Anticlotting mechanisms I: physiology and pathology

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Key points
Anticlotting mechanisms are important in restricting clot formation to the site of injury. The two major systems are the anticoagulant and fibrinolytic systems. The anticoagulant system comprises four enzyme pathways whose function is to reduce thrombin production, limit its activity, or both. Plasmin is the major enzyme of the fibrinolytic system and will ‘dissolve’ already formed clots by degrading fibrin. Diseases of the anticoagulant system may be inherited or acquired and predispose to thromboembolic events. Inherited disorders of the fibrinolytic system are very rare. The clinician is more likely to encounter acquired hyperfibrinolysis that can cause severe haemorrhage.

When animals evolved beyond a certain size, it became necessary for them to have a circulation. One disadvantage of possessing a circulation was possible traumatic exsanguination, so it is not surprising that mechanisms to reduce this risk, what we refer to as ‘haemostasis’, evolved simultaneously. Haemostasis creates its own problems, such as the need to restrict haemostatic mechanisms to a site of injury. This review focuses on the mechanisms for limiting clot formation to the site of injury in humans and the ways in which these mechanisms can fail.

There are two main systems:
- The anticoagulant system that limits fibrin formation.
- The fibrinolytic system that ‘dissolves’ clots that have already formed.

Procoagulant factors are also ‘washed away’ from the site of vascular injury. The resultant dilution means that sufficient concentration to achieve clot formation is limited to the site of production. A second non-specific regulatory mechanism is the adsorption and containment of serine proteases by polymerized fibrin. The high-affinity binding of thrombin by fibrin was originally termed ‘antithrombin I’. This is compromised in fibrinogen deficiency states allowing thrombin and FXa to spill into the circulation.

Anticlotting physiology
The goal of coagulation is fibrin formation. Activation of the coagulation cascade leads to the production of thrombin (FIIa). Thrombin then converts fibrinogen (FI) to fibrin. Physiological anticogulation mechanisms act to reduce thrombin production or reduce the effects of thrombin (Fig. 1).

Antithrombin–glycosaminoglycan pathways
Antithrombin (AT; previously known as antithrombin III) is the main physiological inhibitor of thrombin. Other thrombin inhibitors are heparin cofactor II (HCII), α₂-macroglobulin and α₁-antitrypsin. AT is a circulating glycoprotein of hepatic origin. It is a serine protease inhibitor (SERPIN) that is not vitamin K dependent.

Actions
AT has the following anticoagulant actions:
- it mainly inhibits thrombin and FXa.
- less importantly, it inhibits FIXa and FVIIa. The enzymatic activity against all these substrates of AT is increased at least 1000-fold in the presence of heparin. However, in vivo endogenous heparin does not play a significant role as its plasma level is too low. Antithrombin activity is augmented instead by a glycosaminoglycan called heparan sulphate found on the endothelial cell surface. The binding of AT to heparan sulphate produces the following effects:
  - The anticoagulant activity of AT is enhanced by at least 1000-fold.
  - It triggers the production of prostacyclin that causes vasodilatation and inhibits platelet aggregation.

In addition to anticoagulant activity, AT also has anti-inflammatory effects. By inhibiting thrombin and FXa, it prevents the thrombin-FXa-mediated release of the pro-inflammatory cytokines IL-6 and IL-8. HCII is another circulating SERPIN of hepatic origin. It is also not vitamin K dependent. It specifically inhibits thrombin and appears to have little or no anti-FXa activity. The rate of thrombin inhibition by HCII proceeds slowly but is increased at least 1000-fold when HCII binds to dermattan sulphate, a glycosaminoglycan synthesized by sub-endothelial fibroblasts.

Protein C pathway
Factors Va and VIIIa are potent procoagulants involved in the propagation phase of thrombin production. FVIIIa with FIXa form tenascet that activates FX. FVIIa binds with FXa to form...
prothrombinase that converts prothrombin to thrombin. This propagation phase takes place at the surface of an activated platelet. Their activity is limited by the protein C pathway that comprises four key elements (Fig. 2):

1. Protein C is a circulating vitamin K-dependent serine protease that is synthesized in the liver. It is converted to activated protein C (APC) by thrombin. APC is a potent anticoagulant and degrades FVa and FVIIIa (with protein S and phospholipid as cofactors), thereby limiting coagulation. APC also exhibits anti-inflammatory and anti-apoptotic properties. The action of APC is limited by protein C inhibitor, α2-macroglobulin, and α1-antitrypsin.

