Platelet Glycoprotein IIb/IIIa Inhibitors in Cardiovascular Disease

A. Michael Lincoff
Eric J. Topol
Editors

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Edited by

A. Michael Lincoff, MD

Eric J. Topol, MD

The Cleveland Clinic Foundation, Cleveland, Ohio

Humana Press

Totowa, New Jersey

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

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PREFACE

The last two decades have witnessed a burgeoning interest in the development and application of antithrombotic approaches to the treatment of cardiovascular diseases, based on recognition of the key roles played by the arterial platelet-thrombus in the pathogenesis of acute coronary syndromes, ischemic complications of percutaneous coronary revascularization, coronary artery bypass graft disease, and even *de novo* native and peripheral artery atherosclerosis. Aspirin celebrated its 100th anniversary as a therapeutic agent in 1997, although only recently has some of its mechanism of action been elucidated and its efficacy demonstrated in controlled studies. Heparin too has been employed since its introduction in 1916 as an anticoagulant, but again with only recent and relatively limited controlled evaluation for the treatment of cardiovascular disease. In the last few years, the pharmacologic armamentarium directed against vascular thrombosis has been substantially enriched, with introduction into practice of new fibrinolytic agents, low molecular weight heparins, direct thrombin inhibitors, antagonists to various pathways of platelet activation, and the platelet glycoprotein IIb/IIIa inhibitors.

Despite this recent expansion in the number of alternatives for antithrombotic therapy, the clinical efficacy of such compounds in the management of cardiovascular disease has been at times disappointing. Among the agents directed against the activity and generation of thrombin, heparin has been used most broadly, but with unproven or limited effectiveness in many settings. For example, although employed universally during percutaneous coronary interventional procedures, heparin has never been tested in a placebo-controlled fashion among these patients. Moreover, small trials of heparin in the settings of unstable angina or acute myocardial infarction have suggested only modest efficacy of this agent in limiting ischemic complications. Hirudin and hirulog, potent direct inhibitors of thrombin activity that overcome many of the intrinsic limitations of heparin, have only modestly improved clinical outcome compared with heparin in large-scale trials of coronary intervention or acute ischemic syndromes. Similarly, despite the theoretical advantages of low molecular weight heparins, the efficacy of these agents has proven to be variable, with only enoxaparin showing evidence of clinical benefit over unfractionated heparin in controlled studies. The strategy of clot lysis with fibrinolytic or "thrombolytic" compounds has been clearly demonstrated in large-scale trials to reduce mortality by reestablishing coronary patency during acute myocardial infarction. Yet even the newest generation of these agents remains limited by an apparent "ceiling" of reperfusion rates, as well as the consistent failure of these compounds to improve clinical outcome (with trends toward more frequent ischemic and bleeding complications) in the settings of unstable angina or percutaneous coronary intervention.

Approaches to inhibition of the platelet have met with somewhat more uniform success in the treatment of arterial thrombosis, a finding that suggests that platelet deposition, activation, and aggregation are the crucial initiating components of the response to arterial injury. Placebo-controlled trials have consistently demonstrated that therapy

with aspirin will reduce the risk of ischemic events by up to 30-40% across the broad spectrum of atherosclerotic diseases of the arterial tree. Through its irreversible inactivation of platelet cyclo-oxygenase, aspirin inhibits the thromboxane-mediated mechanism of platelet activation; compounds directed against other activation pathways of the platelet have generally exhibited efficacy comparable but not markedly superior to that of aspirin. The thienopyridines, ticlopidine and clopidogrel, prevent platelet activation via inhibition of the adenosine diphosphate receptor, with a recent large-scale trial demonstrating modest benefit of clopidogrel over aspirin in reducing the long-term risk of ischemic events among patients with cardiovascular, cerebrovascular, or peripheral vascular atherosclerosis. Clinical experience with the thienopyridines administered *in addition* to aspirin among patients undergoing coronary stent implantation, however, has suggested that clinical efficacy may be substantially enhanced when more than one mechanism of platelet activation is inhibited.

The limitations of compounds directed against individual pathways of platelet activation may therefore be overcome by approaches aimed at the "final common pathway" of platelet aggregation, the surface glycoprotein IIb/IIIa receptor. Multiple mechanisms of platelet activation in response to different agonists all "converge" to render this transmembrane complex competent to bind circulating adhesion molecules and crosslink adjacent platelets. The clinical hemorrhagic syndrome caused by a rare inherited defect in this receptor (Glanzmann's thrombasthenia), characterized by mucocutaneous and postsurgical bleeding, but infrequent spontaneous organ (particularly central nervous system) bleeding, suggested that therapeutic inhibition of this receptor might be a potent, yet well-tolerated, means of treating thrombotic disorders. Following the initial development by Coller of a monoclonal antibody that blocks the interaction of this receptor with adhesion molecules, peptide and nonpeptide synthetic molecules with similar activity were also designed. Systematic programs of controlled clinical trial evaluation have demonstrated that these agents are markedly effective and safe in reducing the risk of adverse ischemic events among a broad spectrum of patients with atherosclerotic cardiac disease. In 1995, the first glycoprotein IIb/IIIa antagonist, abciximab, received marketing approval as an adjunct to percutaneous coronary revascularization, with two additional agents, eptifibatide and tirofiban, approved by the Food and Drug Administration in 1998 for the management of patients with unstable angina. This class of therapy is now increasingly employed by physicians caring for patients with stable and unstable coronary syndromes.

Platelet Glycoprotein IIa/IIIb Inhibitors in Cardiovascular Disease is a comprehensive, definitive, and detailed overview of the preclinical and clinical development of the class of glycoprotein IIb/IIIa receptor antagonists. The goal of the book is to elucidate the theoretical basis for inhibition of platelet aggregation in the treatment of coronary syndromes, to present and synthesize the evidence demonstrating the efficacy of glycoprotein IIb/IIIa blockade in inhibiting ischemic complications of coronary intervention and the acute coronary syndromes, to provide guidelines for the use of this class of agents in the clinical management of cardiovascular disease, and to provide a speculative view

of other potential applications of this class of therapy. In every case chapters have been authored by acknowledged experts in the field, including the pioneers in the discovery and characterization of cell surface adhesion molecules and the glycoprotein IIb/IIIa receptor and the principal investigators for the major clinical trials of the receptor antagonists. The most current data are included, producing a complete body of knowledge of the contemporary "state of the art" in this field.

Part I of the book concentrates on the basic pathophysiology underlying the theoretical usefulness and development of this class of agents. In Chapter 1, Drs. Tolleson and Harrington provide the underpinnings for antithrombotic therapy in cardiovascular disease by reviewing the role of thrombosis and platelet activity in the pathophysiology of acute ischemic syndromes or complications of coronary intervention. The function of cell surface adhesion molecules in mediating platelet adhesion, the essential reaction for the hemostatic function of platelets, is discussed by Dr. Plow in Chapter 2, followed by a detailed description of the structure and functions of the glycoprotein IIb/III receptor by Drs. Law and Phillips. Dr. Coller then recounts the "bench to bedside" development of the first agent directed against this receptor, the monoclonal antibody fragment abciximab, and summarizes the pharmacologic properties of the currently available compounds of this class.

Part II concentrates on the clinical setting in which the role of glycoprotein IIb/IIIa blockade has been most intensively studied thus far: percutaneous coronary revascularization. The three agents that have been evaluated for this indication (abciximab, eptifibatide, and tirofiban) are discussed in Chapters 5–8, focusing on data derived from the pivotal Phase III and IV trials. In Chapter 5, I review the EPIC, EPILOG, and EPISTENT trials, which together established the efficacy of abciximab among the broad spectrum of patients undergoing coronary balloon angioplasty, atherectomy, or stent implantation. Drs. O'Shea and Tcheng discuss the IMPACT II trial testing eptifibatide among patients undergoing coronary intervention for a variety of indications in Chapter 6, whereas Drs. Salame, King, and Chronos summarize the RESTORE trial of tirofiban in patients treated by revascularization for acute ischemic syndromes in Chapter 7. Chapter 8 by Drs. van den Brand and Simoons focuses on the strategy tested in the CAPTURE trial of pretreatment by glycoprotein IIb/IIIa blockade with abciximab for refractory unstable angina. Part II concludes with a summary chapter by Dr. Topol and myself integrating the results of the major trials of glycoprotein IIb/IIIa during coronary intervention, comparing the different agents and providing practical guidelines for clinical use.

Part III focuses on the emerging role of glycoprotein IIb/IIIa blockade during the acute coronary ischemic syndromes of unstable angina and acute myocardial infarction. In Chapter 10, Drs. Moliterno and White provide a comprehensive review and synthesis of the results of the four major trials (PURSUIT, PRISM PLUS, PRISM, and PARAGON), which demonstrated the role of empiric therapy with eptifibatide, tirofiban, or lamifiban among patients with unstable angina or myocardial infarction without ST-segment elevation. Drs. Greenbaum, Harrington, and Ohman discuss in Chapter 11 the applications of

glycoprotein IIb/IIIa blockade to the treatment of acute myocardial infarction with ST-segment elevation as an adjunct to mechanical or pharmacologic reperfusion.

Part IV provides a view into the future development and application of this class of agents to the treatment of vascular disease. The medicoeconomic aspects of this therapy in the settings of coronary intervention or acute ischemic syndromes are analyzed by Dr. Mark in Chapter 12. Drs. Kleiman, Mazur, and Graziadei describe and evaluate the various techniques of monitoring platelet function in Chapter 13, an issue that is becoming increasingly important for the optimization of platelet inhibitor dosing, particularly with long-term oral therapy. The "new frontier" of chronic antiplatelet therapy with oral glycoprotein IIb/IIIa agents is discussed by Drs. Kereiakes and Cannon in Chapter 14, a strategy that holds promise as a means of extending the efficacy of parenteral agents or providing long-term protection against ischemic events in high-risk individuals. Dr. Sila writes from the neurologist's viewpoint in Chapter 15, first summarizing the intracranial hemorrhagic risk associated with these agents and then speculating on the potential applications of this class of therapy to the treatment of cerebrovascular disease. Finally, Dr. Califf summarizes his overview and perspective on the advances made in the management of vascular thrombosis and new directions for progress in this field.

The pharmacologic inhibition of the platelet glycoprotein IIb/IIIa receptor represents one of the most exciting fields of cardiovascular research, with rapid, logical, evidence-based development from the laboratory to broad clinical use. The information described in this book provides a compelling body of data supporting the effectiveness and safety of these agents in the treatment of thrombotic vascular disease. In the setting of coronary intervention, glycoprotein IIb/IIIa blockade represents the most important advance in pharmacotherapy since aspirin and, together with stenting, has established a new standard of efficacy for this procedure. In the acute coronary syndromes, these agents provide protection against important ischemic events among patients treated conservatively or by revascularization. The rationale for application of these agents to reperfusion therapy for myocardial infarction is sound, and data from Phase II studies in this setting are encouraging. The potential clearly exists to extend these therapies to the management of cerebrovascular and peripheral vascular disease. The field will continue to advance by development of new parenteral and oral agents, refinement of techniques for platelet function monitoring, and evaluation of the combination of glycoprotein IIb/IIIa blockade with novel inhibitors of thrombin or other components of the coagulation cascade.

I acknowledge and express my appreciation for the superb contributions by the chapter authors of this book, who drew on their considerable expertise and first-hand experience to produce a truly up-to-date and comprehensive discussion of this field. Additionally, the publisher and production staff at Humana Press, including Paul Dolgert, Fran Lipton, and Susan Giniger, made exceptional efforts for the timely completion of this project. Robin Moss deserves recognition for her imaginative book cover artwork. My sincere gratitude goes to my coeditor, Eric J. Topol, who not only provided experience and

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A. Michael Lincoff, MD

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CONTRIBUTORS

ROBERT M. CALIFF, MD, *Professor of Medicine, Duke University Medical Center; Director, Duke Clinical Research Institute, Durham, NC*

CHRISTOPHER P. CANNON, MD, *Assistant Professor of Medicine, Harvard Medical School, Brigham & Women's Hospital, Boston, MA*

NICOLAS A. CHRONOS, MD, *Assistant Professor of Medicine; Director, Clinical Research, The Andreas Gruentzig Cardiovascular Center, Emory University Hospital, Atlanta, GA*

BARRY S. COLLER, MD, *Chairman, Department of Medicine, Mt. Sinai Hospital, New York, NY*

NIKKI GRAZIADEI, BS, *Research Technologist, Section of Cardiology, Baylor College of Medicine, Houston, TX*

ADAM B. GREENBAUM, MD, *Fellow in Cardiology, Duke Clinical Research Institute, Duke University Medical Center, Durham, NC*

ROBERT A. HARRINGTON, MD, *Assistant Professor of Medicine, Duke Clinical Research Institute, Duke University Medical Center, Durham, NC*

DEAN J. KEREIAKES, MD, *Medical Director, The Lindner Center for Clinical Cardiovascular Research; Professor of Clinical Medicine, University of Cincinnati College of Medicine, Cincinnati, OH*

SPENCER B. KING, III, MD, *Professor of Medicine, The Andreas Gruentzig Cardiovascular Center, Emory University Hospital, Atlanta, GA*

NEAL S. KLEIMAN, MD, *Associate Professor of Medicine, Baylor College of Medicine; Assistant Director, Cardiac Catheterization Laboratories, The Methodist Hospital, Houston, TX*

DAVID F. KONG, MD, *Fellow in Cardiology, Duke University Medical Center, Durham, NC*

DEBBIE A. LAW, MD, *Scientist, Department of Thrombosis, COR Therapeutics, Inc., South San Francisco, CA*

A. MICHAEL LINCOFF, MD, *Associate Professor of Medicine, Department of Cardiology; Director, Experimental Interventional Laboratory, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, The Cleveland Clinic Foundation, Cleveland, OH*

DANIEL B. MARK, MD, *Professor of Medicine; Director, Outcomes Research and Assessment Group, Duke University Medical Center, Durham, NC*

WOJCIECH MAZUR, MD, *Fellow in Cardiology, Baylor College of Medicine, Houston, TX*

DAVID J. MOLITERNO, MD, *Director, Angiographic Core Laboratory, Department of Cardiology, The Cleveland Clinic Foundation, Cleveland, OH*

E. MAGNUS OHMAN, MD, *Codirector, Clinical Trials; Associate Professor of Medicine, Duke Clinical Research Institute, Duke University Medical Center, Durham, NC*

CONOR O'SHEA, MD, MRCPI, *Fellow in Cardiology, Duke University Medical Center, Durham, NC*

DAVID R. PHILLIPS, MD, *Principal Research Scientist, COR Therapeutics, Inc., South San Francisco, CA*

EDWARD F. PLOW, MD, *Head of Research, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, The Cleveland Clinic Foundation, Cleveland, OH*

MAHOMED Y. SALAME, MD, *Interventional Cardiology Fellow, The Andreas Gruentzig Cardiovascular Center, Emory University Hospital, Atlanta, GA*

CATHY A. SILA, MD, *Associate Director of the Cerebral Vascular Center, Department of Neurology, The Cleveland Clinic Foundation, Cleveland, OH*

MAARTEN L. SIMOONS, MD, *Professor of Cardiology, University Hospital Rotterdam, Rotterdam, The Netherlands*

JAMES E. TCHENG, MD, *Associate Professor of Medicine, Department of Medicine, Duke University Medical Center, Durham, NC*

THADDEUS R. TOLLESON, MD, *Fellow in Cardiology, Duke Clinical Research Institute, Durham, NC*

ERIC J. TOPOL, MD, *Professor of Medicine, Chairman, Department of Cardiology; Director, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, The Cleveland Clinic Foundation, Cleveland, OH*

MARCEL J. B. M. VAN DEN BRAND, MD, *Interventional Cardiologist, University Hospital Rotterdam, Rotterdam, The Netherlands*

HARVEY D. WHITE, MD, *Director of Coronary Care & Cardiovascular Research, Green Lane Hospital, Epsom, Auckland, New Zealand*

I Basic Principles

1 Thrombosis in Acute Coronary Syndromes and Coronary Interventions

Thaddeus R. Tolleson, MD
and Robert A. Harrington, MD

CONTENTS

INTRODUCTION

Acute coronary syndromes (ACS), including unstable angina, non-Q-wave myocardial infarction, and ST-elevation myocardial infarction, are the most commonly encountered conditions facing clinical cardiologists today, accounting for more than 650,000 hospitalizations annually *(1)*. The pathophysiology of ACS is now well described, beginning with disruption of an atheromatous plaque, with subsequent platelet aggregation and thrombus formation. The resulting clinical syndromes vary, and depend upon multiple related factors.

The treatment of ischemic coronary disease, including the ACS, by percutaneous interventions has dramatically increased over the past decade. The number of coronary artery lesions treated by percutaneous transluminal coronary angioplasty (PTCA) is expected to exceed 1,000,000 annually in the next several years. Though its success rate is now greater than 90%, acute periprocedural occlusion continues to complicate approximately 6% of all procedures *(1)*. The vast majority of acute occlusions are due, at least in part, to intracoronary thrombosis.

