STUDIES AND PERSPECTIVES OF PLASMINOGEN ACTIVATORS

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ABSTRACT

• *The fibrinolytic system participates in a variety of physiological and pathological processes. It consists of an inactive proenzyme, plasminogen, which can be activated to the active serine protease plasmin by the action of different types of plasminogen activators. The main function of the fibrinolytic system is to dissolve fibrin deposits in blood vessels. Thrombogenesis can be influenced by an insufficient or ineffective fibrinolytic system. The tissue-type plasminogen activator (t-PA) receives considerable attention since its deficiency has been shown to be a leading cause for thrombophilic situations. Increases of its main inhibitor (PAI) probably play a similar role. Using the advantages of recombinant DNA technology modem thrombolytic drugs based on the structure of plasminogen activators are applied for the therapy of thromboembolic diseases. Structure and function of the fibrinolytic system are outlined in the following review. Diagnostic evaluation of the fibrinolytic system and therapeutic considerations are discussed.*

INTRODUCTION

• The principal role of the hemostatic mechanism is to protect the integrity of the vascular system. Blood coagulation and fibrinolysis are well-coordinated systems and the hemostatic balance depends upon a variety of interrelations including cellular (thrombocytes, endothelial cells, leukocytes) and humoral (activators, inhibitors) components (1).

The purpose of this review is to consider how the fibrinolytic system participates in these processes under physiological and pathological conditions. It covers the nature of plasminogen activators (PA) and plasminogen activator inhibitors (PAI) and their involvement in various pathological situations. While the picture is far from being complete, diagnostic and therapeutic opportunities of plasminogen activators are discussed.

THE FIBRINOLYTIC SYSTEM

• The central reaction of the fibrinolytic system is the conversion of plasminogen, an inactive zymogen, to the active serine protease plasmin, which in turn degrades proteolytically a variety of substrates, including fibrin, fibrinogen and clotting factors Va and Villa (2).

Two main pathways of plasminogen activation can be distinquished. Analogous to blood coagulation they are referred to as intrinsic and extrinsic pathways.

Intrinsic activation (contact system-dependent pathway): The intrinsic activation of the fibrinolytic system is schematically shown in Figure 1.

Factor XII (Hageman factor) is converted to its activated form (Xlla) when exposed to negatively charged surfaces. Factor Xlla activates prekallikrein to kallikrein. Kallikrein converts, possibly using the activation of another yet unidentified pro-activator, plasminogen to plasmin (3). The importance of this activation pathway is not yet clarified, however, it should be noted, that patients with Hageman factor deficiency may be at risk for thrombosis.

EXTRINSIC ACTIVATION

• This pathway has been receiving considerable attention. It consists of two activators and different types of inhibitors. The main components of the extrisic activation pathway are shown in Figure 2.

The most important activator of plasminogen is the tissue type plasminogen activator (t-PA), which has been extensively characterized (4) and is recently being pro-
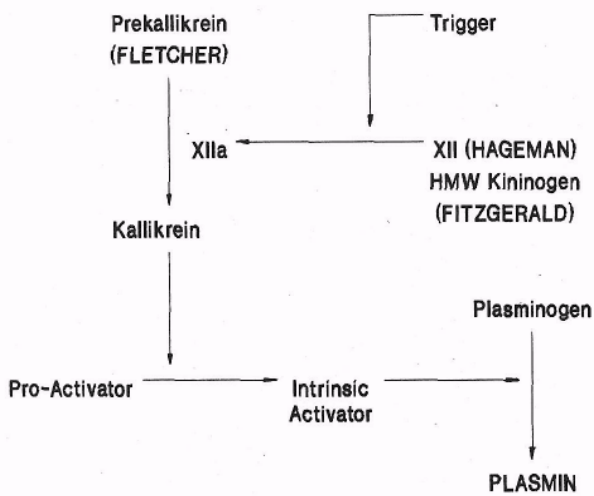
Figure 1: Intrinsic activation of the fibrinolytic system

Figure 2: Extrinsic activation of the fibrinolytic system

duced by recombinant DNA technology (5). Vascular endothelial cells are the main source of plasma t-PA, although it has been shown in thrombocytes, too. T-PA is secreted in its single chain form from the circulation. This release can be induced by various substances (thrombin, adrenaline, histamine), exercise and venous occlusion. Once in circulation, single-chain t-PA is quickly converted into its two-chain, disulfide-linked form by minimal amounts of free plasmin. Both forms of t-PA possess enzymatic activity, that is greatly enhanced in the presence of fibrin. The assembly of t-PA and plasminogen onto a fibrin surface accelerates the fibrinolysis and secures a relative clot specificity.

