Review

Platelet–Endothelial Interactions in Atherothrombotic Disease: Therapeutic Implications

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Summary: The role of the platelet and the endothelium in the pathogenesis of atherosclerosis and subsequent ischemic events has been the subject of extensive investigation. Arterial sites where endothelial function is severely impaired are often the sites of atheroma development. Lesion evolution impairs endothelial function, leading to a self-perpetuating cycle of growth. During early lesion development, overt thrombotic events are rare. However, rupture of an advanced, necrotic plaque or intimal ulceration triggers arterial thrombosis, at which point the importance of platelet function may be seen clearly. The Antiplatelet Trialists' Collaboration meta-analysis demonstrated the benefit of antiplatelet therapy to patients with atherosclerotic disease. Aspirin is the most widely studied agent and is considered the standard of antiplatelet therapy. Newer agents that intervene at different stages of the platelet activation pathway have been developed. Clopidogrel, a new adenosine diphosphate receptor antagonist, is more effective than aspirin in reducing vascular events in patients with prior myocardial infarction, stroke, or established peripheral arterial disease. The glycoprotein IIb/IIIa antagonists such as abciximab have proven effective in the setting of active arterial thrombosis and percutaneous revascularization, but their value in secondary prevention remains unknown. All patients with atherosclerosis should be treated with an antiplatelet drug. Current evidence suggests that either aspirin or clopidogrel are appropriate first-line agents. There is urgent need for an analysis of the risk/benefit ratio in various populations and clinical settings to determine the most appropriate type and intensity of therapy for a given patient.

Key words: atherosclerosis, endothelium, platelet, aspirin, clopidogrel, ticlopidine, glycoprotein IIb/IIIa

Introduction

Atherosclerosis is one of the leading causes of death and disability in the United States. In 1995 there were 481,287 deaths attributable to coronary heart disease. The American Heart Association estimates that 1,100,000 Americans will experience an unstable coronary syndrome this year. Within 6 years of a myocardial infarction (MI), almost a third of survivors will experience a second event, a fifth will be limited by congestive heart failure, and a tenth will suffer sudden cardiac death.¹ These sequelae of the thrombotic progression of atherosclerosis have stimulated research leading to an improved understanding of the complex processes involved in endothelial function, plaque instability, platelet activation, and the formation of thrombi. This review will describe the underlying pathophysiology of the atherothrombotic process and discuss the role of antiplatelet therapy in the prevention of ischemic events.

Early Atherosclerosis

Advanced atherosclerotic lesions responsible for acute ischemic events or significant blood flow limitation are the culmination of a process that may begin as early as childhood. Abnormal endothelium or normal endothelium exposed to physical or biochemical stresses adversely affecting its function is believed to play a central role in the development of these lesions. Endothelium maintains vascular integrity, regulates the passage of macromolecules and inflammatory cells, and may influence the growth and behavior of arterial wall components through signaling molecules such as endothelin, prostacyclin, and nitric oxide (NO).^{2,3} More important, in the setting of established atherosclerosis, normal endothelium works in a complex fashion to provide tonic inhibition to pro-coagulant forces present in blood.^{4,5}

Perturbed endothelium relaxes its discretion in maintaining vascular integrity and regulating macromolecule transport.

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Oxidized low-density lipoprotein (LDL) accumulates within the arterial intima, and expression of complex molecules such as P-selectin promote platelet and inflammatory cell adherence.^{4, 6, 7} This facilitates the ingress of macrophages intent upon clearing excess lipid.⁸ The macrophage becomes engorged, giving its cytoplasm a foamy appearance on light microscopy.⁹⁻¹¹ Sites of lipid/foam cell accumulation are visible grossly as yellow streaks on the intimal surface of large- and medium-sized arteries and are known as fatty streaks. The fatty streak is the initial lesion of atherosclerosis. It does not represent a genuine obstruction to blood flow. It does, however, represent the beginning of an atherosclerotic lesion and constitutes a site of persistent endothelial dysfunction.⁷

Conditions Associated with Endothelial Dysfunction

The initiator of endothelial dysfunction and atherosclerotic plaque formation has not been identified with certainty. Epidemiologic studies have identified a variety of risk factors that are associated with atherosclerotic events. These include elevated serum lipid levels, oxidation of LDL particles, elevated intra-arterial pressure, presence of catecholamines, infection, cigarette smoking, and others.^{4, 9, 10, 12} Findings from the Bogalusa Heart Study argue that, in addition to their association with acute events, many of these risk factors are critical to the development of early atherosclerosis.¹³

Hypercholesterolemia, hypertension, homocysteinemia, diabetes mellitus, and cigarette smoking are all associated with reduced endothelial NO production, which, whether measured directly or using surrogate methods such as acetylcholine-induced or flow-mediated vasodilation, is the most readily accessible means of assessing endothelial function (Fig. 1).^{4, 14-20} Prothrombotic and antithrombotic regulatory mechanisms are altered by each of these risk factors.^{4, 21-27} Treatment of elevated LDL cholesterol improves endothelial

function.¹⁶ In addition, cholesterol lowering with statin drugs results in a reduction in the rate of acute ischemic events that are not linearly related to any measurable change in the angiographic appearance of diseased coronary vessels.²⁸⁻³⁰ The natural conclusion to draw from such observations is that endothelial injury or dysfunction, if not the root cause, is the primary accelerant of the atherosclerotic process and that treatment directed to restoring normal endothelial function will slow disease progression.

