The Role of Platelet Activation in the Pathogenesis of Atherothrombosis

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The Role of Platelet Activation in the Pathogenesis of Atherothrombosis
A CME-certified Monograph

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Learning Objectives
After participating in this activity, learners will be able to:

- Understand the integral link between platelet aggregation and the coagulation cascade
- Recognize the role of platelet activation and aggregation in atherothrombosis
- Assess the rationale for therapeutic interventions to prevent platelet activation and aggregation

Program Overview
Platelets are key players in the events leading to atherothrombosis and the clinical manifestations of acute coronary syndromes (ACS). They are early participants in inflammation leading to plaque formation, produce many of the factors that promote platelet aggregation, and are central to coagulation. In this module, participants will examine the steps leading to platelet adhesion, activation, aggregation and eventual thrombus formation. Participants will also consider the platelet’s role in coagulation and rationale for current antiplatelet therapies.

Target Audience
This educational activity has been developed for physicians and other health care providers in primary care, cardiology, and emergency medicine who manage ischemic risk in patients with ACS in the acute, periprocedural, and chronic settings.

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She has received grant support from and served on a speakers’ bureau for Schering Corporation, and has served as a consultant for Bristol-Myers Squibb and sanofi-aventis.

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• Is vetted through a process to resolve any conflicts of interest of planners, presenters, or authors
• Is evaluated for its effectiveness in meeting identified educational needs
• Provides information toward improvements in the quality and safety of health care

An environment that
• Supports learners’ abilities to meet their individual educational needs
• Respects and attends to any special learner needs
• Respects diversity among learners
• Is free of promotional, commercial, and/or sales activities
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Introduction
Atherothrombosis, the formation of a thrombus at the site of atherosclerotic plaque disruption, can occur in any of the 3 arterial trees—cerebral, coronary, or peripheral—and results in clinical manifestations of stroke, acute coronary syndromes (ACS), or peripheral arterial disease. Except for the extraordinary flu of 1918, atherothrombosis has been the major cause of death in the U.S. for every year since 1900.  
Platelets are key players in the events leading to atherothrombosis: they are early participants in inflammation leading to plaque formation, produce many of the factors that promote platelet aggregation, and are central to coagulation.  
Platelets usually flow over the endothelium and are not ordinarily attracted to it. The normal vascular endothelium releases several different antithrombotic and fibrinolytic factors, including the small molecules nitric oxide (NO) and prostacyclin (PGI2), an ectoADPase (CD39), tissue factor pathway inhibitor-1 (TFPI-1), and tissue plasminogen activator (t-PA). Platelets are programmed to respond to vascular injury, whether resulting from trauma or from plaque disruption. Injury exposes the prothrombotic subendothelium and promotes platelet recruitment, adhesion, and spreading. Adhesion to the endothelial substratum or subendothelium, in turn, promotes platelet activation and release of prothrombotic agents, ultimately resulting in platelet aggregation and thrombus formation. The coronary arterial tree is probably the best understood of the 3 arterial trees, so an exploration of the role of platelets in ACS will serve to illustrate this process. We will examine the role of platelets in each of the steps, adhesion, activation, aggregation, and thrombus formation resulting in ACS, to help us understand how antiplatelet agents are used in preventing and treating atherothrombotic disease.

Platelet adhesion
Damage to the endothelial lining of the arterial wall exposes collagen of the subendothelium. Within seconds, circulating von Willebrand factor (vWF) binds to exposed collagen, and platelets are recruited and initially tethered to the vWF through their surface glycoprotein (GPIIb. Under conditions of high shear stress, seen in small arteries partially obstructed by atherosclerosis, circulating vWF can also initiate direct platelet aggregation. This mechanism is probably involved in the increased risk of acute coronary events seen in patients with angina and elevated levels of circulating vWF.
Following platelet tethering via vWF and GPIb, more stable interactions take place between collagen and platelet collagen receptors, particularly the immunoglobulin superfamily receptor GPVI and the integrin αIIbβ3. These interactions adhere the platelets closely to collagen and lead to signaling and activation.

