot lysis depends on binding of plasminogen to the clot surface. Plasminogen binds to lysine residues within fibrin via lysine-binding sites contained in plasminogen’s kringle domains.2 As plasmin cleaves fibrin by hydrolysis of peptide bonds adjacent to lysine residues, C-terminal lysine residues are gen-
erated, to which plasminogen binds with higher affinity than to internal lysine residues, which further accelerates fibrinolysis by promoting plasminogen binding to the dissolving clot. Thrombin activatable fibrinolysis inhibitor (TAFI), which circulates in plasma as a zymogen and is activated proteolytically by thrombin, is a basic carboxypeptidase that cleaves C-terminal lysine residues from fibrin. Consequently, activated TAFI inhibits fibrinolysis by inhibiting binding of plasminogen to the partially degraded fibrin surface. Because of its activation by thrombin, TAFI constitutes a major site of cross-talk between the blood coagulation and fibrinolysis systems.

Not only does TAFI allow thrombin to modulate fibrinolysis but also it provides a mechanism by which deficient or excessive thrombin generation, resulting from, for example, hemophilia, protein C deficiency, or factor VLeiden, may contribute to pathologic bleeding or thrombosis, ie, by upregulating or downregulating TAFI activation and fibrinolysis.

Comparison of Murine and Human Fibrinolytic Systems

Lijnen et al performed an interesting and important study in which they purified the main components of the mouse fibrinolytic system and studied their function in vitro in autologous and heterologous biochemical and clot lysis assays involving mouse and human fibrinolytic components. While the biochemical characteristics of individual components of the PA system are largely overlapping between mice and humans, there are important differences. Most significantly, the murine plasma fibrinolytic system is more resistant to activation (ie, the generation of plasmin activity) than the human system, which appears to be mediated by a relative resistance of murine plasminogen to activation by murine t-PA and a shorter plasma half-life of murine t-PA than that of human t-PA attributable to apparent inhibition of mouse t-PA by plasma inhibitors other than PAI-1. In regards to cross-species interactions of fibrinolytic components, murine plasminogen is activated much more slowly by human t-PA than by murine t-PA. This issue is important in regards to interpretation of studies involving administration of recombinant human t-PA to mice, eg, in studies of pharmacological thrombolysis. The concentration of some components of the PA system in specific biologic compartments can differ between mice and humans. For example, the concentration of PAI-1 in mouse platelets is markedly lower than its concentration in human platelets. On the whole, the differences between the human and murine PA systems are minor, but must be kept in mind when designing and interpreting specific experiments. It is also important to note that the human PA system has some differences not only from the murine PA system but also from those of other animals used in laboratory research, such as the rat, rabbit, dog, and pig.

Insights Into Regulation of Intravascular Fibrinolysis From Murine Knockout Models

Complete fibrinogen deficiency results in spontaneous bleeding in mice, although the severity of the bleeding defect varies in different mouse strains. Complete fibrinogen deficiency leads to severe generalized thrombosis in mice, which demonstrates the critical role of fibrinogen in fibrin homeostasis. Mice completely lacking t-PA exhibit delayed clearance of intravascular fibrin after vascular injury compared with wild-type mice, which suggests that the main inhibitor of fibrinolysis is present in the intravascular compartment. Mice completely lacking PAI-1 exhibit delayed thrombus formation and accelerated thrombolysis after vascular injury, underscoring the key role of PAI-1 in regulating intravascular fibrinolysis.

Homozygous α2-antiplasmin-deficient mice do not exhibit abnormal bleeding, but do demonstrate diminished thrombolysis after vascular injury and accelerated lysis of experimental pulmonary emboli. These results suggest that the main in vivo function of α2-antiplasmin is to regulate circulating plasmin activity and intravascular fibrinolysis. Dewerchin et al studied mice with combined deficiency of PAI-1 and α2-antiplasmin in several bleeding and thrombosis models and compared double-deficient mice to mice with isolated deficiency of each factor to study the relative roles of these inhibitors in regulating fibrinolysis in vivo. Their results suggested that the higher endogenous fibrinolytic capacity observed in mice with combined...
PAI-1 and α2-antiplasmin deficiency is mainly caused by the lack of α2-antiplasmin, whereas PAI-1 plays a less important role in controlling intravascular fibrin turnover. Initial mouse studies suggested that TAFI deficiency, which would be hypothesized to downregulate thrombosis (because of upregulated fibrinolysis), had no effect of clot formation after either arterial or venous injury.35 However, TAFI-deficient mice exhibit accelerated fibrinolysis in pulmonary embolism models,36,37 and potato carboxypeptidase inhibitor, which inhibits activated TAFI, decreases thrombus formation in the inferior vena cava of mice after ferric chloride injury.38 Therefore, TAFI can modulate endogenously mediated fibrin clearance in vivo. While blood coagulation and fibrinolysis are usually considered as distinct enzymatic pathways that intersect at the level of fibrin, several studies involving genetically modified mice have demonstrated the interconnected function of the blood clotting and lysis pathways. For example, mice expressing the murine homologue of factor VLeiden demonstrate dampened fibrinolysis, which appears to be mediated by enhanced thrombin formation and TAFI activation.16