Urine-type plasminogen activator (urokinase, u-PA) has been originally isolated from urine (6). It is produced by many different cell types (7). Single-chain and two-chain forms of urokinase are known. Single-chain urokinase is, contrary to single-chain t-PA, an inactive molecule. After conversion to the two-chain form it expresses fibrinolytic activity. Urokinase probably plays a minor role in the intravascular fibrinolysis and is rather important for pericellular proteolysis (cell migration, tissue destruction, tumor growth, tumor metastasis). Its usefulness as tumor marker has been reported (8).

REGULATIOM OF THE FIBRIHOLYTIC SYSTEM

• The fibrinolytic system is regulated by a fine network of plasminogen activators, plasminogen activator inhibitors and plasmin inhibitors. The two most important inhibitors are plasminogen activator inhibitor 1 (PAI-1) and alpha-2-antiplasmin. They belong to the serpin pro-tein family (SERine Protease INhibitors).
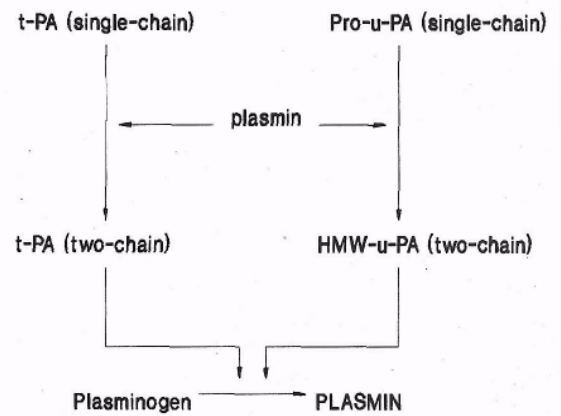
At least four different plasminogen activator inhibitors have been described:

PAI-1 endothelial cells, thrombocytes
PAI-2 placenta, monocytes
PAI-3 = protein C-inhibitor
Nexin fibroblasts

PAI-1 has been discovered in 1983 (9,10). PAI-1 is synthesized and secreted mainly from endothelial cells. It has been found also in thrombocytes, monocytes, smooth muscle cells and hepatocytes. It is biochemically well characterized (11). PAI-1 inactivates rapidly t-PA and u-PA in the circulation. The functionally active form of PAI-1 in plasma is bound to the plasma protein vitronectin, possibly to stabilize the active conformation and to protect the molecule against oxidation, which causes irreversible inactivation.

A second type of plasminogen activator inhibitor, PAI-2, has been recently isolated from placenta. It seems to be mainly an urokinase inhibitor (12). Its role in physiology and pathology is not yet understood.

PAI-3 is identical to the protein C inhibitor. It is rather a weak inhibitor of plasminogen activators. In addition, the potein C - thrombomodulin system promotes fibrinolysis by inactivating PAI-1 through activated protein C (13). The importance of this slow reaction is not yet clarified.

The fibroblast-derived protease nexin possesses PAI activity. It is absent from plasma and therfore rather linked to extravascular fibrinolysis.

Alpha-2-antiplasmin is the main plasmin inhibitor in plasma and inactivates free plasmin very rapidly by

formation of 1:1 complexes. It is secreted by the liver and circulates at concentration of 70 mg/1.

ROLE OF THE FIBRIfJOLYTIC SYSTEM IN THROMBOGEHESIS

• The most important role of the fibrinolytic system is the degradation of fibrin deposits in blood vessels. Therefore it seems to be reasonable to link a defective or insufficient fibrinolytic system with the pathophysiology of thrombosis and thrombogenesis itself.

