

binds to the receptor, but should be used only once for patients undergoing percutaneous coronary intervention. Eptifibatid and tirofiban are licensed for high-risk patients with unstable angina or non-Q-wave myocardial infarction.

Platelet activation is also induced by ADP released from degranulating platelets. Clopidogrel and ticlopidine inhibit the binding of ADP to its platelet receptor. These two drugs are licensed for the prevention of ischaemia, stroke or myocardial infarction in at-risk patients.

### Fibrinolytic and antifibrinolytic drugs

Fibrin and fibrinogen are broken down by the protease plasmin, which is produced by the conversion of inactive plasminogen. Drugs that activate plasmin, break up thrombi and are used in the treatment of life-threatening venous thrombosis and pulmonary embolism. Streptokinase, extracted from  $\beta$ -haemolytic streptococci, binds to the plasminogen exposing its active site and thus inducing plasmin-like activity. Antibodies to streptokinase appear after about 4 days and the drug should not be used again for at least 1 year. Alteplase, reteplase and tenecteplase are recombinant tissue plasminogen activators that are not antigenic. Alteplase is unmodified human tissue plasminogen activator while the others have some amino acid sequences to increase their elimination half-life to allow bolus administration. They are more selective for fibrin-bound plasminogen than for free plasminogen and are thus claimed to be clot selective.

Inhibition of plasminogen activation can be achieved with drugs such as tranexamic acid, which are used in conditions where there is a risk of haemorrhage such as prostatectomy and dental extraction for patients with haemophilia. Aprotinin inhibits proteolytic enzymes and is used for patients at risk of significant blood loss during surgery. ◆

### FURTHER READING.

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# Haemostasis, blood platelets and coagulation

David S Minors

### Haemostasis

Haemostasis is the stemming of blood loss following damage to a blood vessel; it normally involves three processes (Figure 1):

- constriction of the damaged vessel
- formation of a temporary platelet plug
- clotting (coagulation) of blood at the site of damage.

The first two of these are normally responsible for the initial cessation of blood loss, and the blood clot provides more permanency. Thus, a deficiency of blood platelets (thrombocytopenia) is characterized by ease of bleeding, even with minor damage, especially in skin and epithelial surfaces where it forms bruises (purpura). By contrast, a deficiency of the clotting mechanism often results in bleeding into deep tissues and bleeding may initially cease due to the formation of the platelet plug.

### Platelet function and formation of the platelet plug

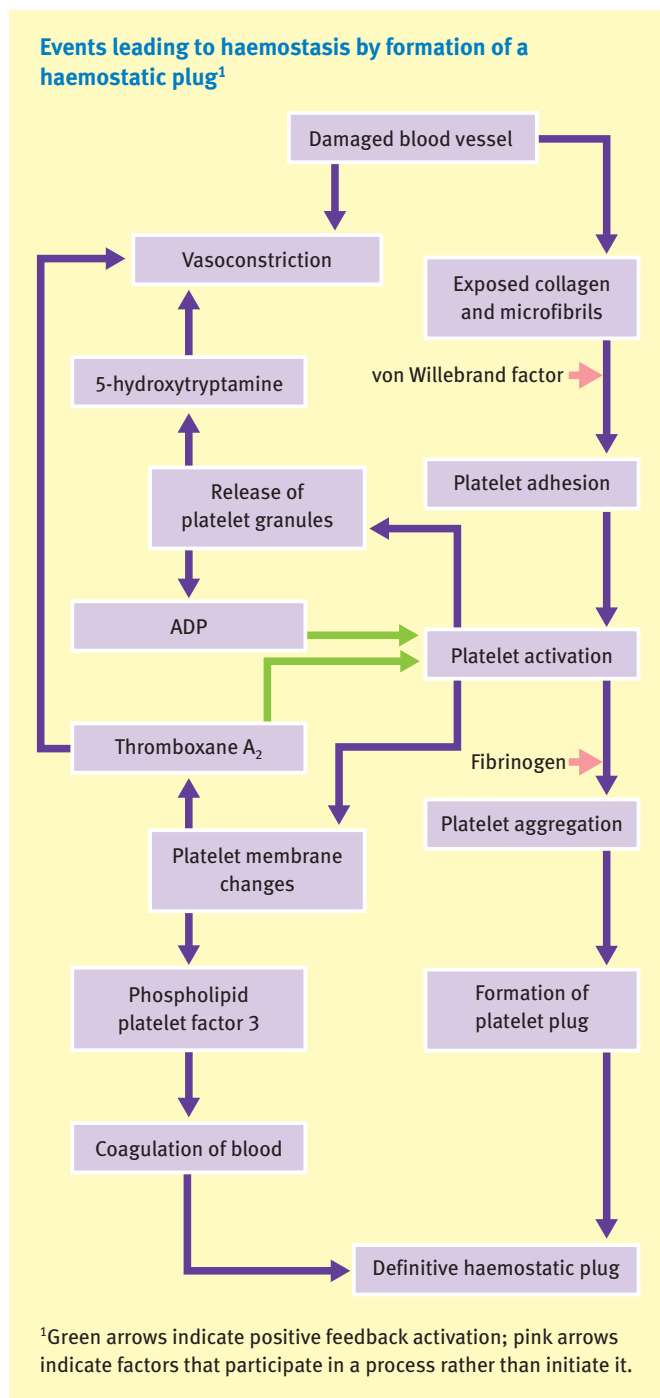
The normal platelet count is about  $3 \times 10^{11}$ /litre. Platelets are incomplete cells formed from megakaryocytes in the bone marrow. Formation of platelets involves 'pinching off' bits of cytoplasm from the megakaryocyte, with each megakaryocyte giving rise to about 1000 platelets.

