Scientific basis

Biological role of fibrinolysis

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Fibrinolysis is a basic defence mechanism of the organism designed to control the deposition of fibrin in the vascular system and elsewhere. When thrombin is generated by the coagulation system it acts on fibrinogen and 'precipitates' it out of solution as a polymer—fibrin. This polymer deposition is regulated by the fibrinolytic system which acts on the insoluble protein fibrin and, by splitting a limited number of peptide bonds, renders it soluble. This solubilisation mechanism which liquifies the fibrin clot is the fibrinolytic system.

Fibrinolysis was first described in a rudimentary form over a century ago. Nowadays the term fibrinolysis is generally restricted to the 'fibrinolytic' action of the plasminogen-plasmin system, but fibrin is of course removed by other means such as phagocytosis and proteolysis by other enzymes.

Plasminogen-plasmin

The key component of the fibrinolytic (plasminogen-plasmin) system is the single-chain glycoprotein plasminogen, which is present in plasma and in most tissues. From this polypeptide proenzyme the highly proteolytic serine protease plasmin is formed by limited proteolysis. Plasmin has broad substrate specificity but *in vivo* it is rendered relatively selective for fibrin by the nature of the molecular biology of the fibrin, plasminogen, plasmin, and anti-plasmin interactions.

Activators

The conversion of plasminogen to plasmin is brought about by plasminogen activators which are in two categories, firstly, extrinsic plasminogen activators which are extrinsic to the plasma and, secondly, intrinsic plasminogen activators which are intrinsic to the plasma.

The extrinsic activators are the tissue activators, which are firmly fixed in tissues, and the endothelial cell activator—vascular activator—which is found in the vascular endothelium and released into the

blood. The intrinsic activators are, in the main, components of the factor XII activation system produced by activation of the intrinsic coagulation mechanism. The activity of these activators is regulated by inhibitors, but our knowledge of the anti-activators is less clear than our knowledge of the anti-plasmins. The activity of vascular activator is also regulated by its high affinity for fibrin.

Role of fibrin

Fibrin is central to the whole mechanism of fibrinolysis because the fibrin surface provides a special milieu with optimum conditions for the reactions of the plasminogen-plasmin system. Plasminogen and plasminogen activator bind selectively to fibrin and on the fibrin surface plasmin is formed which immediately interacts with the fibrin. This interaction occupies the active sites of the plasmin and renders them unavailable for interaction and neutralisation by the anti-plasmin in the surrounding plasma. Plasmin formed away from the fibrin surface immediately interacts with the fast anti-plasmin α_2 -plasmin inhibitor (α_2 -PI) and is inhibited. This inhibition reaction is somewhat unique in its speed of action, being the fastest protein-protein interaction known. This remarkable speed of reaction gives an indication of the biological importance of controlling plasmin activity. In this way plasmin-mediated proteolysis is made highly specific for fibrin and is contained within the immediate environment of the clot. Like several parts of the coagulation mechanism it is a surface-linked phenomenon.

Only when excessive amounts of plasmin are formed or the α_2 -PI concentration in the plasma is deficient can the fibrinolytic process extend out into the general circulation and generalised fibrinolysis ensue.

Fibrinolysis interactions

Fibrinolysis is indeed a complex co-operative

reaction at the fibrin surface of activator, plasminogen, plasmin, and α_2 -PI which, should it spill over into the surrounding plasma, will be met by a massive potential of anti-plasmin activity primarily in the form of α_2 -PI and secondarily as α_2 -macroglobulin (α_2 -M).¹ These molecular interactions have recently been elucidated for fibrinolysis in blood and it is assumed that fibrinolysis in tissues and non-vascular spaces follows a similar pattern.

Biological principles of fibrinolysis

The biological principles which operate the fibrinolytic system are basically the same as those for the coagulation system. An initiating or trigger mechanism is required to 'turn on' the system and thereafter serine proteases are generated to achieve the system's biological function. While proteases can generate biological functions they can also destroy them, because proteolysis is an irreversible process and proteases are not endowed with repair functions. A 'switch off' mechanism or an inhibition mechanism is, therefore, essential to limit proteolysis.

This on-and-off mechanism is controlled by different switches. The on switch is the release, or perhaps activation, of plasminogen activators. The off switch is largely the containment of the reaction by the inhibitor α_2 -PI supplemented, if necessary, by α_2 -M. It is aided by the constraints of a surface mediated reaction, and the run down of available surface for the fibrinolytic reaction as fibrin is digested. The on switch of activation is also regulated by anti-activators. Unlike the α_2 -PI mechanism, the mechanism of these anti-activator reactions is poorly understood and is still the subject of some controversy.

In addition to the switch on, switch off mechanisms, the fibrinolytic system has an inbuilt self-acceleration process. When plasminogen activator meets plasminogen, if the conditions are right, plasmin is formed. This plasmin, in addition to attacking fibrin, feeds back into the system and greatly accelerates the conversion of plasminogen to plasmin.

Fibrinolysis activation

Our knowledge of the trigger mechanism is very limited, although we know there are several types of plasminogen activators.² The tissue type of plasminogen activator is firmly fixed in tissues and can be extracted and studied. It is a stable protein which is probably very closely related to vascular, or endothelial, activator and to the urine activator urokinase. It is found in most body tissues and in greatest concentration in tissues such as the uterus,

suprarenals, thyroid, and ovaries and in lowest concentration in liver, testes, and spleen.