Lesion Progression

Within an early, established atherosclerotic lesion, lipid accumulation and inflammatory cell recruitment may further influence endothelial function, leading to a self-perpetuating cycle of lesion growth. In advanced lesions, visible superficial platelet accumulation is extremely common, suggesting that the intimal site that has become hostile to normal endothelium allows or encourages intermittent platelet adherence. Cytokine release and adhesion molecule expression may accentuate further macrophage accumulation, vascular smooth muscle proliferation, and collagen deposition.³¹

Within diseased intima, remodeling enzymes produced by activated macrophages erode connective tissue, allowing lesion expansion and cellular migration and destroying normal arterial architecture.⁹ The circumference of the artery may actually increase while lesion expansion maintains a stable central lumen (the Glagov phenomenon).^{32, 33} Within the lesion or lesions, inflammatory cell accumulation, lipid accumulation, and cell death produces a central zone of necrosis heavily invested with inflammatory mediators, lysosomal contents, and procoagulant molecules such as tissue factor.^{9, 31, 34} Wound healing processes that coexist with inflammation or follow it result in the accumulation of vascular smooth muscle cells and ground substance that will eventually be replaced by a dense collagenous stroma. The resulting lesion resembles an abscess with a liquefied, necrotic core bounded by scarred, fibrotic intima whose least stable portion abuts the arterial lumen.

Arterial remodeling described by the pathologist has clinical relevance with respect to the predictive value of traditional imaging studies.³⁵⁻⁴⁰ Angiographic observations from the Coronary Artery Surgery Study have shown that while sites of severe stenosis are most likely to become completely occluded with time, the majority of occlusions, or sites of progression, occur at sites that did not appear to be "critically" diseased.³⁹ In 298 patients observed for more than 5 years, 24% of unbypassed arterial segments with an $\geq 80\%$ diameter stenosis were found to be completely occluded. Only 13% of arterial segments with less severe stenosis were found to be occluded. However, when all sites of total occlusion were examined, 94% of them had $< 80\%$ diameter stenosis at the time of the original angiogram. At first glance, this seems counterintuitive. Arterial sites that appear to be less severely diseased should be less likely to display aggressive evolution. However, remodeling of the vessel allows accommodation of even extensive atherosclerotic lesions while maintaining a lumen that may appear relatively free of disease. Thus, angiography

FIG. 1 Conditions associated with endothelial dysfunction. Adapted from Ref. No. 3.

may quantify the number and severity of stenoses providing a rough estimate of disease burden, but it cannot reliably identify arterial segments that are potentially unstable.

Thrombosis

Lesions that have developed a central necrotic zone are poised on the verge of rapidly increasing their rate of progression. The lateral border or shoulder regions of the lesion cap are well populated with activated macrophages. Should the destructive activity of activated macrophages exceed the rate of new connective tissue matrix deposition, the structural integrity of the lesion cap will be reduced, resulting in rupture of the lesion and exposure of the necrotic core to blood elements. The contents of the necrotic core are an intense stimulus for platelet accumulation and thrombosis. The volume of thrombus that forms is dependent upon the stimulus intensity, blood flow characteristics, and platelet and coagulation enzyme function. This sudden increase in lesion volume frequently critically limits or completely obstructs coronary blood flow resulting in unstable angina or acute MI.^{5, 10, 31, 41, 42}

Inflammation and necrosis may set the stage for lesion rupture and dramatic disease progression. However, this scenario may not be a universal requirement for thrombus formation.^{43, 44} One of the early models of arterial thrombus formation, the Folts model, involved the placement of a constricting ligature around a normal vessel to produce severe stenosis.⁴⁵ Resulting flow aberrations marked by high shear rates produced endothelial injury and accentuated platelet adherence and aggregation that would intermittently cause complete occlusion of the vessel. The cyclic character of arterial flow through the vessel closely resembled the type of flow presumed to be present in patients with unstable coronary syndromes, centering attention on platelet function in unstable angina and acute MI.

Autopsy studies and coronary atherectomy specimens from patients with unstable angina and acute MI have disclosed sites of platelet deposition and arterial thrombosis without plaque rupture or with only intimal hyperplasia, believed to result from mural thrombus accumulation.^{31, 43, 44} This suggests that some episodes of atherosclerosis progression and unstable angina do not require plaque rupture but are critically dependent on endothelial dysfunction, flow aberration, and platelet accumulation. Based upon the Folts model, this form of lesion progression is most likely to occur at sites of severe preexisting stenosis.

Equally important are the observations of Paris Constantinides, whose animal model of plaque rupture and arterial thrombosis has recently been reproduced.⁴⁶⁻⁴⁸ Searching for a reproducible model of atherosclerosis-related thrombosis, Constantinides fed experimental animals a diet that produced advanced atherosclerotic lesions. To his surprise, he observed very few episodes of plaque rupture and thrombosis. The addition of vasoactive substances believed to trigger acute events, such as epinephrine, did little to improve his yield. However, induction of a hypercoagulable state by using Russel's Viper

Venom followed by a vasoactive amine reliably resulted in atherosclerotic plaque rupture and arterial thrombosis.

Therefore, the two mechanisms by which atherosclerotic disease progresses, plaque rupture with explosive thrombosis and smaller volume mural thrombus formation without plaque rupture, may be interrelated (Fig. 2). Lesions with a necrotic core enlarge through rupture of their surface cap stimulating large volume thrombus accumulation. Digestive enzymes produced by macrophages within the plaque cap are believed to be responsible for a predisposition to rupture. The observations of Constantinides in an animal model suggest that erosion of the plaque cap is not part of the normal evolution of an atherosclerotic lesion but requires a stimulus. His experimental stimulus was the induction of a hypercoagulable state and the administration of a vasoactive amine. While these observations in an animal model may not accurately reflect the disease process in humans, an intriguing possibility is raised. Plaque rupture may require antecedent mural thrombus formation.