The structure of platelets is complex. Normally they are discoid in shape and bounded by a complex plasma membrane. This membrane is extensively invaginated, forming a canalicular system, and contains numerous glycoproteins (GP), including GPIa, GPIb and GPIIb/IIIa, that act as adhesion molecules and play an important part in platelet function. Intracellularly, the platelet contains numerous microfilaments, a dense tubular system and two types of granules:

- $\alpha$ -granules, which contain fibrinogen, von Willebrand factor (vWF), a heparin antagonist (PF<sub>4</sub>) and a platelet growth factor
- dense-granules, which contain certain adenosine nucleotides (including ADP) and 5-hydroxytryptamine (5-HT).

Normally, platelets do not adhere to the smooth endothelial lining of blood vessels. When the vasculature is damaged, however, this exposes the blood to subendothelial collagen and microfibrils. Platelets bind to this collagen via GPIa and further binding is facilitated by vWF. vWF forms a bridge between subendothelial microfibrils and platelets via the membrane GPIb. Binding of GPIa and GPIb exposes GPIIb/IIIa, which binds fibrinogen and vWF. Following this adhesion, the platelet becomes activated, changes shape to become more spherical, and sends out pseudopodia that

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enhance platelet–platelet interaction (platelet aggregation). Platelet aggregation is aided by fibrinogen that binds to GPIIb/IIIa and forms a bridge between adjacent platelets. On activation, platelets also release their granules (platelet release reaction). The 5-HT that is released acts as a potent vasoconstrictor. In addition, there is exposure of a membrane phospholipid, platelet factor 3 (PF<sub>3</sub>) and release of arachidonic acid from the phospholipids. The arachidonic acid is converted within the platelet to thromboxane A<sub>2</sub> (TxA<sub>2</sub>), which is a potent vasoconstrictor and aids the localized vasoconstriction. In addition, TxA<sub>2</sub>, together with ADP released from the dense granules, further enhances platelet activation, aggregation and release (Figure 1). In this way, an aggregate of

platelets that plugs the damaged blood vessel is rapidly built up. Spreading of this plug away from the site of injury is prevented by the production of prostacyclin (PGI<sub>2</sub>), which is a potent inhibitor of platelet aggregation and release, in the normal endothelium in adjacent areas. (The release and aggregation reactions are also inhibited by aspirin, which inhibits TxA<sub>2</sub> synthesis, and thus explains, in part, the anticoagulant activity of aspirin.)

