Multiple Mechanisms of Thrombosis Complicating Atherosclerotic Plaques

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Summary: Atherosclerosis is a highly prevalent disorder and remains a leading cause of morbidity and mortality in the United States. Recent advances in vascular biology have led to a better understanding of the mechanisms underlying atherogenesis. The central role played by plaque disruption, and by adhesion, activation, and aggregation of platelets that trigger activation of the coagulation cascade in the pathogenesis of acute thrombotic events is also better understood. Combination antithrombotic therapy targeting various platelet activation mechanisms and the coagulation cascade may help optimize the management of atherosclerosis.

Key words: acute coronary syndromes, plaque, thrombosis, platelet

Introduction

Atherosclerosis is a systemic disease that affects not only the coronary circulation, but extracerebral, intracerebral, and peripheral vessels as well. Our understanding of the pathogenesis of atherosclerotic lesions continues to grow while a unified picture of unstable plaque structure, inflammation, matrix degradation, and prothrombotic activity develops. Over the past decade, age-adjusted mortality rates from coronary artery disease have steadily declined in the United States.¹ Modifications in the American lifestyle, as well as improvements through the beneficial use of antiplatelet and chole-

sterol-lowering agents, account for much of the decline.^{1,2} A basic understanding of the unstable atherosclerotic lesion must be translated into a clinic benefit beyond these recent advances.³ Mastery of the cellular and molecular biologies of atherosclerosis will permit the development of novel strategies for mitigating this prevalent disease.⁴

Pathogenesis of the Acute Coronary Syndromes

Atherosclerotic lesions may remain clinically dormant for decades, producing no symptoms. However, suddenly without warning, a clinically silent lesion may precipitate an acute manifestation of atherosclerosis.⁴ The transition from the chronic phase of atherosclerosis to the acute manifestations constitutes a major preoccupation of clinical cardiology. Flow-limiting stenoses were formerly considered the cause of the acute coronary syndromes. Recent studies, however, have shown that most myocardial infarctions result from thrombi that occur on the substrate of a lesion that does not cause a critical narrowing of a coronary artery.⁵ We now appreciate that the acute coronary syndromes frequently result from physical disruption of the atherosclerotic plaque (Fig. 1).⁶

Plaque disruption takes two major forms: a frank fissuring of the plaque's fibrous cap and a superficial erosion of the intimal surface.⁷ In the case of a ruptured cap, blood coagulation factors come into contact with the plaque's lipid core, which is rich in tissue factor and considered to be the major procoagulant in this situation. In the case of superficial erosion, platelets can contact subendothelial basement membrane and collagen within the plaque, which may trigger platelet aggregation. In addition, smooth muscle cells underlying the endothelium can also express tissue factor, further contributing to thrombus formation.⁷

Several features exist that distinguish plaques that are vulnerable to rupture from those that are stable (Fig. 2).^{8–10} Compared with stable plaques, vulnerable plaques have a large lipid-rich core, a thinner fibrous cap, and more numerous lymphocytes, macrophages, and activated vascular smooth muscle cells. The inflammatory cells are concentrated at the shoulders of plaques, where wall stress is also higher and where rupture of the plaque cap is more commonly observed.⁵

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FIG. 1 Summary of the pathophysiology of plaque rupture. Atheromatous lesions are especially prone to disruption. ET = endothelial activation, MMP = matrix-degrading metalloproteinases, NO = nitric oxide activity, TIMP = tissue inhibitors of metalloproteinases. Reprinted from Ref. No. 6 with permission.