Platelet activation
Following platelet adhesion to the endothelium, interactions between collagen and platelet GPVI and with other platelet surface receptors such as GPIIb/IIIa cause activation, platelet secretion, and, via inside-out signaling, a conformational change in GPIIb/IIIa that encompasses a host of intracellular signals termed platelet activation.
Increases in cytosolic calcium and remodeling of the cytoskeleton allow platelets to change shape from a disc, seen in freely flowing blood, to a sphere that rolls along the exposed subendothelium in contact with vWF, until it is firmly but reversibly attached to the surface through αIIbβ3 (GPIa-IIa) and GPVI. The attached platelet then flattens, extensively spreads, and irreversibly adheres to the surface through activated GPIIb/IIIa (Figure 1).
Platelet activation causes microtubular contraction, generates arachidonic acid, and induces the release of endogenous granules containing adenosine diphosphate (ADP) and serotonin, as well as growth factors, cytokines, adhesive molecules, and coagulation factors into the extracellular milieu. These factors promote smooth muscle cell migration and proliferation, endothelial cell activation as well as platelet— platelet and platelet—leukocyte interactions.
The amplification of activation is facilitated through molecular binding to 3 main receptor types on the platelet surface: the P2Y₁ and P2Y₁₂ ADP receptors, the thromboxane A₂/prostaglandin H₂ (TH) receptor, and the thrombin protease-activated receptors (PARs) (Figure 2).¹⁴

P2Y₁₂ receptors amplify and sustain platelet activation in response to ADP binding, and these receptors are key components for platelet aggregation.¹⁴ ADP is thought to potentiate both collagen- and thrombin-stimulated thrombin generation through the P2Y₁₂ receptor.¹⁵ Because of this role in platelet aggregation, the P2Y₁₂ receptor has been of considerable interest as a target for the development of antiplatelet agents. For more information on P2Y₁₂ receptor antagonists and other antiplatelet agents, please view Module 3, Novel Antiplatelets in Development, of the Coronary Invaders interactive CME activity at www.coronaryinvaders.com.

Platelets have 2 different types of thrombin receptors, PAR1 and PAR4. PAR1 is considered to be the major site through which thrombin promotes platelet activation. Injured endothelium releases tissue factor (TF), which promotes thrombin generation. The released thrombin binds to platelet PARs, cleaving the N-terminus and by a tethered ligand mechanism activates the PAR1 and PAR4 receptors mediating a potent platelet response.⁴,¹⁷,¹⁸

In all, platelet activation releases adhesion molecules, chemokines, coagulation and fibrinolytic factors, growth factors, and transmitters, resulting in a change in platelet morphology and, particularly, membrane characteristics that permit and enhance ligand binding to activated membrane GPIIb/IIIa molecules.¹⁹,²⁰

Activated platelets release arachidonic acid, leading to thromboxane A₂ (TxA₂) generation and further platelet activation through the TH receptor.⁶ Aspirin, the most widely used antiplatelet agent, inhibits production of TxA₂, thereby reducing the amount of this potent agonist available to bind to the TH receptor.¹⁶


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Platelet aggregation
The initial platelet monolayer formed by interaction with the endothelial substratum allows for the recruitment and aggregation of additional platelets, resulting in thrombus growth. Key in this process is the activation of GPIIb/IIIa to a high-affinity state.

Initial platelet-platelet interactions are likely similar to initial platelet interaction with the endothelial substratum; transient adhesion of one platelet to another triggers release of ADP and TxA₂ in the immediate vicinity, leading to sustained activation of the recently adherent platelet, and forming a firmer adhesion.¹⁸

Signals generated within activated platelets cause conformational changes in transmembrane GPIIb/IIIa, exposing previously cryptic fibrinogen and vWF binding sites. Dimeric binding of fibrinogen (and other adhesive proteins such as fibronectin) or multimeric binding of vWF crosslinks adjacent platelets, allowing the buildup of microaggregates containing as many as 20 cells.²¹⁻²³ Sustained activation results in the formation of irreversible aggregates or the thrombus.