Though the inciting event leading to plaque rupture is different in ACS and percutaneous interventions (spontaneous versus iatrogenic rupture), the resulting arterial pathophysiology is similar for both events. The complex interaction of the exposed endothelium, atherosclerotic plaque, platelets, and coagulation cascade results in thrombosis with compromise in the patency of the vessel in a percentage of patients. Thus, in both ACS and percutaneous interventions, therapies targeting thrombosis have

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proven efficacious, even as new therapies are developed and undergoing clinical trials. This chapter will discuss the pathophysiologic mechanisms responsible for these interactions.

HISTORY

In 1912, Herrick, an American physician, concluded from his clinical experience and research that the common feature in acute myocardial infarction (AMI) was sudden occlusion of a coronary artery *(2)*. Though not readily accepted at first, Herrick's observations slowly gained acceptance over the next 50 years. The clinical syndromes of AMI were described and linked to coronary thrombosis by Levine in Boston *(3),* as well as Parkinson and Bedford in Europe *(4)*. Not long after, two groups of researchers in the United States described the clinical syndrome now known as unstable angina, and again linked its pathophysiology to coronary thrombus *(5,6)*. Heparin was used in some form as early as the 1920s *(7),* and by the late 1930s was being used to treat venous thrombosis *(8)*. Wright is generally credited with being the first American physician to treat a patient with heparin *(9),* and was probably the first to use dicoumarol therapeutically as well *(10)*. He subsequently headed up the American Heart Association Trial of anticoagulation in AMI in 1948, which showed a significant mortality benefit in the treated group *(11)*.

The clinical experience with the use of acetylsalicylic acid (ASA) in the treatment of AMI actually began in the 1940s. Craven, an otorhinolaryngologist in private practice in California, noted that his tonsillectomy patients who chewed excessive amounts of aspergum for pain relief had excessive bleeding *(9)*. He then began to treat all his older male patients with ASA to prevent myocardial infarction (MI). He subsequently published two papers, reporting on more than 8400 treated patients, in whom he found no MI among treated patients *(12,13)*. However, his work appears to have been largely ignored at the time.

Though thrombolytic therapy is often viewed as a relatively recent advancement in the treatment of ACS, reports of the proteolytic activity of urine (later known as urokinase) were first described as early as 1861 *(14)*. The first report of MI being treated with a thrombolytic agent, streptokinase, was in 1958 by Sherry and Fletcher *(15)*.

At the end of the 1950s, with these important clinical findings known, the medical community appeared poised to make significant advances in the treatment of ACS. Instead, in a remarkable turnaround in conceptual understanding and therapeutic approach, physicians began to use less anticoagulation in treating the acute ischemic syndromes over the next two decades. Several key factors likely contributed to this, including misinterpretation of autopsies and the conclusion that coronary thrombus follows, rather than precedes MI; inadequate clinical trials employing nonrandomized designs with insufficient sample sizes; and increasing focus on approaches to limiting infarct size by reducing oxygen demand.

In 1969, Gifford and Feinstein *(16)* reviewed the trials of anticoagulation in AMI, noting the numerous design deficiencies and the paucity of randomized data. Although numerous trials of thrombolytic therapy were conducted between 1960 and 1980, none demonstrated a definitive benefit of therapy. Thus, by the early 1980s, the role of thrombosis in ACS, and hence the value of anticoagulation and thrombolytic therapy, remained controversial.

DeWood *(17),* in a landmark paper, showed that total coronary occlusion was present in 87% of patients in the early hours of transmural myocardial infarction. His group believed that patients in the early hours of infarction were best treated by emergency bypass surgery; hence these patients all had coronary arteriography in the acute phase of their infarction. Subsequent autopsy studies further elucidated the now reemerging concept of thrombosis as the predominant cause of ACS *(18,19)*. Ambrose then correlated the progression from stable angina to unstable coronary syndromes with angiographic evidence of intraluminal thrombus *(20)*. Falk showed through pathologic studies that patients experiencing sudden death preceded by episodes of unstable symptoms often had occlusive thrombus composed of layers of platelet thrombi in various stages of organization *(21)*.

As the pathophysiologic mechanism of ACS became better understood, treatment strategies to alter the natural course of this event soon followed. Thus the understanding of thrombosis as the underlying mechanism in unstable coronary syndromes, as well as in acute occlusion following percutaneous coronary interventions, led to new therapeutic developments aimed at preventing or inhibiting this phenomenon. Once these therapies reached the clinical phase, randomized controlled clinical trials (RCT) have formed the basis of evaluating the efficacy of these treatments.

COMPONENTS OF THE THROMBOTIC PROCESS

Fissuring or rupture of an atherosclerotic plaque, with subsequent thrombus formation reducing or obliterating coronary blood flow, is accepted as the primary mechanism responsible for the development of unstable angina and MI. The pathologic response that follows rupture of an atherosclerotic plaque is similar to the physiologic response to any vascular injury. Whether in ACS or coronary interventions, the process that ultimately results in arterial thrombosis involves the complex interactions of four components of the thrombotic process: the endothelium; the atherosclerotic plaque; platelets; and the coagulation cascade.

The Endothelium

The vascular endothelium controls normal vessel responsiveness and thromboresistance (Fig. 1). It is a multifunctional organ system composed of metabolically active and physiologically responsive component cells that meticulously regulate blood flow. The endothelium forms an obligate monolayer, which lines the entire arterial tree, representing the principal barrier between the blood and the arterial wall. As an active site of protein synthesis, endothelial cells synthesize, secrete, modify, and regulate connective tissue components, vasodilators, vasoconstrictors, anticoagulants, procoagulants, fibrinolytics, proteins, and prostanoids.

The most important function of the vascular endothelium is to prevent the initiation and development of nonphysiologic thrombi. The endothelium normally provides a nonthrombogenic surface because of its surface coat of heparin sulfate and its capacity to form prostaglandin derivatives, particularly prostacyclin (PGI2), a potent vasodilator and effective inhibitor of platelet aggregation *(22)*. Endothelial cells also produce the most potent natural vasodilator known, endothelial-derived relaxing factor (EDRF), a thiolated form of nitric oxide *(23)*. EDRF formation by the endothelium is critical in maintaining a balance between vasoconstriction and vasodilation in the process of arte-

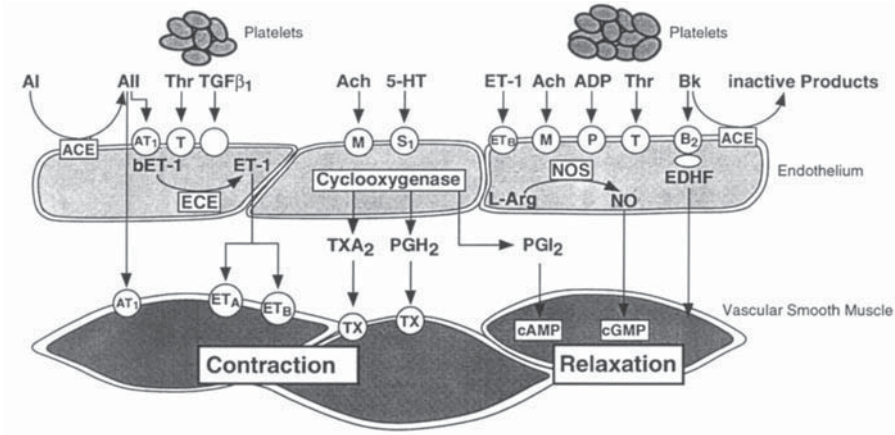

Fig. 1. Endothelium-derived vasoactive substances. The endothelium is a source of relaxing (right part) and contracting factors (left part). AI/AII = angiotensin I/II; Ach = acetylcholine; ADP = adenosine diphosphate; Bk = bradykinin; cAMP/cGMP = cyclic adenosine/guanosine monophosphate; ECE = endothelin-converting enzyme; EDHF = endothelium-derived hyperpolarizing factor; ET-1 = endothelin-1; bET-1 = big endothelin-1; 5-HT = 5-hydroxy-tryptamine (serotonin); L-Arg = *L*-arginine; NO = nitric oxide; PGH_2 = prostaglandin H_2; PGI_2 = prostacyclin; $TGF\beta_{-1}$ = transforming growth factor-β_{-1}; Thr = thrombin; TXA_2 = thromboxane A_2. Circles represent receptors: B_2= bradykinergic receptor; M = muscarinic receptor; P = purinergic receptor; T = thrombin receptor; S = serotonergic receptor.

rial homeostasis *(24)*. Endothelial cells also secrete a number of vasoactive agents—such as endothelin, angiotensin-converting enzyme, and platelet-derived growth factor—that mediate vasoconstriction *(25)*. In addition, these cells secrete agents that are effective in lysing fibrin clots, including plasminogen, as well as procoagulant materials such as von Willebrand factor (Table 1) *(26)*.

In addition to the numerous substances produced and subsequently secreted, endothelial cells also possess receptors for many different molecules on their surface. These include receptors for LDL *(27),* growth factors, and various pharmacological agents. This receptor-ligand interaction on the surface of the endothelial cell serves as the initial substrate for thrombus formation. In addition, the interaction of endothelial cells with cellular elements of the blood, specifically platelets, macrophages, and thrombin, is critical in pathologic thrombus formation in ACS and coronary interventions.

Atherosclerotic Plaque Formation

Our understanding of the pathophysiology of coronary atherosclerosis has changed dramatically in the last few years. The types of atherosclerotic lesions, the mechanisms of progression of coronary atherosclerosis with plaque instability and rupture, and the subsequent thrombotic phenomenon leading to ACS, have now been more clearly elucidated. In 1995, a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis of the American Heart Association *(28)* advised a new classification for atherosclerosis in which lesions are divided into six types (Figs. 2 and 3).

Type I lesions consist mainly of adaptive thickening secondary to smooth muscle cell proliferation in lesion-prone locations in coronary arteries. Type II lesions consist of both macrophages and smooth-muscle cells with extracellular lipid deposits, and type III

Table 1
The Endothelial Cell and Hemostasis

Product	*Properties*
Ectonucleotidases	Surface enzymes that regulate breakdown of platelet-active and vasoactive nucleotides
Nitric oxide	Labile-secreted inhibitor of platelet aggregation and adhesion; vasodilator
Plasminogen activator inhibitor	Secreted, circulating and matrix-bound inhibitor of tPA
Platelet-activating factor	Secreted and cell surface-associated platelet and leukocyte stimulant
Prostacyclin	Labile secreted inhibitor of platelet aggregation; vasodilator
Tissue factor (thromboplastin)	Procoagulant only expressed on activated endothelium
Tissue plasminogen activator (tPA)	Stored and secreted regulator of fibrinolysis
Thrombomodulin	Surface-expressed anticoagulant
Von Willebrand factor and clotting Factor VIII	Stored and secreted cofactor for platelet adhesion

lesions consist of smooth-muscle cells surrounded by some extracellular fibrils and lipid deposits. This type III lesion, or fatty streak, is the earliest grossly detectable lesion of atherosclerosis *(29)*. By age 25, most individuals in Western society have fatty streaks, which consist of an accumulation of lipid (mainly oxidized LDL) within macrophages or foam cells, and mostly in the extracellular space of the intima. Type IV lesions are confluent cellular lesions with a great deal of extracellular lipid, whereas type V lesions consist of an extracellular lipid core covered by a thin fibrous cap. The type VI, or complicated lesion, occurs as a result of rupture or fissure of a nonseverely stenotic type IV or V lesion. Depending on changes in the geometry of the disrupted plaque, and particularly whether the subsequent thrombus completely occludes the artery, the event may be catastrophic, resulting in MI and/or death, or be clinically silent *(30)*.

In the last few years, a number of studies have demonstrated that arteriographically mild coronary lesions may be associated with significant progression to severe stenosis or total occlusion *(31–33)*. These lesions may account for as many as two-thirds of the patients in whom unstable angina or other ACS develop (Table 2).

The nonlinear and episodic progression seen in coronary artery lesions likely results from disruption of type IV and V lesions, with subsequent thrombus formation leading to either ACS or asymptomatic plaque growth. Following plaque disruption, hemorrhage into the plaque, luminal thrombosis, or vasospasm may cause sudden flow obstruction, giving rise to new or changing symptoms. The magnitude of thrombotic response following plaque rupture may depend on the thrombogenicity of the exposed plaque components, local flow conditions determined by the severity and geometry of the luminal stenosis, and the systemic thrombotic-thrombolytic milieu at the time of plaque rupture *(34)*.

What factors predispose a percentage of these plaques to become unstable and rupture? Understanding this requires a closer look at the makeup of these lesions. Plaques are composed of a variable amount of lipid core and a connective tissue matrix cap. The

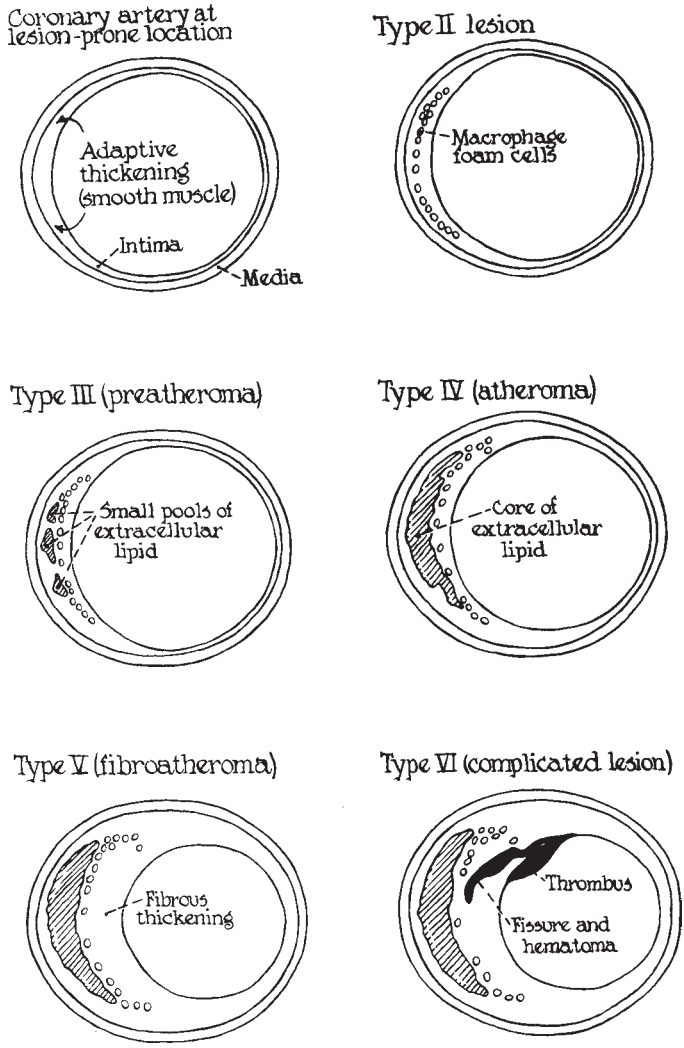

Fig. 2. Cross-sections of identical, most proximal part of six left anterior descending coronary arteries. The morphology of the intima ranges from adaptive intimal thickening always present in this lesion-prone location to a type VI lesion in advanced atherosclerotic disease. Other cross-sections show sequence of atherosclerotic lesion types that may lead to type VI. Identical morphologies may be found in other lesion-prone parts of the coronary and many other arteries. From ref. *28*.

lipid component is derived mainly from plasma LDL, which has been oxidized and subsequently taken up by monocyte-derived macrophages, now known as foam cells. Within the core is a soft, hypocellular, and avascular "gruel" containing cholesterol *(35,36)*. The composition of the cholesterol is critically important, as atheromatous gruel containing cholesterol esters is soft and prone to disruption *(37)*. Once plaque rupture occurs, the thrombogenic components of the gruel (tissue factor, collagen, foam cells, etc.) provide a substrate for subsequent thrombus formation.