Activator is also found in human milk, tears, saliva, cerebrospinal fluid, and bile. A trigger that can initiate fibrinolysis is therefore available throughout most body tissues and body fluids. The activation of this trigger appears to be the result of fibrin deposition, ischaemia, hypoxia, or a combination of these, but our knowledge of this is very scanty.

The extrinsic activation mechanism of fibrinolysis seems to play an important role in keeping body cavities and channels free from unwanted fibrin. Thus the urinary tract activator urokinase helps to keep the urinary tract free of fibrin. Various duct systems like the lacrimal ducts and bile ducts have plasminogen activator activity which helps to keep them patent. Fibrinolysis also plays an important role in reproduction. It contributes to maintaining the patency of reproductive channels and plays a role in fertilisation. It is also an important component in the mechanism of menstrual bleeding.

The trigger mechanism for activating fibrinolysis in blood is more complicated. Blood contains some extrinsic activator in the form of vascular endothelial activator which is synthesised in the endothelial cells of the small veins, stored there, and released on demand. The concentration of this activator in blood has a pronounced diurnal variation, being lowest in the morning. Its release can be greatly enhanced by vascular stasis, ischaemia, and exercise.

Blood also contains two types of intrinsic activators—one which is associated with activation of factor XII (the factor XII dependent pathway) and the other which is independent of factor XII (the factor XII independent pathway). It now seems quite clear that when intrinsic coagulation is activated fibrinolysis is activated at the same time. There is much still to be learned about the various plasminogen activators and how they become activated.

The dynamic hypothesis

Many years ago it was proposed that there was a dynamic balance between coagulation and fibrinolysis in the vascular system. Thus it was suggested that fibrin was being continuously laid down from low grade activation of the coagulation system and continuously cleared by low grade activation of the fibrinolytic system. Present evidence to support this concept is scanty and it must now be accepted that systemic intravascular fibrin deposition is normally extremely limited. This, however, does not exclude the possibility of localised fibrin deposition as an everyday occurrence and as an expression of normal 'wear and tear'.

Biological role of fibrinolysis

Clinical problems arise not only from excessive or unlimited activation of the fibrinolytic system but probably also from reduced fibrinolytic activity. It seems quite clear that a proportion of patients with recurrent venous thrombosis have reduced levels of vascular activator.³

Therapeutic possibilities

What are the possibilities for therapeutic manipulation of the fibrinolytic system? The system can be activated therapeutically by infusing either streptokinase or urokinase and inducing hyperfibrinolysis. This form of treatment, however, has been available for well over 10 years but has found only limited application in anti-thrombosis therapy. During such fibrinolytic therapy the plasminogen may fall to zero, and it may be beneficial to give simultaneously an infusion of plasminogen.

Fibrinolysis can also be augmented by oral anabolic steroid therapy, which enhances the production of vascular endothelial activator. This form of treatment, however, has never really progressed beyond the experimental stage.

Inhibition of fibrinolysis, the topic of this symposium, can be readily achieved by therapeutic intervention. Plasminogen carries lysine-binding sites which react with fibrin and play a key role in the fibrinolysis reaction by attaching plasminogen to fibrin. Lysine or related amino-acids, epsilon-aminocaproic acid, or tranexamic acid can occupy these lysine sites and thus prevent the binding of plasminogen to fibrin. This phenomenon explains the considerable anti-fibrinolytic properties of these amino-acids *in vivo*.

Fibrinolysis: experiments of nature

In the past year there have been reports of congenital abnormalities of the fibrinolytic system which serve to emphasise the importance of its biological role. As with congenital deficiencies of the coagulation system these are proving interesting models of defective fibrinolytic systems.

Koie *et al.*,⁴ Aoki *et al.*,⁵ and Kluft *et al.*⁶ have described cases of congenital deficiency of α_2 -PI. Patients with this deficiency have a severe haemorrhagic diathesis which is most probably due to premature lysis of haemostatic plugs resulting from relatively uninhibited local *in-vivo* fibrinolysis occurring in the absence of α_2 -PI. Particularly interesting also is that the administration of a lysine amino-acid, tranexamic acid, reduced the incidence and severity of the haemorrhagic diatheses in these patients.

This experiment of nature indicates the very

important biological role of α_2 -PI in haemostasis.

Aoki *et al.*⁷ and Wohl *et al.*⁸ have reported hereditary molecular abnormalities of plasminogen in patients with a history of thrombosis. In Aoki's⁷ cases the abnormality was a depressed level of plasminogen activity in plasma although the plasma plasminogen antigen concentration was normal. In Wohl's detailed report⁸ plasminogen variants named Chicago I and Chicago II are described which have impaired activator-binding properties, subnormal functional plasminogen values, and subnormal plasmin generation rates. The authors suggest that 'the occurrence of variant plasminogens could be common'.

This further experiment of nature indicates the very important biological role of plasminogen in haemostasis.

Conclusion

The biological role of fibrinolysis is therefore that of a closely controlled fibrin clearing 'machine' which has a potentially massive activating mechanism and at the same time a massive reserve of inhibitors.

If the machine is triggered into action then its chemical design largely serves to contain it in the immediate environment of its target fibrin.

The lysine amino-acids, epsilon-aminocaproic acid or tranexamic acid, are powerful synthetic fibrinolytic inhibitors which are available should fibrinolysis escape from its normal control mechanism or should supplementary inhibition be required.

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