Inflammation and Atherosclerosis

Inflammation is a key feature of plaque disruption in the acute coronary syndromes. Histologically, atherosclerotic lesions contain immunocompetent cells, including macrophages and T lymphocytes, in addition to smooth muscle cells of the arterial wall, accumulations of lipids, and extracellular matrix components.^{8, 11} Because acute coronary events commonly result from thrombosis triggered by disruption of an atherosclerotic plaque, functional attributes—rather than the degree of stenosis alone—determine the propensity of atherosclerotic plaques to provoke acute manifestations. Evidence supports the role of the potent procoagulant tissue factor in inciting the thrombosis that causes most acute coronary syn-

dromes.¹² Macrophage content and expression of tissue factor correlate with rupture and instability of the atherosclerotic plaque.⁸ Macrophage-derived matrix metalloproteinases can digest the plaque's extracellular matrix and thus impair its stability and permit access of blood coagulation proteins to tissue factor in the lipid-rich core, which incites thrombosis.⁷

Various studies have suggested the involvement of humoral and cellular immune reaction at all stages of arteriogenesis. In addition to macrophages, T cells localize in regions of plaque rupture. T lymphocytes can make up nearly 20% of the cell population in regions of plaques.¹³ Data also suggest that smooth muscle cells, attracted from the media into the intima, enter the intima after lymphocytes. The majority of T cells express two markers of activation, including the major histocom-

FIG. 2 Characteristics of vulnerable and stable plaques. Reprinted from Ref. No. 8 with permission. SMC = smooth muscle cells, HLA-DR = human leukocyte antigen-DR.

patibility complex class II HLA-DR antigen, the interleukin-2 receptor, and integrins.¹⁴

The CD40 Signaling Pathway

T lymphocytes, endothelial cells, smooth muscle cells, platelets, and macrophages within the atherosclerotic vessel wall express the surface molecule CD40 ligand,⁷ known to play a major role in several immunologic pathways.¹⁵ Ligation of CD40 on endothelial cells and macrophages activates several functions related to atherogenesis, including the expression of adhesion molecules, cytokines, and matrix metalloproteinases,

thus indicating a role of CD40–CD40 ligand interactions in atherosclerosis. CD40 and CD40 ligand localize in situ in human and mouse atherosclerotic lesions.¹⁶ Recent investigation has shown that inhibition of CD40 signaling reduced lesion formation in vivo in a mouse model of atherosclerosis.¹⁷ Furthermore, experiments have demonstrated that CD40 signaling by T cells mediates the contact-dependent expression of active tissue factor by vascular cells involved in atherogenesis.¹⁸

The CD40 signaling pathway may mediate cellular interactions and inflammatory responses involved in atherogenesis by regulating antigen-specific T-cell responses to yield activation. The presence of functional CD40 ligand on nonleukocytic cells associated with atherosclerotic lesion indicated a novel T cell-independent route of inflammatory activation, an important component of atherogenesis. CD40–CD40 ligand interactions may play a crucial role in atherosclerosis.⁷

Thrombosis and Healing: A Dynamic Regulation

Evidence exists that plaque rupture occurs frequently, even in asymptomatic individuals.⁷ The majority of plaque disruptions do not produce clinical manifestations. However, most arterial thrombi either fail to occlude the vessel or are transitory. A healing thrombus, although clinically silent, may still contribute to plaque growth. Platelets contain potent fibrogenic mediators, including platelet-derived and transforming growth factor. Thrombin and activated factor X can stimulate smooth muscle cell proliferation by direct and/or indirect routes. At a molecular and cellular level, elements interplay to govern the thrombogenicity of the atherosclerotic plaque (Fig. 3).⁸ Local prevailing hemostatic and fibrinolytic balance likely proves decisive in determining the fate of a given disruption.

FIG. 3 Interplay of elements that govern the stability of the atherosclerotic plaque. IFN- γ = interferon- γ , CD40L = CD40 ligand, IL-1 = interleukin-1, TNF- α = tumor necrosis factor- α , MCP-1 = macrophage chemoattractant protein-1, M-CSF = macrophage colony-stimulating factor. Reprinted from Ref. No. 8 with permission.

Mechanisms of Plaque Disruption Leading to Thrombus Formation

Three mechanisms of plaque rupture exist: rupture of the fibrous cap, superficial erosion of the endothelial layer, and iatrogenic plaque disruption. Rupture of a thin fibrous cap resulting in exposure of flowing blood to the lipid core of the plaque engenders the vast majority of coronary thrombi, accounting for about two thirds of fatal myocardial infarctions (MIs).^{6,8} Endothelial erosion accounts for at least one fourth of significant coronary thrombi and is observed more often in younger individuals and in women with less luminal narrowing and calcification.¹⁰ Symptoms can result when larger areas of endothelial erosion give rise to thrombus formation without hemorrhage into the plaque. In addition, iatrogenic plaque disruption is the result of percutaneous coronary interventions. Platelets bind to the strut of the stent and combine with the caseous plaque material herniating through the struts. The resulting effect is platelet activation leading to thrombus formation.