Platelets and coagulation
Although coagulation was originally depicted as a proteolytic enzyme cascade, more recently, Hoffman and Monroe have proposed that overlapping steps occurring on specific cell surfaces are responsible.²⁴⁻²⁶

For effective coagulation, clot formation must be restricted to the site of the injury and not propagated throughout the vascular tree. This can be accomplished by localizing the coagulation to specific cell surfaces. In their model, Hoffman and Monroe propose that different cells in the vasculature play either procoagulant or anticoagulant roles, maintaining a homeostatic balance between the 2 processes. Adherent, activated platelets and platelet microaggregates play the procoagulant role, and vascular endothelial cells play the anticoagulant role.²⁷

This view of coagulation requires 2 cell types; TF-bearing cells and platelets, which provide the procoagulant surface and several coagulation factors. Physical separation of the 2 cell types keeps coagulation from initiating until there is an injury. Coagulation is hypothesized to occur in 3 overlapping stages: initiation, amplification, and propagation.²⁶
Monocytes and endothelial cells are the 2 cell types outside the vasculature that express TF in the initiation phase. These cells normally convert low levels of prothrombin to thrombin in the extracellular space. This initiation process does not normally lead to clot formation. However, once platelets have adhered to the site of injury, thrombin amplifies the prothrombinase complex on the platelet surface through several different pathways. Thrombin activates nearby platelets through the PAR1 receptor, it activates factors V and VIII on the platelet surface, and it releases factor VIII bound to vWF. These activities result in large amounts of thrombin generated at the platelet surface.

During the propagation phase, thrombin leaves the platelet and stabilizes the forming clot by cleaving peptides from fibrinogen to allow polymerization into insoluble fibrin. Fibrin binds thrombin, which is incorporated into the enlarging clot and acts to increase thrombin production. Thrombin binding to both PAR1 and PAR4 results in a potent platelet activation response. PAR4 requires the increased levels of thrombin produced during the propagation phase. This crucial role of thrombin has lead to the development of pharmacologic agents that prevent the cellular affects of thrombin by blocking PAR1.

**Platelets and atherothrombosis**

Coagulation is a normal response to vascular injury, arresting hemorrhage and promoting wound healing. In the homeostatic state, prothrombotic pathways are kept in check by antithrombotic pathways, and unneeded intravascular thromboses are avoided. This balance is upset in atherothrombosis—disruption of an atherosclerotic lesion with subsequent thrombus formation—and procoagulant pathways become dominant.

Although the role of platelets in thrombus formation is well established, there is evidence that platelets may also play a role in the inflammatory process that produces the initial atherosclerotic plaque. Atherosclerosis has many procoagulant characteristics, including excess TF production, decreased amounts of NO, exposed collagen, and vWF expression.

Platelets are thought to play a key role in initiation of atherosclerosis through their ability to attract monocytes into the arterial wall, thereby creating endothelial dysfunction. In addition, platelets produce inflammatory mediators, growth factors, and adhesion molecules that play a role in this process. Two platelet inflammatory mediators in particular, RANTES (regulated on activation, normal T-cell expressed and secreted) and CD40 ligand, have been singled out as important in atherothrombotic disease.

There is evidence that heightened platelet reactivity plays a part in the atherothrombotic process. Circulating levels of vWF, fibrinogen, and platelet factor 4 are increased in patients with ACS.

