

From Fibrinolysis to the Plasminogen–Plasmin System and Beyond: A Remarkable Growth of Knowledge, with Personal Observations on the History of Fibrinolysis*

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Abstract

Great advances have been made in our understanding of the fibrinolytic system from the initial discovery of proteolysis of fibrin by plasmin to the multifaceted and complex role of the plasminogen–plasmin (P–P) system. We now know that the P–P system is composed of several serine proteases and their inhibitors (serpins). This system is involved in many physiological functions, including embryogenesis, cell migration, and wound healing. They also play an important role in the pathogenesis of many diseases, including atherosclerosis, obesity, cancer, and even autoimmune disorders, and neuronal degeneration. Knowledge of their role in cancer enables their use as a prognostic factor. Therapeutic use of various forms of proteases derived from this system has been employed as thrombolytic agents. In addition, small molecules designed to inhibit many of the components of the P–P system are now available for clinical trial, aimed at treatment of these various disorders. The history of such remarkable development of our knowledge on fibrinolysis is reviewed in this article.

Keywords

- ▶ fibrinolysis
- ▶ plasminogen
- ▶ plasminogen activator
- ▶ PAI-1

Among the many advances in our understanding of the various physiologic phenomena, few can match that of fibrinolysis. This is the process in which a fibrin clot is removed by the body when its hemostatic function is completed. The fibrinolytic system plays an important role in many of the physiologic functions of the body as well as in the pathogenesis of many diseases. Our knowledge of this system, also known as the plasminogen–plasmin system (P–P system), has made enormous advances from regulation of hemostasis to the pathogenesis of a wide range of diseases including atherosclerosis, obesity, cancer, and even autoimmune disorders, as well as neuronal degeneration. New findings recently obtained have enormous therapeutic implications. Components of the fibrinolytic system can be selectively

targeted by new inhibitors. On the 40th anniversary of *Seminars in Thrombosis & Hemostasis*, it seems appropriate to provide our readers with a historical overview of this remarkable development, along with some interesting personal observations and an outlook into the enormous translational potential of this system.

Early Observations

Postmortem Fibrinolysis

This phenomenon was first observed in postmortem blood by both Morgagni¹ in 1769 and later John Hunter² in 1794. The latter wrote:

In many modes of destroying life the blood is deprived of its power of coagulation, as happens in sudden death produced by many kinds of fits, by anger, electricity or

* This article is dedicated to my mentor Alexander J. S. McFadzean, FRCP, who started it all for me.

lightning; or by a blow on the stomach, etc. In these cases we find the blood, after death, not only as fluid a state as in the living vessels, but it does not even coagulate when taken out of them.

Virchow noted that “capillary blood in the cadaver was always fluid and incoagulable and that the blood in the veins was more often than not incoagulable.” From this astute observation, he hypothesized that liquefaction of blood originated from the endothelium.³ Morawitz observed that in sudden death, there was no fibrinogen in the blood, that such blood contained a lysin that could destroy the fibrinogen and fibrin in normal human blood.⁴ Yudin made use of these discoveries and published the results of transfusion of cadaver blood in Russia in 49 clinical cases by selecting subjects that died from sudden death.⁵ Blood was collected without preservatives from the jugular vein to avoid the infected mesenteric blood and could be stored in the refrigerator for up to 4 weeks. He and his assistants Skundina and Rusakov were able to observe the process of fibrinolysis in postmortem blood under a microscope.

In Vitro Observations

In 1838, Denis observed that blood collected by wet-cupping first clotted and then spontaneously dissolved in 12 to 24 hours.⁶ In 1887, Green noted that fibrin disintegrated in saline without obvious bacterial action.⁷ In 1893, the term “fibrinolysis” was first given by Dastre,⁸ who noted that the process was a source of error in the measurement of fibrin in plasma. With dog’s blood, he found an average loss of 8% in weight of fibrin on incubation for 18 hours. While many investigators were pursuing the isolation of the enzyme “fibrinolysin” from the plasma,^{9–11} Tillet and Gardner made a notable contribution by observing that a filtrate from streptococcus could activate the process of fibrinolysis and went on to isolate the enzyme streptokinase (SK).¹² Milstone showed that the globulin fraction of plasma could produce fibrinolysis.¹³ Kaplan and later Christensen and McLeod found that this protein is inactive itself, but it is the precursor of the protease. The precursor of this was then termed plasminogen and the active enzyme plasmin.^{14–16} Biochemical studies led to discovery of plasminogen activators (PAs). They are naturally occurring within our bodies as tissue plasminogen activator (tPA) and urokinase, also known as urokinase-type plasminogen activator (uPA). They are also found in bacteria, such as SK in β -hemolytic streptococcus and staphylokinase in staphylococcus, and in other animals, such as desmoteplase in the vampire bat saliva. They were put to therapeutic use for thrombolysis, with SK being first used to break down fibrinous pleural adhesions in 1949¹⁷ and in acute myocardial infarction.¹⁸