The process of plaque disruption can best be understood in terms of factors that increase the vulnerability of the plaque to rupture, and stresses or strains imposed on the

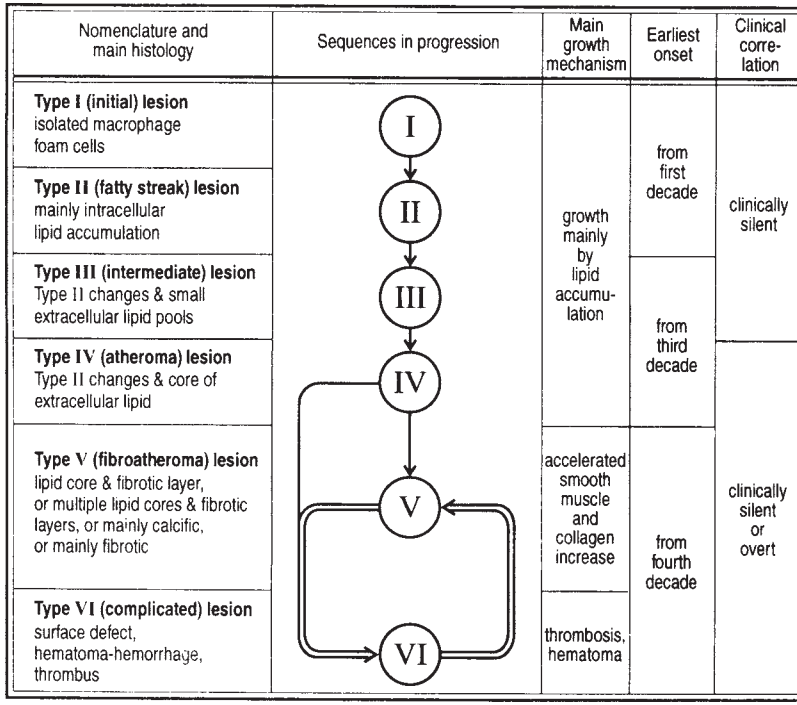

Fig. 3. Flow diagram in center column indicates pathways in evolution and progression of human atherosclerotic lesions. Roman numerals indicate histologically characteristic types of lesions enumerated in Table 2 and defined at left of the flow diagram. The direction of arrows indicates sequence in which characteristic morphologies may change. From type I to type IV, changes in lesion morphology occur primarily because of increasing accumulation of lipids. The loop between types V and VI illustrates how lesions increase in thickness when thrombotic deposits form on their surfaces. Thrombotic deposits may form repeatedly over varied time spans in the same location and may be the principal mechanism for gradual occlusion of medium-sized arteries. From ref. *28*.

plaque, referred to as "triggers". Plaque vulnerability to rupture is dependent on at least three interrelated factors: lipid content, the thickness of the fibrous cap, and subintimal inflammation within the plaque (Fig. 4).

Lipid Content of the Plaque

The size and consistency of the atheromatous lipid core are important factors for the stability of a plaque. In general, the bigger the lipid core, the more vulnerable (rupture-prone) is the plaque *(38)*. Data from Ambrose et al. suggest that in ulcerated, ruptured plaques, the size of the lipid pool exceeds 40% of the total plaque area in more than 90% of cases *(19)*. At body temperature, the cholesterol is in liquid or crystalline form *(39)*; the ratio of liquid to crystalline cholesterol has been hypothesized to influence the propensity for plaque disruption *(40)*.

Thickness of the Fibrous Cap

As this highly thrombogenic lipid core underlies the fibrous cap, the integrity of the fibrous cap determines the stability of an atherosclerotic plaque. Disruption of the fibrous cap usually occurs at the point where the cap is thinnest, most commonly at

Table 2
Angiographic Severity of Culprit Coronary Artery Stenosis Before the Development of Acute Coronary Syndromes[a]

		Percentage of patients with diameter stenosis of culprit vessel		
		<50%	*50%–70%*	*>70%*
Clinical presentation				
Unstable angina				
Ambrose et al. *(3)*	*N* = 25	72	16	12
Acute myocardial infarction				
Ambrose et al. *(3)*	*N* = 23	48	30	22
Little et al. *(82)*	*N* = 41	66	31	3
Giroud et al. *(50)*	*N* = 92	78	9	13
Nobuyoshi et al. *(94)*	*N* = 39	59	15	26
Average of pooled results		65	20	15

[a]From ref. *34*. References in table are found in ref. *34*.

the border of the plaque with the normal wall or in the center of the cap overlying a lipid pool *(41)*.

A dense, fibrous extracellular matrix is the main component of the fibrous cap of atherosclerotic plaques. The principal constituents of this extracellular matrix are types I and III collagen (a triple-helical coil derived from specific procollagen precursors), elastin, and proteoglycans (Fig. 5) *(42)*. Interferon-B (INF-B) elaborated by activated T-cells reduces collagen synthesis by causing smooth muscle cell apoptosis and by specifically inhibiting collagen synthesis in smooth muscle cells. Additionally, the matrix metalloproteinases, such as collagenase and stromelysin, which facilitate intercellular matrix degradation, are released by lipid-laden macrophages under the influence of cytokines such as INF-B, macrophage colony-stimulating factor (M-CSF), macrophage chemoattractant protein-1 (MCP-1), and interleukin-1 (IL-1). These metalloproteinases can also be expressed by endothelial and smooth muscle cells in the plaque, after being activated by cytokines *(23)*. Libby has shown that cytokines do not appear to affect the synthesis of tissue inhibitors of matrix metalloproteinases *(43)*. This lab has also helped to define the role of another important cytokine, interferon gamma (INF-γ). Among the cells found in human atherosclerotic plaques, only activated T-lymphocytes can elaborate INF-γ. This interferon markedly decreased the ability of human smooth muscle cells to express the interstitial collagen genes when exposed to transforming growth factor-B (TGF-β), the most potent stimulus for interstitial collagen gene expression known for these cells *(25)*.

Subintimal Inflammation

Atherosclerosis has been characterized as a chronic inflammatory disease of the intima *(44)*. Chronic inflammatory cells are commonly noted on histologic sections of advanced lesions and at sites of plaque disruption, as well as being a constant feature at the site of thrombosis *(45)*.

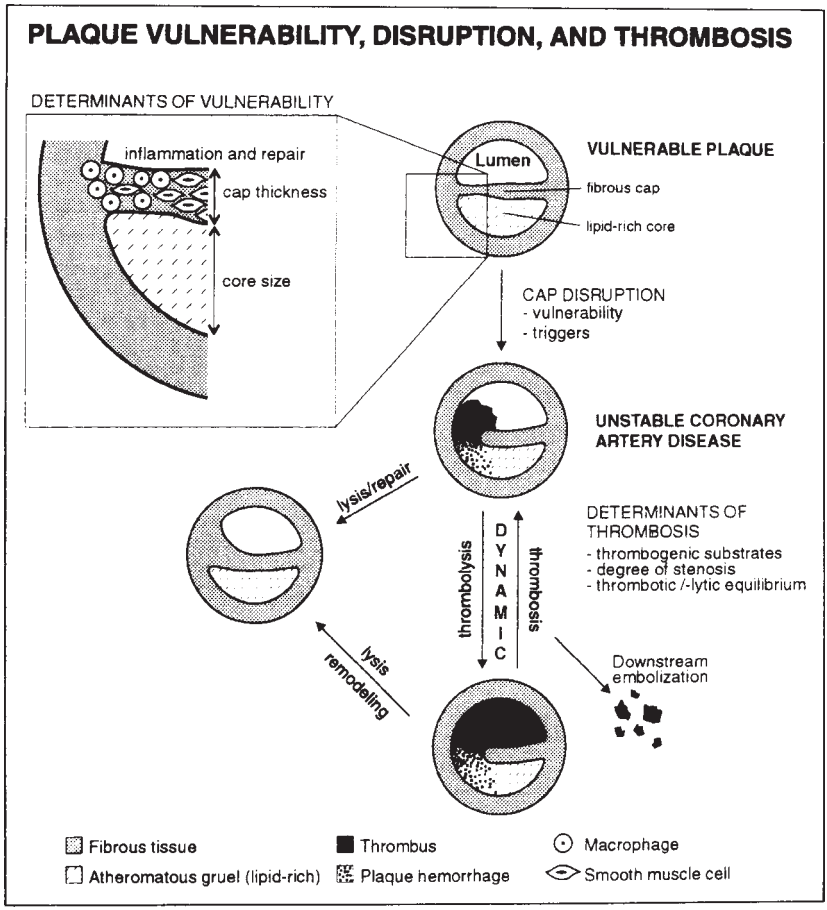

Fig. 4. Schematic illustrating pathophysiologic mechanisms. Reprinted with permission from ref. *73*.

Inflammatory cells, especially activated macrophages and foam cells, play an important role in plaque disruption by inducing breakdown of various matrix proteins in the fibrous cap, causing the cap to thin, thereby making it vulnerable to disruption *(16)*. Macrophages have the capacity to synthesize matrix-degrading neutral metalloproteinases (MMPs), including interstitial collagenase (MMP-1), gelatinase-A (MMP-2), gelatinase-B (MMP-9), and stromelysin (MMP-3) *(16)*. This family of enzymes, which have been detected in foam cells in atherosclerotic plaques taken from human as well as animal subjects, can digest all the structural matrix components of the fibrous cap *(46)*. The enzymes are usually localized to the rupture-prone shoulder regions and to sites of enhanced circumferential stress *(47)*. Thus, the processes of plaque inflammation and integrity of the fibrous cap are intimately related, and one cannot properly discuss one or the other without a clear understanding of both processes.

Triggers

Events triggering plaque rupture have been intensely investigated. Coronary plaques are constantly stressed by a variety of mechanical and hemodynamic forces that may trigger disruption of vulnerable plaques *(48)*. These include plasma catecholamine surges

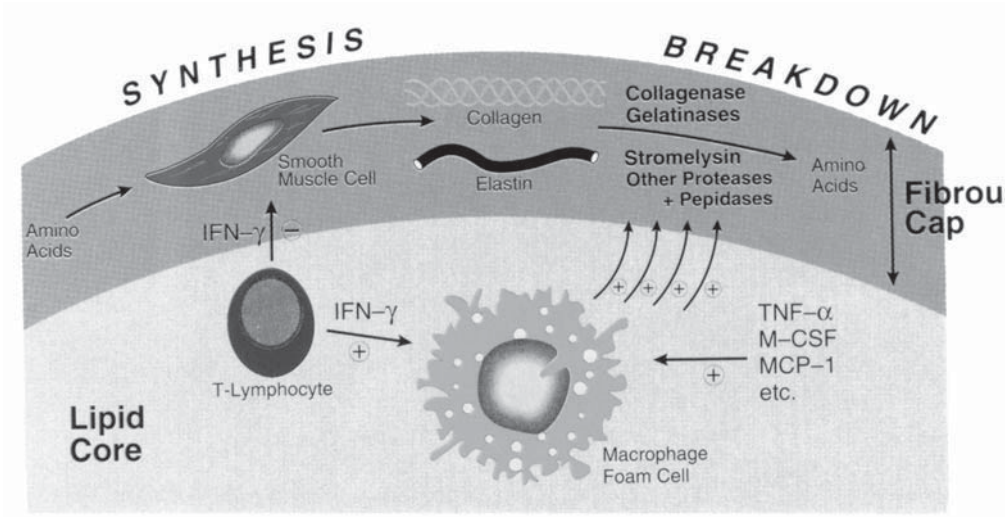

Fig. 5. Black-and-white diagram showing metabolism of collagen and elastin in the plaque's fibrous cap. The vascular smooth muscle cell synthesizes the extracellular matrix protein, collagen, and elastin from amino acids. In the unstable plaque, interferon-γ (IFN-γ) secreted by activated T cells may inhibit collagen synthesis, interfering with the maintenance and repair of the collagenous framework of the plaque's fibrous cap. The activated macrophage secretes proteinases that can break down both collagen and elastin to peptides and eventually amino acid. This breakdown of these structural molecules of the extracellular matrix can weaken the fibrous cap, rendering if particularly susceptible to rupture and precipitation of acute coronary syndromes. IFN-γ secreted by the T lymphocytes can in turn activate the macrophage. Plaques also contain other activators of macrophages, including g tumor necrosis factor-α (TNF-α), macrophage colony-stimulating factor (M-CSF), and macrophage chemoattractant protein-1 (MCP-1), among others. From ref. *42*.

and increased sympathetic activity *(49),* blood pressure surges, exercise *(50),* changes in heart rate and contractility affecting the angulation of coronary arteries, coronary vasospasm *(51),* and various hemodynamic forces *(52,53).* Hence, plaque rupture is a function of both internal plaque changes (vulnerability) and external stresses (triggers). Vulnerability is more important than triggers in determining the risk of a future event, because if no vulnerable plaques are present in the coronary arteries, there is no rupture-prone substrate for a potential trigger to affect *(54).*

Platelets

Once plaque rupture occurs, exposing thrombogenic substances to flowing blood, the interaction of platelets and coagulation factors, namely thrombin, determines the magnitude and extent of thrombosis at the site (Fig. 6). Platelet deposition occurs almost instantaneously after deep arterial injury. Triggered by damage to the vessel wall and local exposure of the subendothelial matrix, platelets adhere to subendothelial collagen. At least two rheological factors potentiate early platelet binding to the subendothelium. First, platelets are unevenly distributed in flowing arterial blood. Owing to the dynamics of laminar flow and the relative densities of the blood corpuscles, platelets tend to concentrate at the periphery, directly adjacent to the endothelium *(55).* This enhances maximal platelet-collagen interaction and facilitates binding in areas of endothelial

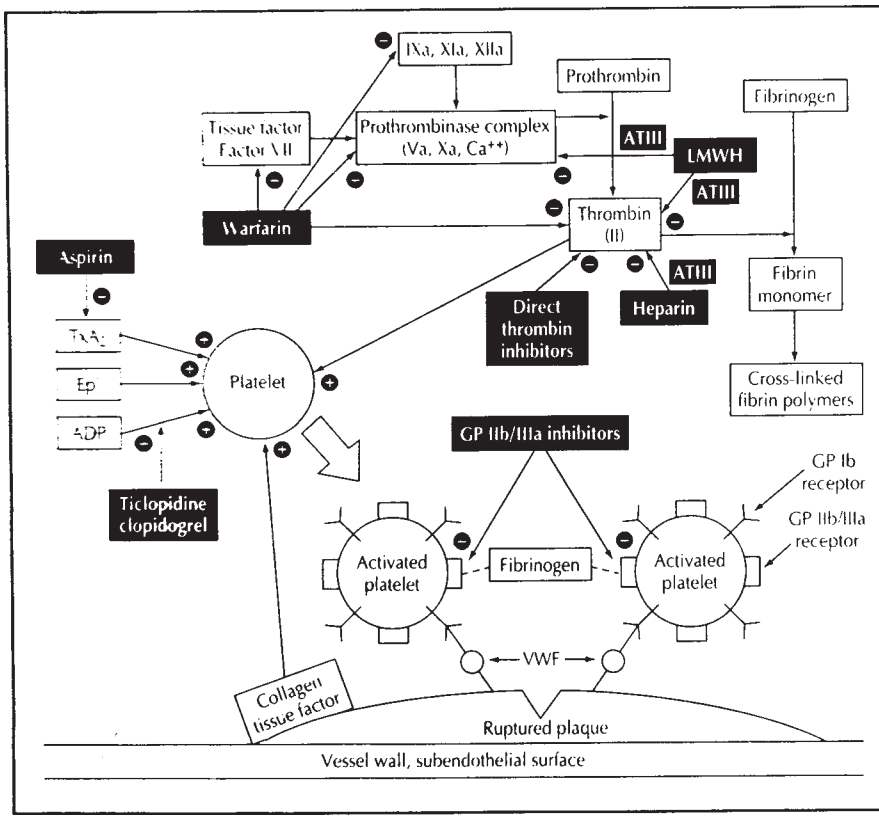

Fig. 6. Interaction between antiplatelet and antithrombin agents and the process of thrombosis. ADP = adenosine diphosphate; ATIII = antithrombin III; Epi = epinephrine; GP Ib receptor = glycoprotein Ib receptor; GP IIb/IIIa receptor = glycoprotein IIb/IIIa receptor; GP IIb/IIIa inhibitors = glycoprotein IIb/IIIa inhibitors; LMWH = low-molecular-weight heparin; TxA_2 = thromboxane A_2; VWF = von Willebrand's factor. Reprinted with permission from ref. *74*.

injury. Second, through poorly understood mechanisms, high shear stress is known to increase activation of platelets *(55)*. Platelets traveling through an area rendered acutely stenotic by plaque rupture are transiently exposed to high shear stress forces and may become activated. Adherence is mediated primarily by the von Willebrand Factor (vWF), a multimeric glycosylated protein that is synthesized in endothelial cells and secreted into the subendothelium, where it binds to collagen *(55)*. Coverage of the exposed site by platelets depends on the recognition of adhesive proteins by specific platelet-membrane glycoproteins, many of which are integrins *(56)*.

The glycoprotein (GP) Ib receptor, which exists in a complex with glycoprotein-IX and glycoprotein-V on the platelet surface, binds vWF and is the principal glycoprotein involved in the initial contact between platelets and the vessel wall *(57)*. Because GP Ib is a constitutively expressed integrin, resting platelets can bind vWF and thereby adhere to collagen without first being activated. Platelets then continue accruing at the site of endothelial cell denudation until the entire area of injury is covered by a platelet monolayer, anchored to the subendothelium via the GP Ib-vWF bond.