The common denominator of platelet thrombus formation offers an explanation for observed associations between ischemic events and environmental triggers.⁴⁹⁻⁶¹ An advanced lesion with a necrotic core that is devoid of endothelial protection allows small volume platelet accumulation which, in turn,

FIG. 2 Thrombotic lesion progression. Vessel segments with severe stenosis, disturbed flow patterns, and endothelial dysfunction (top left) may allow significant platelet accumulation (top right) without plaque rupture. Alternatively, an advanced plaque devoid of endothelial protection may allow platelet accumulation and subsequent inflammatory cell recruitment (middle left). Attempted clearance of mural thrombus and "healing" (bottom left) may result in temporary weakening of the cap, increasing the likelihood of rupture (bottom center). Finally, inflammation within the necrotic core region (middle right) may recruit and stimulate macrophage activity within the plaque cap (bottom right) resulting in rupture (bottom center). PLT = platelet, MP = macrophage, PMN = polymorphonuclear leukocyte.

offers a stimulus and site of attachment for inflammatory cells. The "healing" process, clearing the thrombus zone in preparation for connective tissue matrix deposition, may stimulate inflammatory cell activity within the neighboring plaque cap, reducing structural integrity and increasing, temporarily, the probability of rupture. Thus, the advanced lesion represents the ever-present potential for rupture and thrombosis. The final progression to lesion rupture requires the loss of endothelial protection or augmentation of platelet function which may, in many instances, be the result of triggers such as infection, morning arousal, and unusual physical or emotional stress.

It is conceivable that platelet inhibition of sufficient potency could actually prevent plaque rupture events and substantially reduce atherosclerosis progression. However, several independent mechanisms of platelet function would require treatment including initial adhesion, activation, and aggregation. The cost in terms of hemorrhagic risk would be substantial. What seems more feasible based upon theoretical considerations and clinical observations is that antiplatelet therapy is best suited to limit the volume of thrombus once initiated. The most effective reduction in disease progression will require limited reduction in platelet function combined with restoration of normal or near normal endothelial function.

Platelet Function-Activation Pathway

Intact endothelial cells favor the fluid phase of coagulation equilibrium by two primary mechanisms. First, they act as a physical barrier that prevents exposure of blood to the thrombogenic subendothelial constituents of the vessel wall. Second, normal endothelium synthesizes and releases prostacyclin (PGI₂), NO, and adenosine diphosphatase (ADPase) that prevent or limit platelet activation in their immediate vicinity. Surface expression of molecules such as thrombomodulin, heparinoids, protein S, and tissue factor pathway inhibitor all contribute to control of coagulation.⁵ In addition, tissue plasminogen activator and urokinase-type plasminogen activator, both secreted by endothelial cells, promote fibrinolysis.⁵

Platelets circulate in a relatively inactive state, discoid in appearance, with a surface receptor population seeking out sites of adherence. Circulating activators such as epinephrine increase readiness, but true expression of platelet function is best seen upon attachment to connective tissue matrix such as collagen or bound von Willebrand factor (vWF).^{62, 63} Endothelial cell damage or loss at the site of arterial injury breaches the barrier separating the vessel wall contents from circulating blood. Upon finding a site of attachment and with the encouragement of locally produced thrombin, thromboxane A₂ (TXA₂), and ADP, platelets release their storage granule contents, become spiculated in appearance, alter their membrane to act as a catalyst for coagulation enzymes, and increase the surface density of glycoprotein IIb/IIIa (GP IIb/IIIa).^{64, 65} Glycoprotein IIb/IIIa binds fibrinogen which may in turn be bound by a similar receptor on a neighboring platelet producing platelet-platelet crosslinking and aggregation.^{66, 67} Another important part of this activation process is the release of ADP and the production of TXA₂ which serve to stimulate local platelet activation further.⁶⁴

As the platelet thrombus grows and the lumen is compromised, blood flow velocity increases, accentuating the difference in flow rates between the vessel wall and the central lumen. A high shear rate promotes further platelet aggregation through physical effects upon platelet diffusion and vWF. Thromboxane A₂ and ADP-dependent activation of platelets are predominant in shear-induced platelet stimulation.⁶⁸ Stimulation of normal endothelium by high shear stress stimulates the synthesis of antiplatelet molecules such as PGI₂ and NO, which limit platelet accumulation. When endothelial cells are lost or damaged, as in the Folts model of cyclic flow variation, the thrombotic stimulus of shear stress is unopposed.⁶⁹

Antiplatelet Therapy in the Prevention of Ischemic Events

Several drugs targeting individual steps in normal platelet function have been developed (Fig. 3). Virtually all of these

FIG. 3 Steps of platelet activation targeted in the development of antiplatelet agents. PGs = prostaglandins, NO = nitric oxide, GpIb = glycoprotein Ib, vWF = von Willebrand factor, NSAIDs = nonsteroidal anti-inflammatory drugs, TxS = thromboxane synthase, GpIIb-IIIa = glycoprotein IIb/IIIa, TXA₂ = thromboxane A₂. Adapted from Ref. No. 71.

agents have been shown to reduce the risk of thrombotic events in patients with atherosclerosis.

