Commentary

Past, present, and future of anti-platelet therapy

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Abstract

Introduction: Anti-platelet drugs are useful in preventing unwanted clots, but the complexities of platelet activation and clot formation are challenging.

Background: Platelets can be activated by a variety of agents, including natural biomolecules, foreign materials, and drugs. Calcium mediates a number of the intracellular processs Once activated, platelets release factors that act on other circulating cells and vascular endothelial cells to promote formation of a clot. The original anti-platelet drug, aspirin, inhibits cyclooxegenase, interfering with a crucial step in the biochemical cascade. Aspirin is cost-effective but limited in its application. Newer drugs, ticlopidine and clopidogrel, act on the activation pathway at different points, so they can supplement aspirin.

New Directions: Abciximab represents a new generation of antiplatelet drug, being an antibody that binds to platelet surface receptors, thus inhibiting growth of thrombus. Other potential sites for antibody intervention are extracellular matrix and endothelial surface components. As new drugs are developed it becomes more imperative to find assays of platelet function that are sensitive and cost-effective.

Conclusion: Although much progress has been made in anagement of clotting significant opportunities and challenges remain, both in treatment and in measurement of treatment effectiveness.

Key words: platelet, clotting, anti-platelet drugs.

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Abbreviations, in the order used in this report.

IP3:	inositol 4, 5 bisphosphate
GP:	glycoprotein
vWF:	von Willebrand factor
COX:	cyclooxygenase
ADP:	adenosine diphosphate

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Introduction

Platelet activation is a topic of major concern to stroke specialists because of its roles in producing thrombus, contributing to atherosclerotic plaque, and sealing off a bleeding vessel. The mechanisms involved are complex, which, on the one hand, means that there are many biochemical opportunities to regulate the processes, but on the other hand, provides many points of autoregulation that may interfere with our attempts to manage the system. In this brief review we will first consider the known mechanisms of platelet function and then address our progress in developing anti-platelet drugs.

Background

Platelet Mechanisms

Blood platelets interact with a variety of soluble agonists, such as epinephrine and adenosine diphosphate, and many insoluble cell matrix components including collagen, laminin, and biomaterials used for the construction of invasive medical devices.[1] These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and non-integrins) on the plasma membrane and lead to the activation of intracellular effector enzymes.

The majority of regulatory events appear to require free calcium. Ionized calcium is the primary bioregulator, and a variety of biochemical mechanisms modulate the level and availability of free cytosolic calcium. Major enzymes that regulate the free calcium levels via second messengers include phospholipase C, Phospholipase A2, and Phospholipase D, together with adenylyl and guanylyl cyclases. Activation of phospholipase C results in the hydrolysis of phosphatidyl inositol 4, 5 bisphosphate and formation of second messengers 1, 2–diacylglycerol and inositol 4, 5 bisphosphate (IP3). Diglyceride induces activation of protein kinase C, whereas IP3 mobilizes calcium from internal membrane stores. Elevation of cytosolic calcium stimulates phospholipase A2 and liberates arachidonic acid. Free arachidonic acid is transformed to a novel metabolite, thromboxane A2 by fatty acid synthetase (Cox-1, cyclooxygenase). Thromboxane A2 is the major metabolite of this pathway and plays a critical role in platelet recruitment, granule mobilization, and secretion.

Platelet secretory granules contain a variety of growth factors, mitogens and inflammatory mediators. Secretion of granules promotes p-selectin expression on the platelet membrane. Furthermore, activation also promotes the expression of acidic

lipids on the membrane and tissue factor expression, thus making these cells pro-coagulant. Fully activated platelets can modulate the function of other circulating blood cells such as leukocytes, monocytes, and macrophages as well as vascular endothelial cells. Agonist-mediated stimulation of platelets promotes the expression of an epitope on glycoprotein (GP) IIb/IIIa receptors. Activation of this receptor is essential for the binding of circulating fibrinogen.

Fibrinogen forms a bridge between individual platelets and facilitates thrombus formation. Unlike fibrinogen that can bind platelets at low shear, von Willebrand Factor (vWF) binds platelet GP IbIX complex only under high shear conditions. Up-regulation in signaling pathways will increase the risk for clinical complications associated with acute coronary events. Down-regulation of signaling pathways may precipitate bleeding diathesis or hemorrhagic stroke. Based on this knowledge about various activation mechanisms current anti-platelet drugs are being developed for therapeutic applications.