Platelet activation follows adhesion and can be initiated by several mechanical and

Table 3
Platelet Granule Content

Alpha granules	*Dense granules*	*Lysosomes*
Fibrinogen	Serotonin	Glucose-6 phosphatase
Thrombospondin	Calcium	Acid phosphatase
Beta thromboglobulin	ATP	Platelet Factor 3
Platelet Factor 4	ADP	B-*N*-Acetyl-galactosominidase
Albumin	Pyrophosphate	α-Arabinosidase
vWF		
Fibronectin		
Factor V		
∞ 2 Macroglobulin		
Vitronectin		
α_1-Proteinase inhibitor		
Histidine-rich glycoprotein		
Platelet-derived growth factor		

chemical stimuli. The presence of thrombin and the adhesion of platelets to collagen and other components of the subendothelial matrix are among the strongest stimulators of platelet activation. Though they are capable of little or no protein synthesis, platelets contain, sequestered in their granules, numerous prepacked extraordinarily potent molecules (Table 3) *(58)*. Once bound and subsequently activated, these platelets release the contents of their storage granules, which contain, in addition to potent growth factors (mitogens), various other substances such as serotonin, ADP, thromboxane A2, and epinephrine that are capable of activating additional nearby platelets. Irrespective of the agonist, the final common pathway leading to the formation of the platelet plug is platelet aggregation. These activated platelets, however, do not bind via the aforementioned GP Ib-vWF interaction, as these sites are already occupied by the initial platelet monolayer. Instead, further platelet recruitment depends upon the expression of a second platelet receptor, the glycoprotein IIb/IIIa (GP IIb-IIIa) receptor complex (Fig. 7).

The GP IIb-IIIa receptor belongs to the integrin family of heterodimeric adhesion molecules, which are formed by the noncovalent interaction of a series of alpha and beta subunits *(59)*. Integrins are found on virtually all cell types and mediate a diversity of physiologic responses. Multiple integrins (Ia/IIa, Ic, IIa, receptor for Laminin, etc.) are present on the surface of the platelet and play a role in platelet adhesion *(60)*. The GP IIb-IIIa receptor is the most abundant on the platelet surface, with approximately 50,000 copies per platelet. Though clearly the most clinically important interaction is with fibrinogen, the receptor has also been shown to bind other adhesive proteins involved in aggregation, such as fibronectin, vitronectin, and vWF *(61,62)*.

The recognition specificity of the GP IIb-IIIa receptor is defined by two peptide sequences. The Arg-Gly-Asp (RGD) sequence was initially identified as the adhesive sequence in fibronectin, but is also present in fibrinogen, vWF, and vitronectin. All these ligands contain at least one RGD sequence, whereas fibrinogen contains two RGD sequences per half-molecule *(42)*. The second sequence involved is the Lys-Gln-Ala-Gly-Asp-Val sequence, located at the extreme carboxy terminus of the gamma chain of fibrinogen *(63,64)*. Unlike the RGD sequence, this sequence is found only in fibrinogen.

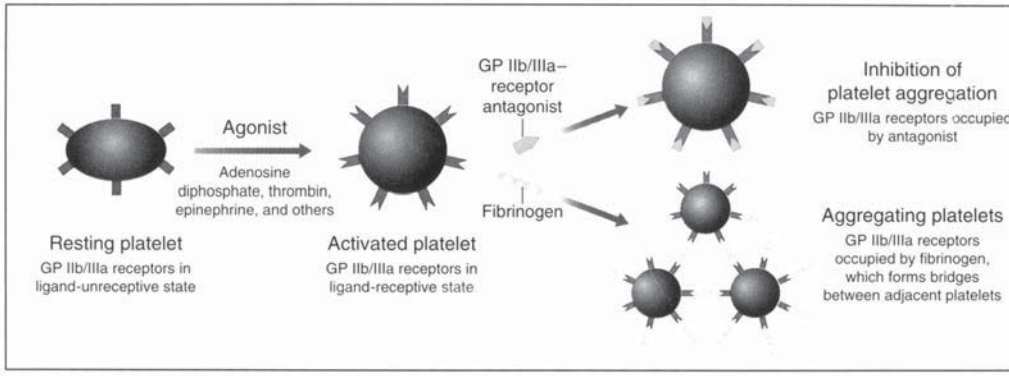

Fig. 7. Overview of the processes of platelet activation and aggregation and the inhibition of platelet aggregation by inhibitors of glycoprotein (GP) IIb/IIIa receptors. Platelet activation causes changes in the shape of platelets and conformational changes in GP IIb/IIIa receptors, transforming the receptors from a ligand-unreceptive to a ligand-receptive state. Ligand-receptive GP IIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of GP IIb/IIIa receptors also bind to GP IIb/III receptors, blocking the binding of fibrinogen and thus preventing platelet aggregation. From ref. *57*.

Electron microscopic (EM) and immunological studies suggest that this second sequence is the predominant site for fibrinogen-GP IIb-IIIa binding *(65,66)*.

Resting platelets do not express GP IIb-IIIa in a configuration suitable for ligand binding, but upon platelet activation this complex undergoes a conformational change that allows it to avidly bind fibrinogen *(67)*. Once activated, the original platelet monolayer recruits additional platelets, eventually forming a platelet plug via GP IIb-IIIa-fibrinogen-GP IIb-IIIa bridging. This process replicates itself as new platelets enter the injured vascular bed, become activated, expressing GP IIb-IIIa receptors in the appropriate conformation, and become incorporated into the growing plug. The area of previously denuded endothelium is thereby quickly covered by the growing platelet plug. Left unchecked with no compensatory mechanisms, this process would lead to arterial lumen occlusion. This is prevented as neighboring endothelial cells secrete anti-aggregatory agents including prostacyclin, endothelial derived relaxation factor (EDRF) or nitric oxide (NO), as it is now known, and ADPase *(34)*.

The Coagulation Cascade

The initial flow obstruction after arterial injury is usually due to platelet aggregation. However, a pure platelet thrombus is very unstable, and may be easily dislodged unless it is subsequently reinforced by fibrin cross-linking. Therefore, both platelets and fibrin play intimate roles in thrombus formation.

Following arterial injury, in vivo coagulation is initiated by the tissue factor-dependent pathway. This system becomes activated when tissue factor comes in contact with circulating factor VII, a plasma zymogen (Fig. 8). Recall that the coenzyme tissue factor is found on the surface of macrophages, fibroblasts, smooth muscle cells, and activated endothelial cells. The atherosclerotic plaque itself also contains abundant tissue factor, which is synthesized by lipid-laden foamy macrophages and is predominantly localized in the necrotic core of the plaque *(68)*.

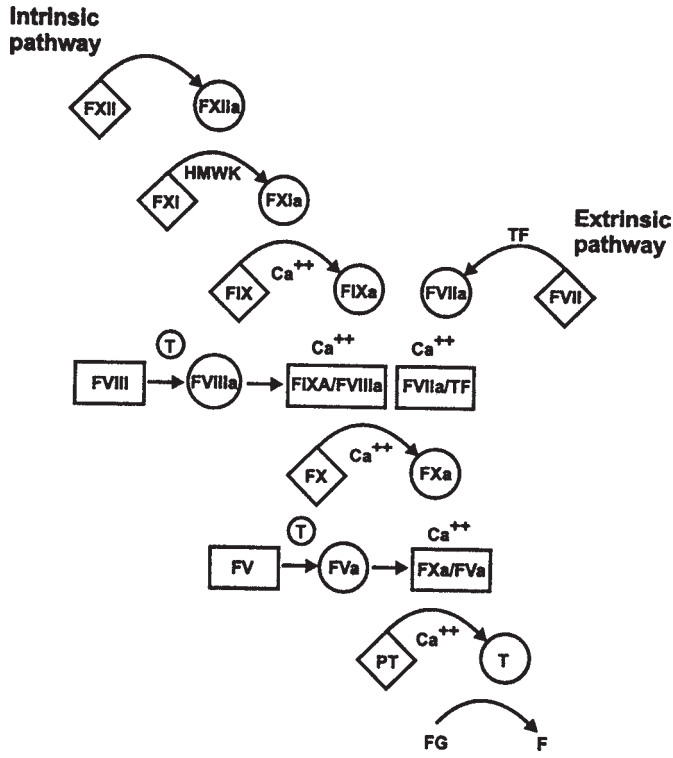

Fig. 8. Platelet structure. Legend: FX = factor X; TF = tissue factor; PT = prothrombin; T = thrombin; F = fibrin; FG = fibrinogen; HMWK = high molecular weight kininogens; Ca^{++} = calcium. Reprinted with permission from ref. *75*.

The tissue factor (TF)-VII complex undergoes limited autoactivation, generating the more potent TF-VIIa complex. This process then replicates, leading to a self-amplification of the TF-VIIa complex *(35)*. After self-amplification, the TF-VIIa complex then forms an additional complex with plasma coagulation factor X on the surface of activated platelets. This is thought to occur on activated platelets because the platelet membranes contain essential phospholipids that are in the correct configuration to support coagulation. Once factor X is converted to Xa by limited proteolysis, it disengages from the complex, then reassembles on the platelet with factor V to form the so-called "prothrombinase" complex (Xa-V). However, in its inactive form, this complex is inefficient in converting prothrombin to thrombin. The mechanism of thrombin-mediated positive feedback is not fully understood, but moderate levels of thrombin may be capable of activating factor V to a more potent cofactor, factor Va *(69)*. This then assembles with Xa to form the Xa-Va prothrombinase complex, with dramatically more thrombin-generating capability *(70)*. Increased levels of thrombin also enhance the conversion of factor VIII to VIIIa, which then combines with factor IXa on the platelet surface, dramatically increasing the conversion of factor X to Xa, and subsequently generating even more prothrombinase complex *(71)*.

Each of the reactions following the TF–VII interaction occurs on the surface of activated platelets. Hence, thrombin-mediated platelet activation can be considered another of the positive feedback mechanisms responsible for the dramatic increase in thrombin

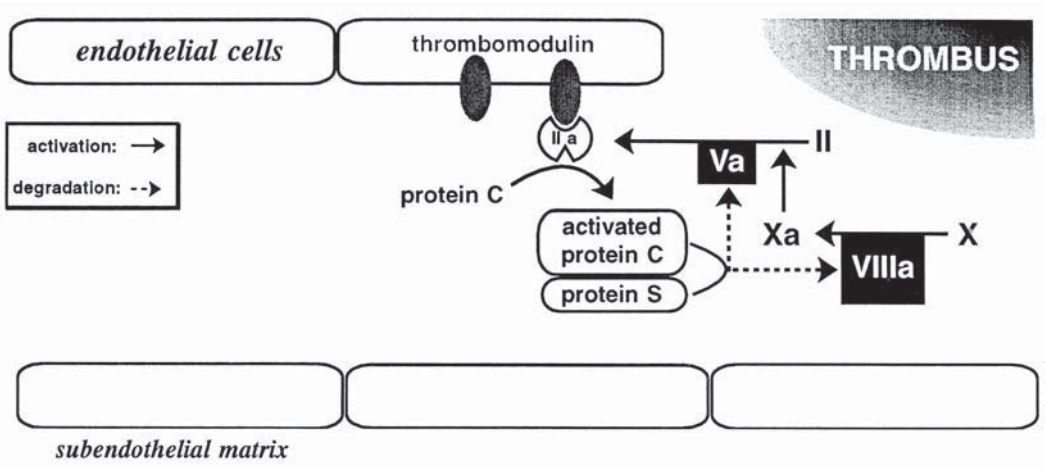

Fig. 9. Feedback mechanism for the inactivation of factors Va and VIIIa. Thrombin (IIa) bound to thrombomodulin specifically activates protein C. Activated protein C, in concert with protein S, efficiently cleaves and thus inactivates the coagulation factors VIIIa and Va required for the assembly of the ternary complexes to accelerate the thrombin generation. This potent feedback mechanism may be essential to confine e excessive thrombin generation to severely injured, deendothelialized parts of the blood vessel. From ref. *54*.

generation leading to clot formation. More intense platelet recruitment increases the available platelet surface area, thus enhancing thrombin generation. Thrombin presumably plays a key role in additional platelet recruitment, as thrombin itself is the most potent physiological activator of platelets *(35)*.

Similar to the mechanisms that limit platelet growth to areas of endothelial injury, there are also mechanisms to limit thrombin generation and subsequent fibrin formation. The two principal inhibitors are the heparin sulfate-antithrombin III (AT III) system and the thrombomodulin-protein C-protein S system. AT III binds circulating thrombin, inactivates it, and eventually clears it through the reticuloendothelial system. Once bound by heparin, AT III undergoes a conformational change that greatly enhances its affinity for thrombin *(72)*.

In the presence of ongoing thrombin generation, neighboring endothelial cells increase their concentration of a membrane-bound protein receptor, thrombomodulin (Fig. 9). This protein is capable of modifying the function (specificity) of thrombin. Whereas the preferred substrates for free thrombin are factors V, VIII, fibrinogen, and possibly factor XI, thrombin associated with endothelial-cell thrombomodulin alters its affinity so that it binds specifically (and virtually exclusively) to a plasma protein referred to as protein C *(35)*. Limited proteolysis of protein C by thrombomodulin-bound thrombin activates protein C, which then associates with protein S. This complex is then able to inhibit factors Va and VIIIa, abolishing the procoagulant properties of these cofactors.

THE PROCESS OF THROMBUS FORMATION

Though it is conceptually useful to view the components of the thrombotic process individually, it must be understood that these processes occur simultaneously and in concert. As mentioned previously, without the appropriate substrate (a vulnerable plaque), the various triggers known to be associated with unstable coronary syndromes

are of no clinical consequence. Once plaque rupture does occur, the response to this perturbation is multifactorial, with the final clinical outcome dependent on the interplay of the components described above, namely, the (dysfunctional) endothelium, the procoagulant constituents of the exposed plaque, activated platelets, and tissue factor-dependent coagulation mediated primarily by thrombin. It appears as if most ruptured plaques are resealed by a small mural thrombus, and only infrequently does a major luminal thrombus evolve. The factors that seem to determine the thrombotic response include the character and extent of the exposed thrombogenic substrate; local flow disturbances secondary to degree of stenosis and surface irregularities; and thrombotic-thrombolytic equilibrium at the time of rupture *(58),* incorporating the dynamic interaction between activated platelets and components of the coagulation cascade.

Based on these mechanisms of thrombus formation and propagation, treatment strategies utilizing an understanding of the molecular pathophysiology of this process continue to be developed. These then form the underpinnings of randomized controlled trials to evaluate the clinical outcomes of these therapies in populations of patients undergoing percutaneous interventions or with ACS.

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2 Mechanisms of Platelet Adhesion

Edward F. Plow, PhD

Contents

INTRODUCTION

Platelets are born from megakaryocytes and are bred to adhere. As such, these anuclear particles represent one of the most highly specialized cells within the body. This functional dedication to adhesion is essential in order to prevent excessive bleeding from sites of vascular injury. The rapidity with which platelets seal an injured vessel is a remarkable testament to their adhesive specialization and is essential to the maintenance and/or restoration of vascular integrity. As with many physiological systems, an overly exuberant cellular response can have major pathophysiological consequences. In the case of platelet adhesion, the out-of-control response can be devastating, thrombosis.

Platelet attachment and spreading reactions, together referred to as platelet adhesion, are essential for the hemostatic function of platelets. Platelet adhesion depends primarily upon interaction of these cells with components of the subendothelial matrix that become exposed as a consequence of injury to the endothelial cell lining of the vessel wall. Engagement of these substrates is mediated by an array of adhesion receptors which are displayed upon the cell surface. Platelet–platelet interactions, platelet aggregation, are essential for formation of a complex thrombus, and, thereby, for the thrombotic properties of platelets. Platelet aggregation depends primarily upon the engagement of the plasma proteins, fibrinogen, and von Willebrand factor (vWF), by the cell-surface glycoprotein, GPIIb-IIIa (integrin $\alpha_{IIb}\beta_3$). In theory, the thrombotic function of platelets can be inhibited without severely compromising their hemostatic function by blockade of GPIIb-IIIa. Direct evidence that this theory can, in fact, be reduced to practice has been provided from studies in animal models and from clinical trials in humans.

From one perspective, this volume can be viewed as a testimonial to the principle that the adhesion and aggregation functions of platelets are sufficiently separable that GPIIb-IIIa can be targeted for antithrombotic therapy without causing excessive bleeding. This

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chapter focuses on the mechanisms underlying platelet adhesion. The major receptor systems and their ligands will be discussed briefly. GPIIb-IIIa and its role in platelet aggregation are considered in Chapter 3. Unfortunately, the adhesive and aggregation functions of platelets are not entirely separable, and a bleeding risk may be intrinsic to effective blockade of GPIIb-IIIa. This overlap may be due to the requirement for some platelet aggregation for effective hemostasis or, as discussed below, the role of GPIIb-IIIa not only in platelet aggregation but also in platelet adhesion. It is the severity of the bleeding risk relative to the antithrombotic efficacy that will shape the future of GPIIb-IIIa antagonists.