### Coagulation

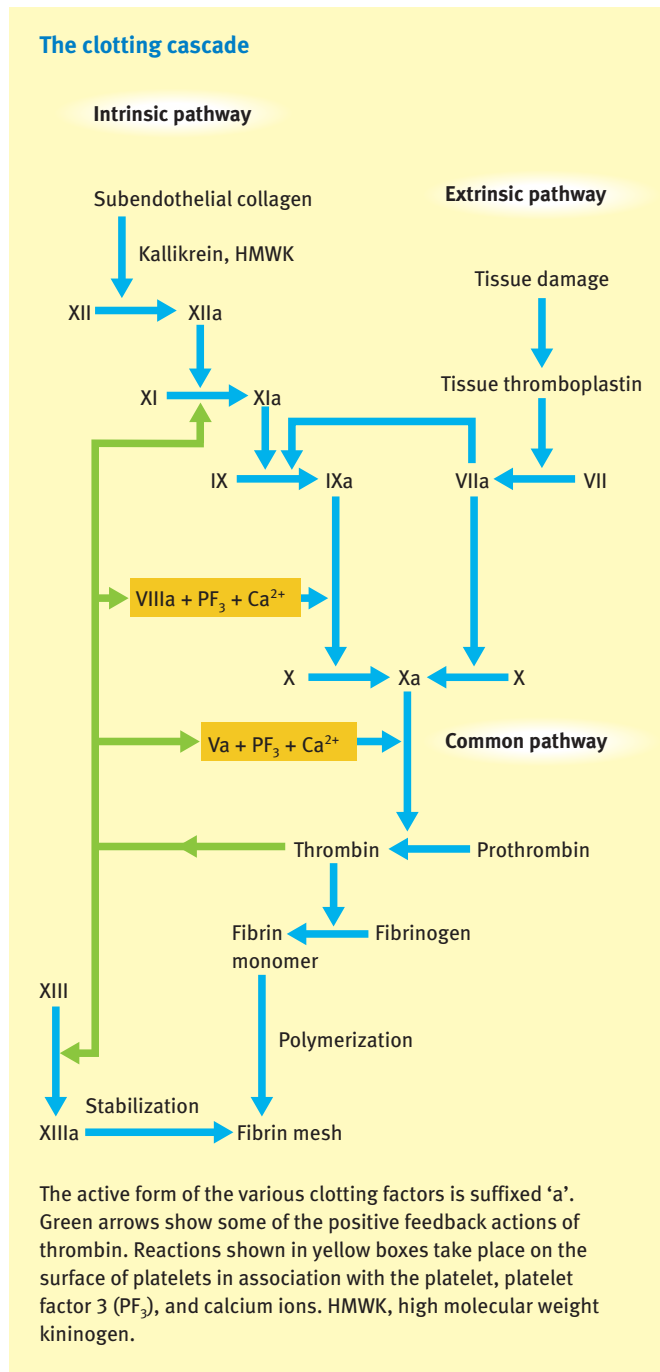
The platelet plug provides a nidus around which the blood clots, so forming a more definitive haemostatic plug. The fundamental reaction in the formation of the blood clot is the conversion of the soluble plasma protein fibrinogen into insoluble fibrin under the action of thrombin. Thrombin splits off two polypeptide chains from the fibrinogen molecule to form fibrin monomer, which polymerizes to form a meshwork of strands. Initially, the strands are held together weakly by hydrogen bonds, but activation of factor XIII by thrombin leads to the formation of covalent cross-bridges between fibrin chains, thus strengthening them.

The conversion of prothrombin (the inactive precursor of thrombin) to thrombin involves a series of plasma serine proteases (collectively referred to as clotting factors) that normally exist in an inactive, proenzyme form, becoming activated in a cascade sequence. As shown in Figure 2, classically, the activation of the clotting factors proceeds down two pathways that converge at factor X to a common pathway. The intrinsic pathway can be activated *in vivo* by exposure of blood to collagen in the subendothelium. This activates the first factor in this pathway, factor XII, which can also be activated by exposure of blood to any electronegative wettable surface (e.g. glass). The extrinsic pathway is activated by release of lipoproteins, referred to as tissue thromboplastin or tissue factor, from damaged tissue. Although initially it was thought that the two pathways operated independently, this is now known not to be the case and interactions occur. Thus, as indicated in Figure 2, active factor VII formed in the extrinsic pathway can directly activate factor IX, thus bypassing the early stages in the intrinsic pathway. In addition, thrombin has positive feedback effects, since thrombin activates factors V and VIII, which act as co-factors in the formation of thrombin and factor Xa, respectively, and factor XI. Blood platelets also play an essential role in clotting since they provide phospholipid PF<sub>3</sub> and activation of factor X by factor IX and the conversion of prothrombin to thrombin by factor X take place, in part, on the surface of platelets in association with PF<sub>3</sub>. In this association, the relevant co-factor (V or VIII) in the reaction is bound to PF<sub>3</sub> along with calcium ions, and greatly increases the activity of the co-factor.

### Tests for clotting deficiency

Three tests are commonly used to detect clotting deficiency.