The Antiplatelet Trialists' Collaboration

The Antiplatelet Trialists' Collaboration meta-analysis analyzed the results of 145 randomized clinical trials of prolonged antiplatelet therapy in 70,000 high-risk patients (defined as having vascular disease or other conditions implying an increased risk of occlusive vascular disease) and 30,000 low-risk patients from the general population.⁷⁰ Among high-risk patients, antiplatelet therapy provided an overall risk reduction of 27% in vascular events (nonfatal MI, nonfatal stroke, or vascular death) (Table I). The most widely studied antiplatelet regimen was medium-dose (75–325 mg) aspirin. An analysis of all aspirin trials demonstrated a 25% odds reduction in vascular events. Other regimens studied included aspirin plus dipyridamole, aspirin plus sulfipyrazone, and monotherapy with sulfipyrazone, dipyridamole, ticlopidine, or sulotidil.

Antiplatelet therapy was consistently effective in a wide range of patient populations. The odds reductions by patient subtype were: 29% in 20,000 patients recovering from an acute MI; 25% in 20,000 patients with a history of MI; 22% in 10,000 patients with past history of stroke or transient ischemic attack (TIA); and 32% in 20,000 patients with other relevant history (e.g., unstable angina, stable angina, vascular surgery, angioplasty, atrial fibrillation, valvular disease, and peripheral vascular disease). Total mortality was significantly reduced in high-risk patients (odds reduction 17%; $p < 0.01$).⁷⁰

Individual Antiplatelet Agents

New thrombin antagonists (e.g., hirudin, hirulog, hirugen, and argatroban) interfere with thrombin-induced platelet activation as well as limiting the generation of thrombin. These agents, however, will probably be used mainly as an alternative to heparin. The following will review the safety and efficacy of available antiplatelet agents and other agents now in development.

Aspirin: Aspirin has been used as an antithrombotic agent for nearly half a century, and until recently it was the only antiplatelet agent in general use. Its efficacy, relative safety, and low cost have made it the standard of antiplatelet therapy. The antiplatelet activity of aspirin derives from its ability to inactivate cyclooxygenase irreversibly, leading to inhibition of platelet TXA₂ synthesis. Although this action has no effect on initial platelet adhesion to the vessel wall, it partially inhibits the final step in platelet aggregation.⁷¹ The effect of this inhibitory action on platelet TXA₂ production occurs within 15 to 30 min of oral administration.⁷² Reduction in platelet aggregability persists for 4 to 7 days after a single dose. Aspirin also inhibits the production of prostacyclin, the major product of vascular endothelium.⁷³

As an antiplatelet agent, aspirin has several important clinical limitations. Some individuals develop resistance, necessi-

TABLE I Patients suffering vascular events: Antiplatelet Trialists' Collaboration meta-analysis

High-risk category	Antiplatelet therapy	Control	p Value (2P)
Acute MI (%)	10	14	<0.00001
History of MI (%)	13	17	<0.00001
History of stroke (%)	18	22	<0.00001
Other relevant history (%)	6–9	8–14	<0.00001

Source: Ref. No. 70.

Abbreviation: MI = myocardial infarction.

tating dose escalations to achieve inhibition of platelet aggregation. Inability to achieve inhibition of aggregation has been observed even at high dosages (1300 mg/day).^{74, 75} Several mechanisms of resistance have been suggested, including the inability of aspirin to inhibit platelet activation induced by processes independent of TXA₂, specifically thrombin and shear stress.^{68, 76, 77} There is also evidence that in some individuals inhibition of cyclooxygenase by aspirin may increase the conversion of arachidonic acid to 12-hydroxyeicosatetraenoic acid (12-HETE), a compound that appears to promote platelet adhesion.⁷⁸ In a study by Buchanan and Brister, patients undergoing coronary artery bypass grafting (CABG) were evaluated to determine the effect of aspirin on hemostasis (measured by bleeding time) and platelet function (measured by 12-HETE synthesis and ex vivo adhesion).⁷⁸ Patients had been taking aspirin (325 mg/day) for at least 6 months; it was discontinued 7 days before surgery. When aspirin therapy was reintroduced, 58% of patients responded; bleeding time was prolonged, and platelet biochemistry and function tests demonstrated platelet inhibition. In nonresponders (42%), platelet 12-HETE and adhesion were increased. The differential effects of aspirin on the arachidonic acid system offer a pharmacodynamic explanation for cases of individual aspirin resistance.

Gastrointestinal side effects may also limit the use of aspirin in some patient populations. Aspirin therapy can induce bleeding ulcers and/or erosions, even at lower doses. Buffered aspirin does not lessen gastric toxicity, and enteric-coated preparations seem to be protective only in short-term use.⁷⁹

Cyclooxygenase blockers have been observed to counteract the antihypertensive efficacy of the angiotensin-converting enzyme (ACE) inhibitors. A daily dose of 300 mg of aspirin has been reported to inhibit enalapril-induced reduction of mean arterial pressure by over 20% in 60% of patients with mild hypertension, 57% of patients with moderate hypertension, and 50% of patients with severe hypertension. When counteraction of the enalapril effect occurred, it averaged 60% in mildly hypertensive, 70% in moderately hypertensive, and 90% in severely hypertensive patients.⁸⁰