2. Thrombomodulin (TM) is a trans-membrane receptor found on endothelial cells. TM binds with thrombin. Thrombin activation of protein C proceeds slowly but after formation of the TM–thrombin complex there is at least a 1000-fold increase in the production of APC such that thrombin effectively acts as an anticoagulant. This prevents clot formation in areas of undamaged endothelium.

3. Endothelial protein C receptor (EPCR) is another trans-membrane receptor found on endothelial cells. Its function also enhances the process of protein C activation. EPCR binds to protein C and presents the molecule optimally to the TM–thrombin complex for cleavage leading to APC formation.

4. Protein S is a vitamin K-dependent glycoprotein, synthesized by both endothelial cells and hepatocytes. Protein S functions as cofactor to APC in the inactivation of FVa and FVIIIa. It exists in plasma in a free form (40%) and a complex form (60%) bound to C4b-binding protein (C4b–BP). Only the free form has anticoagulant activity. C4b–BP is an inhibitor of the complement system and is up-regulated in inflammation. The increased levels of C4b–BP lead to reduced protein S activity and a resulting procoagulant state. The complement inhibitory activity of C4b–BP is however not reduced by its binding to protein S. Protein S also has anticoagulant properties that are independent of APC. This includes direct reversible inhibition of the prothrombinase (FVa–FXa) complex.
Tissue factor pathway inhibitor

Tissue factor (TF) pathway inhibitor (TFPI) is a polypeptide produced by endothelial cells. It is found on the endothelial cell surface and also circulating in plasma. TFPI is the main inhibitor of the TF pathway of coagulation (formerly known as the extrinsic pathway). The trigger for the coagulation cascade in vivo is the binding of circulating FVIIa to exposed TF. TFPI alone has little effect on FVIIa. It first binds to FXa forming a TFPI–FXa complex in which FXa is reversibly inhibited. The TFPI–FXa complex then subsequently binds to TF–FVIIa forming a quaternary complex in which both FVIIa and FXa are inhibited. 3

Step 1: TFPI + FXa ⇌ TFPI-FXa.
Step 2: TFPI-FXa + TF-FVIIa ⇌ TFPI-FXa-TF-FVIIa.

Protein S enhances the interaction of TFPI with FXa in the presence of calcium ions and phospholipid. This action is also independent of APC.

Protein Z-dependent protease inhibitor/protein Z

This is the most recently described component of the anticoagulant system. Protein Z (PZ)-dependent protease inhibitor (ZPI) is a plasma enzyme that is produced in the liver. It inhibits FXa in a reaction that requires the presence of PZ and calcium. The ability of ZPI to inhibit FXa is increased 100-fold in the presence of PZ. 4

PZ is a vitamin K-dependent glycoprotein and functions as a cofactor for ZPI. ZPI also inhibits factors Ixa and Xia, but this mechanism does not require PZ.

Diseases of the anticoagulant system

Diseases of the anticoagulant system belong to a group of thrombophilic conditions associated with an increased predisposition to venous thrombosis. In 5–10% of patients presenting with clinical features of venous thromboembolism, there will be an underlying deficiency of one or more components of the anticoagulant system. These are all inherited as autosomal-dominant conditions.

AT deficiency

AT deficiency is a strong risk factor for thrombotic disease and may be inherited or, more commonly, acquired. Several studies suggest that a decrease to 60–70% of normal levels critically predisposes to venous thrombosis.

There are two forms of inherited AT deficiency.

Type 1 AT (quantitative) deficiency is characterized by a decrease in both the concentration and the activity of AT in the blood. Homozygous Type 1 AT deficiency is presumed to be incompatible with life as there are no known cases of this condition in humans. Heterozygous Type 1 AT deficiency has prevalence in the general population of ~1 in 2000 and is associated with a 10-fold increased risk of thrombosis.

Type II AT (qualitative) deficiency is characterized by a normal AT concentration in the blood but with reduced activity. 5

Acquired AT deficiency may be because of

- decreased production
  - liver cirrhosis
- increased loss
  - nephrotic syndrome
  - protein losing enteropathy
- enhanced consumption
  - sepsis
  - burns
  - disseminated intravascular coagulation (DIC)
  - cardiopulmonary by-pass surgery
  - prolonged unfractionated heparin therapy.

HCII deficiency

Inherited HCII deficiency is a rare autosomal-dominant disorder. Interestingly, it is not strongly associated with venous thrombosis. The view of most authors presently is that HCII deficiency contributes only to a thrombotic risk when combined with other deficiencies. 6

Protein C deficiency

Protein C deficiency is a pro-thrombotic condition and hereditary protein C deficiency has a wide spectrum of clinical presentation. The range is from the completely asymptomatic to a severe form that occurs in homozygous states and presents with life-threatening DIC in neonates termed neonatal purpura fulminans. In between is the patient with an increased risk of venous thrombosis and, in females, recurrent abortions. Its prevalence in the general population is 0.2–0.4%.

