

Aprotinin and hemostasis in cardiopulmonary bypass

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Aprotinin and Hemostasis in Cardiopulmonary Bypass

Impaired hemostasis has been associated with cardiopulmonary bypass (CPB) from its very beginning, contributing significantly to the morbidity and mortality related to cardiac surgery. During the past 40 years, however, technically improved CPB circuits strongly diminished these problems of hemostasis. Nevertheless, impaired hemostasis has not yet been eliminated entirely. This is demonstrated by a more recent achievement, proving that blood loss can be reduced to about half in routine cardiac surgery by using the protease inhibitor aprotinin.

The use of CPB causes massive contact-phase and complement activation as the blood interacts with the nonphysiological surfaces of the CPB circuit. Contact-phase activation starts the cascade of the kinin-generating system, the intrinsic coagulation pathway, and the fibrinolytic system. As massive activation of the **intrinsic** coagulation pathway leads to systemic thrombin generation, patients undergoing CPB have to be heparinized to prevent disseminated intravascular clotting. Heparinization also makes retransfusion of pericardially shed blood possible, which, by contact with tissue, has additionally been activated via the **extrinsic** coagulation pathway. So far, no systematic attempts have been made to inhibit the activation of the kinin-generating and fibrinolytic pathways or the complement system. As specific assays to determine the effect of blood activation during CPB on the different pathways are now available, insight into systemic blood activation, particularly into the major factors leading to impaired hemostasis and the effects of aprotinin during CPB, has increased.

Hemostatic Mechanisms at the Bleeding Sites during Surgery

Upon incision and thus the cutting of blood vessels, platelets immediately adhere onto the de-endothelialized vascular wall structures, such as collagen fibers, basement membranes, and microfibrils. This prompt reaction is caused by various

accessible glycoprotein receptors on platelets for von Willebrand factor (vWF), collagen, and laminin. For instance, glycoprotein (GP) Ib receptors on the platelet membrane bind to collagen fibrils via tissue-bound vWF.

At the same time, tissue factor activates the extrinsic coagulation pathway, which generates thrombin. Thrombin binds readily to the platelets by a specific thrombin receptor. This binding induces further platelet activation, such as a conformational change of the GPIIb-IIIa receptor and a "flip-flop" reaction of the platelet membrane. In this activation process, the cyclo-oxygenase pathway is involved. This process is, therefore, inhibited by aspirin. The conformational change of the GPIIb-IIIa complex transforms it into the main fibrinogen receptor. It thus enables platelet-platelet interaction, which leads to platelet aggregation.

The "flip-flop" reaction involves the transportation of negatively charged phospholipids from the inner side of the phospholipid bilayer to the outer layer of the platelet membrane, thus exposing a negatively charged, procoagulant phospholipid surface. This amplifies thrombin formation by providing the appropriate phospholipid surface for cofactors (factor Va and factor VIIIa) that cause a strong, positive feedback activation of the coagulation factors (factors IXa and Xa). Thus, thrombin sparks thrombin generation explosively.

The activation of platelets is also followed by a change in the shape of platelets (i.e., pseudopod formation) and microvesicle shedding, which enlarges the procoagulant surfaces, contributing to the explosive increase of thrombin generation. Thrombin also induces a release of the platelets' granule contents, which play a role in local hemostasis. Adenosine diphosphate (ADP), released from the dense bodies, stimulates platelet aggregation, whereas serotonin, released from the granules, causes local vasoconstriction.

Thrombin that is generated within the platelet plug, polymerizes fibrinogen into fibrin, which leads to the formation of a more solid, hemostatic plug. At the site of an arterial lesion, local thrombin generation should be explosive. If not, thrombin would be washed-out by the fast blood flow. However, explosive thrombin generation should only lead to local hemostatic plug formation, not to disseminated intravascular coagulation. Therefore, the positive feedback activation of cofactors (factors Va and VIIIa) should be counteracted by activation of negative feedback systems. This is effectuated by tissue factor plasma inactivator (TFPI) and anti-thrombin III but particularly by the protein C and protein S systems. The protein C system is activated by the binding of thrombin to the receptor thrombomodulin on the surface of endothelial cells. During this process, thrombin loses its procoagulant properties. In conjunction with protein S and the phospholipid platelet membrane, activated protein C inactivates Va and VIIIa into Vi and VIIIi, thereby ending further prothrombin conversion.