Concomitant use of enalapril and aspirin has also been shown to reduce the survival benefit of enalapril. In an analysis of data from 6,797 patients in the Studies of Left Ventricular Dysfunction (SOLVD) trial, enalapril failed to improve survival in patients receiving an antiplatelet agent (aspirin or

dipyridamole) at baseline. Enalapril therapy, however, significantly reduced a combined endpoint of death or hospital admission for heart failure. The authors concluded that use of aspirin or dipyridamole is associated with "retained but reduced" benefit from enalapril.⁸¹ These findings are consistent with those of a previous study showing that aspirin attenuated the favorable effect of enalapril on systemic vascular resistance, left ventricular pressure, total pulmonary resistance, and cardiac output in patients with severe congestive heart failure.⁸² A reanalysis of data from the Cooperative New Scandinavian Enalapril Survival Study (CONSENSUS II) found the effect of enalapril on mortality after acute MI less favorable among patients taking aspirin at baseline.⁸³ Aspirin and other nonsteroidal anti-inflammatory drugs have a propensity to increase blood pressure and impair renal function.^{84,85} Although the interaction between aspirin and enalapril is not understood, ACE inhibitors stimulate prostaglandin synthesis by impeding the degradation of bradykinin. Aspirin may inhibit the production of beneficial vasodilator prostaglandins.⁸⁵

Dipyridamole: Dipyridamole appears to inhibit platelet aggregation by direct stimulation of prostacyclin synthesis, inhibition of phosphodiesterase, elevation of platelet cyclic adenosine monophosphate (AMP) levels, and blockade of adenosine uptake into blood cells. In the secondary prevention of stroke, aspirin-dipyridamole combination therapy has proved effective compared with placebo and aspirin alone. In the European Stroke Prevention Study 1 (ESPS-1), combination therapy with aspirin (330 mg t.i.d.) and dipyridamole (75 mg t.i.d.) demonstrated a 38% reduction compared with placebo ($p < 0.001$) in recurrent stroke in patients with prior ischemic stroke or TIA treated for 2 years.⁸⁶ The European Stroke Prevention Study 2 (ESPS-2) evaluated the safety and efficacy of aspirin alone (50 mg/day), dipyridamole alone (400 mg/day), and combination therapy for the prevention of stroke, death, TIA, and other vascular events in more than 6,000 patients with previous TIA or ischemic stroke.⁸⁷ Compared with placebo, stroke risk was reduced by 18% with aspirin alone, by 16% with dipyridamole alone, and by 37% with combination therapy. Although data from the ESPS-1 and ESPS-2 suggest improved efficacy with dipyridamole and aspirin combined, questions raised regarding the study design, the low aspirin dosage, discontinuation due to adverse events, and compliance have cast doubt on the reliability of these results.

Dipyridamole may also add to the benefits of aspirin in preventing the complications of peripheral arterial disease (PAD).⁸⁸ Dipyridamole does not, however, add to the beneficial effects of aspirin in secondary prevention of MI or in maintenance of patency in CABG.⁷¹ Adverse events associated with dipyridamole, such as gastrointestinal symptoms, headache, and a rare coronary "steal" that may induce myocardial ischemia, limit its usefulness as an antiplatelet agent.^{71, 89, 90} On the basis of studies to date, the use of dipyridamole as an antiplatelet agent is questionable.

Ticlopidine: The first available thienopyridine derivative, ticlopidine, inhibits ADP-induced platelet aggregation by blocking the binding of ADP to platelet receptors. It also inhibits aggregation due to collagen, epinephrine, arachidonic

acid, thrombin, and platelet-activating factor.^{91, 92} Ticlopidine-induced inhibition of fibrinogen binding to GP IIb-IIIa receptors is due to ADP receptor blockade.⁹³ In contrast to aspirin, ticlopidine inhibits platelet aggregation due to shear stress *in vitro*.⁹⁴

The Ticlopidine Aspirin Stroke Study (TASS), a multicenter, double-blind, randomized trial, compared the effect of ticlopidine (500 mg/day) with that of aspirin (1300 mg/day) on the risk of stroke or death in patients with prior TIA or minor stroke within 3 weeks of enrollment. At 3 years, ticlopidine had a consistent advantage over aspirin. The rate of nonfatal stroke or death from any cause was 17% for the ticlopidine group and 19% for the aspirin group, a risk reduction of 12% ($p = 0.048$) for ticlopidine. The rate of fatal and nonfatal stroke at 3 years was 10% for the ticlopidine group and 13% for the aspirin group, a risk reduction of 21% ($p = 0.024$).⁹⁵ Greater efficacy was observed for ticlopidine over aspirin whether the endpoint was ischemic events alone ($p = 0.007$), or a composite of ischemic event plus death and/or stroke ($p < 0.001$).⁹⁶

The Canadian-American Ticlopidine Study (CATS) found ticlopidine superior to placebo in the secondary prevention of stroke.⁹⁷ Ticlopidine monotherapy also demonstrated efficacy in preventing aortocoronary bypass graft occlusion, slowing progression of nonproliferative diabetic retinopathy, and preventing thrombotic events in patients with peripheral vascular disease.⁹⁸⁻¹⁰¹ Therapy with ticlopidine reduces rates of vascular surgery in patients with intermittent claudication by 50% ($p < 0.001$).¹⁰⁰

Thrombotic occlusion, as well as hemorrhagic and peripheral vascular complications due to intensive anticoagulation, have seriously limited the benefits of intracoronary stenting. The Intracoronary Stenting and Antithrombotic Regimen (ISAR) trial compared antiplatelet therapy with ticlopidine plus aspirin with a conventional anticoagulant regimen (heparin followed by phenprocoumon and aspirin). With the antiplatelet regimen, there was an 82% lower risk of MI and a 72% lower need for repeated interventions. Hemorrhagic complications occurred only in the anticoagulant group.¹⁰² In a subgroup of patients considered at high risk for subsequent adverse cardiac events, the incidence of such events was 12.6% with anticoagulant therapy and 2.0% with ticlopidine plus aspirin. The rate of stent vessel occlusion was 11.5% and 0%, respectively.¹⁰³