Role of PA System in Controlling Intimal Hyperplasia After Vascular Injury

In addition to functioning within the vascular lumen to control fibrinolysis, the PA system is active within the blood vessel wall, where it plays an important role in controlling vascular remodeling. The development of intimal hyperplasia after vascular injury is diminished in plasminogen-deficient mice, supporting the concept that plasmin associated with vascular smooth muscle cells (VSMCs) enhances cell migration by fostering extracellular matrix degradation, either directly or indirectly by activating matrix metalloproteinases.39,40 VSMCs express u-PA and its receptor (Figure 2). Urokinase (u-PA) deficiency and pharmacological inhibition of u-PA receptor, but not t-PA deficiency, inhibit neointima formation in mice, suggesting that u-PA–triggered plasmin formation drives VSMC migration.28,41,42 Murine studies examining the role of PAI-1 in the development of intimal hyperplasia after vascular injury in mice with normal lipid metabolism have not yielded concordant results. Some studies found that endogenous PAI-1 expression promotes neointima formation after vascular injury,43,44 others concluded that endogenous PAI-1 inhibits neointima formation,45–47 and one study found no effect of PAI-1 on neointima formation.48 Two studies examined the role PAI-1 in promoting intimal hyperplasia in hyperlipidemic mice, induced by FeCl3-induced injury of the common carotid artery, with both studies finding that PAI-1 promoted intimal hyperplasia.22,49 PAI-1 has the potential to modulate neointima formation by multiple mechanisms. By stabilizing intravascular fibrin, which often forms in response to vascular injury and which can be invaded by VSMCs and other cell types to form a neointima, PAI-1 may promote intimal hyperplasia.7 Consistent with this hypothesis, hyperfibrinogenemic mice exhibit enhanced neointima formation after carotid artery ligation compared with wild-type mice,50 and depletion of plasma fibrinogen by administration of ancdor reduces intimal hyperplasia in mice after carotid artery ligation.51 However, α2-antiplasmin deficiency, which promotes fibrinolysis in vivo,52 has no effect on neointima formation in mice,52 suggesting that stabilization of fibrin by PAI-1 and/or α2-antiplasmin does not play an essential role in murine vascular remodeling.

Figure 2. PAI-1 stabilizes the provisional fibrin matrix. Inhibition of fibrinolysis by PAI-1 can promote accumulation of intravascular fibrin, which may be invaded by proliferating VSMCs and circulating progenitor cells to form a cellular neointima.

Figure 3. Inhibition of VSMC migration by PAI-1. PAI-1 inhibits u-PA bound to u-PA receptor (uPAR) on the surface of VSMC, which reduces plasmin formation and degradation of extracellular matrices. PAI-1 also binds to the amino-terminus of vitronectin, to which (uPAR) and αβ3 also bind, thereby inhibiting vitronectin-dependent migration of VSMC.

Vitronectin

Plasminogen

VSMC

αβ3

uPAR

PAI-1

u-PA

Plasmin

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PAI-1 promotes VSMC proliferation and inhibits apoptosis,\textsuperscript{53,54} which could promote neointima formation. PAI-1 may reduce neointima formation by: (1) inhibiting u-PA and, consequently, cell-associated plasmin formation; and (2) binding to extracellular matrix vitronectin (Figure 3). The binding domain on vitronectin for PAI-1 overlaps with the binding domains on vitronectin for vitronectin receptors present on VSMC, ie, $\alpha_v\beta_3$ and u-PA receptor.$^{55-58}$ Consequently, binding of PAI-1 to vitronectin can block binding of VSMCs to vitronectin and inhibit VSMC migration through extracellular matrices, which could inhibit neointima formation. PAI-1 also modulates the activation and vascular effects of transforming growth factor-$\beta_1$, which has important, though pleiotropic, effects on VSMC proliferation and migration.$^{56,59}$