Acquired protein C deficiency may occur in

- vitamin K deficiency
- warfarin therapy
- DIC
- sepsis
- liver cirrhosis.

APC resistance

In this thrombotic disorder, there is failure of APC-mediated degradation of FVa, FVIIIa, or both. It may be hereditary or acquired. Factor V Leiden is the commonest hereditary cause of APC resistance and also the commonest of the inherited thrombophilia disorders. The genetics are interesting, having occurred around 30,000 years ago and spread by a founder effect after the out-of-Africa divergence that occurred around 100,000 years ago. Consequently, it is rare in Asian, African, and Australian populations. Its frequency in white populations is 2–15%, but it is found in 15–25% patients with DVT. It involves a mutation in Factor V that affects the response to APC in two ways:
Factor V Leiden is resistant to cleavage by APC. This leads to persistently high FVa levels and a hypercoagulable state.

After FVa is inactivated by APC the product of APC-mediated FVa cleavage serves as cofactor in the subsequent degradation of FVIIIa by APC. So, factor V Leiden also causes persistently high FVIIIa levels.

Some individuals display a genetic predisposition to elevated FVIII levels. In 50% of these individuals, there is an association with elevated levels of von Willebrand factor (vWF). However, elevated vWF is not the only explanation and the condition is still not fully understood.

The elevated FVIII levels cause a state of relative APC resistance.

Acquired conditions that cause elevated FVIII levels can also predispose to APC resistance. These include:

- pregnancy
- surgery
- obesity
- diabetes mellitus
- malignancy.

**Protein S deficiency**

Protein S deficiency is associated with increased risk of thrombosis and may be acquired or hereditary. The latter is four to five times commoner than protein C deficiency.

Causes of acquired protein S deficiency are similar to those of acquired protein C deficiency and include vitamin K deficiency, warfarin therapy, liver cirrhosis, pregnancy, and chronic disease, for example human immunodeficiency virus.

**The fibrinolytic system**

**Plasminogen/plasmin**

Plasmin is the main enzyme of the fibrinolytic system. It is synthesized in the liver as the proenzyme plasminogen and then released into the circulation. Plasminogen is a glycoprotein and the native form (Glu-plasminogen) has glutamic acid at its N-terminal. Plasminogen cannot cleave fibrin, but has an affinity for fibrin and is thus incorporated into the clot. It is then converted to plasmin by two distinct activators:

1. Tissue-type plasminogen activator (t-PA).
2. Urokinase-type plasminogen activator (u-PA).

Plasmin then functions as a serine protease.

**Actions**

- It cleaves fibrin to soluble fibrin degradation products.
- Plasmin stimulates further plasmin production by producing more active forms of t-PA and u-PA.
- Plasmin converts Glu-plasminogen to modified forms with lysine at the N-terminal (Lys-plasminogen). Lys-plasminogen is a more favourable substrate for plasminogen activators. Plasmin thereby has a positive feedback on its own production.

**Plasminogen activators**

Tissue-type plasminogen activator (t-PA) is a serine protease that is released slowly into the blood by damaged endothelial cells. It has the capacity to bind to fibrin where it converts clot-bound plasminogen to plasmin. Owing to its fibrin-binding capacity, the main role of t-PA-mediated plasminogen activation is dissolution of fibrin in the circulation and maintenance of vascular patency.

Urokinase-type plasminogen activator (u-PA) was originally isolated from human urine but is found in several locations including the blood and extracellular matrix. It does not bind directly to fibrin (unlike t-PA) and plays a minor role in comparison. Instead u-PA binds to a specific cell surface receptor called the urokinase-type plasminogen activator receptor (u-PAR). This binding is essential for u-PA to then activate cell-bound plasminogen.

**Fibrin as cofactor**

In the absence of fibrin, t-PA displays low activity towards plasminogen. Both t-PA and plasminogen have the ability to bind to fibrin, and this tertiary complex formed with fibrin is necessary for plasmin generation. Fibrin thus functions both as a cofactor for plasminogen activation and a final substrate for plasmin.

Fibrin which has been partially degraded by plasmin presents a more efficient binding surface for plasminogen leading to accumulation of plasminogen on the clot surface, enhanced plasmin formation, and enhanced clot lysis (Fig. 3).