Among patients with successful stenting after acute MI, those receiving ticlopidine plus aspirin had a lower clinical event rate (3.3 vs. 21.0%) and stent vessel occlusion rate (0 vs. 9.7%) during the first 30 days than did patients receiving anticoagulant therapy. After 6 months, survival free of recurrent MI was higher with ticlopidine plus aspirin (100 vs. 90.3%) and stent vessel occlusion was lower (1.6 vs. 14.5%).¹⁰⁴ Other studies have also demonstrated the benefit of aspirin plus ticlopidine in reducing complications post stenting.¹⁰⁵⁻¹⁰⁸ In a study of the effect of aspirin-ticlopidine therapy on platelet function, the combination was shown to be superior to aspirin monotherapy in inhibiting platelet surface expression of the activated fibrinogen receptor and of P-selectin after coronary stenting.¹⁰⁷

The use of ticlopidine has been limited by its side effects, particularly rare but severe neutropenia, which occurs in about 0.8% of patients. Neutropenia tends to occur within 3 months of the initiation of treatment and is usually reversible after discontinuation. Diarrhea has been observed in 12.5% of patients, resulting in drug discontinuation in 6.3%.¹⁰⁹ Other common side effects include increased serum cholesterol, thrombocytopenia, and abnormal liver function. Ticlopidine interacts with other drugs metabolized by hepatic microsomal enzymes, including cimetidine, digoxin, theophylline, phenobarbital, and phenytoin.¹⁰⁹

Clopidogrel: Clopidogrel is a new ADP-receptor antagonist. It selectively and irreversibly inhibits the binding of ADP to its platelet receptor, thereby preventing platelet activation and amplification, and ultimately the activation of the GP IIb-IIIa receptor. Its molar potency is greater than that of ticlopidine, and it is better tolerated.¹¹⁰ Clopidogrel appears to have a faster onset of action than ticlopidine. A 75 mg once-daily dose inhibits ADP-induced platelet aggregation within 2 h of the first dose and reaches steady state within 3 to 7 days. In addition to inhibiting platelet adhesion and aggregation, clopidogrel blunts platelet activation secondary to shear stress in in vitro models and blunts myointimal proliferation in animal models.^{111–113} A study by Bachmann *et al.* showed that a 375 mg loading dose of clopidogrel followed by 75 mg/day for 10 days inhibited ADP-induced platelet aggregation by 55% at 1 h and 80% at 5 h.¹¹⁴

Clopidogrel (75 mg once daily) was compared with aspirin (325 mg once daily) in the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial, a randomized, double-blind, multicenter study of 19,185 patients. The trial was designed to assess the relative safety and efficacy of clopidogrel in reducing the risk of vascular events in patients with a recent ischemic stroke or MI, or symptomatic PAD. Patients were followed up for 1 to 3 years (mean 1.9 years).¹¹⁵

In an intention-to-treat analysis, the annual risk of primary ischemic events (fatal or nonfatal ischemic stroke, MI, or other vascular death) in the clopidogrel group was 5.32%, compared with 5.83% for the aspirin group. This amounts to a relative risk reduction (RRR) of 8.7% ($p = 0.043$) in favor of clopidogrel. Analyzing the outcome for MI, the risk reduction for clopidogrel was 19.2% ($p = 0.008$) over aspirin (Fig. 4). A subgroup analysis of CAPRIE data found that for 8,446 patients presenting with MI or with a past history of MI on the stroke or PAD cohorts, the RRR was 7.4% in favor of clopidogrel; in 10,047 patients with any history of cardiovascular disease, the RRR was 7.6% in favor of clopidogrel.¹¹⁶ Therefore, in a population of atherosclerotic patients similar to CAPRIE, clopidogrel would be expected to prevent an estimated 24 major clinical events (ischemic stroke, MI, vascular death) per 1,000 patients per year, compared with an estimated 19 events per 1,000 patients taking aspirin.

The side effects of clopidogrel are generally mild, and tolerability is similar to that of aspirin. In the CAPRIE trial, the most commonly reported adverse effects in clopidogrel-treated patients were gastrointestinal upset, bleeding disorders, rash, and diarrhea. Gastrointestinal hemorrhage occurred in

2.0% of patients treated with clopidogrel, compared with 2.7% of those treated with aspirin ($p < 0.05$); intracranial hemorrhage occurred in 0.35% of patients in the clopidogrel group compared with 0.49% for those taking aspirin (not statistically significant). The incidence of neutropenia ($< 200/\text{mm}^3$) in the clopidogrel group (0.10%) was similar to that of the group taking medium-dose aspirin (0.17%). Thrombocytopenia occurred in 0.26% of patients in both treatment groups. Routine hematological monitoring is not required for patients treated with clopidogrel. There were no elevated liver function tests, and no clinically significant pharmacodynamic drug interactions have been reported to date.^{115, 117}

The results of CAPRIE demonstrate that long-term administration of clopidogrel is more effective than aspirin for reducing the combined risk of ischemic stroke, MI, or vascular death in patients with a recent MI, a recent stroke, or established PAD. Clopidogrel may have beneficial effects in combination with aspirin in patients undergoing coronary stenting and other interventional procedures, although this awaits clinical studies.