When thrombin is generated systemically, the level of activated protein C increases before any change in the level of factor V, fibrinogen, or platelets can be observed. This regulation of protein C seems to play a pivotal role in preventing disseminated intravascular coagulation.

In addition, by neutralizing the inhibitor of the tissue-type plasminogen activator (PAI), activated protein C stimulates fibrinolysis. Fibrinolysis, i.e., the splitting of polymerized fibrin strands, is caused by the proteolytic enzyme plasmin. Plasmin arises from plasminogen, a process that is induced by plasminogen activators such as tissue-type plasminogen activator (tPA). The release of tPA from endothelial cells is stimulated by various factors that also activate clotting. Although tPA by itself is a poor plasminogen activator, counteracted in plasma

by plasminogen-activator inhibitors like PAI-1, its activity increases hundredfold in the presence of fibrin. This explains why tPA is primarily effective in hemostatic plugs.

When tPA is present, fibrin monomers on the platelet surface accelerate local plasminogen activation to form plasmin, which internalizes the GPIb receptors on platelets.² This might help to prevent circulating platelets from continuing to adhere and participate in the initial, local hemostatic process. In addition to splitting fibrin, plasmin cleaves fibrinogen and the GPIb receptors.

All these features of plasmin dynamically balance the hemostatic plug formation induced by thrombin. Local hemostatic plug formation is further controlled by intact endothelial cells. These produce heparan sulfate, which enhances the inhibiting effect of downstream antithrombin III on thrombin. Stimulated by thrombin, the intact endothelial cells also release prostacyclin (PGI₂), a potent inhibitor of platelet activation that stops the positive feedback activation of thrombin by platelets.

The above-described, classical picture of hemostasis at the site of vascular damage has been extended by the more recent demonstration of the profound effects of shear-stress on the processes leading to thrombus formation.²⁰ At surgical bleeding sites (predominantly vasoconstricted capillaries and arterioles), flowing blood is exposed to high shear-forces. These shear-forces induce the release of the intracellular multimeric vWF of platelets, which immediately binds to the GPIb receptors on the platelets' surface. This binding causes the transmembrane calcium channels to open, leading to an influx of calcium into the platelets. This calcium influx activates processes that lead to a functional change of GPIIb-IIIa, enabling its binding to vWF.

The repeating subunit structure of these large multimers of vWF offers an array of interaction sites, making multivalent binding to the GPIIb-IIIa receptors on the platelet membrane possible, thereby increasing the number of contact points and the affinity (strength) of interaction.³ As a result, the overall force, linking platelets to the surface or to one another or to both, is increased. This then effectively opposes the shear-stress that flowing blood exerts on the platelet plug formation. In contrast to thrombin-induced platelet aggregation, shear-induced platelet aggregation is independent of the cyclo-oxygenase pathway¹⁷ and, therefore, not inhibited by aspirin.

When platelets in flowing blood are not activated and shear-forces are low, platelets can adhere to and spread on a surface in case the GPIb platelet receptors "recognize" immobilized vWF. In addition, GPIIb-IIIa receptors can bind to fibrinogen, and cohesion with other platelets can proceed. These processes can occur even when platelets are inhibited by aspirin. However, when GPIb binds to immobilized vWF or soluble vWF (at higher shear-rates), or when thrombin binds to thrombin receptors independent of shear-forces, platelets do become activated. Both strong platelet adherence and platelet aggregation to oppose high shear-forces are particularly effectuated by activated GPIIb-IIIa receptors binding to multimeric vWF.

It is clear that the local circumstances at the site of vascular damage determine effective hemostatic plug formation, which primarily depends on platelet adhesion and aggregation. It is important to realize that two platelet mechanisms contribute to hemostasis: the ligand- (thrombin)-induced platelet activation and the shear-induced platelet activation. The first depends on the cyclo-oxygenase pathway; the second does not.