SUBSTRATE PROTEINS FOR PLATELET ADHESION

The Subendothelial Matrix

In addition to serving as a physical barrier, endothelial cells synthesize and elaborate components, notably PGI_2, NO, and enzyme(s) that degrades ADP *(1,2),* which prevent platelet activation and adhesion. When the endothelium is disrupted, these platelet suppressive activities are lost, and components of the subendothelium which support platelet adhesion become exposed. As discussed shortly, there are a variety of subendothelial cell constituents that serve as substrates for platelet attachment and spreading. Furthermore, the matrix is a mutable surface. It can be remodeled as a consequence of its interaction with plasma proteins and/or by proteolysis, and these alterations influence its capacity to support platelet adhesion. Another relevant consideration is that, since the phenotypic properties of endothelial cells from different blood vessels varies, the composition of the matrix they deposit will vary as well. Accordingly, certain proteins may play a dominant role in supporting platelet adhesion in certain blood vessels. Moreover, even at the same anatomical location, the composition of the atherosclerotic plaque and the nature of the fissure or injury to the endothelium will expose different substratum.

Some of the major subendothelial matrix proteins that support platelet attachment and/or spreading reactions are listed in Table 1. From the extent of this list, it is clear that platelets can adhere to a variety of substrates once the endothelium has been disrupted. Not all of the adhesive proteins listed in Table 1 support the same spectrum of platelet responses. For example, under some conditions, platelets attach to but do not spread on laminin *(3),* whereas vWF and fibronectin appear to support both cell attachment and spreading *(4–6)*. Certain collagen types not only support the attachment and spreading of platelets but also evoke platelet secretion *(7)*. In turn, the secretory response, which can be induced by a variety of platelet agonists, directly influences the adhesive properties of platelets by altering their surface. Specifically, as a consequence of the secretory response, certain adhesion receptors become expressed (e.g., P-selectin) *(8)* and the density and/or distribution of others becomes altered [e.g., GPIIb-IIIa *(9),* GPIb-IX *(10)*]. Of the multiple forms of collagen, types I, III, IV, and VI are regarded as being particularly important in supporting platelet adhesion *(11,12)*.

Although endothelial disruption is the primary stimulus for platelet adhesion, there is also developing evidence that platelets can interact with activated endothelial cells *(13)* and neutrophils, in turn, can interact with the surface of such adherent platelets *(14,15)*. The role of such cell–cell interactions in thrombus formation remains to be determined. Although not interacting directly with platelets, erythrocytes also exert a

Table 1
Major Subendothelial Proteins that Support Platelet Adhesion

Matrix constituent	*Comment*
Collagens	Large family of proteins that can support platelet adhesion aggregation, and secretion
von Willebrand factor	Large multimeric protein critical for the hemostatic function of platelets
Fibronectins	Dimeric or multimeric proteins that support attachment and spreading of platelets
Thrombospondins	Trimeric proteins exhibiting both adhesive and antiadhesive properties for platelets
Laminins	Proteins supporting platelet adhesion

marked effect on adhesion of platelet to the vessel wall *(16)*. Thus, the subendothelial matrix, and perhaps even intact endothelium, provides multiple substrates and mechanisms to support platelet adhesion, and other vascular cells can modulate these interactions.

Nonmatrix Adhesive Proteins

In addition to the subendothelial matrix, proteins that support platelet adhesion can be derived from two other biologically relevant sources: blood, which comes in contact with the injured vessel; and platelet alpha-granules, which secrete their content of adhesive proteins when stimulated by agonists. The sources of key platelet adhesive proteins are listed in Table 2. Some of the platelet adhesive proteins (e.g., vWF and fibronectin) reside in all three locations. Fibrinogen and vitronectin are present in platelets and plasma, but are apparently not synthesized by the endothelial cells. However, they are components of the provisional matrix formed at sites of vascular injury and are, therefore, particularly relevant substrates for platelet adhesion. It should be noted that the molecular forms of the adhesive proteins derived from these different sources are not identical. For example, the degree of vWF multimerization differs for the plasma and platelet forms *(17),* and the splicing variants of fibronectin differ depending upon their sites of synthesis *(18)*. As still yet another variable, a variety of these adhesive proteins interact with one another. For example, vWF, fibronectin, and thrombospondin all bind to collagen *(19–21)*. These interactions may indirectly bridge platelets to a matrix protein or may modulate the adhesive properties of the matrix.

Shear and Platelet Adhesion

Shear rate developed by flowing blood varies with vessel caliber. Local turbulence may develop as blood flows across the irregularities of an atherosclerotic plaque or a developing thrombus. Variations in the fluid dynamics of blood greatly influences platelet adhesion *(22–24)*. Over the past decade, more and more studies of platelet adhesion have been conducted under flow conditions to help ensure the biological relevance of the analyses. Shear is particularly important in defining the contribution of vWF to platelet adhesion. In in vitro experiments, a role of vWF in platelet adhesion is readily demon-

Table 2
Origins of Platelet Adhesive Proteins

Adhesive protein	*Matrix*	*Plasma*	*Platelets*
von Willebrand factor	+	+	+
Fibronectin	+	+	+
Thrombospondin	+	–	+
Vitronectin	–[a]	+	+
Fibrinogen	–[a]	+	+
Laminin	+	–	–
Collagen	+	–	–

[a]Constituent of provisional matrix.

strable at high but not at low shear or under static conditions *(5,25)*. [By contrast, a role for fibronectin in supporting platelet adhesion can be demonstrated at both high and low shear *(26)*.] Nevertheless, patients with von Willebrand Disease have a major bleeding diathesis, attesting to the importance of vWF and shear in supporting platelet function. Shear may exert its influence on platelet:vWF adhesion by affecting the conformation of the adhesive protein or its platelet receptor, GPIb, or both *(24,27,28)*.

PLATELET ADHESION RECEPTORS

The individual adhesive proteins discussed above interact with platelets by serving as ligands for specific cell-surface receptors. The major platelet adhesion receptors are listed in Table 3. Several nomenclature systems have been used to identify the same membrane proteins of the platelet. The original nomenclature was based on electrophoretic mobility, giving rise to glycoprotein (GP) I, II, III, etc., with GPI having the highest molecular weight. As gel separation and protein detection systems became more sophisticated, several proteins were discerned with similar electrophoretic mobilities (e.g., GPI became GPIa, GPIb, and GPIc). Several of the membrane proteins exist on the platelet surface as noncovalent complexes; thus, GPIb-IX, GPIc-IIa, GPIIb-IIIa, should be viewed as single membrane proteins with multiple subunits. Despite these ambiguities, the nomenclature based on electrophoretic properties remains widely used. Other nomenclature systems have arisen from the fact that several platelet membrane proteins are present and have been assigned different names on other cell types. This is the basis for the very late antigens (VLAs) and leukocyte differentiation (CD) designations, which now receive limited usage in the platelet literature. Some receptors also are identified on the basis of their function (e.g., the vitronectin and the fibronectin receptors). A functional designation has a major limitation since multiple membrane proteins can function as receptors for the same ligand, e.g., vitronectin ($\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$) and fibronectin ($\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$), and several platelet membrane proteins can function as collagen receptors (see below). Beyond creating a nomenclature complexity (nightmare), the functional redundancy of the platelet receptors enables the cell to establish multiple contacts with a single matrix constituent. In turn, a single ligand may initiate several distinct functional responses by engaging multiple receptors. The major platelet adhesion receptors are discussed as follows.

Table 3
Major Platelet Adhesion Receptors

Receptor	*Common alternative designations*	*Major adhesive ligands*
GPIa-IIa	$\alpha_2\beta_1$, VLA-2	Collagen
GPIb-IX-V		vWF
GPIc-IIa	$\alpha_5\beta_1$, VLA-5, fibronectin receptor	Fibronectin
GPIIb-IIIa	$\alpha_{IIb}\beta_3$	Fibrinogen, vWF, fibronectin, vitronectin, thrombospondin
GPIV	GPIIIb, CD36	Collagen, thrombospondin
GPVI		Collagen
$\alpha_6\beta_1$		Laminin
$\alpha_V\beta_3$	Vitronectin receptor	Vitronectin, fibrinogen, fibronectin, vWf thrombospondin, osteopontin

Platelet Integrins

Many of the adhesive protein receptors on platelets, including GPIIb-IIIa, are integrins. The integrins are a broadly distributed family of heterodimeric cell-surface molecules that share certain structural, immunochemical, and functional properties *(29–33)*. A prototypic integrin structure is illustrated in Fig. 1A. The β-subunits, of which eight are known currently, are highly homologous to one another, exhibiting extensive (35–45%) identity at the primary amino acid sequence level. The α-subunits, of which 15 are presently known, are also similar to one another, but exhibit less extensive sequence identity. The α-subunits are synthesized as single-chain polypeptides; but some, such as GPIIb (α_{IIb}), are proteolytically processed to a two-chain, disulfide linked form *(34–37)*. Each α-subunit has a short cytoplasmic tail, a single transmembrane segment and a large extracellular portion, which contains several cation binding sites. Each β-subunit is the product of a separate gene and combines in a noncovalent complex with an α-subunit to form an adhesive protein receptor. Each β-subunit also spans the membrane once and typically has a short cytoplasmic tail. The extracellular domain is formed by complexation of the α- and β-subunits, and high affinity binding of adhesive proteins depends upon contributions from both subunits. A single β-subunit can combine with several α-subunits, with each complex having distinct functional properties. The cytoplasmic tails of the subunits link to the intracellular cytoskeleton and to signaling pathways, interactions that determine the roles of integrins in cell adhesion and signal transduction. Key to the regulation of the ligand binding function of many integrins is their activation state. Stimulation of cells by a variety of agonists can generate intracellular signals, which are transmitted from within the cell to the extracellular domain of integrins, enhancing their affinity for ligands (inside-out signaling). Similarly, occupancy of integrins can generate signals that are transmitted across the membrane and initiate intracellular responses (outside-in signaling). Such bidirectional signaling as it relates to GPIIb-IIIa function are discussed separately in Chapter 3 and reviewed elsewhere

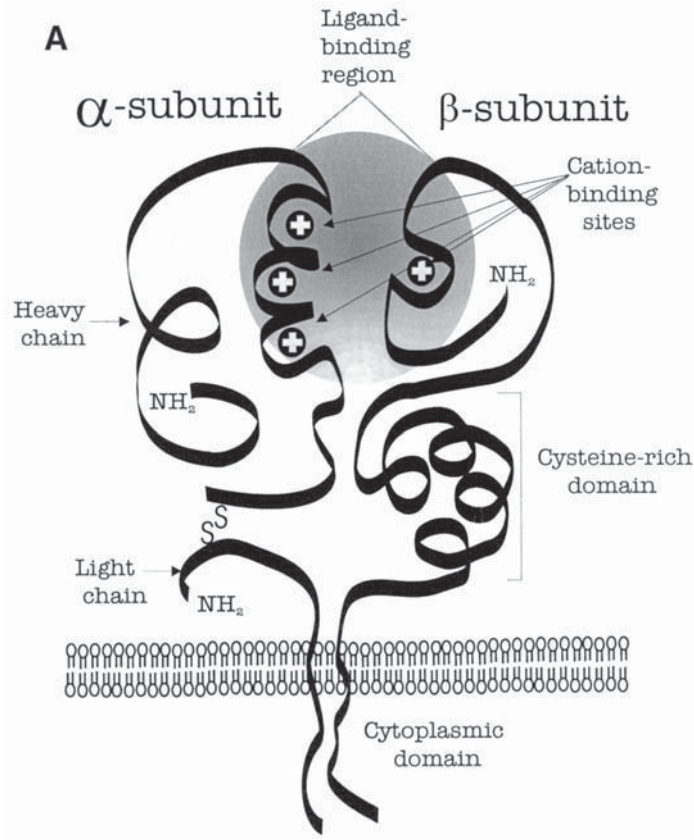

Fig. 1. (A) Schematic depiction of the structure of a prototypic integrin adhesion receptor.

(38,39). Although it is clear that the other integrins that are present on platelets can be activated in other cell types, the importance of such activation to the hemostatic function of platelets is uncertain.

Platelets express two major β-subunits, β_1 and β_3 (β_2 has been reported to be present at low levels), and five α-subunits (Table 4). Of the integrins expressed on blood cells, GPIIb-IIIa ($\alpha_{IIb}\beta_3$) is the most narrowly distributed and is restricted predominantly to platelets/megakaryocytes. GPIIb-IIIa may contribute to the association of platelets with tumor cells *(40–42),* an interaction important to the metastatic dissemination of neoplastic cells *(43,44)*; and certain tumors have been reported to express GPIIb-IIIa *(41)*.

Particularly relevant to the consideration of GPIIb-IIIa blockade is its sister receptor, $\alpha_v\beta_3$, which shares the same β_3 subunit *(45–47)*. The ligand repertoires of $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ are overlapping but not identical, and monoclonal antibodies and low molecular weight antagonists have been developed, which are selective for each of the β3 integrins. $\alpha_v\beta_3$ is expressed at low levels on platelets (50–500 copies) *(48,49)* in contrast to GPIIb-IIIa (40,000–80,000) *(50)*. However, in vitro studies have ascribed a functional role of $\alpha_v\beta_3$ in platelet adhesion *(51,52)*. Furthermore, $\alpha_v\beta_3$ is broadly distributed and is present at high copy number on many vascular cells including endothelial cells, smooth muscle cells, and certain leukocytes [*see* in *(47,53)*]. Its role in the biology of these cells include contributions to angiogenesis, chemotaxis, cell adhesion, and migration *(54,55)*. Thus,

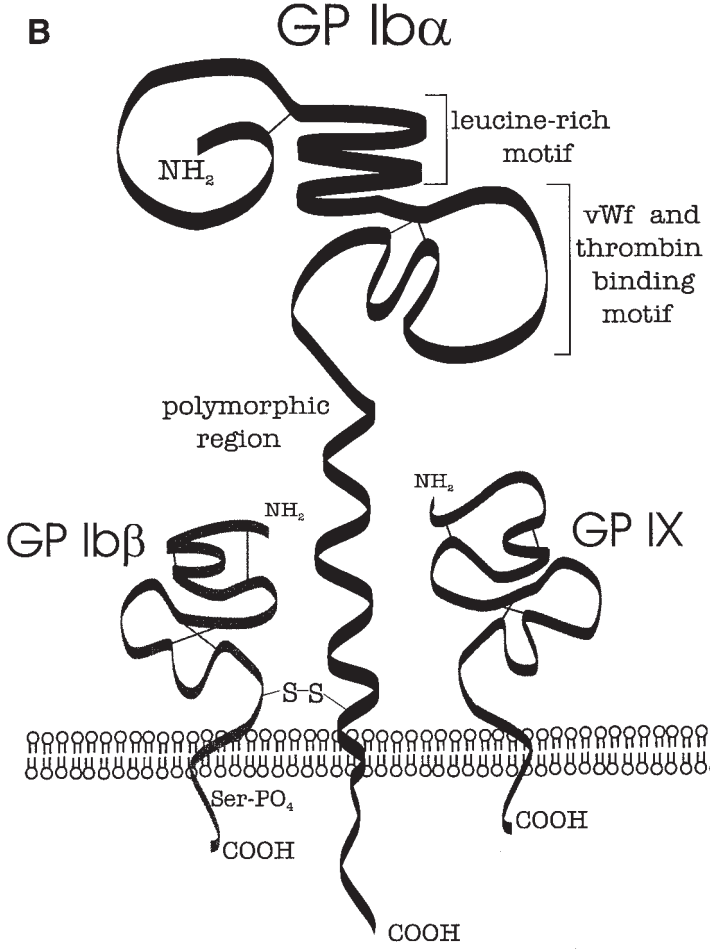

Fig. 1. (B) Schematic depiction of the GPIb-IX complex on the platelet surface [adapted from ref. *(24)*].

cross reactivity of GPIIb-IIIa antagonists with $\alpha_V\beta 3$ is an important consideration although the consequences of such cross-reactivity (beneficial, deleterious, or neutral) have not been resolved.

Role of GPIb-IX in Platelet Adhesion

GPIb-IX is critical to the hemostatic function of platelets. Patients with Bernard-Soulier syndrome lack GPIb-IX and have a marked bleeding diathesis *(56,57)*. The major role of GPIb-IX in hemostasis can be traced to its function as a receptor for vWF *(22,24,58–60)*. vWF is composed of multiple functional domains, including domains involved in binding to GPIb *(61,62)* and to matrix collagen *(63)*. Thus, vWF in the matrix may directly mediate platelet adhesion, and the plasma and/or platelet forms of the molecule may bridge the cells to other matrix components. In vitro, vWF does not interact directly with GPIb on platelets. An interaction can be measured in the presence of ristocetin *(64),* an antibiotic, or botrocetin *(65),* a snake venom peptide. High shear appears to serve as the physiologic counterpart of these agonists *(25)*. High shear alters the

Table 4
Platelet Integrins

Integrin	*Ligands*
$\alpha_2\beta_1$	Collagen
$\alpha_5\beta_1$	Fibronectin
$\alpha_{IIb}\beta_3$	Fibrinogen, fibronectin, vitronectin, von Willebrand factor, thrombospondin, factor XIII, prothrombin
$\alpha_V\beta_3$	Vitronectin, fibrinogen, von Willebrand factor, thrombospondin, fibronectin, prothrombin, osteopontin
$\alpha_6\beta_1$	Laminin

conformation of vWF and/or GPIb-IX, permitting their productive interaction *(27,66–68)*. Such high shear stress can be attained in the microcirculation or in narrowed arteries.