Glycoprotein IIb-IIIa inhibitors: The final common pathway of platelet aggregation is GP IIb-IIIa receptor platelet crosslinking mediated by a shared fibrinogen molecule. Several antagonists of the GP IIb-IIIa receptor have been developed and tested: a modified monoclonal antibody to GP IIb-IIIa, abciximab (c7E3 Fab), peptide (peptidomimetic) antagonists (e.g., eptifibatide), and nonpeptide (nonpeptide mimetic) antagonists.¹¹⁸ These are potent inhibitors of platelet aggregation and prolong bleeding time.^{70, 71} Today, GP IIb-IIIa compounds have only been evaluated in acute treatment (intravenous administration) in phase III trials. No data are available for chronic therapy.

In the EPIC (Evaluation of c7E3 in Preventing Ischemic Complications), EPILOG (Evaluation of PTCA to Improve Long-term Outcome by c7E3 GP IIb-IIIa Receptor Blockade), EPISTENT (Evaluation of Platelet Inhibition in Stenting), and CAPTURE (c7E3 Antiplatelet Therapy in Unstable Refractory Angina) trials, intravenous abciximab was shown to be effective in reducing early ischemic events (with need for revascularization or nonfatal MI or death).^{119–122} This effect was sustained and durable in patients undergoing coronary angioplasty and directional atherectomy.^{119, 120} In the EPIC trial,

FIG. 4 Reduction of outcome events: Stroke, myocardial infarction (MI), and vascular death. Reproduced from Ref. No. 115 with permission.

abciximab was associated with a significant increase in both major and minor bleeding events compared with placebo. Thrombocytopenia and the need for platelet transfusion were also more likely in patients treated with abciximab. In the EPILOG trial, use of low-dose weight-adjusted heparin lowered major bleeding to a level comparable to that seen in placebo-treated patients.¹²⁰

Eptifibatide, a peptide GP IIb-IIIa antagonist, was evaluated clinically in IMPACT II (Integrilin to Minimize Platelet Aggregation and Coronary Thrombosis II). This was a double-blind, placebo-controlled, multicenter trial of 4,010 patients undergoing coronary intervention. There were three treatment arms: placebo, high-dose eptifibatide, and low-dose eptifibatide. The low- and high-dose eptifibatide groups had significant reductions in the composite endpoint (death; MI; urgent/emergency revascularization, CABG, or stent replacement) by 30 days. Eptifibatide did not increase major bleeding rates; however, thrombocytopenia occurred more frequently at the higher dose.¹²³

In PRISM, a phase III randomized, double-blind trial of more than 3,200 patients with unstable angina, tirofiban reduced the risk of death, MI, and refractory ischemia by 36% at 48 h and reduced the risk of death at 30 days by 39% compared with heparin.¹²⁴ Treatment with tirofiban plus heparin was evaluated in 1,570 high-risk patients in the PRISM-PLUS trial.¹²⁵ Tirofiban-plus-heparin therapy reduced the combined risk of MI and refractory ischemia by 23% at 30 days and the combined risk of heart attack and death by 31% compared with heparin alone.¹²⁵ Preliminary results of treatment with tirofiban given over 36 h to patients undergoing angioplasty have shown little reduction of event rate at Day 30. Major bleeding occurred in 6% of patients receiving tirofiban, and 3.9% required transfusions, but there was no increase in thrombocytopenia.¹²⁵

Nonpeptide IIb/IIIa antagonists are not susceptible to intestinal peptidases. Oral administration results in reversible, dose-dependent GP IIb-IIIa receptor blockade.⁶⁵ Several nonpeptide derivatives suitable for oral administration are under development. Lamifiban is an orally active nonpeptide that is almost 1,000 times more active in inhibiting platelet aggregation in human platelet-rich plasma than a peptide consisting of the amino acid sequence that binds fibrinogen to the GP IIb-IIIa receptor.⁶⁵ When tested against placebo in unstable angina, higher doses of lamifiban reduced the combined cardiovascular event rate from 8.1 to 2.5% at 1 month. Bleeding time was prolonged to 25 min at effective doses. Major bleeding events occurred in 2.9% of patients receiving lamifiban. Thrombocytopenia occurred in patients also receiving heparin.¹²⁶ Other oral agents, such as sibrafiban and xemilofiban, are in development.^{127, 128}

The safety and optimal administration of oral GP IIb-IIIa inhibitors are as yet unknown. Several issues have been raised by preliminary data, including the degree of long-term receptor inhibition needed for the prevention of ischemic events, potentiation of oral agents by prior treatment with intravenous GP IIb-IIIa receptor antagonists, the safe level and duration of receptor blockade, and the optimal duration of treatment after

an acute coronary event.¹²⁹ Clinically significant bleeding has been observed with the administration of oral agents, requiring close monitoring for bleeding events and platelet function. Acute severe thrombocytopenia has also been observed. Because the effect of GP IIb-IIIa inhibitors is reversible, peak-trough levels are also a critical issue in disease management. Background aspirin therapy may be necessary to protect against thrombosis should platelet function be restored at drug trough levels. The long-term safety and efficacy of oral agents have not been established. Further investigation is needed to resolve these issues.

Conclusions and Clinical Recommendations

Endothelial dysfunction manifest as abnormal signal transduction, surface receptor expression, or protein production is a central component in the development and progression of atherosclerosis. The mechanisms producing abnormal function are not well defined but are clearly related to the pathologic biochemical abnormalities accompanying the disease states or behaviors that are closely associated with the development of vascular disease, namely hypercholesterolemia, diabetes mellitus, cigarette smoking, and hypertension. Treatment of hypercholesterolemia and diabetes mellitus, and abstinence from cigarette smoking slow the progression of atherosclerosis presumably, at least in part, by favorably influencing endothelial function.