In Vivo Observations

Many studies were performed during this period in experimental animals, showing that fibrinolytic activity can be induced by anaphylactic shock,^{19,20} severe hemorrhage,²¹ and electric convulsion.²² In man, increased fibrinolytic activity was found during surgical operations,^{23,24} severe

hemorrhage,^{21,23} strenuous exercise, and the injection of adrenaline.²⁵ It was believed that adrenaline might have been responsible, as conditions such as alarming suggestions under hypnosis, anxiety in students about to take part in examinations, and patients awaiting gastroscopy, could all induce fibrinolysis. The finding that excessive fibrinolysis could cause major clinical bleeding was observed in many disorders, and was referred to as “fibrinolytic purpura.”²⁶ It was seen in transfusion reactions,²⁷ severe burns,²⁸ metastatic carcinoma of the prostate,²⁹ obstetrical complications such as abruption placentae and amniotic fluid embolism,^{30–33} and in many types of surgical operations,^{33–35} some complicated with fatal intraoperative hemorrhage. In liver diseases, spontaneous plasma fibrinolysis was first noted by Goodpasture.³⁶ He devised a simple test for fibrinolysis by observing the dissolution of a clot formed from recalcified blood over 24 hours. This “Goodpasture test” was used for many years in North America. Soon after, Ratnoff confirmed presence of fibrinolytic activity in cirrhosis but not in acute hepatitis.³⁷

Personal Observations

My introduction to this topic was serendipitous. In 1955, while performing the one-stage thrombin time, using Quick original method, as part of the work up of a cirrhotic patient with massive intraoperative hemorrhage, I encountered difficulty in obtaining the end point. A thin wisp of fibrin was formed but quickly disappeared under my eyes. Such was the dramatic effect of the excessive fibrinolysis activated by surgery in cirrhosis,^{38,39} thus confirming observations by others.^{36,37} As the origin of the fibrinolytic activity was unknown at the time, we used direct approaches studying venous blood in vivo in man and found that fibrinolytic activity could be released from veins after various stimuli.^{40,41} Likewise, similar results were found in experimentally induced venous thrombi in rabbits.⁴² As discussed earlier, Virchow, by noting that blood in small blood vessels was more likely fluid and incoagulable than blood collected from larger vessels,³ had suspected that blood vessels were the origin of fibrinolysis. But our findings were the first direct observation that fibrinolytic activity was derived from veins.

Several interesting aspects of our studies are noteworthy. First, we observed that stimulation of one venous segment could release fibrinolytic activity from another vein located far from the site of stimulation, indicating that the stimulus could be transmitted via perivascular sympathetic nerves.⁴¹ At the time, we were mystified and had no other explanation for this phenomenon. Some 50 years later, Jim O’Rourke came to me at a meeting and excitedly told me that he had demonstrated that the perivascular sympathetic pathway was indeed responsible for this signal transmission.^{43,44}

In our studies on animals, experimentally induced venous thrombi were produced in the marginal veins of rabbit’s ears.⁴² Parallel studies using these stimuli were done using the lysis of the thrombi as the end point. The findings verified that we had the ones observed in the human veins.^{42,45}

Next Five Decades (1960–2010)

While Astrup and his coauthors were able to show the amounts of PA in different organs by extraction,^{46–54} the pursuit of knowledge soon turned to finding components of the P–P system at the cellular level. A fibrin slide method for histologic localization was first designed by Todd,⁵⁵ who used a modification of Astrup fibrin plate method for pinpointing areas of lysis in histologic sections. With this method, fibrinolytic activity was localized to the endothelium in both normal^{56–59} and pathologic tissues.^{60–63} It appeared that the activity was most intense in young regenerating endothelial cells, as shown in newly formed capillaries growing with granulation tissues,⁵⁹ in revascularized myocardium following infarction⁶⁰ and in coronary atherosclerotic lesions.⁶² These findings were subsequently confirmed by more sophisticated methods such as in situ hybridization.

