INTRODUCTION

The hematologic management of the cardiac surgical patient entails a complex balance between extreme degrees of anticoagulation and the restoration of normal hemostasis after the procedure. These two opposing processes must be managed carefully and modified with respect to preoperative disease state, duration of cardiac surgery, use of extra corporeal circulation and the desired hemostatic outcome ***(Shore-Lesserson, 2005).***

Skillful surgery combined with blood saving methods and careful management of blood coagulation will help to reduce unnecessary blood loss and transfusion requirements. Excessive surgical bleeding causes hypovolemia, hemodynamic instability, anemia and reduced oxygen delivery to tissues with a subsequent increase in post operative morbidity and mortality.

Adverse effects of allogenic blood transfusion include transmission of infectious diseases, immunosuppression, transfusion related acute lung injury, transfusion reactions and graft versus host reactions ***(Mahdy and Webster, 2004).***

Since excessive fibrinolysis during cardiac surgery is frequently associated with abnormal perioperative bleeding, many authors have advocated prophylactic use of antifibrinolytic drugs to prevent hemorrhagic disorders ***(Valter et al., 2000).***

The prophylactic use of aprotinin is effective in decreasing blood loss and transfusions and is most widely tested drug to date ***(Ferraris et al., 2007).***

Several studies with tranexamic acid have demonstrated a significant reduction of perioperative bleeding and the need for transfusion. However, the efficacy of tranexamic acid in direct comparison to aprotinin has been minimally investigated ***(Ferraris et al., 2007).***

AIM OF THE WORK

The aim of the work is to compare the efficacy of the use of aprotinin and tranexamic acid in reducing blood loss after cardiopulmonary bypass (CPB) and their effects on the coagulation profile when given to patients under going coronary artery bypass grafting (CABG).

Chapter I

PHYSIOLOGY OF HEMOSTASIS AND COAGULATION MECHANISM

The coagulation system is considered by many clinicians to consist just of platelets and clotting factors. For sometime, however, it has been recognized that many more cellular and molecular components participate in the coagulation process, there by forming a multifaceted, well balanced system called hemostasis. Moreover, the coagulation system is not only made for forming clots but is also involved in a variety of defence systems, including tissue repair, defence against microorganisms, autoimmune processes, arterioslerosis, tumour growth and metastasis ***(Bombeli and Spahn, 2004).***

Hemostasis is the body's normal response to vascular injury and involves a complex interplay of systems within the body that helps to seal the endovascular defect and prevent exsanguination. The three major components of hemostasis include the vascular endothelium, the platelets, which constitute primary hemostasis and the coagulation cascade glycoproteins, which constitute secondary hemostasis.

Fibrinolysis is a normal physiologic response to clot formation, which ensures that coagulation remains localized to the area of vascular injury ***(Kaplan et al., 2008).***

Components of hemostasis:

1. Vascular constriction
2. Vascular endothelium
3. Platelet plug
4. Coagulation cascade
5. Modulators of coagulation and fibrinolysis

1. Vascular constriction

Immediately after a blood vessel has been cut or ruptured, the trauma to the vessel wall it self causes the smooth muscle in the wall to contract, this instantaneously reduces the flow of blood from the ruptured vessel.

This contraction results from (1) local myogenic spasm (2) local autacoid factors from the traumatized tissues and blood platelets (e.g., thromboxane A_2). (3) nervous reflexes. The spasm can last for many minutes or even hours, during which time the processes of platelet plugging and blood coagulation can take place ***(Guyton, 2006).***

2. Vascular endothelium:

The endothelium is a simple squamous epithelium that overlies connective tissue collagen and other proteins that are capable of activating platelets to begin clot formation. Thus an intact endothelium physically separates the blood from collagen and other platelet activators in the vessel wall.

In addition, the endothelial cells secrete prostacyclin (PGI_2), a type of prostaglandin and nitric oxide (NO) ***(Irafox, 2006).***

Nitric oxide vasodilates blood vessels and inhibits platelets. Its mechanism involves activation of guanylate cyclase with eventual uptake of calcium into intracellular storage sites. Prostacyclin (PGI2) possesses powerful vasodilator and antiplatelets properties. Endothelium-derived prostacyclin (PGI2) opposes the vasoconstrictor effects of platelet produced thormboxane A2 (TxA_2) ***(Kaplan et al., 2008).***

The enzyme cyclooxygenase (abbreviated Cox) is required for prostaglandin formation. There are two major forms of this enzyme termed Cox-1 and Cox-2.

The formation of thromboxane A2 by platelets is catalyzed by Cox-1 and the formation of prostacyclin by an intact endothelium is catalyzed by Cox-2. Aspirin inhibits (Cox-1) (as well as Cox-2) and by this means reduces platelet aggregation. Since platelets are not complete cells, they cannot regenerate new enzymes.

