Fibrinolysis—A Review

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ABSTRACT

The function of fibrinolysis is to dissolve fibrin clots. The agent of fibrinolysis is plasmin, a glycoprotein with gram molecular weight (GMW) of 90,000. Under natural conditions, plasminogen is converted to plasmin by tissue plasminogen activator (TPA). Activation occurs on the fibrin surface, thus confining proteolytic activity to the appropriate site. Tissue plasminogen activator, produced by monoclonal methods, has recently been made available for limited therapeutic use. Currently streptokinase and urokinase are widely used therapeutically to activate plasminogen. These agents cause plasmin to be formed which is free in the circulation as well as bound to fibrin, resulting in proteolysis of circulating plasminogen and clotting factors. Fibrinolytic therapy has proven to be more beneficial than anticoagulation alone for deep vein thrombi and for pulmonary emboli. During therapy, laboratory studies demonstrate reduced concentrations of plasminogen, fibrinogen, and of alpha-2 plasmin inhibitor, and prolongation of activated partial thromboplastin time and thrombin time. Laboratory findings must be correlated with the clinical course. Demonstration of circulating plasmin-antiplasmin complex may be a useful indicator of active fibrinolysis.

Introduction

The principal function of the fibrinolytic system is to dissolve fibrin, counterbalancing the coagulation system. Fibrinolysis acts simultaneously to permit the existence of useful clots and to prevent catastrophic generalized systemic coagulation. (The coagulation scheme itself has properties which tend to limit and localize fibrin production).

Plasmin is the agent of fibrinolysis. Plasminogen and its activator(s) are constantly present in plasma, but activation of plasminogen requires the presence of a fibrin strand. Thus fibrin serves as a sort of rate-limiting catalyst for the production of the enzyme which destroys it. Dissolution of fibrin causes release of plasmin into the circulation where it is immediately bound by antiplasmin(s). The strong non-specific proteolytic action of plasmin is normally localized to the clot as a consequence of these interactions (figures 1, 2, 3, and 4).

Physiology

Plasminogen is a glycoprotein with GMW of 90,000. It is made in the liver.
and has a half-life of two days. The amino acid sequence has been completely determined. The basic structure is a coiled chain which undergoes activation by shortening of the N-terminal end in several steps. The molecule undergoes subsequent autocatalytic cleavage into two chains which are connected by SH bonds. The A-chain is the heavier of the two and is derived from the N-terminal portion of the parent molecule, while the B-chain represents the C-terminal portion. The A-chain contains several lysine-binding sites by which plasminogen forms specific attachments to fibrin. The lysine-binding sites on the plasminogen molecule may also combine with the principal plasmin inhibitor, alpha-2 antiplasmin, and with therapeutic antiplasmins such as epsilon amino caproic acid (EACA). Thus both natural and therapeutic inhibitors act by competing with fibrin for the lysine-binding sites on plasminogen.

**Plasminogen Activators**

Plasminogen may be activated by intrinsic, extrinsic, or exogenous (usually therapeutic) agents. Intrinsic activation begins, as in the coagulation scheme, by contact activation of Hageman Factor XII. The intrinsic fibrinolytic system is poorly understood. However, its importance is suggested by the fact that Hageman factor deficient individuals and patients with acquired inhibitors of Factor XII activators both have thrombotic and not hemorrhagic tendencies.

Plasminogen activators are found in many tissues and secretions. The best known is tissue plasminogen activator (TPA). While the site of manufacture is not certain, TPA is present in endothelial cells, especially within veins. It is released into the circulation in response to a variety of stresses including venous stasis, ischemia, and exercise, and in response to vasoactive substances such as epinephrine and histamine. The half-life of TPA in the circulation is 15 minutes. There is significant diurnal variation with maximal activity at 4:00 P.M. and minimal activity at 4:00 A.M. Tissue plasminogen activator is a serine protease with GMW of 60,000. Although TPA is normally present in the plasma, its concentration is diminished in patients with venous thrombosis or myocardial ischemia. Although large amounts of TPA are released into the blood on exercise, plasmin generation does not result because of the absence of fibrin. Thus TPA and plasminogen coexist in normal plasma but do not interact. Both have strong affinity for fibrin surfaces where TPA acts upon plasminogen, initiating its conversion to plasmin. Here, too, plasmin is protected from its inhibitors by its attachment to fibrin. Note that this mechanism serves to protect circulating fibrinogen and other bystander proteins from proteolysis by plasmin.

Exogenous activators of plasminogen include urokinase, which is made by epithelial cells of the urinary tract, and streptokinase, a bacterial product. Urokinase is a serine protease; its action is similar to TPA. Its presence in the urinary tract may be necessary to maintain the patency of the renal tubules and col-
Fibrinolysis, in contrast, is not a protease but binds to the plasminogen molecule, deforms it, and causes it to become autocatalytic. Note that both urokinase and streptokinase are capable of activating circulating (as opposed to fibrin-bound) plasminogen, resulting in activated plasmin which is not bound to fibrin. Free plasmin, if present in excess of its inhibitors, will attack fibrinogen and other proteins nonspecifically. Thus, activation of plasminogen by urokinase or streptokinase will result in depletion of fibrinogen as well as clot dissolution and may impair the normal coagulation process. The plasmin which is formed under these conditions is systemic and diffuse and, therefore, apt to be less potent at the thrombotic site than TPA-activated plasmin.