Using cell cultures in vitro, studies of the PAs from various cells were made to determine their function in physiology and in pathology.⁶⁴ These studies revealed that fibrin is not the sole substrate for plasmin. As a protease, plasmin can break down extracellular matrix, thereby enabling cell movements. Plasmin can also activate latent metalloproteinases. Thus, plasmin participates in a wide range of processes that involve cell migration. As the role of plasmin is not limited to the lysis of fibrin, the fibrinolytic system is more appropriately referred to as the P–P system.⁶⁵ This period of development is also noted for the discovery of other members of the P–P system,^{65,66} including cell surface receptor for uPA (uPAR),⁶⁷ for tPA, known as annexin A2^{68–70} as well as a surface protein S-100A10 that is colocalized on the cell surface with receptors for plasminogen.^{71,72} In addition, the inhibitors of fibrinolysis were also identified, including several PA inhibitors (PAI),^{73–76} of which PAI-1 has an especially important biological role. The present day concept of the P–P system is shown in ► Fig. 1.

Through the proteolytic action of plasmin, the P–P system was found to be regulating many physiologic processes,

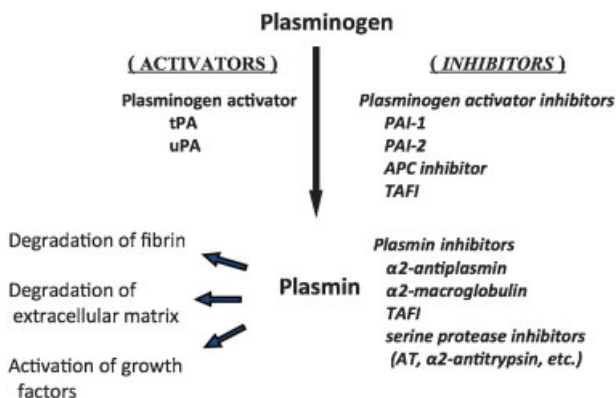


Fig. 1 The present day concept of the plasminogen–plasmin system. The factors listed on the left are activators and those on the right (in italics) are inhibitors, kept in balance under physiologic conditions. APC, activated protein C; AT, antithrombin; PAI, plasminogen activator inhibitor; TAFI, thrombin-activable fibrinolysis inhibitor; tPA, plasminogen activator; uPA, urokinase-type plasminogen activator.

including embryogenesis,⁷⁷ ovulation,^{78,79} neuron growth,^{80,81} brain function,^{82–84} catecholamine secretion,⁸⁵ activation of inflammatory cells,⁸⁶ wound healing,^{87,88} and skeletal muscle regeneration.⁸⁹ Notable advances were made in linking the function of this system with the pathogenesis of a wide range of diseases, among which are cancer^{90–92} and vascular diseases.⁹³ Many new thrombolytic agents were also developed.^{94,95} Much information was made available in several reviews^{65,96} including an issue of the *Seminars of Thrombosis & Hemostasis* in 1991⁹⁷ and more recently in 2013.⁹⁸ In the following sections, progress in several of these developments has been selected to be reviewed in greater depth.

Cancer

The earliest observation of association of fibrinolysis and cancer was made by Carrel and Burrows, who observed liquefaction of growth media by malignant tumors,⁹⁹ whereas clinical record of fibrinolytic bleeding was made by Tagnon et al in patients with metastatic carcinoma of prostate.²⁹ Evidence of a possible causative role of PA in malignancy was shown by the sharp increase in fibrinolytic activity in viral transformed fibroblasts.¹⁰⁰ This PA was identified by Astedt and Holmberg as uPA.¹⁰¹ Subsequently, uPA, uPAR, and PAI-1 were also found by tissue extraction, immunohistochemical staining, and in situ hybridization to be greatly increased in many forms of cancer. These include cancer of the breast, stomach, colon and rectum, esophagus, pancreas, glioma, lung, kidney, prostate, uterine cervix, ovary, liver, and bone.^{92,100,102} The P–P system participates in multiple steps in cancer from carcinogenesis to growth and metastasis.^{92,103,104} The complex interactions involved in these processes are beyond the scope of this article.

The levels of uPA, uPAR, and PAI-1 have been found to be correlated to the aggressiveness and the metastatic potential of many tumors both in tumor cell cultures and in tumor tissues.^{104,105} These are used as biomarkers in the risk stratification of several cancer, especially in carcinoma of breast. Higher levels of uPA and PAI-1 are associated with worse prognosis in carcinoma of breast.^{106–109} The incorporation of the uPA/PAI-1 status into the treatment algorithms has been shown to be helpful in deciding which patients can be spared from the more aggressive chemotherapy.¹¹⁰ Clinical validation of the usefulness of these biomarkers are currently being performed in other types of cancer as well.^{104,105,111–113} In carcinoma of pancreas, the postoperative survival of those with high expression of uPA and uPAR was 9 months compared with 18 months in those without expression of both markers or of only one marker.¹¹⁴ In small cell carcinoma of lung, those with high levels of uPAR predicted poor response to chemotherapy.¹¹⁵ More studies are needed to verify the clinical utility of these biomarkers.