Therefore, the Cox-1 enzyme inhibited by aspirin is inhibited for the life of the platelets and thus aspirin can significantly prolong bleeding time ***(Irafox, 2006).***

The plasma membrane of the endothelial cells contains an enzyme known as (CD39) which has its active site facing the blood. The CD39 enzyme breaks down ADP in the blood to AMP and Pi.

ADP is released by activated platelets and promotes platelet aggregation and this protective mechanism is needed to ensure that platelets don't stick to the vessel wall and to each other ***(Irafox, 2006).***

3. Formation of the platelet plug

Platelets (also called thrombocytes) are minute discs 1 to 4 micrometers in diameter. They are formed in the bone marrow from megakaryocytes, which are extremely large cells of the hematopoietic series in the marrow, the megakaryocytes fragment into the minute platelets either in the bone marrow or soon after entering the blood, especially as they squeeze through capillaries. The normal concentrations of platelets in the blood is between 150.000 and 300.000 per microliter ***(Guyton, 2006).***

Platelets have multiple and over expanding role in hemostasis. They are recruited not only when vascular integrity is disturbed, but also they maintain the integrity of normal endothelium, as evidenced by the tendency of the patient with platelet deficiencies to develop purpuric bleeding ***(Edward and Juan, 2000).***

The platelets respond through three steps as follows:

1. Adhesion:

When the vascular endothelium becomes denuded or injured, the platelet has the opportunity to contact von willibrand factor (vWF), which is bound to the exposed collagen of the subendothelium. A platelet membrane component, glycoprotein (GP) 1b, attaches to vWF, thus anchoring the platelet to the vessel wall. Independently, platelet membrane GpIa and GPIIa and IX may attach directly to exposed collagen, furthering the adhesion stages ***(Kaplan et al., 2008).***

Adhesion requires margination of platelets: high hematocrites concentrate red blood cells in the central regions of the vessel, promoting marginal placement of platelets. Dilute hematocrite after cardiopulmonary bypass (CPB) impairs this effect, thus adversely affecting platelet adhesion. For this reason, red blood cell transfusion alone may improve hemostasis. However, transfusion should not be used primarily to achieve this goal since red cell concentrates carry a high concentrations of cytokines and platelet activating factor (PAF), which may contribute to platelet dysfunction and consumptive coagulopathy ***(Kaplan et al., 2008).***

2. Activation/ secretion:

Following adhesion, platelets undergo a shape change from a disc to a sphere, spread along the subendothelium of

their cytoplasmic granules, i.e., the (δ) dense granules containing ADP and serotonin and the (α) granules containing (platelet-derived growth factor, platelet factor 4 (heparin antagonist), β-thromboglobulin, fibrinogen, vWF, fibrionectin and other factors.

The release of ADP leads to a conformational change in the fibrinogen receptor, the glycoprotein IIb-IIIa complex (GPIIb-IIIa), on the surface of adherent platelets allowing it to bind to fibrinogen ***(Kumar and Clark, 2007).***

3. Aggregation:

When platelets secrete large amounts of ADP and thromboxane A_2, the ADP and thromboxane in turn act on nearby platelets to activate them as well, the stikiness of these additional platelets causes them to adhere to original activated platelets ***(Guyton, 2006).***

Aggregation occurs also when fibrinogen released from α granules from molecular bridges between (GPIIb/IIIa) receptors of adjacent platelets (the final common platelet pathway) ***(Kaplan et al., 2008).***

Further platelet membrane receptors (e.g., P_2Y_{12}) are exposed during aggregation providing a surface for the interaction of coagulation factors, this platelet phospholipids activity is referred to as platelet factor 3 (PF-3). The presence of thrombin encourages fusion of platelets and fibrin formation

reinforces the stability of the platelet plug ***(Kumar and Clark, 2007).***

4. The coagulation cascade

All research workers in the field of blood coagulation agree that clotting takes places in three essential steps:

1. In response to rupture of the vessel or damage to the blood it self, a complex cascade of chemical reactions occurs in the blood involving more than a dozen blood coagulation factors. The net result is formation of a complex of activated substances collectively called (prothormbin activator).

2. The prothrombin activator catalyzes the conversion of prothrombin into thrombin.

3. The thrombin acts as an enzyme to converts fibrinogen into fibrin fibers that collect platelets, blood cells and plasma to form the clot ***(Guyton, 2006)***.