- The activated partial thromboplastin time (APTT) is a measure of the integrity of the intrinsic pathway and is performed by mixing blood with kaolin (which provides an electronegative surface), a phospholipid (as a substitute for PF<sub>3</sub>) and calcium. The normal APTT is 30–40 seconds. This test is used to monitor anticoagulant therapy with unfractionated heparin.
- The prothrombin time measures the integrity of the extrinsic pathway and is performed by adding blood to tissue thromboplastin derived from brain tissue and calcium. Since there are differences in the efficacy of different batches of the thromboplastin, the



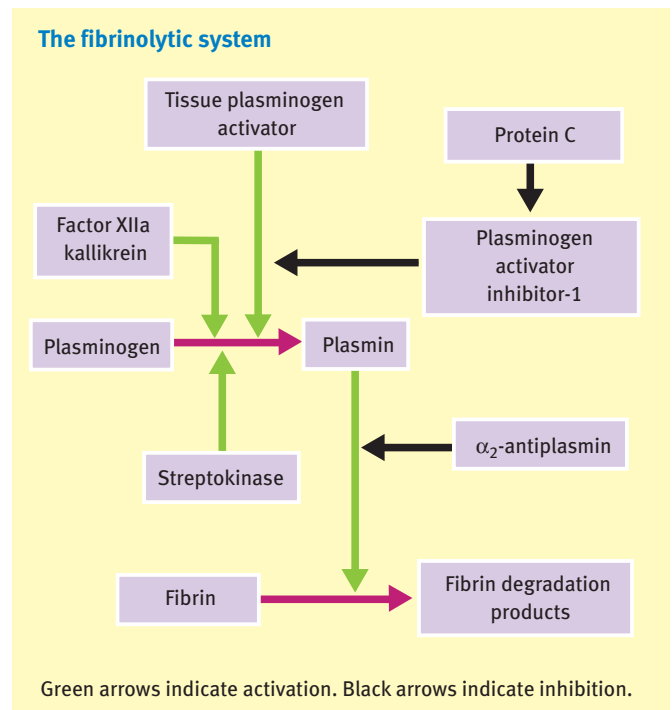
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test is also run against an international reference standard. The prothrombin time is then expressed as a ratio, the international normalized ratio (INR), in which the time for the sample blood to clot is expressed relative to the control sample. In oral anticoagulant therapy with warfarin the dose is adjusted to maintain the INR in the range 2–4.

- The thrombin time measures a deficiency of fibrinogen or inhibition of thrombin and is measured by adding citrated blood to bovine thrombin. The normal thrombin time is 14–16 seconds.

#### Anticlotting mechanisms and fibrinolysis

*In vivo*, there is a natural tendency for blood to clot. This is balanced by various naturally occurring anticoagulants. The most



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important of these is antithrombin III, which inhibits the activity of factors IX, X, XI and XII as well as thrombin. This inhibition is greatly facilitated by heparin.

Coagulation is also inhibited by thrombomodulin, which is secreted by intact endothelial cells and binds thrombin. The thrombomodulin–thrombin complex then activates a proenzyme protein C, found in plasma, to its enzymatic form and this, together with a co-factor, protein S, in plasma, results in the inactivation of factors V and VIII. Therapeutically, warfarin is used as an anti-coagulant. It is a vitamin K antagonist – vitamin K being required in the synthesis of several of the clotting factors (prothrombin and factors VII, IX and X).

Fibrinolysis (the dissolution of fibrin) also occurs, through the action of plasmin, which degrades fibrin and fibrinogen into fibrin-degradation products (Figure 3). Plasmin is present in plasma as an inactive precursor, plasminogen. Its conversion to plasmin is brought about primarily by tissue-type plasminogen activator (t-PA), a protein released from endothelium, in addition to limited conversion by factor XIIa and kallikrein. Bradykinin is a powerful stimulator of t-PA release, whereas the activity of t-PA is inhibited in plasma due to the presence of plasminogen activator inhibitor-1 (PAI-1). However, this inhibitor is itself inhibited by activated protein C. Thus activated protein C stimulates fibrinolysis. The action of plasmin is limited to the site of the clot since any plasmin free in plasma is inactivated by the enzyme  $\alpha_2$ -antiplasmin found in blood. Therapeutically, for example in the early treatment of myocardial infarction, clots can be dissolved by administration of streptokinase, which promotes conversion of plasmin to plasminogen. This drug is being replaced by use of recombinant t-PA. ♦

#### FURTHER READING

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