**Inhibitors of fibrinolysis**

The plasmin generating potential of plasma is sufficient to completely degrade all of the fibrinogen/fibrin in the body in a very short time, so fibrinolytic activity has to be carefully controlled. Fibrinolysis is limited by:

- plasminogen activator inhibitors (PAIs) that prevent further conversion of plasminogen to plasmin.
- circulating substances that directly inhibit plasmin.
- thrombin activatable fibrinolysis inhibitor (TAFI) that makes fibrin unfavourable for degradation by plasmin.

**Plasminogen activator inhibitors**

Up to three types of PAIs have been described, but PAI type 1 (PAI-1) is considered the main physiological inhibitor. It is a glycoprotein member of the SERPIN family and is synthesized by a variety of cells including megakaryocytes, endothelial cells, hepatocytes, and adipocytes. PAI-1 down-regulates fibrinolysis by rapidly and irreversibly inhibiting both t-PA and u-PA. The inhibitor is consumed in the process leading to its description as a ‘suicide inhibitor’. 
Plasmin inhibitors

α₂-Antiplasmin (α₂-AP) is the primary inhibitor of plasmin. It is a circulating glycoprotein SERPIN that is primarily synthesized by the liver. It rapidly inhibits plasmin in one of the fastest protein–protein interactions.

α₂-Macroglobulin is considered the secondary inhibitor of plasmin in plasma. It is a large plasma protein synthesized in the liver. It also inactivates plasminogen activators, APC, and thrombin.

Thrombin activatable fibrinolysis inhibitor

TAFI is a plasma proenzyme that is synthesized by the liver. TAFI has been variously described as procarboxypeptidase U (unstable procarboxypeptidase), plasma procarboxypeptidase B, or procarboxypeptidase R. It is activated to a carboxypeptidase by thrombin. Thrombin alone is a weak activator of TAFI but when thrombin binds to TM this activation is accelerated by a factor of more than 1000-fold.

TAFIa has a half-life of about 10 min and is an important link between the coagulation and fibrinolytic systems.

Diseases of the fibrinolytic system

Congenital disorders of the fibrinolytic system are rare. If the defect produces a hyperfibrinolytic state, then it manifests as a bleeding disorder. On the other hand, mutations that down-regulate the fibrinolytic system will predispose to thromboembolic disease.

Plasminogen deficiency

The prevalence in the general population is ~0.5% and there are two forms:

- Type I plasminogen deficiency, also known as hypoplasminogenemia, is a quantitative disorder characterized by a reduction in quantity and activity of plasminogen. In severe cases, it is associated with hydrocephalus and a rare congenital conjunctivitis.
- Type II plasminogen deficiency, or dysplasminogenemia, there is markedly reduced activity as a result of abnormalities of the plasminogen molecule. Surprisingly, isolated plasminogen deficiency is not considered as a risk factor for thromboembolic disease.

PAI-1 deficiency

Congenital PAI-1 deficiency is a rare disorder associated with mild–to-moderate bleeding that is usually provoked by trauma or surgery. It has also been implicated in some cases of menorrhagia.

Elevated PAI-1 levels have been detected in various disease states such as diabetes, obesity, and coronary artery disease, and may be linked to the increased risk of arterial thrombotic events in these conditions.

α₂-Antiplasmin deficiency

Hereditary α₂-AP deficiency, also known as Miyasato disease, is a rare autosomal disorder characterized by bleeding as a result of excessive fibrinolysis.
Dysfibrinogenaemia

Dysfibrinogenaemia is a disorder characterized by the presence of abnormal fibrinogen. The clinical manifestation is varied and some patients are asymptomatic. Those that develop symptoms present with a tendency to bleed or a tendency to thrombosis with a small group having a predisposition to both bleeding and thrombosis. In dysfibrinogenaemia with a tendency to thrombophilia, the mechanisms include defective clot lysis because of plasmin resistance and defective assembly of the fibrinolytic system.

Congenital dysfibrinogenaemia is rare. Acquired dysfibrinogenaemia may be present in conditions such as liver cirrhosis and multiple myeloma.

Acquired hyperfibrinolysis can occur in trauma patients, liver cirrhosis amniotic fluid embolism, and disease states in which there is massive activation of t-PA. This can lead to DIC and severe haemorrhage. It is a complex area that cannot be covered in this review.

Conclusions

Anticlotting mechanisms are essential to maintain the fluid state of the blood and prevent excessive clotting. The process of elucidating and further clarifying the delicate balance between haemostasis and clotting and also roles of the various systems involved is continuous.

Declaration of Interest

None declared.

References


Please see multiple choice questions 13–16.