GPIb-IX is multifunctional, as it can also serve as a binding site for thrombin *(69,70)*. It also is a target for certain of the drug-induced platelet antibodies and bears several alloantigens *(24)*. The full primary structures of both GPIb and GPIX was determined from cDNA cloning approaches *(71,72)*. GPIb is composed of a heavy chain (α) and a light chain (β), and both span the platelet membrane once (*see* Fig. 1B). GPIbα associates with actin-binding protein *(73),* thereby establishing a linkage with the cytoskeleton of the platelet *(74)*. The extracellular portion of the α-chain is highly susceptible to proteolysis, and a large proteolytic fragment, glycocalicin, can be detected in the plasma of some patients with thrombocytopenic disorders *(75)*. GPIX is a small, single-chain polypeptide with a molecular weight of 20,000. All three subunits of the GPIb-IX complex are structurally related and contain leucine-rich structural motifs *(28)*. GPV also is deficient from Bernard-Soulier platelets and forms a loose association with GPIb-IX *(76),* but its role in the function of GPIb-IX is uncertain. An important consequence of occupancy of GPIb-IX by vWF is the induction of intracellular signaling events, which ultimately lead to activation of GPIIb-IIIa and then platelet aggregation *(77)*. GPIb-IX has been shown to associate with 14.3.3 *(78),* a protein that has been implicated in signaling pathways in many cells. Also, the cytoplasmic tails of the GPIb-IX complex are subject to phosphorylation of specific serine residues *(79),* modifications that may control its interactions with actin binding protein *(73)* and, consequently, with the cytoskeleton and signaling pathways. Recent data indicate that vWF:GPIb-IX may play a significant role not only in platelet adhesion and activation of GPIIb-IIIa, but also in mediating direct platelet–platelet interactions *(80)*.

Platelet Collagen Receptors

If functional redundancy is an indication of importance, then collagen must be a particularly important substrate for platelet adhesion [reviewed in *(11,12)*]. In addition to the bridging of collagen to platelets via vWF (see above) and fibronectin, no fewer than three other distinct collagen receptors, GPIa-IIa (integrin $\alpha_2\beta_1$) *(81),* GPIV *(82,83)* and GPVI *(84),* exist on platelets. Natural deficiencies of all three of these receptors have been reported to reduce platelet adhesion to collagen *(85–87),* and the deficiencies of GPIa-IIa and GPVI lead to bleeding diatheses. Recent studies have suggested coopera-

tive roles for these receptors in platelet adhesion *(88)*. GPIa-IIa and GPIV *(82)* are involved in the initial adhesion of platelets to collagen [although the relative contribution of these receptors is controversial *(86,89)*], and GPVI is primarily involved in platelet activation and aggregation induced by collagen. Collagen structure also plays a significant role in influencing platelet reactivity *(90,91)*. The triple helical structure of collagen *(92)* is important in triggering platelet adhesion and secretory responses as is the degree of multimerization (monomeric vs fibrillar collagen). Superimposed on these variables are differential effects of shear rates on platelet adhesion to collagens *(93)*. These variables are considered in detail in several recent articles and reviews *(11,12,88)*.

GPIIb-IIIa as an Adhesion Receptor

In addition to its role in platelet aggregation, GPIIb-IIIa is a platelet adhesion receptor. This adhesive function reflects the capacity of GPIIb-IIIa to recognize multiple ligands, which are present in matrices. These ligands include fibronectin *(94),* thrombospondin *(95),* and vWF *(96)*. Fibrin(ogen) *(97)* and vitronectin *(98),* which deposit at sites of vascular injury, also support platelet adhesion via GPIIb-IIIa. Without exception, all of these ligands have alternative and/or cooperative platelet adhesion receptors. As an illustrative example, thrombospondin is recognized by GPIIb-IIIa *(95)* and $\alpha_V\beta_3$ *(51),* with both integrins probably recognizing an RGD sequence within the ligand. GPIV recognizes a distinct site within thrombospondin *(99–101)* and the integrin-associated protein (IAP) cooperates with GPIIb-IIIa to develop a tight interaction of thrombospondin with the platelet surface *(102,103)*. Also without exception, recognition of these adhesive ligands by GPIIb-IIIa is blocked by the same set of monoclonal antibodies *(104)* to the receptor and by the RGD and gamma chain ligand peptides *(105–108)*. Accordingly, although not systematically explored in the literature, it is a reasonable prediction that all of the GPIIb-IIIa antagonists developed to date will block recognition of these adhesive substrates as well as blocking the binding of soluble ligands to the receptor. With the availability of other platelet receptors to recognize these ligands, such blockade may not compromise hemostasis; however, numerous in vitro studies [e.g., *(109–111)*] conducted under both static and flow conditions have demonstrated a role of GPIIb-IIIa in platelet adhesion to various substrates. Thus, the consequences of GPIIb-IIIa blockade on platelet adhesion in the setting of human therapy remains uncertain.

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3 Glycoprotein IIb-IIIa in Platelet Aggregation and Acute Arterial Thrombosis

Debbie A. Law, PhD
and David R. Phillips, PhD

Contents

GLOSSARY

ADP	Adenosine diphosphate, a platelet agonist
CD40L	This membrane protein is a ligand for CD40 and a member of the nerve growth factor superfamily. It is expressed on activated platelets. CD40L binding to CD40 results in intracellular signaling being initiated
CHO cells	Chinese hamster ovary cells
CIB	Calcium and integrin binding protein—a protein that was identified as associating with the cytoplasmic domain of GP IIb using a yeast two-hybrid screen

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EF hand	Structural motif forming a calcium binding site typified by helices E and F of parvalbumin
FACS	Fluorescent activated cell sorter
FAK	Focal adhesion kinase—a cytoplasmic tyrosine kinase
Fcγ	A protein found associated with the Fc receptor complexes FcγRIII and FcMRI, which contains an ITAM (see below)
GP	Glycoprotein
IP3	Inositol triphosphate
ITAM	Immune receptor tyrosine activation motif—a consensus motif containing two tyrosine residues which, when phosphorylated, are important in initiating signals through a number of cell surface receptors present on cells of the immune system
Kd	Equilibrium dissociation constant
KQAGDV	Peptide sequence consisting of the amino acids lysine, glutamine, arginine, glycine, aspartic acid, and valine
LIBS	Ligand-induced binding sites—neoepitopes expressed on GP IIb-IIIa following the binding of ligands like fibrinogen and GP IIb-IIIa antagonists and recognized by LIBS antibodies
Mac-1	The leucocyte integrin CD11b/CD18
MIDAS domain	Metal ion-dependent adhesion site—structural motif identified first in integrin α-subunits
Mr	Apparent molecular weight of a protein determined by electrophoresis on a reduced SDS-polyacrylamide gel
nmr	nuclear magnetic resonance spectroscopy
P2X1	ADP-activated ligand gated ion channel that directly increases intracellular calcium levels
P2Y1	ADP-activated, Gq-coupled seven-spanning transmembrane receptor
PAR1, PAR3	Members of the protease activated receptor family that function as thrombin receptors in platelets. Activated by cleavage of their amino-terminii leading to the exposure of a new amino acid sequence that acts as a "tethered ligand" to activate the receptors
PDGF	Platelet derived growth factor
PKC	Protein Kinase C
Pl^{A2}	A GP IIb-IIIa isotype
PLC	Phospholipase C—a phospholipase isoform which, when activated, can lead to phosphoinositide hydrolysis, diacylglycerol production, and increases in intracellular calcium levels
Raf-1	A serine/threonine kinase
RGD	Peptide sequence consisting of the amino acids arginine, glycine, and aspartic acid
SHC/GRB2	Two adaptor proteins capable of binding to phosphotyrosine residues of signaling proteins. SHC can associate with GRB2, which can then associate with SOS (see below) linking cell surface receptors to the Ras signaling pathway
SOS	Son of sevenless—a guanine nucleotide exchange factor that activates Ras
Src-family	A family of at least 11 cytoplasmic tyrosine kinases whose prototypic member is Src
Syk	Spleen tyrosine kinase—also known as p72, a tyrosine kinase found in hematopoietic cells. Known to bind to phosphorylated tyroinse residues in ITAM motifs via its two SH2 regions
TGFβ	Transforming growth factor β

TRAP	Thrombin receptor activation peptide—a peptide corresponding to the amino acid sequence of the "tethered ligand" of the PAR1 thrombin receptor. This peptide can activate PAR1 in the absence of thrombin cleavage
VWF	von Willebrand factor

INTRODUCTION

Glycoprotein (GP) IIb-IIIa [$\alpha_{IIb}\beta_3$ in integrin nomenclature *(1)*], the most abundant protein on the platelet surface, is the primary receptor mediating platelet aggregation, a process central to acute arterial thrombosis and to hemostasis. Indeed, its central role in aggregation positions GP IIb-IIIa at the heart of thrombosis and has directed aggressive strategies into developing a new class of drugs, termed GP IIb-IIIa antagonists, which block the binding of adhesive proteins to GP IIb-IIIa thus preventing platelet aggregation. GP IIb-IIIa anatagonists have been shown to therapeutically regulate platelet function to prevent, for example, the thrombotic complications associated with coronary artery disease *(2–4)*. The pivotal role that GP IIb-IIIa serves in platelet aggregation arises due to the dynamic nature of this receptor, which displays several functional activities that are important not only in understanding how this receptor is involved in platelet aggregation and thrombosis, but also, ultimately, for understanding how GP IIb-IIIa antagonists can be more effectively utilized to optimize their antithrombotic activities.

The first of these activities, and the most well known, is inside-out signaling through GP IIb-IIIa to generate the receptor function for soluble fibrinogen. On unstimulated platelets, GP IIb-IIIa has low affinity for soluble fibrinogen, i.e., it is in an "inactive" conformation. However, following stimulation of platelets by a variety of agonists, GP IIb-IIIa undergoes a rapid conformational change such that it can now bind soluble fibrinogen. This "activation" of the receptor function of GP IIb-IIIa occurs as a result of "inside-out" signaling events transduced by platelet agonists such as thrombin, adenosine diphosphate (ADP) or collagen, agonists generated at sites of vascular injury such as occurs during percuteaneous interventions, upon rupture of an atherosclerotic plaque, or from external trauma. Upon binding to GP IIb-IIIa, the bivalent fibrinogen can further mediate platelet aggregation by crosslinking the surfaces of activated platelets. Several other ligands can also bind to active GP IIb-IIIa including fibronectin, vitronectin, and von Willebrand factor (vWf). Indeed, the binding of vWf may have a unique role in mediating aggregation under conditions of high shear such as would occur in coronary arteries.

A second activity does not require prior platelet stimulation. It is now recognized that when fibrinogen binds to GP IIb-IIIa on the platelet surface, or otherwise becomes immobilized on artificial surfaces, it undergoes a conformational change that allows it to bind to "inactive" GP IIb-IIIa on unstimulated platelets. This binding results in "outside-in" signals being transduced via GP IIb-IIIa, which, in turn, can lead to further platelet activation including the activation of other GP IIb-IIIa molecules on the platelet surface, the induction of platelet secretion, and the consolidation and stabilization of platelet aggregates. These events also occur when fibrinogen is immobilized on the vessel wall. While the signaling pathways initiated by ligand binding to GP IIb-IIIa are only now being elucidated, it is apparent that the cytoplasmic domains of both GP IIb and GP IIIa are involved in these processes. Proteins have been identified that bind to these regions and are currently being evaluated for their potential roles in GP IIb-IIIa

signal transduction. These outside-in signaling events are also involved in processes such as clot retraction where GP IIb-IIIa serves as the transmembrane link between extracellular fibrin and intracellular cytoskeletal proteins. Outside-in GP IIb-IIIa signaling also induces the release of platelet alpha granules, to link platelet aggregates to coagulation, inflammation, and cell proliferation responses.

Additional functions for the extracellular regions of GP IIb-IIIa include the reversible binding of five moles of divalent cations, important not only in the structure and adhesive protein binding activities of GP IIb-IIIa, but also in the binding of GP IIb-IIIa antagonists. It is now recognized that GP IIb-IIIa undergoes structural transition(s) following the binding of either adhesive proteins or GP IIb-IIIa antagonists resulting in the expression of neoepitopes, which can be detected by ligand-induced binding site (LIBS) antibodies, a process that may have profound implications for chronic treatment with GP IIb-IIIa antagonists.

Furthermore, a number of other GP IIb-IIIa responses have to be taken into account when considering the role of GP IIb-IIIa in platelet aggregation. For example, the concentration of GP IIb-IIIa on the platelet surface can be markedly increased within sites of thrombosis as platelet stimulation can increase GP IIb-IIIa surface expression by as much as 50%. Also, GP IIb-IIIa has been shown to shuttle between the plasma membrane and the alpha granule membrane, providing a mechanism for the packaging of fibrinogen in alpha granules during megakaryocytopoesis and an as-yet-unknown function in circulating platelets.

Finally, genetic analysis of GP IIb-IIIa continues to be an active area of investigation: multiple point mutations have been identified that result either in defective expression or function of this receptor resulting in the Glanzmann's thrombasthenia phenotype: polymorphisms have been identified and it has been proposed that the Pl^{A2} isotype is associated with accelerated coronary artery disease.

This chapter summarizes recent studies pertaining to the structure and function of GP IIb-IIIa. The themes to be developed are twofold. First, GP IIb-IIIa is not a static receptor, but one whose adhesive functions respond to and initiate signal transduction processes within the platelet and whose distribution changes during platelet stimulation. Second, the central role of GP IIb-IIIa in aggregation indicates that it is an attractive target for effective therapeutic regulation of platelet aggregation and thrombosis. Several reviews of this subject have been published *(5–7)*: the emphasis here will be on concepts relevant to the role of platelets in arterial thrombosis and on recent advances.

GP IIb-IIIa IN PLATELET AGGREGATION

On unstimulated, discoid platelets as normally exist in circulation, GP IIb-IIIa is distributed between the plasma membrane (~80,000 copies per platelet), the alpha granule membrane (~40,000 copies per platelet), and the dense body membrane (trace amounts) *(8,9)*. Only limited receptor functions have been attributed to the GP IIb-IIIa on the surface of unstimulated platelets. One is to bind immobilized fibrinogen—a reaction that may assist the recruitment of platelets to damaged vessel walls or to platelet aggregates *(10)*. Another function of GP IIb-IIIa on unstimulated platelets is to bind prothrombin, which may contribute to the procoagulant activity of platelets *(11)*. However, following platelet stimulation, which also may involve alpha granule secretion, not only is there an up to 50% increase in the GP IIb-IIIa expressed on the surface membranes

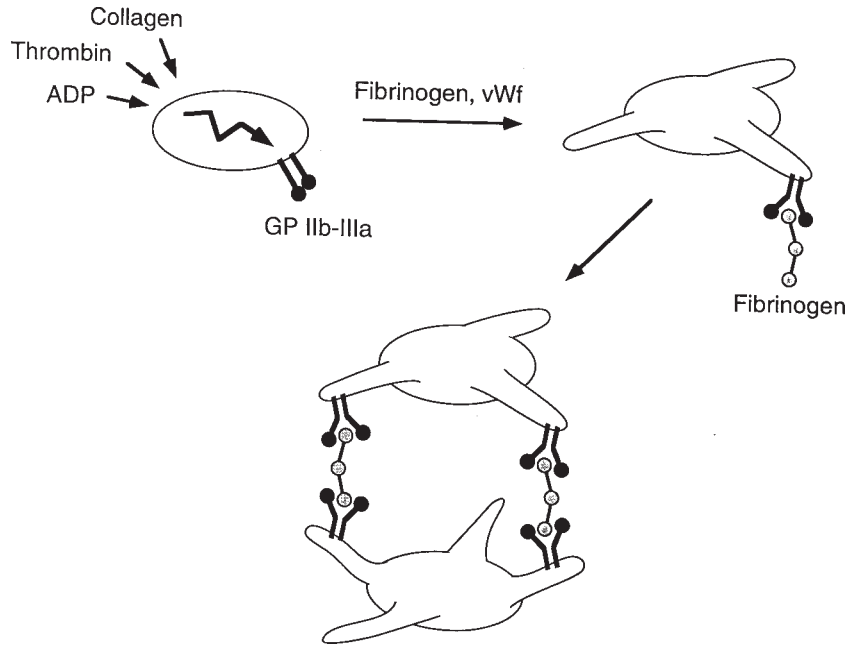

Fig. 1. Schematic illustrating the role of GP IIb-IIIa in platelet aggregation. On resting, discoid platelets the GP IIb-IIIa is present in an "inactive" conformation and is unable to bind soluble fibrinogen. Upon activation by classical agonists such as collagen, thrombin, or adenosine diphosphate (ADP) the platelet undergoes a shape change and the GP IIb-IIIa is activated such that it can now bind its soluble ligands, fibrinogen, and von Willebrand factor (vWf). Platelet aggregation is mediated by the bivalent fibrinogen forming bridges between GP IIb-IIIa molecules on the surfaces of neighboring, activated platelets.

of platelets, but GP IIb-IIIa becomes a receptor for the soluble forms of several adhesive proteins, including fibrinogen, vWf, fibronectin, and vitronectin. The affinity of fibrinogen for GP IIb-IIIa on stimulated platelets (Kd ~500 nmol/L) and the high concentration of fibrinogen in plasma (~11 μmol/L) predicts that GP IIb-IIIa rapidly becomes saturated with fibrinogen upon platelet stimulation. With time, the initial reversible binding of fibrinogen to GP IIb-IIIa becomes irreversible, possibly contributing to the stability of platelet aggregates *(12)*. GP IIb-IIIa also shuttles between the plasma membrane and the alpha granule membrane, which is independent of platelet stimulation *(13)*. This shuttling allows for the packaging of fibrinogen in alpha granules during megakaryocytopoiesis *(14)* and for the uptake of GP IIb-IIIa ligands in mature platelets *(15)*. The function of GP IIb-IIIa shuttling in circulating platelets could potentially allow for the uptake of GP IIb-IIIa antagonists during antithrombotic therapy, a possibility not yet fully explored *(16)*.