Plasmin itself may catalyze the conversion of plasminogen to plasmin under some circumstances.3

Plasmin Inhibitors

Plasmin, in addition to its fibrinolytic ability, is capable of non-specific proteolysis similar to trypsin activity. After plasmin is set free into the circulation by the dissolution of fibrin, it is rapidly combined with natural inhibitors. These are normally present in excess of the available plasmin and plasminogen reservoir. This is the final and necessary step in the process of protecting against undesirable proteolysis.

Plasmin inhibitors include alpha-2 antiplasmin, alpha-2 macroglobulin, and alpha-1 antitrypsin, all of which are glycoproteins.1 While alpha-2 antiplasmin is present in lower concentration than alpha-2 macroglobulin, it has much higher affinity for plasmin. Alpha-2 antiplasmin, which is also called alpha-2 plasmin inhibitor, binds specifically to plasmin in a 1:1 ratio. The larger pool of alpha-2 macroglobulin acts as a reserve inhibitor.

Plasmin has several actions in addition to fibrinolysis. It serves to activate Factor XII, thus amplifying the intrinsic scheme for both fibrinolysis and coagulation and indirectly activating prekallikrein. It also nonspecifically activates C1, C3, and C5.3 Therefore, plasmin mediates interaction between fibrinolysis, fibrinogenesis and inflammation.

It has been hypothesized that the body normally sustains a constant low level of continuing systemic fibrinogenesis which is balanced by ongoing fibrinolysis. Turnover studies with labelled components of the coagulation and fibrinolysis systems do not support this view. It is now held that both systems are activated only according to need.26

Clinical Applications

Coagulation has been manipulated therapeutically for over a generation. Fibrin formation has been inhibited with anticoagulants and enhanced with clotting factors. Yet regulation is by no
means satisfactory. The fibrinolytic system offers the possibility of another dimension of control. Thus, treatment for excessive or untimely clotting would include reinforcement of fibrinolysis, while hemorrhage resulting from impaired clotting might be treated by therapeutic reduction of fibrinolysis.

There is currently much interest in treating very fresh coronary and cerebral thromboses and pulmonary emboli with local fibrinolytic compounds. Recent technical advances in vascular catheterization have made possible the local delivery of exogenous plasminogen activators. Both local and systemic therapy have repeatedly proven to be superior to anticoagulation alone. However, there are several caveats. Both urokinase and streptokinase are associated with increased bleeding, approximately twice that which is seen with anticoagulation alone. Central nervous system hemorrhage occurs in about one percent of cases. Urokinase, which is derived from human cell cultures, is extremely expensive. Streptokinase is less expensive but is strongly antigenic. Anti-streptokinase (antistreptolysin) is almost universally present in adults owing to previous Streptococcal infections. These antibodies must be overcome before streptokinase therapy will be effective. Allergic side effects including anaphylaxis occur, and rheumatic myocarditis has been observed. Bleeding complications may be treated with EACA which blocks the action of plasmin. Anticoagulants and antiplatelet drugs should be avoided during thrombolytic therapy; however, heparin is usually given immediately afterward to prevent rethrombosis. Bolus intravenous therapy has shown some success as an alternative to catheter placement of fibrinolytic compounds and offers the advantages of economy and speed, which are of the essence.

Genetic engineering and monoclonal techniques have recently been applied to TPA production. Tissue plasminogen activator is newly available in pharmacological amounts and may be the ideal therapeutic agent. It offers the following advantages relative to urokinase and streptokinase: (1) Its action is localized to the clot, sparing circulating fibrinogen and coagulation factors V and VIII from proteolysis. This should reduce the frequency of hemorrhagic complications; (2) Since TPA-activated plasmin is formed at the fibrin surface rather than in the circulation, there is no need to overcome the circulating anti-plasmins before the new plasmin becomes effective. (3) TPA is non-allergenic. At least two clinical trials of TPA have just begun, one testing it with freshly evolving cases of myocardial infarction, the other with peripheral arterial occlusions.

Thrombophlebitis leading to pulmonary embolism is still a frequent and disastrous complication of surgery. There is evidence that the postoperative patient is at particular risk because of depression of fibrinolysis following surgery. This may be due to depletion of TPA during surgery or to elevated alpha-2 antiplasmin. Plasmin inhibitor concentrations rise somewhat postoperatively, increasing the danger of thrombosis. Prophylactic enhancement of the fibrinolytic system may be indicated for selected patients.

