Thrombin During Cardiopulmonary Bypass

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Cardiopulmonary bypass (CPB) ignites a massive defense reaction that stimulates all blood cells and five plasma protein systems to produce a myriad of vasoactive and cytotoxic substances, cell-signaling molecules, and upregulated cellular receptors. Thrombin is the key enzyme in the thrombotic portion of the defense reaction and is only partially suppressed by heparin. During CPB, thrombin is produced by both extrinsic and intrinsic coagulation pathways and activated platelets. The routine use of a cell saver and the eventual introduction of direct thrombin inhibitors now offer the possibility of completely suppressing thrombin production and fibrinolysis during cardiac surgery with CPB.

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Blood is a tissue designed to sustain all cells by continuous circulation within a vast labyrinth paved with endothelial cells. These unique cells simultaneously maintain the fluidity of blood and ensure integrity of the vascular system. Contact with the surgical wound and diversion of blood into the heart-lung machine trigger an angry defense reaction that evolved to protect the body from pathogens, injuries, and noxious substances. The major participants are blood cells and five plasma protein systems, which generate a massive reaction by upregulating cellular receptors and by releasing a potpourri of vasoactive, cytotoxic, and cell-signaling substances into the circulation. Platelets, neutrophils, monocytes, and endothelial cells are the major cellular actors; and complement, contact, intrinsic coagulation, extrinsic coagulation, and fibrinolytic protein systems are the primary plasma participants. When activated, these cells and proteins initiate complex and overlapping reactions and interactions with a multitude of target molecules to create a "whole body inflammatory response" [1]. Anticoagulation is required, and unfractionated heparin has been exclusively used for over half a century with generally satisfactory results.

To simplify presentation, we have arbitrarily separated the defense reaction into thrombotic and acute inflammatory responses. This review does not attempt to catalog the changes in particular hormones, peptides, cytokines, proteases, metalloproteinases, phospholipids, eicosanoids, reactive oxygen and nitrogen species, free radicals, cytotoxins, signaling proteins, and cellular receptors that occur during cardiopulmonary bypass (CPB). Instead we concentrate on the primary mechanisms involved in the generation of thrombin, which is the key enzyme involved in the thrombotic portion of the defense reaction during CPB.

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Methods

An Internet search was made using the keywords *thrombin*, *thrombosis*, *tissue factor*, and *fibrinolysis* on Google, Ovid, Pub Med, and Scirus. The number of hits for *tissue factor* varied between 4826 (Ovid) and "about 24,900,000" (Google). The other keywords produced hits within this range. Thus, we retreated to traditional sources, which included textbooks [2, 3]; original references and reviews (see subsequent sections); and more than three decades of collaborative, government-funded, active research.

Heparin

A review of the thrombotic response to CPB and open heart surgery (OHS) is a study of defense reactions to heparinized blood circulated and exposed outside the body. Very high doses of unfractionated, standard heparin are required to maintain the fluidity of blood during CPB and OHS, but even these doses fail to completely inhibit thrombin formation. Standard heparin is required for CPB [4], but acts as a cofactor by accelerating the actions of the natural plasma protease, antithrombin (antithrombin III) [5].

Antithrombin is an abundant (140 μg/mL), large molecule (58 kDa) that binds thrombin to form the thrombin-antithrombin complex (TAT), which is rapidly cleared from the circulation by the liver. Antithrombin also inhibits factor Xa, which forms part of the prothrombinase complex. Earlier in the coagulation cascade, antithrombin inhibits factors XIIa, XIa, and IXa and essentially all serine proteases involved in coagulation [6].

Heparin increases antithrombin-mediated inhibition of thrombin and factors IXa and Xa more than 1000-fold, but the mechanisms of inhibition differ. In the case of thrombin, a bridging effect predominates, whereas with factor Xa and factor IXa, the major effect is an allosteric expanse of a reactive loop [7].

Heparin has advantages and disadvantages. The most notable advantages are rapid thrombin inhibition and reversal by protamine. Protamine does not reverse low-

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Table 1. Hemostatic Actions of Thrombin

Procoagulant
Cleaves fibrinogen to fibrin
Activates factor XIII to cross link fibrin
Activates platelet PAR-1 (at low concentration) and GPIb and PAR-4 receptors
Causes platelet shape change, expression of pseudopods
Causes release of α-granule contents: ADP, serotonin, chemokines, growth factors, and tissue factor
Causes release of thromboxane A2
Stimulates surface expression of P-selectin and CD 40 adhesive ligands
Stimulates expression of GPIIb/IIIa receptors
Activates factors V, VIII, XI
Activates factor VII in wounds
With thrombomodulin activates TAFI
Stimulates endothelial cells to produce tissue factor, von Willebrand factor
Stimulates subendothelial smooth muscle constriction
Anticoagulant
Stimulates ECs to synthesize and release t-PA and u-PA
Binds to thrombomodulin, which inhibits platelet activation and cleavage of fibrinogen and factors Va and VIIIa
Binds to thrombomodulin for capture of protein C
Activates protein C
Stimulates ECs to produce nitric oxide and prostacyclin
Stimulates ECs production and release of TFPI

ADP = adenosine diphosphate; ECs = endothelial cells; GPIb = glycoprotein 1b; PAR-1 = proteinase activated receptor-1; TAFI = thrombin activatable fibrinolysis inhibitor; TFPI = tissue factor pathway inhibitor; t-PA = tissue-type plasminogen activator; u-PA = urokinase plasminogen activator.