The enzymes involved in the blood coagulation belong to a family of proteases known as serine proteases, a class of enzymes with a common mechanism of enzymatic action that requires the catalytic triad of serine, aspartic acid and histidine within the active site (table 1) ***(Mahdy and Webster, 2004).***

Table (1): Clotting factors in blood and their synonyms

Clotting factor	Synonyms
Fibrinogen	Factor I
Prothrombin	Factor II
Tissue factor	Factor III; tissue thormboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thormboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thormboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Flecher factor
High-molecular-weight Kininogen	Fitzerald factor; HMWK (high-molecular-weight) kininogen

(Mahdy and Webster, 2004)

a) Extrinsic pathway: Injury to the arterial or venous wall exposes perivascular, tissue factor expressing cells to blood. Tissue factor (TF) is a cellular receptor for activated factor VII (factor VIIa) and factor VII. Factor VIIa, found in small amounts in normal plasma, binds to exposed TF. Once bound to TF, factor VIIa can catalyse the activation of factor VII, which also binds to exposed TF. The factor VIIa-TF complex then activates factors IX and X in the presence of Ca^{2+}, leading to

the generation of factors IXa and Xa respectively. Activation of factor X through the above mechanism is referred to as the extrinsic pathway.

b) The intrinsic pathway is initiated by activation of factor XII by kallikrein on foreign surfaces or damaged endothelium, and is facilitated by kininogen. The active form of factor XII, factor XIIa, catalyses the conversion of factor XI to its active form, factor XIa. In the presence of Ca^{2+}, factor XIa activates factor IX to its active form, factor IXa. Factor IXa binds to the cofactor factor VIIIa bound on membrane surfaces in the presence of calcium ions to generate a complex with enzymatic activity known as tenase, a nickname for the enzymatic activity that acts on factor X ***(Kumar and Clark, 2007)***.

c) Common pathway: Activation of factor X to factor Xa by the extrinsic or intrinsic pathways is the start of the common pathway of coagulation. The latter pathways is thought to be of little significance in vivo since patients with factor XII, prekallikrein or kininogen deficiency have on bleeding disorders. These proteins are not required for haemostasis. However, they may play a role in fibrinolysis and in fibrin formation during inflammation and wound healing. When factor Xa is generated, it complexes with factor Va, phospholipids and calcium to form the prothrombinase complex, which converts prothrombin to its active form, thrombin. The generated thrombin cleaves fibrinogen, releasing

fibrin then activates factor XII responsible for cross linking the fibrin polymer (Fig. 1) ***(Mahdy and Webster, 2004).***

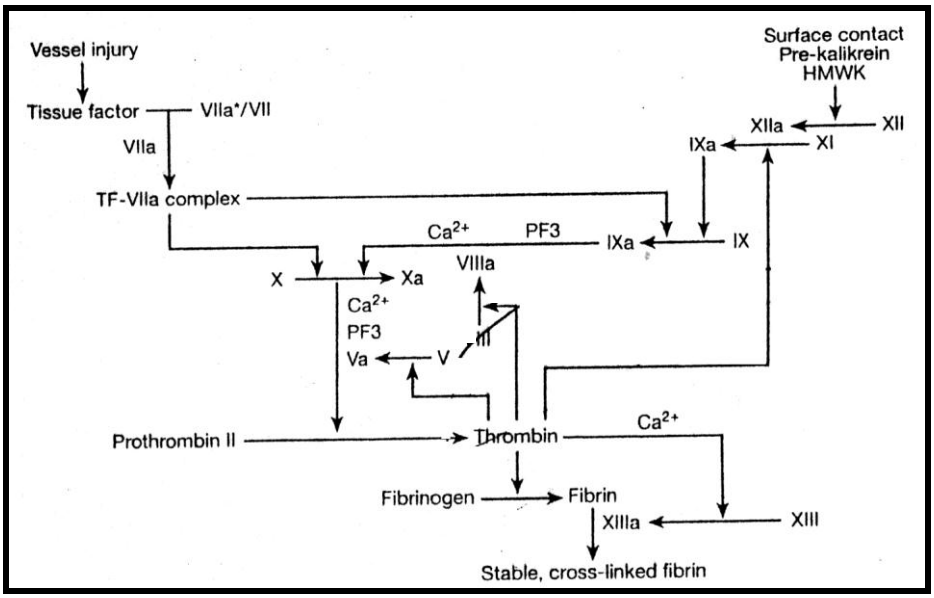

Figure (1): The coagulation cascade. *A small quantity of activated factor VII is physiologically present in the plasma and is responsible for the initial binding with tissue factor (TF). Ca^{2+}, calcium ion; PF3, platelet factor-3; HMWK, high molecular weight kininogen ***(Mahdy and Webster, 2004)***.