Platelet Adhesion and Activation

Platelets have been recognized as being pivotal in the initiation and propagation of coronary thrombosis.¹⁹ At the site of the ruptured plaque, platelets form an initial monolayer and a variety of activators (collagen, adenosine diphosphate [ADP], epinephrine, serotonin) promote platelet activation. Platelets anchor to the arterial subendothelial matrix through the simultaneous interaction of von Willebrand factor protein and glycoprotein (GP) Ib that bind to collagen within the subendothelium. Binding to arterial subendothelium activates platelets, causing change from a smooth discoid shape to a spiculated form and degranulation of the alpha and dense granules, releasing platelet aggregatory and chemoattractant agents.²⁰ The GPIIb/IIIa molecule is activated and undergoes a conformational change, developing a high affinity for fibrinogen. Because fibrinogen is a multivalent molecule, it facilitates platelet cross-linking, creating a growing platelet aggregate.²¹ Degranulated platelets release ADP, which binds to the ADP receptors on the surface of neighboring platelets, initiating an amplification process resulting in their activation and degranulation. Activation of cyclooxygenase in platelets converts arachidonic acid to thromboxane (TX) A₂, which binds to TXA₂ receptors, activating neighboring platelets and potentially resisting thrombolysis. The result of these processes is the creation of platelet aggregates and the formation of a potentially occlusive thrombus.^{22,23}

The coagulation pathway is activated on exposure to tissue factor at the site of ruptured plaque.²⁴ Tissue factor activates factors VIIa and X, leading to the clotting cascade and conversion of prothrombin to thrombin, which converts fibrinogen to fibrin. Thrombin participates in autoamplification via thrombin receptors on platelets that further activate the coagulation cascade. The enlarged thrombus may be completely occlusive,

and it contains cross-linked fibrin strands with entrapped red blood cells. Thus, superimposed on the platelet-rich thrombus, it triggers an acute coronary event, such as MI.^{22,23}

Inhibitors of Platelet Activation

Three primary players are critical to platelet activation: thrombin, TX, and the ADP receptor. Fortunately, antithrombotic agents are available to inhibit each (Fig. 4).²⁵ Aspirin irreversibly acetylates cyclooxygenase, blocking the platelet synthesis of TXA₂. Heparin acts by accelerating the action of two naturally occurring plasma inhibitors: antithrombin III, which inhibits thrombin and activated clotting factors; and at higher dosages, heparin cofactor II, which acts on thrombin decay. GPIIb/IIIa inhibitors block binding of fibrinogen and prevent aggregation of platelets.^{21,26} The thienopyridine class of agents (clopidogrel and ticlopidine) noncompetitively blocks the ADP receptor, inhibiting ADP pathway activation, the key amplification loop in platelet activation.²⁷ A rationale exists for combining antiplatelet therapies to prevent platelet activation via multiple mechanisms (Fig. 4).

Conclusion

In the last decade, intensified clinicopathology investigations, improved animal models, and increased insights at the levels of cell and molecular biology have led to advances in the understanding of atherosclerosis development and its clinical sequelae. It is apparent that we stand on the threshold of a new era of understanding the pathophysiologic mechanisms underlying acute coronary syndromes. This will translate directly into better clinical outcomes through the availability of therapeutic agents targeted at these mechanisms. An effective antiplatelet regimen for the treatment of atherosclerotic vascular disease may include administration of two or more agents, each directed at a specific site of platelet activation.

FIG. 4 Mechanism of action of oral antiplatelet agents. ADP = adenosine diphosphate, TXA₂ = thromboxane A₂, COX = cyclooxygenase. Data adapted from Ref. No. 25.

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