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Hereditary and acquired defects in the fibrinolytic system associated with thrombosis

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A prime physiologic function of the fibrinolytic system is to keep the circulating blood fluid. On one hand, excessive fibrinolytic activity would result in bleeding, while a failure of this function would lead to thrombosis. This was recognized as early when Mole studied postmortem fibrinolysis [1]. He noted that cadaver fibrinolysin failed to appear in patients dying of infection. This was confirmed later in cirrhotic patients in whom increased plasma fibrinolytic activity was inhibited when a serious infection occurred, during the postoperative period, or when corticotrophin treatment was given [2–4]. Such an impairment of fibrinolysis was believed to contribute to the development of portal vein thrombosis in these patients. Plasma from patients with primary carcinoma of the liver could inhibit fibrinolysis *in vitro* [5]. A number of patients with inherited impairment of the fibrinolytic system have been observed to have high risk of thrombosis since then. Likewise, the risk of thrombosis also has been recognized in a number of patients with acquired impairment of fibrinolysis. In this article, both of these conditions will be reviewed.

The fibrinolytic system

Although lysis of fibrin is one of the main functions of the fibrinolytic system, its components are involved in many additional biologic processes [6–8]. Thus, it more suitably is referred to as the plasminogen-plasmin system. The components of this system are shown in Fig. 1. The proteolytic enzyme plasmin is derived from the activation of its precursor, plasminogen, by several activators. In

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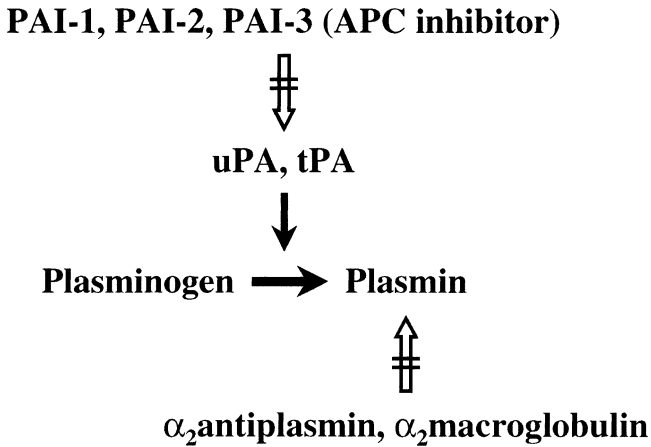


Fig. 1. Components of the plasminogen-plasmin system. Plasmin is formed when plasminogen is activated by uPA or tPA. This action is inhibited by PAI-1, PAI-2, and PAI-3, the latter also known as activated protein C inhibitor. Plasmin is inhibited by α_2 -antiplasmin and α_2 -macroglobulin.

humans, there are two plasminogen activators (PAs), urokinase-type PA (uPA) and tissue-type PA (tPA). Receptors for plasminogen, uPA, and tPA are present on cell surfaces, facilitating the assembly of the system. The proteolytic activities of plasmin and the PAs are modulated by their respective inhibitors. Plasmin inhibitors include α_2 -antiplasmin and α_2 -macroglobulin. PA inhibitors (PAIs) include type 1 (PAI-1), type 2 (PAI-2), type 3 (PAI-3, identical to the inhibitor of activated protein C), and protease nexin. In addition, the fibrinolytic system may be inhibited by a protein activated during clotting. This protein was described recently and termed thrombin-activatable fibrinolysis inhibitor (TAFI) [9,10]. It is a carboxypeptidase B derived from the liver. When cleaved by thrombin, it is converted to the active form (TAFIa) as carboxypeptidase U. TAFIa stabilizes fibrin and inhibits the lysis of fibrin by preventing plasminogen from binding to fibrin.

Following a breach in the vascular lining, normal hemostasis requires a balance between the formation of fibrin and its eventual breakdown. It has become clear that if the fibrinolytic response occurs too early, or if an excessive amount of fibrinolytic protease is present, bleeding will occur. Conversely, to keep the vascular flow intact, the level of fibrinolytic activity has to be kept constantly at a physiologic level and cannot be impaired. There are congenital and acquired disorders associated with impaired fibrinolysis in which the risk of thrombosis is increased greatly.