The binding of fibrinogen and vWf following platelet stimulation causes platelets to aggregate. In a simple aggregation reaction, as occurs with a stirred suspension of platelets in a cuvet during laboratory analysis, it is likely that fibrinogen, which is bivalent, bridges the surfaces of activated platelets (Fig. 1). Aggregation reactions under flow as occurs during thrombosis and hemostasis within the vasculature are undoubtedly much more complicated, primarily because of the effect of shear on cellular interactions. For example, fibrinogen bound to the GP IIb-IIIa on the surface of an aggregated platelet

undergoes a conformational change into an "activated" form, which is capable of mediating further cellular interactions. One is to bind the GP IIb-IIIa on unstimulated platelets *(17)* which, through a process known as "outside-in" signaling, induces platelet binding and subsequent stimulation. Another is to bind Mac-1 on neutrophils to initiate the adhesion of these cells *(18)*. Thus, these activities of bound fibrinogen are important as they allow for the recruitment of unstimulated platelets and neutrophils, respectively, into a growing thrombus. In another example, high shear, as would occur within a constricted coronary artery induces the binding of vWf to the GP Ib-IX-V complex on the platelet surface, resulting in platelet activation including the activation of the receptor function of GP IIb-IIIa, vWf, and fibrinogen binding, and further platelet aggregation *(19,20)*. Both reactions, the interaction of bound fibrinogen with the GP IIb-IIIa on unstimulated platelets and the activation of GP IIb-IIIa by vWf, serve to promote thrombus growth by mechanisms mediated primarily through the binding of adhesive proteins.

The amount of GP IIb-IIIa recruited to the platelet surface during platelet stimulation is a function of the strength of the platelet agonist. Potent agonists like thrombin cause most of the alpha granule GP IIb-IIIa to be recruited with prebound fibrinogen to the platelet surface, whereas weak agonists like ADP only marginally increase the platelet surface GP IIb-IIIa *(21,22)*. This alpha granule pool of GP IIb-IIIa is functionally important. Indeed, thrombin stimulation of platelets in which the plasma membrane GP IIb-IIIa molecules have been rendered inactive can still induce sufficient functional GP IIb-IIIa to the platelet surface to support platelet aggregation *(23)*. Clinical studies have shown that thrombin-induced, but not ADP-induced platelet aggregation, is rapidly restored following discontinuation of infusion of high-affinity GP IIb-IIIa antagonists like the Fab fragment, abciximab, or L-738,167, a synthetic GP IIb-IIIa antagonist *(24,25)*. In these trials, the unbound pool of these antagonists, but not the platelet-bound pool, is rapidly cleared. Because thrombin, but not ADP, induces maximal surface expression of the alpha granule pool of GP IIb-IIIa, thrombin-induced aggregation is more rapidly restored. P-selectin is another alpha granule protein that becomes expressed on the surface of activated platelets *(26)*. Fluorescence-activated cell sorter analysis has shown that thrombosis associated with percutaneous intervention or with unstable angina increases the amount of platelet-associated P-selectin, implying that these clinical settings increase the amount of activated platelets within the circulation, and also providing an assay to measure such platelets *(27)*.

GP IIb-IIIa STRUCTURE

The GP IIb subunit of GP IIb-IIIa consists of two disulfide-linked subunits GP IIbα (Mr = 125 kD) and GP IIbβ (Mr = 22 kDa), which are formed when the precursor GP IIb polypeptide undergoes cleavage into a heavy and light chain. In contrast, GP IIIa (Mr = 105 kDa) is a single polypeptide, which contains intrachain disulfide linkages. In both cases, the proteins have large extracellular domains, single transmembrane domains, and relatively short cytoplasmic domains (47 amino acids for GP IIIa and 20 for GP IIb). The cytoplasmic domains are accessible to signaling proteins within the platelet and are most likely involved in signal transduction reactions transmitted by this integrin. Figure 2 shows a rotary shadowing image of purified GP IIb-IIIa complexes and a depiction of how the primary sequences of GP IIb and GP IIIa may be accommodated into this structure *(28)*. Each complex consists of a globular "head" and two "legs" with hydro-

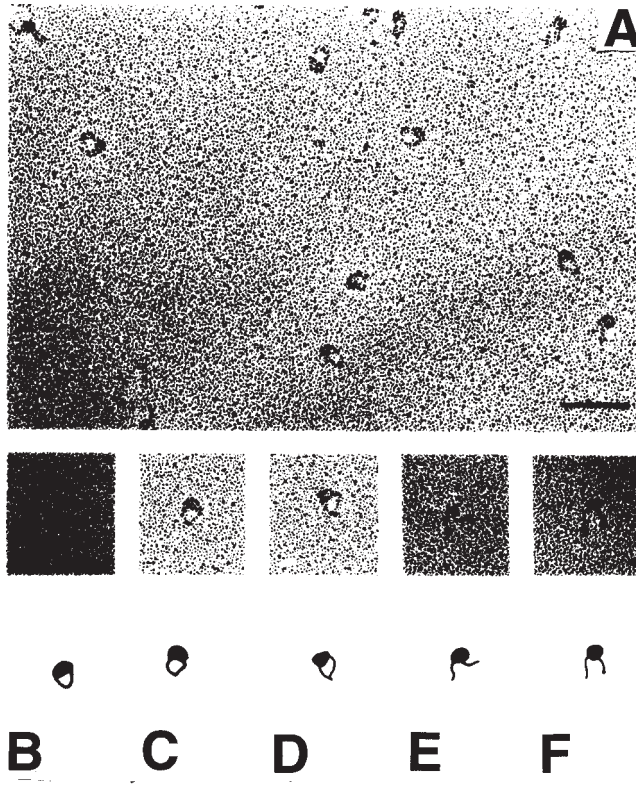

Fig. 2. Electron micrograph of rotary-shadowed GP IIb-IIIa complexes. **(A)** A field of GP IIb-IIIa complexes (bar represents 50 nm). **(B-F)** Examples of GP IIb-IIIa complexes with (below) diagrammatic representations of the micrographs. The globular "head" includes the ligand binding sites in GP IIb-IIIa *(29),* whereas the "tails" appear to have a more flexible structure and are used for membrane insertion. [Figure taken from ref. *(28).*]

phobic feet used for membrane insertion. The fibrinogen binding domain is contained within the head *(29)* and extensive experimental approaches have been used to identify the sequences within the head that constitute the adhesive protein binding pockets including mutational analysis, peptide inhibition, chemical crosslinking, and blocking monoclonal antibodies. Although the consensus from these experiments suggest that the binding pocket consists of the MIDAS (metal ion-dependent adhesion site) domain (residues 110–294 of GP IIIa) *(30–32)* and the second of the four EF hands on GP IIb (residues 297–308) *(33),* this assignment should be viewed with caution, as multiple binding pockets appear to exist (described in the next sections) and definitive assignments await structural analysis of cocrystals of GP IIb-IIIa with its ligands.

LIGAND BINDING PROPERTIES OF GP IIb-IIIa

Although both fibrinogen and vWf are ligands for GP IIb-IIIa, these molecules utilize different sequences to bind to the GP IIb-IIIa and their binding is regulated differently (Fig. 3). Fibrinogen consists of six polypeptide chains, two alpha, two beta, and two gamma, arranged in a symmetrical, bivalent orientation. Because peptides containing

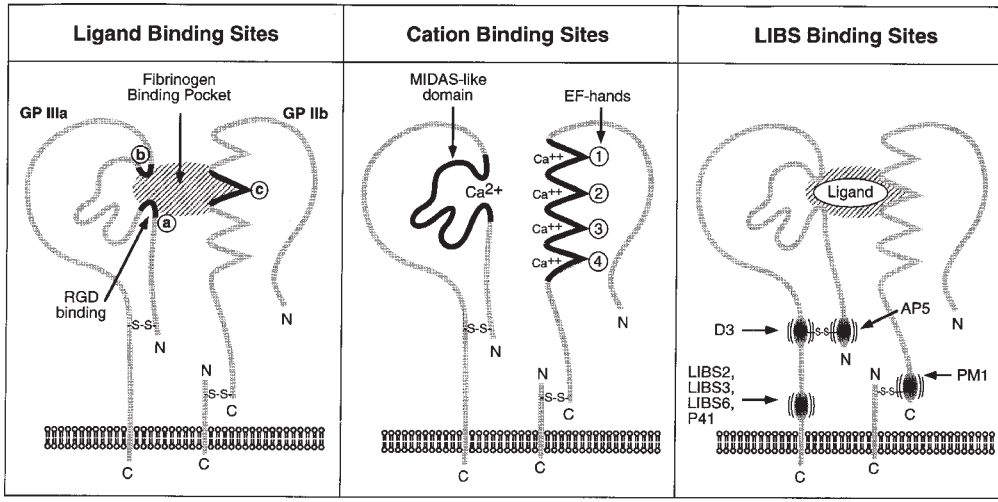

Fig. 3. Model for the structure of GP IIb-IIIa with putative ligand binding sites, cation binding sites, and LIBS epitopes indicated. The first panel indicates fibrinogen binding pocket on GP IIb-IIIa, which includes **(A)** the RGD binding site (residues 109–133) [reviewed in *(33),* **(B)** an additional putative fibrinogen binding site (residues 212-217), as determined by peptide blocking studies and mutagenesis experiments *(189),* and **(C)** the site on GP IIb (residues 294–314) responsible for binding the carboxy terminal hexapeptide (KQAGDV) on the fibrinogen gamma chain *(190)*. Putative Ca^{2+} binding sites are indicated in the middle panel. The four sites on GP IIb are thought to resemble conventional EF-hands *(191)* and involve residues 243–254, 297–308, 365–376 and 426–437, whereas the site on GP IIIa from residues 110–294 is similar to a MIDAS domain *(30)*. Both the MIDAS-like Ca^{2+} binding site on GP IIIa and the second EF-like Ca^{2+} binding site on GP IIb are also implicated in ligand binding. The third panel depicts the regions thought to be recognized by the indicated LIBS (ligand-induced binding site) antibodies. These epitopes become accessible following ligand binding. The sites on GP IIIa were mapped using baculovirus expressed recombinant proteins corresponding to different regions of GP IIIa *(67)* and encompass the following residues: for AP5, 1–6; for D3, 422–490; for LIBS2, LIBS3, LIBS6, and P41, 602-690. The site for PMI1 on GP IIb, residues 844–859, was mapped using synthetic peptides *(192)*.

the Arg-Gly-Asp (RGD) sequence were the first small peptides identified that blocked fibrinogen binding and because the fibrinogen alpha chain has two RGD sequences, it was initially assumed that one or both of these sequences were used to bind GP IIb-IIIa [reviewed in *(7)*]. Surprisingly, however, mutations of either RGD sequence in recombinant fibrinogen has no effect on fibrinogen-GP IIb-IIIa interactions as measured either by platelet aggregation *(34)* or platelet adhesion to immobilized fibrinogen *(35)*. Considerable attention has therefore been directed to the carboxy terminal hexapeptide on the gamma chain, Lys-Gln-Ala-Gly-Asp-Val (KQAGDV), a GP IIb-IIIa binding sequence first identified by Hawiger and coworkers who showed that peptides containing this sequence are effective inhibitors of platelet aggregation *(36)*. This sequence is contained within both outer nodules of fibrinogen, the structural entity of fibrinogen that binds GP IIb-IIIa *(29)*. Additionally, antibodies to this sequence block fibrinogen binding *(37)*, fibrinogen lacking this sequence does not support platelet aggregation and, when mouse fibrinogen is mutated so that it lacks this sequence, a bleeding diathesis is induced *(38)*. The data are compelling, therefore, that fibrinogen binding to GP IIb-IIIa

occurs primarily through the gamma chain sequence. Equilibrium binding has shown that GP IIb-IIIa has one binding site for RGD *(39)*. In contrast, NMR analysis has shown two binding sites for the gamma chain sequence, the second site being induced by the binding of the first *(40)*. Although RGD-containing peptides compete for the initial binding of the gamma chain peptides, it is not yet known whether they bind to the same or different sites, nor is it known how the second gamma chain site relates to the first. GP IIb-IIIa is known to undergo a conformational change upon ligand binding *(41)* and Du et al. *(42)* have found that fixation of platelets with formaldehyde, when GP IIb-IIIa is occupied by a RGD peptide or a peptide from the carboxy terminus of the fibrinogen gamma chain, preserves the fibrinogen binding conformation of GP IIb-IIIa upon peptide elution. Because neither peptide induces fibrinogen binding in the absence of fixation, it is likely that the fibrinogen-binding conformation of GP IIb-IIIa induced by peptide binding rapidly reverses upon ligand dissociation *(43,44)*.

Fibrin harboring a deletion truncation of the gamma chain QAGDV sequence is still capable of supporting clot retraction *(45)*. Because clot retraction is dependent on the fibrin adhesive property of GP IIb-IIIa, this finding suggests that conversion of fibrinogen to fibrin exposes a different GP IIb-IIIa binding site such as RGD or possibly even Lys-Gly-Asp (KGD), a GP IIb-IIIa binding sequence *(46)* found in the beta chain of fibrinogen.

Proteins other than fibrinogen that bind to GP IIb-IIIa, e.g., vWf, fibronectin, vitronectin, prothrombin, most likely do so by the RGD sequences contained within their primary amino acid structures. Mutational analysis of the RGD sequence in vWf support this assignment *(47)*: such data on the other adhesive proteins are not yet available. Of these proteins, the binding of vWf is unique and has features that make it ideally suited to mediate platelet adhesion and aggregation under conditions of high shear as can be found in stenosed coronary arteries. vWf is a large, disulfide-linked multimer of ~220 kDa subunits that has two motifs that bind to receptors on platelets; in addition to the RGD (residues 1744–1746), which binds to GP IIb-IIIa , it also has an A1 domain, a disulfide-linked loop (residues 449–728) that binds the GP Ib-IX-V complex *(48)*. Both motifs in soluble vWf are refractory to unstimulated platelets. However, the conformational change vWf undergoes when it is immobilized on subendothelial structures, exposed to conditions of high shear, or even when exposed to the antibiotic ristocetin or the snake venom protein botrocetin, activates it to bind to the GP Ib-IX-V complex on the platelet surface, an interaction mediated by the A1 domain. GP Ib-IX-V is a signaling receptor and vWf binding induces platelet activation including the activation of the receptor function of GP IIb-IIIa, which allows it to bind the RGD sequence on vWf and also to bind fibrinogen *(49,50)*. The ability of vWf to support platelet aggregation under conditions of high shear *(51)* may be related to these dual binding motifs, repeated on each subunit of vWf, which together would be expected to bind with much higher affinity than either would singly and are thus more capable of binding platelets under conditions of high shear than is fibrinogen. The finding that aggregation initiated by platelet adhesion to vWf is GP IIb-IIIa dependent and is more important at high shear *(20)* suggests that agonist-induced aggregation (e.g., thrombin- or ADP-induced) may be more important at low shear. This may, in part, explain why GP IIb-IIIa antagonists are more effective at preventing arterial thrombosis than are agonist inhibitors like ticlopidine and clopidogrel *(3),* because high shear conditions such as occurs in coronary arteries would increase the importance of GP IIb-IIIa binding to vWf with subsequent GP IIb-IIIa activation—an interaction blocked by GP IIb-IIIa antagonists but not by the inhibitors

that affect ADP-induced platelet stimulation. It may also explain why direct thrombin inhibitors caused bleeding as detected by cerebral hemorrage at doses required to inhibit arterial thrombosis whereas GP IIb-IIIa antagonists apparently do not *(2)*.