In summary, platelet activation and subsequent thrombus formation occur in response to vascular injury via multifaceted adhesive events with cells that modulate inflammation and coagulation. A better understanding of the critical role of the platelet in these events provides a clear rationale for employing antiplatelet agents to selectively harness integral pathways in the pathology of atherothrombosis and prevent its progression.
References

29. Selwyn AP. Prothrombotic and antithrombotic pathways in acute coronary syndromes. Am J Cardiol. 2003;91:3H-11H.
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Post-test
1. Which of the following receptors is responsible for the tethering of platelets to vWF bound to exposed collagen?
   a. P2Y₁₂
   b. GP Ib
   c. GP IIb/IIIa
   d. PAR 1

2. Upon activation, platelets degranulate which of the following?
   a. ADP
   b. Adhesive molecules
   c. Coagulation factors
   d. Serotonin
   e. All of the above

3. Which receptor is responsible for amplifying and sustaining platelet activation in response to ADP binding?
   a. GP Ib
   b. GP IIb/IIIa
   c. PAR 1
   d. P2Y₁₂
   e. PAR 4

4. Injured endothelium releases__________which promotes thrombin generation.
   a. vWF
   b. ADP
   c. Thrombin
   d. Tissue Factor
   e. All of the above

5. Binding of which of the following to the GP IIb/IIIa receptors allows for crosslinking of adjacent platelets?
   a. vWF
   b. Fibrinogen
   c. Thrombin
   d. a and b
   e. All of the above
Evaluation

Please circle the number that best reflects your opinion of the following statements:

<table>
<thead>
<tr>
<th></th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>As a result of participating in this activity, are you now better able to:</td>
<td></td>
<td></td>
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<tr>
<td>Understand the integral link between platelet aggregation and the coagulation cascade?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Recognize the role of platelet activation and aggregation in atherothrombosis?</td>
<td>1</td>
<td>2</td>
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<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Assess the rationale for therapeutic interventions to prevent platelet activation and aggregation?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<th></th>
<th>Poor</th>
<th>Fair</th>
<th>Satisfactory</th>
<th>Very Good</th>
<th>Exceptional</th>
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<tr>
<td>How would you rate the overall quality of this activity?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
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Program Value

1. Please identify the most important thing you learned that will affect the care of patients in your practice:
   - [ ] The link between platelet aggregation and the coagulation cascade
   - [ ] The role of platelet activation and aggregation in atherothrombosis
   - [ ] The rationale for therapeutic interventions to prevent platelet activation and aggregation
   - [ ] Other (please describe): ________________________________

2. As a result of this program, how will you most likely improve care of ACS patients in your practice setting?
   - [ ] I better understand the contribution of platelet hyperreactivity to thrombus formation
   - [ ] I can better explain the rationale for antiplatelet regimens, including dual antiplatelet therapy, to my patients
   - [ ] I better understand the potential therapeutic role of novel antiplatelet agents for my patients with ACS
   - [ ] Other (please describe): ________________________________

3. What barriers prevent optimal, evidence-based care for your ACS patients? (Check all that apply.)
   - [ ] Lack of clear guidelines regarding appropriate treatment for patients at continued risk for thrombotic events despite treatment with available antiplatelet agents
   - [ ] Concerns about bleeding risk
   - [ ] Lack of familiarity with how to apply new clinical trial evidence to practice setting
   - [ ] Financial or time constraints in proper patient assessment
   - [ ] Other (please describe): ________________________________
4. Based on the information presented at this program, will you change your approach to managing patients with ACS?

☐ Definitely ☐ Most likely
☐ Not very likely ☐ No

5. This educational program was fair, balanced, and free of commercial bias.

☐ Yes ☐ No
If no, please explain: ________________________________

6. What additional topics should be covered in future CME activities, to inform and enhance your clinical practice?

________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________

7. What kind of future educational activities can best help you improve ACS patient care in your practice setting?

☐ Case-based simulations that permit application of data and guidelines to real-world practice
☐ Didactic presentations by national experts
☐ Workshop-like activities that simulate application of new data to treatment protocols
☐ Presentations on how other practice settings have successfully improved ACS care
☐ E-based learning that permits personal exploration of data and information
☐ Other (please describe): ________________________________

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