Relationship to the pathophysiology of thrombosis

Endothelial cells provide a major source of fibrinolytic activity in the circulating blood. In endothelial cells, plasminogen and its activators, tPA and uPA, are

synthesized. Following their secretion, these components are assembled on the cell surface (ie, the intimal lining), bound to their respective receptors. Complexes of tPA and PAI-1 and of uPA and PAI-1 also are formed and bound to their respective receptors on the cell surfaces. The assembly of these components is physiologically in a state of balance, so that plasmin is formed only when there is an excess of plasminogen activators. If this balance is disturbed, for example by an excess of PAI-1, fibrinolytic activity is reduced with a tendency for thrombosis. Likewise, if endothelial cells are injured, there will be a lack of local release of plasminogen activators, with the same consequence of a tendency towards thrombosis.

Hereditary defects

Hereditary defects of the fibrinolytic system are uncommon. Recent investigations using “knock-out” mice models suggest that gene deletion of multiple components of this system is compatible with fairly normal and functioning physiology in these animals [6]. This is in part because of the “redundancy” concept that nature endows the body with more than one system in reserve for any particular function. In some congenital deficiencies such as deficiency of α_2 -antiplasmin, bleeding manifests only when the patient is stressed, presumably when plasminogen activators are released in response to the stress. Other hereditary defects are associated with increased risk for thrombosis or thrombophilia. These are defects that may involve several different components of the system, including plasminogen, PA, and PAI.

Plasminogen defects

Plasminogen is synthesized by the liver [11]. Its structure consists of five kringle domains [12]. Of interest, the first 4.5 kringles are homologous to a protein in cancer tissues known as angiostatin, an antagonist of angiogenesis [13]. Quantitative and qualitative defects of plasminogen have been described as associated with thrombophilia. The former also is referred to as type I dysplasminogenemia and the latter as type II dysplasminogenemia.

Type I dysplasminogenemia

Homozygous deficiency of plasminogen is expressed as absence of plasminogen in blood and tissues [14]. This results in failure of the body to remove fibrin deposits in various organs. It manifests as ligneous or pseudomembranous conjunctivitis, hydrocephalus (caused by fibrinous obstruction to the flow of cerebrospinal fluid [CSF]), obstructive airway disorder, and abnormal wound healing. Replacement therapy with plasminogen corrects these defects by allowing the lysis of the fibrinous deposits. Notably, infants with the homozygous defect do not have a higher incidence of thromboembolic events. Likewise, there is no convincing evidence that heterozygosity is associated with increased risk of thrombosis. Shigekiyo et al evaluated two unrelated families comprising 40 subjects, of whom 21 were heterozygotes for plasminogen deficiency and found no

correlation between congenital plasminogen deficiency and the occurrence of thrombosis [15]. This was confirmed by Tait et al, who were unable to demonstrate an increased incidence of thrombosis in patients with isolated heterozygous congenital hypoplasminogenemia, although a synergistic effect with other thrombophilic defects could not be ruled out [16].

Type II dysplasminogenemia

Type II dysplasminogenemia is inherited as a mutation at various loci in the plasminogen molecule leading to functional abnormalities and failure of plasminogen activation. The lack of proteolytic activity has been attributed to a point mutation, G to A substitution in exon 15, resulting in replacement of Ala-601 by Thr in the active center [17,18]. Two other gene mutations accounting for an absence of proteolytic activity also have been characterized: Val-355 to Phe, and Asp-676 to Asn. [17,19]. In studying 129 families with dysplasminogenemia, Tsutsumi et al [19] showed that the vast majority of cases were caused by the Ala-601 to Thr mutation (94.4%). The Val-355 to Phe caused 3.2% of cases, and Asp-676 to Asn caused 1.6%. Even though the mutational defect was detected when the propositi manifested with thromboembolic events in many of the original reports, the majority of their family members though affected, were not symptomatic. Moreover, substitution of Ala-601 by Thr has been reported to be present in healthy subjects in 2.2% and 2.9% of the Japanese and Chinese Han populations, respectively [17,20]. Thus, whether type I or type II congenital plasminogen deficiency is associated with an increased incidence of thrombosis remains unclear.