Anti-platelet Drugs

Aspirin (acetyl salicylic acid), one of the most cost-effective drugs for secondary prophylaxis of vascular diseases, is an irreversible inhibitor of platelet cycooloxygenase (COX). Inhibition of this enzyme prevents the formation of pro-aggregatory metabolites of arachidonic acid, prostaglandin G_2 and prostaglandin H_2. Aspirin found its application as anti-platelet drug even before the discovery of pro-aggregatory prostaglandins.

Aspirin has a short half life in circulating blood. Once it is metabolized the metabolite, salicylic acid, is not an effective inhibitor of platelet function. Furthermore, new platelets with active COX-1 enzyme are added continuously to the circulating blood by the bone marrow. These new platelets can generate pro-aggregatory prostaglandins and activate aspirin-exposed platelets in the circulating blood. Studies from our laboratory in early 1980s demonstrated that in the presence of epinephrine, aspirin-exposed platelets aggregate in response to the action of arachidonate.[2] Arachidonate-induced activation is one of the many pathways of platelet activation, and other agonists can still activate aspirin exposed platelets. Even with these limitations, aspirin at low doses (80-160 mg) has been shown in number of major clinical studies to offer benefit in preventing acute vascular events.

In view of the successful use of aspirin for secondary prophylaxis of vascular diseases, aspirin was adopted as the "gold standard" of anti-platelet therapy. However, as is evident from many clinical studies, a large number of individuals do not seem to get the protection they need from aspirin alone. Therefore, drugs belonging to the thienopyridine group were developed for anti-platelet therapy.[3] Agents such as ticlopidine and clopidogrel exert their anti-platelet activity by binding to the ADP receptor (P2Y12), irreversibly modifying it. An oral clopidogrel loading dose of 300-600 mg produces significant inhibition of ADP-mediated platelet aggregation within 2 hours of administration, which becomes maximal after 6 hours. The parent compounds are not active inhibitors of platelet aggregation; it is their metabolites that are active, hence the delay in the in vivo inhibitory response.

Thienopyridines were considered super aspirins in the earlier clinical trials. However, the results of clinical studies show that compared with aspirin, these drugs are only modestly more effective in preventing serious vascular events in high risk patients. In addition, there is considerable concern about the possibilities of patients developing resistance to thienopyridines after prolonged daily use.

Dual Therapy

Results from the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trail showed that a combination of aspirin and clopidogrel did not demonstrate a statistically significant reduction in the risk of heart attack, stroke or cardiovascular death compared to placebo and aspirin in broad population of patients.[4] However, in those patients undergoing placement of a drug eluting stent, the American Heart Association and American College of Cardiology suggest the use of dual anti-platelet therapy for at least 12 months.[5] Some experts suggest a continued dual therapy until new data with longer follow-up is available. An investigational anti-platelet drug, prasugrel, plus aspirin has been shown to produce a highly significant reduction in risk for coronary stent thrombosis.[6,7] Although prasugrel has been shown to decrease ischemic events, it also has been associated with increased risk of major bleeding especially in patients with a history of stroke or transient ischemic attack.

New Directions

Earlier studies from our laboratory demonstrated that currently available anti-platelet drugs, such as aspirin and clopidogrel do not inhibit platelet interaction with components of the vessel wall or cell matrix, as these interactions are mediated by platelet receptors GP VI and GP1b[8] while the majority of anti-platelet drugs prevent signaling events leading to the activation of GP IIb/IIIa receptors which are essential for fibrinogen binding, cell-to-cell interaction, and thrombus growth. Coller and associates[9] developed a monoclonal antibody (7E3) to platelet GPIIb/IIIa receptor and successfully demonstrated its ability to inhibit platelet aggregation and thrombus growth. Abciximab (previously known as c7E3 Fab) is one of the best examples of development of a drug (*Reopro*) from laboratory to clinical applications.[10] Abciximab is used throughout the world to prevent acute vascular events during interventional procedures.

The success of abciximab therapy during interventional procedures prompted researchers to consider development of receptor blockers that are capable of inhibiting platelet interaction with cell matrix components. In the current issue of the Journal of Vascular and Interventional Neurology, there is a short overview titled "Novel Therapies in the Pipeline: Directions of Research into Platelet Inhibition". The authors speculate that inhibition of vWF interaction with platelet GP Ib receptor may serve as an important mechanism in the management of acute stroke. Similarly there is considerable interest in the development of inhibitors for collagen, another major cell matrix component on the injured vessel surface, and its interaction with platelet receptor GP VI. Preliminary studies by Kleinschintz et al.[11] and Gilebert et al.[12] have explored such possibilities. Large numbers of clinical studies are needed to prove the efficacy, safety and benefit of these approaches. As collagen and vWF are major substrates of interaction for the formation of effective haemostatic plugs for the arrest of bleeding from the blood vessels, one should carefully consider the effect of preventing such interaction on serious bleeding episodes.