Factors Affecting Hemostasis in Cardiopulmonary Bypass

Increasing numbers of patients presenting for coronary artery bypass grafting are treated with **aspirin**. Therefore, hemostasis may already be impaired before surgery, as described in the preceding discussion. In this regard, a prophylactic dose of 100 mg of aspirin does not seem to affect hemostasis additionally in CPB, whereas a higher dose of 325 mg does.^{22,25}

Another anticoagulant that might affect the hemostatic function of platelets is **heparin**, which is routinely used to prevent clotting in the CPB circuit. In about one-third of patients undergoing CPB, heparin interferes with the binding of vWF to GPIb platelet receptors, thereby affecting platelet adhesion.¹¹ **Hemodilution** of about 50%, due to the clear prime of the circuit, affects hemostasis, probably to a small extent.

As mentioned earlier, the use of CPB leads to **massive contact-phase and complement activation**, as blood interacts with the nonphysiological surfaces of the CPB circuit. This leads to activation of the **intrinsic** pathway of the coagulation and fibrinolytic systems and subsequent thrombin and plasmin generation (Fig. 1). Thrombin and plasmin are agonists with a high affinity for binding to the platelet surface, thereby affecting the GPIb receptor (Fig. 2) and, thus, the platelet's hemostatic function.¹

Moreover, at various points of the CPB circuit, high shear-forces are generated, which activate platelets to adhere strongly to thrombogenic surfaces and aggregate. This reduces the number of circulating platelets. In addition, high shear-forces damage erythrocytes, resulting in release of adenosine diphosphate (ADP). ADP is an additional strong agonist for platelet aggregation and further affects the hemostatic capacity of platelets.

FIGURE 1. B-beta₁₅₋₄₂, a specific marker for disseminated intravascular coagulation (systemic thrombin generation), expressed as a percentage of initial values, increased continuously during CPB.

FIGURE 2. Platelet glycoprotein receptor Ib (GPIb), expressed as a percentage of pre-CPB values, decreased by 40% at the onset of CPB.

Another source of blood activation that affects hemostasis during CPB is the translocation of **endotoxin** from the gut into the circulation.^{8,10} This process is likely to be associated with the disturbed microcirculation of the gut mucosa during CPB. Under normal circumstances, the gut mucosa acts as an active barrier to prevent translocation of endotoxin. Endotoxin causes contact activation, stimulates monocytes and macrophages to produce cytokines, and strongly activate endothelial cells to release tPA, all of which interfere with the mechanisms involved in hemostasis.

Systemic blood activation is also caused by the **recirculation of pericardially shed blood** via cardiotomy suction. This blood has been in extensive contact with tissue, which particularly leads to activation of the extrinsic pathways of coagulation and fibrinolysis.²³

A contributing factor that induces systemic blood activation is the **reperfusion** of heart and lungs after CPB.⁷ This is due to the fact that tPA, released following ischemic damage of endothelial cells in these organs during aortic cross clamping, enters the blood circulation, subsequently stimulating fibrinolysis. The administration of **protamine** to neutralize heparin when ending CPB is also known to affect platelet hemostatic function.²⁹

The above factors make it clear that platelet hemostatic function and capacity are affected and fibrinolysis is activated **during** CPB due to multiple factors. These factors together cause impaired hemostasis **after** CPB. Of importance also is that the enzymatic activity of antiproteases, physiologically counteracting the activation of the described plasmatic systems, becomes inhibited by **hypothermia**, routinely employed during CPB.

The reproducible, substantial improvements in hemostasis during and after CPB achieved by administering the antiprotease aprotinin points to a broad protective effect of aprotinin on the mechanisms of hemostasis induced by the multiple factors mentioned. It has been demonstrated that aprotinin prevents the

interaction of heparin with vWF, eliminating the interference of heparin with platelet hemostatic function.¹² In addition, aprotinin in synergism with heparin strongly inhibits the activation of the **intrinsic** coagulation pathway (Fig. 3) that is due to contact activation by blood-material interaction and/or endotoxin translocation.³ Moreover, aprotinin is an effective inhibitor of plasmin. Effective inhibition of thrombin and plasmin by aprotinin leads to preservation of platelet GPIb receptors (Fig. 4).^{6,27,28,32} This importantly contributes to maintain hemostasis (Fig. 5). The strong inhibition of fibrinolysis enhances this effect even more.¹⁶