Plasminogen activator defects

There were several earlier reports of families with a failure of release of fibrinolytic activity following exertion or venous occlusion. Some members of these families were observed to have a propensity to thrombosis. To the authors' knowledge no congenital deficiency of either uPA or tPA has been reported.

Plasminogen activator inhibitor defects

Three polymorphic variations in the human PAI-1 gene have been reported, where specific alleles were associated with altered plasma PAI-1 levels [21]. The first is a *Hind*III restriction fragment length polymorphism; the second is a (C-A)_n dinucleotide repeat polymorphism, and the third is a single nucleotide insertion or deletion polymorphism (4G/5G). The *Hind*III polymorphism develops because a base change in the 3' untranslated region (UTR), where the 1/1 genotype exhibited higher PAI-1 levels than the 1/2 and 2/2 genotypes [22]. Of further interest is the finding that the PAI-1 genotype affected PAI-1 regulation by Lp(a) and hypertriglyceridemic very low-density lipoprotein (VLDL) at a transcriptional level [23]. The smaller alleles of an eight-allele dinucleotide repeat polymorphism also may be associated with increased PAI-1 activity [22]. Regarding the sequence length polymorphism, which occurs in the promoter region of the PAI-1 gene, the (4G/4G) genotype has been found to correlate with

higher PAI-1 activity compared with genotypes possessing a 5G allele [24]. It has been found that the 4G and 5G alleles bind a transcriptional activator, but only the 5G allele binds a repressor protein. As a result, the (4G/4G) genotype has a higher basal PAI-1 transcription rate.

In studies evaluating the association of the (4G/4G) PAI-1 polymorphism with thrombosis, discrepant results have been reported. Eriksson et al evaluated 94 men who had experienced myocardial infarction before the age of 45 and found an increased prevalence of the 4G allele compared with a healthy control population [24]. Notwithstanding, another study confirmed an associated elevation in PAI-1 levels with the 4G/4G genotype but did not find any difference in 4G allele prevalence between patients with myocardial infarction and controls [25]. In addition, a more recent evaluation involving the Physicians' Health Study did not find an increased prevalence of the 4G allele with incidence of myocardial infarction or venous thrombosis [26]. Adding to the controversy, another study revealed that the PAI-1 genotype abnormality represents a risk factor for venous thrombosis in the setting of protein S deficiency [27]. Despite these discrepancies, meta-analysis has indicated that the 4G polymorphism is associated with 1.3-fold increase in coronary events [28].

Acquired defects

Diabetes mellitus

Among diabetic patients, the incidence of coronary artery disease, cerebrovascular accidents, and peripheral artery disease is twofold to fourfold higher than that seen in the nondiabetic population [29,30], making it the leading cause of death in diabetes [31]. Abnormalities in platelet function, coagulation factors, and fibrinolytic activities contribute to the pathogenesis of vascular injury, atherosclerosis, and thrombosis in diabetes. In particular, PAI-1 is being recognized increasingly as a major risk factor in vascular disease [32]. It is inhibitory to apoptosis [33] and facilitates cell migration along with uPA [7,8,34]. When stimulated by cytokines including interleukin-1 (IL-1), tumor necrosis factor- α (TNF α), and insulin, many normal cells, including epithelial and liver cells, express large quantities of PAI-1 [35,36]. Experimental data in vitro and in vivo indicate that PAI-1 is atherogenic by promoting smooth muscle cell migration and inhibiting apoptosis. Plasma fibrinolytic activity, measured by global tests such as euglobulin lysis time, is impaired. This is because of increased PAI-1 levels. In vitro studies have shown that insulin, proinsulin-like molecules, glucose, and VLDL stimulate PAI-1 production [23,37,38]. Glucose-responsive elements are present in the promoter region of the human PAI-1 gene [39]. In normal nondiabetic subjects, a high combined level of insulin, glucose, and triglycerides induced experimentally has been shown to increase the plasma PAI-1 levels at least twofold [40]. The plasma PAI-1 levels have been correlated strongly with insulin resistance and plasma insulin levels. In a multicenter study involving 1551 subjects, statistically significant correlation has been found between PAI-1 and fibrinogen levels and levels of insulin and its precursors [41].