The use of inhibitors in retarding tumor growth has some successes in experimental animals. For example, transfection of PAI-1 to prostate cancer cells impaired growth and metastasis in mice.¹¹⁶ Anti-uPAR antibody blocks prostate cancer invasion, migration, growth, and experimental skeletal metastasis in vitro and in vivo.¹¹⁷ The development of drugs

targeting uPA, uPAR, and PAI-1 has become an exciting area of investigation. A wide spectrum of monoclonal antibodies, targeted toxins, synthetic small molecules and peptides, and antisense molecules are now known to have antitumor effects in human cancer. Several promising drugs include a small molecule targeting the active site in the S1 pocket of uPA. This agent, known as WX 671, has antitumor activity in carcinoma of head and neck,¹¹⁸ pancreatic carcinoma,¹¹⁹ and carcinoma of breast.¹²⁰ Hopefully in the near future, more agents will become available, and prove to be effective as anticancer agents.

Thrombolysis

The earliest thrombolytic agent was SK, an activator of plasminogen.^{12,17,121,122} It was first used to breakdown fibrinous pleural exudates.¹⁷ This same agent was then employed in thrombolysis of experimental clots in rabbits,¹²³ and SK-activated plasmin (also known as “fibrinolysin”) was subsequently used to lyse experimental venous and arterial thrombi in animals.^{124,125} In man, SK-activated plasmin was found to be effective in lysis of thrombi in several clinical studies.^{18,126–128} One notable study was performed on human volunteers, in whom experimental thrombi were produced by a dental broach in an arm vein,¹²⁹ a feat unlikely to be repeated today.

The delivery of the thrombolytic agents was for many years performed by the intravenous route. Before long, this method was found to be problematic, as the plasmin was rapidly inhibited by the circulating antiplasmin. The next phase was the development of several PAs, including tPA and uPA. Again, their therapeutic life span was short lived as they are also promptly inhibited by PAI-1 and antiplasmin. The inhibition, however, can be reduced if the agent is rapidly bound to the fibrin thrombus thereby improving its thrombolytic efficacy.⁹⁴ Many mutants of recombinant tPA were developed to improve these pharmacologic features. Successful thrombolysis depends on age and content of the thrombus, and accessibility of the thrombolytic agent. One notable example for the importance of early thrombolysis is that of ischemic stroke, with convincing evidence that brain function restoration occurs only in early lysis within 3 hours.^{130–132}

To circumvent the inhibitors of fibrinolysis, the thrombolytic agents can be delivered by a catheter directly to the thrombus. Direct delivery of thrombolytic agents to the occluded coronary artery was first performed by Boucek and Murphy in 1960¹³² and in peripheral arterial thrombosis by Dotter in 1974.¹³³ The practice today uses improved techniques such as percutaneous endovascular insertion of a catheter advanced to the site of the thrombus, under direct radiologic imaging. The catheter can be left in place for a slow delivery of the agent.^{134,135} Catheter-directed thrombolysis can also be performed in conjunction with thrombectomy or with high-frequency low-intensity ultrasound waves to accelerate clot dissolution by dissociating the fibrin strands.¹³⁵ Today, the indications for thrombolysis cover practically all forms of thrombi. Further discussion of this topic is beyond the scope of this article.

Inhibitors of PAI-1 as New Drug Target

Among the various components of the P–P system, PAI-1 has been found to be involved in the pathogenesis of a variety of disorders.^{136–139} These include thrombotic disorders, cancer, metabolic syndrome and obesity, polycystic ovarian disease, alopecia, pulmonary fibrosis, nephrosclerosis, and myelofibrosis. It is also implicated in aging and Alzheimer disease. Thus, intensive efforts are being performed in the search for an inhibitor of PAI-1. Both neutralizing monoclonal antibodies against PAI-1¹⁴⁰ and small peptide molecules^{141,142} have been developed. Their clinical activities are yet to be determined.

Conclusion

Since the observations of postmortem fibrinolysis over two centuries ago, our understanding of the fibrinolytic system has evolved immensely. From having a limited function of dissolution of a fibrin clot, the components of the P–P system are now known to be involved in many physiologic and pathologic processes. Such knowledge has enabled the development of effective treatment of many thrombotic disorders. It will be of great interest to watch further development of new drugs based on our increasing knowledge of the action of the components of this system, in particular of PAI-1.

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