The mechanism by which protamine affects platelet function is unclear, and studies on the effect of aprotinin in this regard are needed. Interestingly, improved hemostasis by aprotinin administration can also be achieved in patients using prophylactic aspirin (Figs. 6 and 7).^{22,25} An explanation of this effect is that shear-induced platelet reactivity, which is independent of the cyclo-oxygenase pathway, could be preserved by aprotinin. This emphasizes the essential role of this mechanism in platelet plug formation at multiple bleeding sites during surgery.²⁰

Shear-induced platelet reactivity can be measured by doing a Thrombostat test.¹³ This is an *in vitro* bleeding test, perfusing whole blood through an artificial arteriole (ID 190 μm) under constant pressure (40 mm Hg). This generates a shear-stress of 128 dyn/cm², which can be compared with the value estimated at the surgical bleeding site. By the induced shear-stress, platelets are activated and stick to the collagen filter at the end of the capillary, thus forming a platelet hemostatic plug. It is important to add ADP onto the filter to prevent an abnormally prolonged test result being obtained in patients treated with aspirin.¹⁴ The binding of released platelet-vWF induced by ADP seems to play a dominant role in platelet plug formation by this specific shear-rate, which is not affected by aspirin.¹⁷

Although the results of the *in vivo* bleeding test correlate with the *in vitro* bleeding test,¹³ the *in vivo* test is more sensitive for detecting the various factors influencing hemostasis.¹⁹ For example, the clotting system and the cyclo-oxygenase pathway tend to affect the *in vivo* test.^{19,30} Furthermore, CPB influences the *in vivo*

FIGURE 3. Generation of the B-beta₁₅₋₄₂ fragment, the molecular marker for disseminated intravascular coagulation, was completely inhibited by aprotinin administration (6 million KIU).

FIGURE 4. Platelet glycoprotein receptor IB (GPIb) changes during CPB, expressed as a percentage of pre-CPB values in aprotinin-treated patients given only 2 million KIU in the pump prime or combined with an additional bolus of 2 million KIU given at induction of anesthesia and a continuous infusion of 2 million KIU until protamine administration (total, 6 million KIU) or placebo. GPIb receptors remained preserved with aprotinin independent of the dose during CPB.

test, depending on various factors, e.g., skin temperature, vasotonic medication, and blood pressure.²⁶ Therefore, the *in vitro* test during CPB is more specifically useful for monitoring the shear-induced pathway of platelets, which seems to play a major role in hemostasis, particularly in aspirin-treated patients.

In a recent study, we demonstrated the alteration in hemostasis induced by the shear-induced platelet activation during CPB in aspirin-treated and non-aspirin-treated patients. Before CPB, the bleeding volume during the Thrombostat test was equal for both groups. Systemic heparinization influenced these test results only

FIGURE 5. Blood loss, expressed in grams of hemoglobin content (g Hb), during and after operation in patients treated with 6 million KIU and 2 million KIU of aprotinin was equally reduced and significantly ($*p < 0.05$) lower than in patients receiving placebo.

slightly, which signals that the effect of the clotting system is only minor.²⁴ After starting CPB, bleeding volume in both groups increased abruptly. This indicates that the hemostatic capacity of platelets rapidly and strongly diminishes. During CPB, however, the hemostatic capacity of the platelets was significantly more affected in the aspirin-treated patients than in the non-aspirin-treated patients.

The reported, initial decrease of platelet membrane glycoprotein antigens (GPIb) during CPB^{27,32} might partly reflect the loss of the hemostatic capacity of platelets during the Thrombostat test. In both groups, the shear-induced platelet aggregation recovered partly after CPB, but the hemostatic capacity of the platelets remained significantly more affected in aspirin-treated patients than in placebo-treated patients. This higher vulnerability of aspirin-treated patients for impaired hemostasis induced by CPB, however, can be modified by aprotinin administration.^{22,25}

FIGURE 6. Platelet glycoprotein receptor (GPIb) was significantly ($*p < 0.05$) reduced at the onset or CPB in non-aprotinin-treated patients independent of aspirin pretreatment. Aprotinin administration (2 million KIU in the pump prime), both in patients with or without aspirin pretreatment, preserved platelet GPIb receptors during CPB.