Using histochemical methods Isacson and Nilsson demonstrated in 1972 that patients suffering from deep venous thrombosis had decreased content of plasminogen activators in their vessel walls (14). Korninger et al followed a group of 121 patients with recurrent venous thrombosis for some years and found a correlation between the recurrence rate and a defective fibrinolytic system (15).

Methods for the measurements of specific parameters of the fibrinolytic system (activators, inhibitors) became available with the increasing knowledge of both structure and function of the various components of the fibrinolytic sustem. Subsequently, studies of the fibrinolytic system in patients suffering from venous thrombosis were performed (16-19). All these studies revealed that 30 - 40 % of the patients were found to have impaired fibrinolytic activity. The underlying mechanism were different - in some cases low basal t-PA levels or insufficient release were found, other patients showed an increased inhibitor activity. Combination of booth defects were described too. All these studies are retrospective. Prospective studies are needed for the evaluation of the predictive value of the fibrinolytic parameters.

In the past few years there is growing evidence supporting a role of the fibrinolytic system in arterial thrombosis including coronary thrombosis. Hamsten et al. reported in 1985 on increased PAI activities in myocardial infarction patients (20). The connection between increased PAI activities and development of myocardial. infarction has been confirmed (21).

Impairment of the fibrinolytic system due to decreased t-PA or increased PAI represent the vast majority of all cases of hypofibrinolysis. Other possible and described abnormalities leading to decreased fibrinolytic activity are deficiencies or molecular defects of plasminogen (22), increased alpha-2-antiplasmin activity and dysfibrinogenemia. Impaired fibrinolytic activity is associated with some of the well-established risk factors for the development of coronary heart disease such as smoking, hyperlipoproteinemia, diabetes and obesity.

The fibrinolytic system seems to be under the influence of physical exercise and nutritional status. Physical exercise and food rich in fruits and vegetables reduce PAI activity (23,24).

DIAGNOSTIC EVALUATION OF THE FIBRIHOLYTIC SYSTEM

• It is now generally accepted that patients with defective or insufficient fibrinolytic system are at risk for venous thrombosis (25, 26). In addition there is growing evidence supporting the pathogenetic role of decreased fibrinolytic activity in coronary heart disease and acute myocardial infarction (21). Clinical manifestation may vary, but thrombosis in patients younger than 45 years without any underlying disease or risk factor, reccurent thrombotic events and multiple thromboembolic manifestations within a family, should engage the clinician to-perform a complex laboratory program in order to investigate possible causes of inherited or acquired thrombophilia.

Several diagnostic programs have been proposed (27, 28). Diagnostic programs should include investigation of increased coagulation sysem activity, increased platelet activity, coagulation inhibitor deficiency and decreased fibrinolytic system activity (29).

Two general types of tests may be used for the evaluation of the fibrinolytic system

a) screening tests

b) measurements of specific parameters

Screening tests are employed in order to obtain information about the overall activity of the fibrinolytic system. Usually euglobulin fractions of human plasma prior to and after an appropiate stimulation (e. g. venous occlusion, vasopressin analogue) are used. The euglobulin fraction contains fibrinogen, plasminogen, t-PA and a portion of PAI-1. It lacks, however, u-PA, alpha-2-antiplasmin and a fraction of PAI-1.

Using the euglobulin clot lysis time, a significant shortening of the time, necessary to dissolve the clot is measured after venous occlusion (from approximately 4 hours to 20 - 60 minutes). This shortening is less pronounced or absent in patients with insufficient fibrinolytic activity. The eugloblin clot lysis time correlates with increased values of PAI-1.

The fibrin plate method is another widely used screening test (30). Little amounts of euglobulin fractions are applied onto a plasminogen-rich fibrin preparation in flat dishes and the areas of fibrin lysis are measured prior to and after venous occlusion. The increase of lysis area correlates with the t-PA activity of the sample. This test does not depend on the patient's own fibrinogen and plasminogen.