**Role of PA System in Atherogenesis**

Plasminogen deficiency enhances atherosclerosis formation in apolipoprotein E-deficient (apoE$^{-/-}$) mice.$^{60}$ Such an effect could potentially be caused by enhanced fibrin formation in the absence of plasminogen, with increased intravascular fibrin promoting plaque growth.$^{61}$ However, arguing against this hypothesis are the observations that fibrinogen-deficient apoE$^{-/-}$ mice are not protected from atherosclerosis formation compared with apoE$^{-/-}$ mice with normal fibrinogen expression,$^{62}$ and overexpression of fibrinogen does not enhance atherosclerosis development in apoE$^{*3}$-Leiden transgenic mice.$^{63}$ However, fibrinogen promotes atherogenesis in apolipoprotein(a)-transgenic mice,$^{64}$ supporting a role for fibrinogen in atherogenesis, as has been suggested by clinical data in humans.$^{65}$ The capacity of plasmin to activate latent transforming growth factor-$\beta_1$ (which has been reported in some studies to inhibit VSMC proliferation and migration$^{66}$) and to promote apoptosis are potential mechanisms by which plasminogen deficiency may promote atherosclerosis development.$^{65,66}$ While plasminogen deficiency promotes atherogenesis in apoE$^{-/-}$ mice, complete u-PA deficiency (which, like plasminogen deficiency, would be expected to diminish plasmin formation within the vascular wall) does not significantly alter atherosclerosis formation in hyperlipidemic mice.$^{68}$ Because complete plasminogen deficiency leads to multi-organ damage and systemic illness, which could modulate atherogenesis by several pathways, localized perturbation of plasminogen activation within the vascular wall is probably necessary to adequately study the role of the PA system in atherogenesis. Interestingly, macrophage-targeted overexpression of u-PA accelerates atherosclerosis in apoE$^{-/-}$ mice, potentially by promoting destruction of the vascular media.$^{69}$ The role of PAI-1 in murine atherogenesis has been controversial, with different studies concluding that hyperlipidemic mice also deficient in PAI-1 have unchanged,$^{70}$ less,$^{71}$ or more$^{72}$ spontaneous atherosclerosis development within the aorta or at the distal bifurcation of the carotid artery compared with hyperlipidemic mice with normal PAI-1 expression. As the case with studies of PAI-1 and vascular remodeling in mice with normal lipid metabolism, effects of PAI-1 on fibrin homeostasis do not appear sufficient to account for all of its observed effects on atherogenesis. Effects of PAI-1 on infiltration of cells, such as macrophage, into plaque, proliferation, migration, and apoptosis of VSMCs,$^{53,54,73,74}$ and accumulation and composition of extracellular matrix in plaque$^{72}$ appear to represent important mechanisms by which PAI-1 modulates atherogenesis.

**Resolving the PAI-1 Paradox: Clinical Insights, Key Issues, and Potential Future Studies**

The discordant results of mouse studies examining the impact of PAI-1 on intimal hyperplasia and atherosclerosis development are perplexing and raise concerns regarding the potential use of PAI-1 inhibitors as therapeutic agents in patients with vascular disease.$^{75}$ Interestingly, human studies of the role of PAI-1 in restenosis after coronary artery angioplasty have also yielded discordant results. One study found that elevated plasma PAI-1 was associated with increased risk of restenosis.$^{76}$ However, another study found that elevated plasma PAI-1 was associated with a reduced risk of restenosis,$^{77}$ and a third study found that plasma PAI-1 levels were associated with a decreased risk of restenosis if a stent was implanted, but not if only balloon angioplasty (ie, without stent implantation) was performed.$^{78}$ While these human studies examined associations, rather than cause-and-effect relationships, they highlight the complex vascular functions of PAI-1 and support the hypothesis that PAI-1 may inhibit or promote the development of vascular pathology, including in humans, depending on experimental and clinical conditions. For both humans and mice, it is plausible that when vascular injury is associated with activation of the blood coagulation system and fibrin formation, that PAI-1 may promote intimal hyperplasia and atherosclerotic plaque development by stabilizing fibrin, which serves as a matrix to support VSMC migration. Alternatively, in the absence of fibrin formation, cell migration within the vascular wall may be inhibited by PAI-1 through its capacity to inhibit u-PA and/or block interactions between cells and vitronectin in the extracellular matrix. Such an anti-migratory effect of PAI-1 could inhibit not only intimal hyperplasia but also cell content within the fibrous cap of atherosclerotic plaques, which could promote plaque rupture.$^{79,80}$ However, in distinction to migration, the proliferation of VSMC may be promoted by direct effects of PAI-1 on cell division and by PAI-1’s anti-apoptotic properties.$^{53,54}$ Therefore, under conditions in which VSMC proliferation is the dominant mechanism driving vascular pathology, PAI-1 could potentially increase neointimal growth. In fact, these different conditions may exist in the same artery at different time points after vascular injury. Otuka et al$^{40,81}$ have suggested that local levels of transforming growth factor-$\beta_1$ expression may play a key role in determining whether PAI-1 promotes or inhibits neointima formation. Effects of other modifier genes on PAI-1 function likely account for the observed mouse strain effect on PAI-1 function in vivo.$^{72,82}$