FIGURE 7. Postoperative blood loss during 24 hours after operation. Aprotinin significantly decreased blood loss in aspirin-treated and non-aspirin-treated patients ($*p < 0.05$ comparing aspirin-treated patients with aspirin/aprotinin-treated patients; $**p < 0.05$ comparing non-treated patients and aprotinin-treated patients).

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It is interesting that the dose of aspirin seems to cause a different shear-induced reactivity of platelets *in vitro*,¹⁸ which might also be reflected in the clinical CPB situation. Although the mechanism is still unclear, it underlines the broad protective effect of aprotinin. This might also explain the reproducibility of the hemostatic effect of aprotinin in various centers, independent of the differences in the various circumstances that can influence hemostasis.

On the other hand, the broad and potent action of a natural protease inhibitor like aprotinin might also have unwanted side effects. Because of its inhibitory effect on fibrinolysis and the natural anticoagulant protein C system, aprotinin causes a shift in favor of thrombosis. This aprotinin-induced imbalance is likely rebalanced by systemic heparinization during CPB. However, when aprotinin administration is started at the induction of anaesthesia, *i.e.*, before heparin is given, or when aprotinin is still active after protamine has neutralized heparin, the antifibrinolytic properties may tend to work in favor of thrombin (fibrin) formation.

Particularly during coronary artery bypass grafting, there is a risk of thrombosis in the vein grafts, as these grafts are mostly de-endothelialized after implantation, thus forming an ideal activation site for platelet adherence. Moreover, reports of disseminated intravascular clotting after deep hypothermia and circulatory arrest in patients treated with aprotinin³¹ demonstrate the danger of uncontrolled inhibition of the protein C system under these circumstances, despite systemic heparinization. The rationale is therefore to limit aprotinin administration to the period of systemic heparinization and to moderate hypothermia.

Several reports^{21,28} have shown that 2 million KIU of aprotinin added to the pump prime has an effect on hemostasis that is comparable to that for the initially proposed dose scheme of 6 million KIU (which also covers the period before and after heparinization).²⁸ However, the 6-million-KIU dose has an inhibitory effect on the balance of natural anticoagulation, which is present before and after systemic heparinization. Although a dose of 2 million KIU of aprotinin, to be added to the pump prime only, is therefore theoretically safer than the 6-million-KIU dose, this low dose might not protect sufficiently against all the mentioned factors that have the potential to affect hemostasis during CPB.

Shortly after the aorta cross-clamp is released, a sharp rise in tPA and fibrinolytic activity was observed.⁴ One has to realize that these effects can be caused by multiple coinciding factors: recirculation of heart and lungs, increase of cardiomy suction, and end of hypothermia. As these factors might vary from center to center, a variable protective effect of just 2 million KIU of aprotinin in the prime can be expected. Although this might lower the high reproducibility rate and effectiveness of the 6-million-KIU dose scheme, it could prevent the potential side effects of aprotinin and it certainly reduces the costs.

With all this in mind, it is important to realize that efforts to improve hemostasis during CPB are primarily aimed at reducing or, if possible, eliminating the need for blood products because of their inherent risks. To achieve this goal, several cardiac centers have instituted a strict blood salvage protocol, which includes postoperative retransfusion of preoperatively donated autologous blood. Also in this situation, a low dose of aprotinin should be considered, because it can contribute substantially to reducing the use of blood products.²¹ On the other hand, when postoperative retransfusion of preoperatively donated autologous blood leads to the preservation of a substantial number of platelets that do not endure the negative effects of blood activation caused by CPB, the protective effect of 2 million KIU of aprotinin on circulating platelets might not be so impressive anymore.

However, potentiating effects are seen when heparin-coated circuits, the use of which reduces complement activation,⁹ are primed with 2 million KIU of aprotinin.

In a recent study,¹⁵ none of the separate variables, i.e., neither heparin coating nor aprotinin, improved hemostasis, but their combination did. Another observation in this study was that the inflammatory reaction, which determines length of stay in the intensive care unit, was also significantly milder following the combined use of heparin coating and aprotinin.

The gradually expanding knowledge of the mechanisms affecting blood activation during CPB indicates that for CPB techniques, multiple improvements are still required. In regard to the use of drugs such as aprotinin, it is essential to find the optimal dose and to avoid potential side effects. Finally, all other measures that reduce blood activation and limit the use of blood products should also be taken when employing CPB.

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