A recent summary paper from the National Institutes of Health (NIH) on "Thrombolytic Therapy in Thrombosis" has recommended more extensive use of urokinase and streptokinase for the treatment of deep vein thromboses. Anti-coagulation alone does not eliminate the source of subsequent embolization, alleviate hemodynamic disruption owing to embolization, nor does it prevent long-term damage to veins and to the pulmonary vascular bed. The NIH panel noted that thrombolytic therapy, used in conjunction with anticoagulants, can achieve lysis of thrombi and restoration
of circulation to normal with prevention of long-term vascular bed damage. Hemorrhage remains the principal complication. Thrombolytic therapy must be used without delay and should not be thought of as a backup for cases refractory to heparinization. Absolute contraindications to thrombolytic therapy include cerebrovascular accident (CVA) within two months, other active intracranial processes, and active internal bleeding.

Several studies have attempted to identify those surgical patients who are most apt to develop thrombophlebitis and pulmonary embolism following surgery. Such patients might be treated with prophylactic anticoagulation and/or fibrinolysis. Elevated blood levels of plasmin inhibitors have been demonstrated in several clinical situations associated with a high incidence of thromboembolism. One group followed patients undergoing craniotomy. Radioactive fibrinogen was used to detect thrombus formation in the legs. Patients who developed thrombi failed to show the peak in plasmin activity which normally occurs on the second post-operative day. Clots developed in the legs of these fibrinolysis-deficient patients several days later.

Excessive fibrinolysis contributes to hemorrhage in a variety of clinical conditions. Epsilon amino caproic acid is the antifibrinolytic agent which is used therapeutically in the U.S. Tranexamic acid is more potent and is favored by at least one European group. Patients with hepatic cirrhosis have decreased alpha-2 antiplasmin and increased fibrinolytic activity. Persistence of chronic subdural hematoma may be due in part to clot disruption by plasmin resulting in recurrent bleeding. Subarachnoid hemorrhage from ruptured cerebral aneurysm has been treated with EACA resulting in marked reduction of rebleeding in the interval between initial hemorrhage and operation (2). Epsilon amino caproic acid has been used successfully to prevent hemorrhage in hemophiliacs undergoing surgery and dental procedures. Recently EACA has been used in our institution to treat a hemorrhaging patient with amyloidosis and acquired Factor X deficiency (5). Epsilon amino caproic acid is usually considered to be contraindicated in disseminated intravascular coagulation (DIC) in the belief that fibrinolysis in this condition is secondary and salutary and should not be interfered with. The major complication of antifibrinolytic therapy is thrombosis. In the study of perioperative venous thromboses of the legs cited previously, four of five patients treated with EACA developed venous thromboses, while only one of seven comparable patients not receiving EACA developed clots.

**Laboratory Evaluation of Fibrinolysis**

Plasmin is not available for analysis in the serum or plasma for reasons discussed previously. Therefore, fibrinolysis must be evaluated through its reactants, products, or consequences.

The simplest test for increased fibrinolysis is to observe a tube of clotted blood for rapid clot lysis. This is best carried out at 37°C. The test is crude and slow, requiring 24 hours to establish that the clot has normal durability.

The euglobulin clot lysis time is an alternative which provides a result within two hours. The euglobulin fraction contains globulins, fibrinogen, plasminogen, and plasminogen activators. Euglobulin is precipitated from plasma with acid, then diluted and redissolved in barbital-buffered saline, and finally clotted with thrombin. The process of separating the euglobulin removes plasmin inhibitors and heparin from the final reaction mixture. The time required for dissolution of the clot is measured. Specimens from patients with increased fibrinolysis develop euglobulin lysis times of
less than 90 minutes. However, clinical correlation with dissolution of pulmonary and deep venous thrombi is poor. The discrepancy between clinical and laboratory findings may be due to exclusion of alpha-2 antiplasmin or of heparin from the reaction mixture.

Plasminogen and fibrinogen are both consumed during fibrinolysis. Fibrinogen is removed by coagulation and by degradation by the free plasmin which results from therapeutic use of streptokinase or urokinase. Paradoxically, reduced levels of plasminogen and fibrinogen may indicate either active fibrinolysis or reduced fibrinolysis owing to exhaustion of plasminogen. Interpretation depends on knowledge of the baseline coagulation profile and its evolution. Heparin is usually stopped prior to the initial studies.

There is some controversy concerning the frequency and range of laboratory studies required to monitor a course of fibrinolytic therapy. Decreased plasminogen and fibrinogen indicate the presence of the desired "fibrinolytic state." Activated partial thromboplastin time and thrombin time may be used to follow therapy; however, all such tests reflect multiple factors including fibrinogen depletion, the clot-inhibiting effect of fibrin(ogen) split products, etc. One reason to measure fibrinogen is to alert the clinician to begin replacement if levels fall below 75 to 100 mg per dl to minimize the risk of hemorrhage.

Recent laboratory developments include fluorescent substrate assay for plasminogen. The method may be adapted to measure plasmin inhibitor concentration. Also, antibodies have been developed against alpha-2 antiplasmin. These may be used to demonstrate circulating plasmin-antiplasmin complex. Since the complex is short-lived, its presence offers the most direct evidence for active fibrinolysis. Depletion of alpha-2 antiplasmin has also been recommended as an indicator of fibrinolysis.

References