The clot is composed of a mesh work of fibrin fibers running in all directions and entrapping blood cells, platelets and plasma. The fibrin fibers also adhere to damaged surfaces of the blood vessel, therefore, the blood clot becomes adherent to any vascular opening and thereby prevents further blood loss ***(Guyton, 2006).***

Coagulation cascade (new aspects):

Whereas the classic separation of the coagulation pathway into the extrinsic pathway (initiated by tissue factor) and intrinsic pathway (initiated by contact activation) still has

certain validity, the newer time based constructing provides a much more authentic description of the coagulation process. This involves the following steps (Fig. 2):

i. *Initiation.* Tissue factor (TF) expressed by the damaged vascular bed binds FVIIa (which circulates in small quantities), which then triggers coagulation by activating FIX to producing small amounts of thrombin (FIIa). In a much slower reaction, FIXa binds to and activates FX to FXa (3 in Fig. 2). Most coagulation processes in vivo are considered to be initiated by tissue factor, whereas the clinical significance of the contact activation (activation of FXII) is still not yet entirely clear. A recent report, however, has shown that RNA from disrupted cells may be the long sought FXII activator in vivo.

ii. *Amplification*. Because the amount of thrombin generated at this stage is still too small to activate fibrinogen to fibrin, there are several feedback amplification mechanisms. First, generation of FVIIa is increased by activation of FVII bound to tissue factor by FVIIa, FIXa and FXa. Thrombin then activates the non enzymatic cofactors FV and FVIII, which accelerate the activation of FII by FXa and of FXa by FIXa, respectively. In a further feedback loop (2 in Fig. 2), thrombin also activates FXI to FXIa, increasing the generation of FIXa.

iii. Propagation. To maintain continuous thrombin generation, ensuring the formation of a sufficiently large clot, large amounts of FXa are produced by the activation of FX by FIXa

and FVIIa (intrinsic tenase complex). FIXa stems primarily from the activation of FIX by the FVIIa/ TF-complex.

iv. Stabilization. Maximum thrombin generation occurs after the formation of fibrin monomers. Only then is the amount of thrombin high enough to activate FXIII, a transglutaminase, which then cross links the soluble fibrin monomers to a stable fibrin meshwork. In addition, thrombin then activates the thrombin achievable fibrinolysis inhibitor (TAFI) that protects the clot from fibrinolytic attack ***(Bombeli and Spahn, 2004)***.

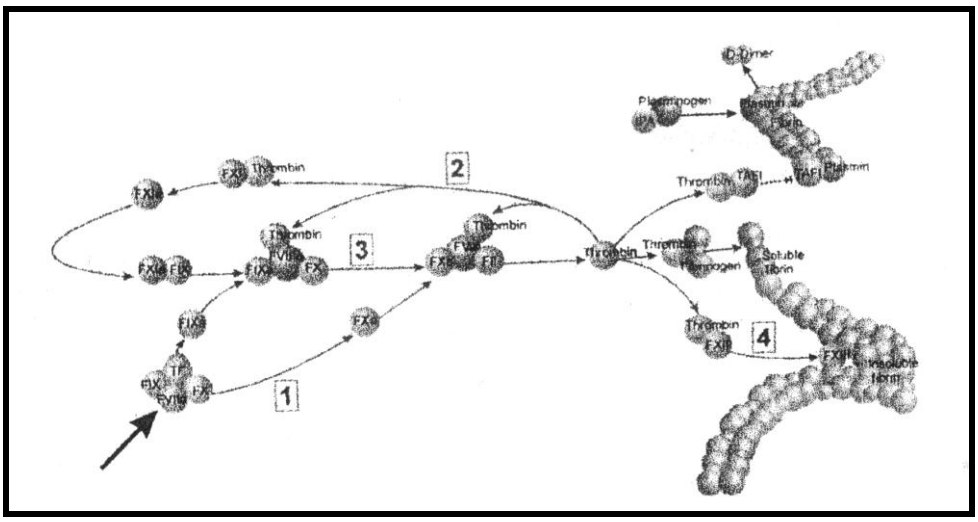

Figure (2): Current model of coagulation and fibrinolysis. In vivo the coagulation process is initiated mainly by FVIIa bound to tissue factor (TF; large black arrow), which then activates both FX (1) and FIX (2) (= initiation phase). To increase thrombin generation further, thrombin activates FV, FVIII and FXI in a feedback-loop (3) (=amplification). Continuation of thrombin generation results mainly from the ongoing generation of FXa by FIXa and FVIIIa (= propagation). Maximum thrombin generation occurs only after the formation of fibrin, leading to the formation of FXIIIa, which then crosslinks the fibrin monomers (4) (=stabilization) ***(Bombeli and Spahn, 2004)***.