Plasminogen activator inhibitor-1 is an etiologic factor in the increased atherosclerosis in patients with insulin resistance, a concept first put forth by Juhan-Vague et al [36]. In atherosclerotic coronary arteries taken from patients with Type 2 diabetes, PAI-1 has been found to be elevated highly in atheromatous tissues when compared with similar material taken from nondiabetic patients. This increase of PAI-1 was found to be disproportionate to the uPA contents, suggesting an increased production of PAI-1 [42].

Circadian variation. Plasma PAI-1 level shows a diurnal variation in normal subjects, with higher levels in late morning hours. This is correlated to a higher incidence of acute myocardial infarction [43], stroke [44], and sudden cardiac death [45] during the same period of the day. This circadian variation is, however, altered in the diabetic patient with a greater frequency of myocardial infarction during the evening hours [46,47]. Interestingly, the circadian variation in PAI-1 levels also is altered in diabetes and shows no increase in the morning hours [48].

Resistance to thrombolysis. A greater resistance to thrombolysis in diabetic patients has been reported in a number of clinical trials of thrombolytic therapy for acute myocardial infarction and for acute peripheral arterial occlusion [49–51]. The rates of incomplete reperfusion and of reocclusion were higher in diabetic patients. The increase in fibrinolytic inhibitor PAI-1 and the increased platelet aggregation in diabetes are believed to be causative factors in the resistance to thrombolysis. Because multiple factors are involved in thrombolytic therapy, however, the role of the thrombophilic factors is not clear. Nonetheless, the outcome of acute myocardial infarction in diabetic patients, in the short- and long-term, is worse than in nondiabetic subjects.

Metabolic control measures—effect on hemostatic and fibrinolytic abnormalities. There are no prospective clinical trials designed to lower the PAI-1 level in diabetes and insulin resistance syndrome. Results of lipid control by the HMG-Co-A reductase inhibitor fluvastatin are encouraging. Factor VII, von Willebrand factor, and PAI-1 levels were lower after reduction of plasma lipids [52]. The use of troglitazone aimed at increasing insulin sensitivity and lowering plasma insulin levels also has resulted in a reduction of PAI-1 levels [53]. Other recommended measures include glycemic control, aiming at maintaining an optimal plasma glucose level. Unfortunately, no anti-PAI-1 agents are available for clinical use. Monoclonal antibodies against the reactive site of PAI-1 and mimetic peptide fragments that inhibit PAI-1 have been used experimentally in vitro and in vivo to enhance thrombolysis, but no long-term studies on the effect on atherogenesis have been published.

Cancer

Since Trousseau's astute observations of "pneumonia alba dolens" in patients with "internal cancerous tumors" [54], the changes in hemostatic factors in the

circulating blood in cancer patients have been documented. These include the abnormally increased levels of coagulation factors and alteration of the fibrinolytic components [55–61]. In the fibrinolytic system, the global test of the euglobulin lysis time and the specific components of plasminogen activators, plasminogen, PAI-1, and α_2 -antiplasmin have been altered towards a state of impaired fibrinolytic activity. Of interest, the increased level of PAI-1, in the blood and in tumor tissues has been found to be an unfavorable prognostic indicator for carcinoma of breast, prostate, and lung [7,62,63].

In terms of thrombogenic risk, PAI-1 has a dual effect. On one hand, it impairs plasminogen activation, thus increasing the thromboembolic risk. On the other hand, it inhibits apoptosis [33]. Because apoptotic cells recently have been observed to generate thrombin [64], this action of PAI-1 indirectly decreases the thrombogenicity of tumor cells.

At the cellular level, tumor cells often contain inhibitors of fibrinolysis. This was first observed in hepatocellular carcinoma [5]. More recently, one of the inhibitors was found to be PAI-1 in many tumor types, including carcinoma of breast, prostate, colon, and squamous cell carcinoma of the skin [7]. Whether abnormalities at the cellular level contribute to increased thrombotic risk in cancer patients remains unclear. On the other hand, in acute promyelocytic leukemia, increased fibrinolytic activity contributes to hemorrhagic complications [65].