Persistent endothelial dysfunction and atherosclerosis progression inevitably result in arterial thrombus formation. The potential for fatal outcome with these events in the coronary circulation has centered clinical attention upon manipulation of enzymes and receptors crucial to the growth and development of thrombus. The greatest impact of this interest in antithrombotic drugs for the treatment of atherosclerosis has been the use of aspirin. Aspirin is a relatively weak antiplatelet drug inhibiting only one of several avenues of platelet activation. Still yet, its administration to patients with a high probability of active, intracoronary thrombus dramatically reduces the incidence of clinical events. Furthermore, aspirin therapy improves long-term survival in patients who are simply at increased risk of developing an arterial thrombus.

Encouraged by the success of aspirin, investigators quickly targeted platelet function as their object of interest. Information was rapidly accumulated detailing the mechanisms of platelet function, particularly the importance of activation mechanisms such as TXA₂ and ADP, and the central role of GP IIb/IIIa in platelet aggregation. In logical succession, new drugs have been developed targeting individual steps in platelet activation and aggregation (Table II). The ADP antagonist clopidogrel is more effective than aspirin and equally well tolerated when used as chronic therapy to reduce the risk of ischemic events. The GP IIb/IIIa receptor antagonists were specifically engineered to provide intense platelet inhibition. Their administration is accompanied by a dramatic reduction in thrombus progression and the risk of ischemic events, especially when given at the time of percutaneous intervention. Oral GP IIb/IIIa receptor antagonists are currently under evaluation.

TABLE II Antiplatelet drugs

Drug	Action	Use	Advantage	Disadvantage
Aspirin	Cyclooxygenase inhibition	ACS, CAD, PVD, ischemic stroke,	Oral Inexpensive	GI ulceration ACE-I antagonism Resistance
Dipyridamole	Increase prostacyclin	Uncertain	Oral	Coronary steal Headache
Ticlopidine	Prevents ADP-induced platelet activation	Ischemic stroke, PVD, coronary stent, SVG patency	Oral	Rash Diarrhea Neutropenia
Clopidogrel	Prevents ADP-induced platelet activation	CAD, PVD Ischemic stroke	Oral	Rash Diarrhea
Abciximab	GP IIb/IIIa antagonist	ACS PTCR	Rapid onset	Intravenous
Eptifibatide	GP IIb/IIIa antagonist	ACS PTCR	Rapid onset Short half-life	Intravenous
Tirofiban	GP IIb/IIIa antagonist	ACS PTCR	Rapid onset Short half-life	Intravenous
Oral non-peptides	GP IIb/IIIa antagonist	ACS ^a	Oral	Dosing uncertainty

^a Limited experience with individual agents, not approved by the U.S. Food and Drug Administration.

Abbreviations: CAD = secondary prevention for coronary artery disease, ACS = acute coronary syndromes, ACE-I = angiotensin-converting enzyme inhibitor, PVD = peripheral vascular disease, SVG = saphenous vein graft, PTCR = percutaneous coronary revascularization, GP = glycoprotein.

This latest step in antiplatelet therapy will probably approach or exceed the boundaries of an acceptable risk/benefit ratio for long-term therapy of most patients. The majority of patients with atherosclerosis do not have a high suspicion for harboring intracoronary thrombus. For these patients we acknowledge the risk of developing thrombus and wish to limit its growth once initiated. Unfortunately, using antiplatelet therapy to prevent ischemic events carries a price. Platelets perform a necessary function in establishing hemostasis. Therefore, an increased risk of spontaneous and post-traumatic hemorrhage will accompany platelet inhibition. Even aspirin, whose antiplatelet effect is modest, carries an increased risk for developing spontaneous intracranial hemorrhage.

Patients with clinical evidence of intracoronary thrombus face a high risk of proceeding to MI and cardiac death. These patients can afford brief exposure to potent antiplatelet therapies and, as a population, see a beneficial effect. On the other hand, patients with clinically stable disease require therapy over a prolonged period of time to enjoy a reduction in ischemic events. This population, whose yearly risk of events is about 5%, cannot afford exposure to a marked increase in the risk of hemorrhagic events.

There is no method available for the reliable identification of a patient with a high short-term risk of developing coronary thrombosis. In the future, perhaps biochemical and genetic studies will allow us to catalogue individual variations in physiology and to estimate their impact upon disease progression and the short-term risk of thrombosis. Specific therapies targeting endothelial health and/or platelet function may then be

tailored accordingly. Currently we must apply therapy based upon a patient's estimated risk of ischemic events using the observations of epidemiologic studies and randomized clinical trials. As a general rule, all patients with established atherosclerosis should be treated with an antiplatelet drug. Aspirin or clopidogrel are the most appropriate first-line agents for the secondary prevention of acute ischemic events. Clopidogrel may be more advantageous in patients with a history of gastrointestinal bleeding believed to result from aspirin therapy or in whom treatment with an ACE inhibitor is required. Combination therapy with clopidogrel or ticlopidine and aspirin may be useful in acute settings or with interventional procedures such as stenting. Patients with unstable disease identified from the clinical history accompanied by electrocardiographic evidence of repolarization abnormalities or ST-segment deviation and those undergoing high-risk percutaneous revascularization should receive temporary, additional therapy with GP IIb/IIIa inhibitors.

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