REVIEWS

molecular-weight heparins. The major disadvantage of heparin is that it fails to completely prevent thrombin formation during CPB [8, 9] and does not inhibit clot-bound thrombin, which antithrombin cannot reach [10]. Heparin concentrations are difficult to monitor in the operating room, and the most common method, activated clotting time, is crude, indirect, and poorly reproducible [11]. Heparin also interacts with platelets and numerous plasma proteins, activates factor XII [12] and complement [13], and induces immunoglobulin G (IgG) antiplatelet PF4 antibodies in some patients [14].

Thrombin

Thrombin is a serine protease that is rapidly inhibited by antithrombin. Its plasma half-life is very short (<1 min) and circulation is evanescent [15]. Normally, the enzyme acts at sites of blood vessel injury and is essential for maintaining the integrity of the vascular network. CPB is unique in that thrombin is continuously generated and circulated to transform a local process into a systemic, iatrogenic disease.

The principal actions of thrombin during applications of extracorporeal circulation are cleavage of fibrinogen into fibrin, activation of factor XIII to crosslink fibrin, activation of platelets by way of the specific thrombin receptors glycoprotein Ib (GPIb) and proteinase activated receptor-1 (PAR-1) and PAR-4, and stimulation of endothelial cells to release tissue plasminogen activator protein (t-PA) and von Willebrand factor [16, 17]. Other important procoagulant and anticoagulant reactions of thrombin are listed in Table 1 and are discussed in the following sections.

Thrombin is probably essential to mammalian life [6] and has a wide range of actions beyond the coagulation system. Thrombin mediates production of a variety of growth factors, which stimulate mitogenesis of smooth muscle cells, endothelial cells, and macrophages, and probably other undiscovered targets as well [18]. At injury sites, thrombin induces the production of cytokines, chemoattractants, and vasoactive substances, which promote neutrophil adhesion, attract macrophages, and increase vascular permeability. Thrombin is also involved in angiogenesis and enhances vascular endothelial growth factor (VEGF) in the growth of tumors and metastases.

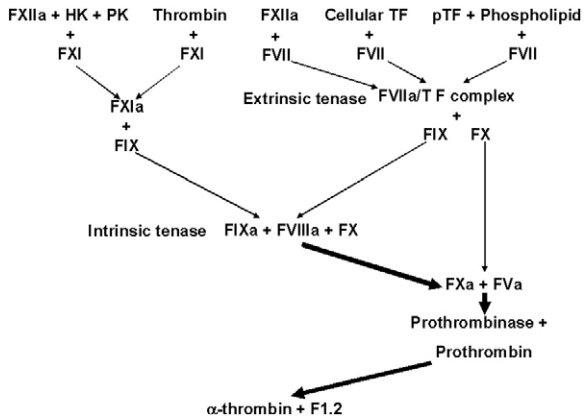

Fig 1. Diagram illustrates the five pathways for generation of thrombin during cardiopulmonary bypass (CPB) and open heart surgery (OHS). The protein or proteins that start each pathway appear at the top of the diagram. Plus signs (+) designate the principal substrate, and arrows show the enzyme produced by the reaction. The contact pathway (far left) is initiated by activation of factor XII, which with cofactors high molecular kininogen (HK) and pre-kallikrein (PK) activates factor XI to factor XIa. Thrombin, once produced, also directly activates factor XI. Factor XIa activates factor IX in the next reaction, which forms part of the intrinsic tenase complex. Although animal studies indicate that factor XIIa activates factor VII, this pathway has not been studied in patients with OHS and CPB. Both cellular tissue factor (TF) and soluble plasma tissue factor (pTF) activate factor VII in the extrinsic coagulation pathway to form the factor VIIa/tissue factor complex or extrinsic tenase. pTF requires a phospholipid cofactor (monocyte, platelet or microparticle). Extrinsic tenase activates both factors IX and X. Activated factor IX (factor IXa) complexes with thrombin activated factor VIII to form intrinsic tenase, which activates factor X. Tissue factor pathways and extrinsic tenase initiate thrombin formation, but once begun, the intrinsic coagulation pathway, aided by activated platelets, is the dominate source of factor Xa. Factor Xa with factor Va forms prothrombinase, which cleaves prothrombin to form α-thrombin and prothrombin fragment, F1.2.