Clinically, thromboembolism is the second most common cause of death in the cancer patients [66]. Among thrombogenic factors, the expression of tissue factor and of cancer procoagulant by tumor cells plays a major role. Evidence of active coagulation, such as presence of fibrinopeptide A levels, are found in over 75% of patients studied [67]. As a result, an adequate fibrinolytic response is required to prevent thrombosis. Thus, the level of fibrinolytic inhibitors found in the cancer patient may be pivotal in determining the occurrence of this complication.

The drug L-asparaginase, used in treatment of lymphocytic malignancies including acute lymphocytic leukemia and some cases of non-Hodgkin lymphoma, has been implicated as a risk factor for thromboembolic disease. This is thought to be through the inhibition of protein synthesis, and as such leading to a reduction in the plasma levels of plasminogen, antithrombin, and various coagulation factors [68,69]. Kucuk et al [70] reported a case of acute lymphoblastic leukemia in which an 18-year-old woman developed a stroke and catheter-related subclavian vein thrombosis after receiving L-asparaginase. At the time of the thromboembolic events, physicians noted she had decreased plasminogen and antithrombin III levels. Thrombolytic therapy for the venous thrombosis with a plasminogen activator was not successful because of the low plasminogen level, until the latter was corrected by the infusion of fresh frozen plasma.

Hormonal effects

Hormonal replacement therapy with physiologic doses of estrogens is associated with decreased cardiovascular events and stroke [71]. There is an increase

in tPA along with reduced PAI-1 level, resulting in an overall increase in fibrinolytic activity [72]. On the other hand, it is clear that the pharmacologic doses of estrogen [73] and selective estrogen receptor modifiers (SERMS) used in cancer patients are associated with increased risk of thromboembolic events [74,75] especially when used in combination with chemotherapy [76]. The role of the fibrinolytic system in this respect is less clear. Estrogens exert effects on the vascular wall and thus at pharmacologic doses may affect the release of plasminogen activators from endothelial cells. It is interesting that with the use of oral contraceptives, changes in levels of the various components of the fibrinolytic system including plasminogen, tissue plasminogen activator, PAI-1, and plasmin-antiplasmin complexes indicated that the overall fibrinolytic activity is increased [75,77–80]. Notably, the level of TAFI is increased in women taking contraceptives containing desogestrel [81], suggesting that when coagulation takes place, TAFI may play a role in inhibiting fibrinolysis.

Management

When a thrombotic event is suspected to be related to a disturbance in the fibrinolytic system, management is generally supportive. Because multiple thromboembolic risk factors may be present in any given patient, it is prudent to rule out any reversible cause and to institute appropriate anticoagulation. In addition, a thorough investigation should be undertaken to document if the phenomenon is secondary to an underlying disorder such as diabetes, occult malignancy, or even pregnancy. Unfortunately, there are no agents that can reverse dysplasminogenemia, plasminogen deficiency, or antagonize the activity of the inhibitors directly.

Summary

The fibrinolytic system plays a pivotal role in the regulation of hemostasis and the prevention of thrombosis. There are no drugs that will increase the plasma fibrinolytic activity for a lasting duration to prevent thrombotic events effectively. Despite the ability of vasoactive agents such as nicotinic acid and metformin to release PA from the vessel wall, this therapeutic effect has not been evaluated adequately. The PAs are short-acting and indicated only for thrombolysis and not for prophylaxis. Future directions are directed at finding agents that can enhance plasminogen activator release or inhibit PAI-1 activity.

As there are multiple factors involved in the pathogenesis of thrombosis, there are a number of conditions in which abnormal fibrinolysis is only a contributory factor. Examples are seen in pregnancy, especially during puerperium, when the thromboembolic risk is at its highest. The levels of inhibitors of fibrinolysis, both PAI-1 and PAI-2, are also at their highest. Another example was seen recently in the antiphospholipid syndrome, where antibodies against Annexin II, a receptor for tPA, were found to be higher than in healthy controls [82]. Thus, a

thorough investigation into other hereditary and acquired risk factors for thrombosis is recommended.

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