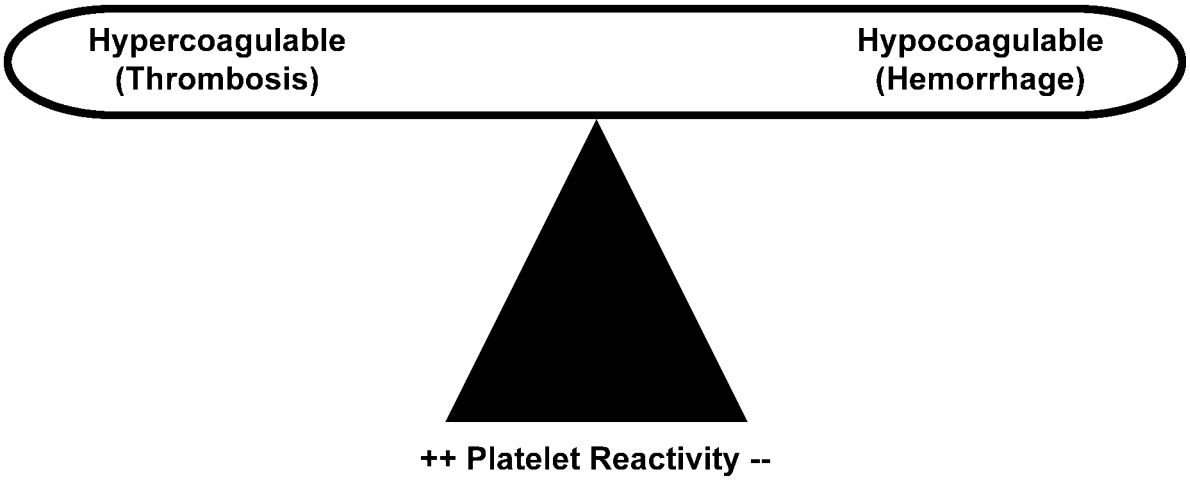

Figure 1. A needed haemostatic profile.

Clinical Monitoring

When considering the future of anti-platelet or antithrombotic therapies, it is important to think about developing assays to monitor the efficacy of such treatments. More than five decades ago the now famous Framingham studies demonstrated the significance of altered lipid metabolism and the associated increased risk for acute vascular events. This awareness promoted the use of lipid lowering drugs, which in turn prompted the development of efficient and sensitive clinical assays to monitor blood lipids. Although anti-platelet drugs have been in use much longer than the lipid lowering drugs, no similar point-of-care assays are available for monitoring effect of anti-platelet and anti-thrombotic therapies. A sensitive and specific, yet rapid and inexpensive screening test that detects predisposition to thrombosis or bleeding, be it sensitive to aspirin, clopidogrel, or GP IIb/IIIa antagonists, would be clinically useful.[13] The test should simply provide the statues of platelet function (as shown in Figure 1) to determine whether the patient is in a hyper- or hypocoagulable state.

Studies are in progress at the Thrombosis Research Laboratories of the University of Minnesota to develop point-of-care assays for monitoring global hemostasis, so that individuals at high risk for developing acute vascular events can be screened and customized therapy provided.

When we think of past efforts in the development of anti-platelet and antithrombotic therapies, aspirin, persantine, heparin, and coumadin come to mind. Current therapy revolves around aspirin, clopidogrel, abciximab, heparin, coumadin, and low molecular weight heparins. Future therapies will involve newer anti-platelet drugs such as receptor antagonists for GP VI and GP Ib. Once we have achieved the ability to monitor global hemostasis and identify those individuals who are at risk for developing thrombotic or hemorrhagic episodes, attention will be given to customized therapy for high-risk individuals.

Conclusion

Although challenging, the cellular and molecular events leading to activation of platelets and formation of clots can be manipulated with drug therapies. Recent developments have led to drugs with a variety of effects, but there is a need for more effective and specific agents. In parallel with drug development, there is a need for analytical tools that can aid drug testing in the laboratory and treatment planning in the clinic.

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