Experiments involving targeted disruption or enhancement of PAI-1 expression within specific cell types, as opposed to total-body PAI-1 deficiency, will likely be necessary to gain a thorough understanding of the role of PAI-1 in neointima formation after vascular injury. Overexpression of PAI-1 within VSMCs reduces the cellularity of neointimal lesions in apoE$^{-/-}$ mice, supporting the hypothesis that enhanced PAI-1 expression in atheroma could promote plaque rupture by decreasing the cellular content of the fibrous cap.$^{80}$ A recent study found that PAI-1 originating from bone marrow-derived cells inhibited neointima formation after ferric...
chloride-induced vascular injury. However, bone marrow cell-derived PAI-1 did not alter plaque size in apoE−/− mice, apparently because of the fact the VSMCs, rather than macrophages, are the dominant source of PAI-1 in atherosclerotic plaque. Because PAI-1 interacts with several molecules, which can produce opposing effects on vascular remodeling (eg, inhibition of fibrinolysis may promote intimal hyperplasia, while inhibition of u-PA and/or binding of PAI-1 to vitronectin may inhibit intimal hyperplasia), the use of a null allele to study PAI-1 function may not be adequate to study the regulatory role of PAI-1 in vivo in specific pathways. One approach to circumvent this potential problem is to inactivate specific functional domains of PAI-1, rather than to completely disrupt PAI-1 expression. PAI-1 mutants lacking antiproteolytic activity, but maintaining normal vitronectin binding (and the converse), have been generated. Transgenic or knock-in strategies could be used to examine the function of these PAI-1 mutants, thereby helping to elucidate the in vivo impact of altering one aspect of PAI-1 function (eg, regulation of VSMC-associated u-PA activity) without disturbing others (eg, regulation of binding of αvβ3 to vitronectin).

Strengths and Limitations of Using the Mouse to Model the Human PA System

In summary, studies involving genetically modified mice have helped to clarify the roles of different PA system components in modulating hemostasis and thrombosis. In general, the results of mouse thrombosis studies from different groups have been concordant, and the murine data have generally been consistent with phenotypic abnormalities observed in humans with spontaneous mutations in PA system genes. Experiments examining the role of the PA system in vascular remodeling and atherosclerosis, while extremely useful and important, have not achieved as high a level of consistency between published studies, nor has the relevance of experimental results to human disease states, such as myocardial infarction, been as high as observed in mouse experiments focusing on acute thrombosis. Several reasons are likely to account for these findings. The scientific variables in studies of vascular remodeling and atherosclerosis, which occur over weeks to months in mice, are likely considerably larger than those in short-term thrombosis experiments, which generally last <30 minutes in mice. The techniques used to trigger vascular remodeling in mice (eg, arterial ligation, and chemical and electrical injury) do not adequately model the more chronic and subtle forms of injury that produce vascular disease in humans. Similarly, murine atherosclerosis models do not generate the complex rupture-prone arterial plaques that are typical of human disease. Vessel size and blood flow are important determinants of vascular function and remodeling, and the cross-sectional area of the human aorta is ~200-fold greater than that of the mouse aorta. Particularly in chronic experiments, the study of mice with total-body deficiency of a PA system component (eg, PAI-1) may not adequately reflect the role of that factor if it acts in different cell types or biologic compartments to produce opposing effects on the parameter being studied (eg, atherosclerosis). Despite these limitations, the laboratory mouse has proved extremely useful in defining the vascular functions of different components of the PA system. Future mouse studies, particularly those involving cell type-specific manipulations of PA system components, as well as those involving introduction of mutations that alter, rather than completely ablate, the function of PA system components, are likely to contribute much more to our knowledge of how the PA system modulates human vascular diseases.

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Disclosures

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References


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