Another appropriate screening test is the fibrin difference assay. The test depends upon the ability of human plasma to dissolve a defined fibrin clot. Normally the test results are between 80 and 100. Values below 30% may indicate a lowered fibrinolytic activity (31, 32).

The three described screening tests allow to differentiate between responders showing impaired basal fi-

brinolytic activity and/or insufficient increase after venous occlusion (33).

Every identification of a hypof ibrinolytic state should be followed by measurements of specific components of the fibrinolytic system.

The principal proenzyme of the fibrinolytic system, plasminogen, is determined by activity meauusements using chromogenic substrates (S-2251). Decreased activity should be clarified (dysproteinemia, hypoplasminogenemia). A few hypofibrinolytic situations due to deficiency or abnormalities of plasminogen have been described (22).

Tissue-type plasminogen activator can be characterized by activity and antigen measurements. Determination of t-PA activity should be preffered since the estimation of t-PA antigen should not necessarily reflect its activity. Measurements of t-PA are usually performed prior to and after venous occlusion. Increase of at least 50% is accepted as marker for appropriate stimulation. Decreased basal activities and/or inappropiate increase of t-PA after stimulation are frequently found in patients with venous thrombosis (34).

Measurement of plasminogen activator inhibitor activity should be performed, since an increased PAI activity could contribute to hypofibrinolysis. Basal PAI activities vary widely. It is recommended that PAI activity should be measured prior to and after venous occlusion (35). High PAI activities after occlusion are thought to overcome the release of t-PA from the endothelial cell, making the fibrinolytic response ineffective. High basal PAI activities have been found frequently in patients suffering from recurrent deep venous thrombosis (32).

The measurement of other components of the fibrinolytic system is either difficult to perform (u-PA) or of suspect clinical significance (alpha-2antiplasmin).

We recommend for the evaluation of the fibrinolytic system first to apply a screening test, and in case of pathological results, the determination of PAI and t-PA prior to and after venous occlusion.

Since the fibrinolytic system is very dynamic, all tests should be applied repeatedly in order to avoid misleading conclusions concerning its activity.

THERAPEUTIC APPLICATION OF PLASM1MOGEH ACTIVATORS

•   Thromboembolic diseases of the cardiovascular system are a main cause of death and disability.

Consequently, therapeutic application of plasminogen activators has been introduced with the increasing knowledge of physiology and pathology of the fibrinolytic system (36). Three generations of fibrinolytic (thrombolytic) drugs based on plasminogen activators can be distinquished:

a) first generation: streptokinase, urokinase

b) second generation: t-PA, single-chain u-PA, acylated plasminogen streptokinase activator complex

c) third generation: gene technology-derived plasminogen activators, hybrid molecules, mutants, antibody-targeted activators

Thrombolytic therapy using streptokinase or urokinase is established as a well-known and realistic form of treatment for venous thrombosis, pulmonary embolism and acute myocardial infarction; however, optimal dose regimen and exact place in the therapy of thromboembolic deseases are still controversial. Disadvantages include difficult dosage and bleeding risk, resulting from non-fibrin specificity.

Initial therapeutic studies with t-PA and single-chain t-PA were performed with their availability in small amounts. Higher fibrin-specific thrombolytic efficacy and absence of systemic fibrinolytic activation were confirmed.

The advantages of recombinant DNA technology contribute to the applicability of plasminogen activators for therapy. Recombinant t-PA (rt-PA) seems to be the drug of choice for treatment of acute myocardial infarction, resulting in successful reperfusion in about 65 % of inf arct-related arteries. Reocclusion rates vary between 10 and 15% in large trials. Therapeutic application of recombinant single chain u-PA in acute myocardial infarction has given similar results.

New trends in thrombolytic therapy include improvement of fibrin specifity of recombinant plasminogen activators and construction of mutants with prolonged in vivo half-life. Several hybrid (chimeric) plasminogen activator molecules have been produced.

Plasminogen activators have been coupled to fibrin-specific antibodies to improve clot-specific lysis (37). These alternatives are currently being investigated. Further developments in this field may be expected from ongoing basic and clinical research.

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