The synthetic GP IIb-IIIa antagonists constitute a wide variety of structures, but as a class do have a common structural feature, a positive and negative charge separated by a distance of 10–20 Å *(52),* a distance similar to that between the positive charge of arginine and the negative charge of aspartic acid on RGD *(53)* and possibly even to that of the positive charge on lysine and the negative charge of aspartic acid on the KQAGDV sequence as it exists in a helical conformation *(40)*. Although it is tempting to speculate that these similarities indicate that synthetic GP IIb-IIIa antagonists bind to the RGD or KQAGDV binding site(s) and are classical competitive inhibitors of fibrinogen binding, data are not yet available to support this competitive mode of inhibition. Most disintegrins, a family of proteins found in snake venom and other hemataophagous organisms express the RGD motif, which causes them to be potent antagonists of the adhesive protein-binding activity of GP IIb-IIIa and other integrins (e.g., $\alpha_v\beta_3$, $\alpha_5\beta_1$) *(54,55)*. Barbourin is a unique disintegrin as it expresses a Lys-Gly-Asp (KGD) sequence, which causes this disintegrin to specifically inhibit GP IIb-IIIa *(46),* a finding that was utilized in the synthesis of the cyclic heptapeptide, eptifibatide *(4,56)*.

Ca^{2+} IN GP IIb-IIIa FUNCTION

The primary amino acid sequences of GP IIb-IIIa predicts five divalent cation binding sites on the GP IIb-IIIa complex: the four EF hands on GP IIb; and the one MIDAS domain on GP IIIa (Fig. 3). Five divalent cations binding to GP IIb-IIIa have indeed been observed and the affinities of these sites for Ca^{2+} predict that all are occupied by this divalent cation when platelets are suspended in plasma *(57,58)*. Divalent cation binding is reversible and reductions in the amount of extracellular cations removes Ca^{2+} from GP IIb-IIIa, dramatically affecting its structure, its binding of adhesive proteins and its binding of antagonists. For example, suspension of platelets in solutions containing ionized calcium concentrations of 40 to 50 μmol/L as achieved in citrate-anticoagulated blood causes partial removal of Ca^{2+} from GP IIb-IIIa, inducing a loss of fibrinogen-binding activity *(59)*. At less than 1 μmol/L Ca^{2+}, as would occur for example in an EDTA-containing buffer, and at 37°C (but to a lesser extent at 25°C), GP IIb dissociates from GP IIIa within the plane of the plasma membrane *(60)*. Dissociated subunits irreversibly loose their structure and their ability to bind fibrinogen and accordingly can no longer mediate platelet aggregation *(61)*. The close proximity of the second EF hand of GP IIb and the MIDAS domain of GP IIIa to the site responsible for binding RGD ligands may account for observations suggesting that binding of Ca^{2+}, RGD ligands and some GP IIb-IIIa antagonists (e.g., eptifibatide) are mutually competitive *(62)*. Thus, platelet assays relying on GP IIb-IIIa function may not be valid when performed in buffers containing less than the 1.1 mmol/L Ca^{2+} normally found in plasma. One illustration of this effect was seen in the pharmacodynamic analysis of eptifibatide. The reduced Ca^{2+} caused by citrate anticoagulation actually increased the binding of eptifibatide but decreased the binding of fibrinogen. The combined result of these two effects is that the apparent inhibitory activity of eptifibatide is markedly overestimated in this condition, an event that led to a dose that gave 40-50% inhibition of platelet aggregation at steady state, versus the 80% inhibition that had been targeted in the IMPACT II trial *(63)*. At

least one additional GP IIb-IIIa antagonist has also been shown to be influenced by this effect of citrate *(64)*; data on others are not yet available.

GP IIb-IIIa LIBS EPITOPE EXPRESSION

Ligand binding to GP IIb-IIIa (e.g., adhesive proteins, GP IIb-IIIa antagonists, antibodies) induces a conformational change in the receptor, resulting in the formation of neoepitopes, collectively termed LIBS epitopes *(65,66)*. Figure 3 diagrammatically illustrates sequences responsible for the various LIBS epitopes and antibodies that recognize these sequences, although additional LIBS antibodies are known whose epitopes have not been identified *(67)*. The response of GP IIb-IIIa to ligands has proven variable in that whereas all ligands and antibodies induce LIBS expression *(68),* the LIBS epitopes expressed are different. For example, while tirofiban and eptifibatide, but not lamifiban *(69),* induced the binding of the LIBS antibody termed D3, both GP IIb-IIIa antagonists induce the binding of mAb15–758 *(70)*.

LIBS epitope expression is increasingly recognized for its potential impact in limiting the use of GP IIb-IIIa antagonists, particularly in long-term, oral administration. Reports have appeared indicating thrombocytopenia in a few individuals as a complication of exposure to orally available or monoclonally derived GP IIb-IIIa antagonists *(71–73)*. Hypotheses for this phenomenon are that the LIBS epitopes expressed either cause platelet clearance directly or induce the production of antibodies to the LIBS epitopes, which secondarily cause platelet clearance by an immune mechanism. Evidence for this comes from animal studies where thrombocytopenia was observed in both chimpanzee and Rhesus monkeys and this condition corresponded to the presence of drug-dependent antibodies against GP IIb-IIIa *(74)*. Accumulation of information on this point for different GP IIb-IIIa antagonists could identify LIBS epitopes critical for this response.

STIMULUS-INDUCED GP IIb-IIIa ACTIVATION

The activation of the receptor function of GP IIb-IIIa (i.e., inside-out GP IIb-IIIa signaling) is known to occur in response to a wide spectrum of physiological and pathophysiological conditions (e.g., vascular trauma to initiate normal hemostasis; atherosclerotic plaque rupture or percutaneous intervention to initiate thrombosis). It is not surprising, therefore, that the stimulus pathways resulting in GP IIb-IIIa activation are redundant. Redundancy is found on at least two levels. First, well known in the platelet literature, GP IIb-IIIa activation occurs in response to multiple agonists. A host of soluble agonists, including thrombin and ADP, several adhesive proteins in the extracellular matrix including collagen, vWf, and fibrinogen and even high shear act on distinct receptors and are each capable of initiating signal transduction pathways to induce activation of the receptor function of GP IIb-IIIa for soluble adhesive proteins [reviewed in *(5,75)*]. This multiplicity of agonists broadens the physiological conditions capable of initiating platelet aggregation to cause thrombosis and hemostasis. It has only recently become appreciated that many of the primary agonists, e.g., thrombin, collagen, and ADP, act on multiple receptors (*see* Table 1). Indeed it appears that there may be a synergy between the different receptors with more that one receptor needing to be activated by a particular agonist for successful platelet activation to occur. In one example, ADP activates at least three receptors on platelets, a ligand-gated ion channel similar to

Table 1
Signal Transduction Coupling to Receptors for Platelet Agonists

Stimulus	*Putative Receptor*	*Second Messenger Pathways Involved*
Adenosine diphosphate (ADP)	$P2Y_{ADP}$, $P2T_{PLC}$, P2Y1	G_q-coupled, activation of PLC
	$P2T_{AC}$, $P2Y_{ADP}$	G_i-coupled, inhibition of adenylyl cyclase
	P2X1-like	Ligand gated ion channel, increase in intracellular Ca^{2+} levels
Thrombin	PAR 1	G_i-coupled, inhibition of adenyly cyclase
		Possibly G_q-coupled (yet to be shown in platelets)
	PAR 3/PAR 4	G_q-coupled
	GP Ib-IX-V complex?	GP Ibβ and GP V bind 14-3-3ζ adaptor protein
		Association with tyrosine kinases and activation of Syk
Collagen	$\alpha_2\beta_1$	Tyrosine phosphorylation and activation of Syk, phosphorylation of $PLC\gamma_2$
	GP VI	Phosphorylation of associated Fcγ chain protein, recruitment and activation of Syk
		Other to be identified
vWF	GP Ib-IX-V complex	Association with 14-3-3 ζ
		Association with tyrosine kinases and activation of Syk
Immobilized fibrinogen	GP IIb-IIIa	Activation of tyrosine kinases (e.g., Src and Syk), activation of PLC, PI3K, SHIP

P2X1 that allows for rapid Ca^{2+} influx, a P2Y1-like receptor (also known as $P2Y_{ADP}$ and $P2T_{PLC}$) that is coupled to phospholipase C and mediates mobilization of Ca^{2+} from intracellular stores and platelet shape change, and a receptor (not yet cloned) that inhibits adenylylcyclase and induces platelet aggregation ($P2Y_{ADP}$ or $P2T_{AC}$) *(76,77)*. The indirect acting thienopyridine-based platelet inhibitors clopidogrel and ticlopidine and the direct acting ATP-based antagonist ARL 66096 inhibit ADP-induced GP IIb-IIIa activation most likely by blocking the latter receptor but have no effect on the P2Y1-like receptor *(76,78)*.

In another example, collagen is now known to bind to at least two receptors, the integrin α2β1, and a membrane glycoprotein termed GP VI *(79–81)*. The two receptors are interdependent as antibodies against either receptor will block collagen-induced platelet activation *(82,83)* and individuals lacking either receptor are defective in collagen-induced GP IIb-IIIa activation and have a bleeding diathesis *(84–86)*. α2β1 appears

to be the high affinity receptor, which tethers collagen so that it can bind GP VI to initiate a signal transduction reaction culminating in GP IIb-IIIa activation.

A third example is thrombin. It is uniformly recognized that proteolysis of protease-activated receptor 1 (PAR 1) by thrombin initiates signal transduction reactions within platelets and GP IIb-IIIa activation *(87)*. However, the inability of PAR 1 antagonists to completely block thrombin-induced human platelet stimulation *(88)* and the differences between thrombin-induced responses compared to those induced by thrombin receptor-activating peptides (TRAP), peptide agonists of PAR 1 *(89)* suggest that the thrombin responsiveness of platelets, in part, may be mediated by a receptor in addition to PAR 1. Possible candidates for a second thrombin receptor include the recently identified PAR 3, although it remains to be established whether this protease activated receptor is expressed on human platelets *(90)*. The GPIb-IX-V complex may also be a candidate since it contains a thrombin cleavage site (in GP V) and a high-affinity thrombin binding site (in GP Ibα), and it is known that this complex has signaling capabilities *(91,92)*.

The second level of redundancy is the signal transduction pathways from agonist receptor stimulation through GP IIb-IIIa activation as different receptors use overlapping signal transduction pathways. For example, many of the agonist receptors on platelets are heptahelical receptors (e.g., PAR 1, thromboxane receptor, ADP receptor), which as a class are known to be coupled to G proteins, a heterotrimeric family of signaling molecules consisting of alpha-, beta-, and gamma-subunits that signal by dissociating into Gα (a GTPase) and beta-gamma dimers, both of which can propagate signals *(93)*. Support for the direct involvement of Gαq in GP IIb-IIIa inside-out signaling is provided by observations showing that platelets from mice made deficient in this alpha-subunit have defective platelet aggregation in response the ADP and thrombin *(94)*. Gαq-deficient mice also have defective thrombosis in response to collagen and have prolonged bleeding times. Interestingly, Gabbeta et al. *(95)* found the platelets from a patient with defective GP IIb-IIIa activation in response to thrombin or ADP were partially deficient in Gαq. Receptors for collagen or for vWf activate different intracellular signaling pathways that ultimately result in GP IIb-IIIa activation. GP VI is normally bound to another membrane protein termed Fcγ *(96)*. Binding of collagen to GP VI causes the phosphorylation of the two tyrosine residues in the ITAM domain on the cytoplasmic face of Fcγ. Phosphorylated Fcγ then recruits Syk to the membrane so that it can be phosphorylated, activating its tyrosine kinase so that it, in turn, can phosphorylate downstream effectors (e.g., phospholipase Cγ) to propagate the signal through to GP IIb-IIIa activation. Platelets from mice in which either the Syk or the Fcγ genes are disrupted are now known to have defective collagen-induced signaling *(97)*. GP Ib, the receptor for activated vWf may also be coupled to Fcγ, as Syk tyrosine phosphorylation following GP Ib signaling has been observed *(98)*. However, the cytoplasmic domains of GP Ibα and GP V in the GP Ib-IX-V complex also bind protein 14-3-3ζ *(92,99,100)*, an adapter protein that binds Raf-1 and PKC, both serine/threonine kinases known to couple receptors to signaling pathways and to phosphatidyl inositol 3-kinase *(101)*. These examples indicate that the intracellular signaling pathways within platelets for GP IIb-IIIa activation can have diverse origins.

As of this writing it is unknown how signal transduction pathways initiated by the various agonist receptors are coupled to GP IIb-IIIa activation. However, key players between agonist receptors and GP IIb-IIIa have been identified. Two are phospholipase C (PLC) and protein kinase C (PKC). PLC exists in several isoforms and functions to

catalyze the hydrolysis of phosphatidylinositol phosphates to produce inositol phosphate (IP3), which induces the release of Ca^{2+} from intracellular stores, and diacylglycerol, which markedly enhances the catalytic activity of protein kinase C (PKC) *(102)*. Phosphatidyl inositol phosphate hydrolysis can be mediated by PLCβ, which is activated by Gαq, or by PLCγ, which can be activated by kinases such as Syk. Support for the role of IP3, Ca^{2+}, and diacylglycerol is derived from studies showing that elevation of the cytoplasmic Ca^{2+} concentration with the calcium ionophore A23187 also induces GP IIb-IIIa activation *(103)* and that the GP IIb-IIIa activation induced by certain platelet agonists can be diminished by PKC inhibitors *(104)*. Another key player between agonist receptors and GP IIb-IIIa activation is phospholipase A2 (PLA2), which catalyzes the release of arachidonic acid from the 2-position of phospholipids. Arachidonate is metabolized to thromboxane A2 (TXA2), the final reaction being catalyzed by cyclooxygenase. TXA2 acts on the TXA2 receptor, a heptahelical receptor, to propagate the signaling response.

The effectiveness of aspirin, a cyclooxygenase inhibitor, to block GP IIb-IIIa activation, particularly at low agonist concentrations, demonstrates the importance of this pathway to GP IIb-IIIa signaling *(105)*. However, the ability of high agonist concentrations to overcome the effects of aspirin inhibition, even when cyclooxygenase is completely inhibited, indicates the redundancy of the signal transduction mechanisms for GP IIb-IIIa activation. Less well understood are the proximal events at GP IIb-IIIa, which result in its activation. Since chemical modifications of GP IIb-IIIa have not been observed during inside-out GP IIb-IIIa signaling, it has been assumed that signaling induces either the association or dissociation of a regulatory protein(s) to the short cytoplasmic domains of GP IIb-IIIa. Although several intracellular proteins have been identified that bind to peptides corresponding to the cytoplamic domains of GP IIb-IIIa including β3-endonexin [to GP IIIa; *(106)*] and CIB [to GP IIb; *(107)*], it is not yet clear whether any of these are responsible for the activation of the receptor function of GP IIb-IIIa following agonist stimulation (*see* Fig. 4 for possible model). Three transmembrane proteins have also been shown to bind GP IIb-IIIa, CD9, a member of the tetraspanin class of proteins *(108,109),* CD47, integrin-associated protein, which may assist in thrombospondin-induced GP IIb-IIIa activation *(110),* and CD98, a potential GP IIb-IIIa modulator *(111)*.

FIBRINOGEN BINDING ON UNSTIMULATED PLATELETS

Although GP IIb-IIIa must become activated to bind soluble fibrinogen, this is not the case for fibrinogen immobilized on surfaces (e.g., vessel walls or plastic) or for fibrinogen bound to GP IIb-IIIa. In both instances, immobilization apparently alters the conformation of fibrinogen so that the gamma-chain sequence becomes capable of binding GP IIb-IIIa on unstimulated platelets *(10,17,112,113)*. Importantly, fibrinogen in this instance becomes a platelet agonist as binding of GP IIb-IIIa by this mechanism initiates an outside-in signal transduction reaction through GP IIb-IIIa, resulting in platelet stimulation (see below for discussion of mechanism). There are potentially three important physiological consequences of this reaction. First, it provides a mechanism for platelet aggregation where fibrinogen is the primary agonist and where the bound fibrinogen is responsible for recruiting and activating platelets by outside-in GP IIb-IIIa signaling. Second, fibrinogen bound to GP IIb-IIIa on surfaces is also capable of binding Mac-1 to

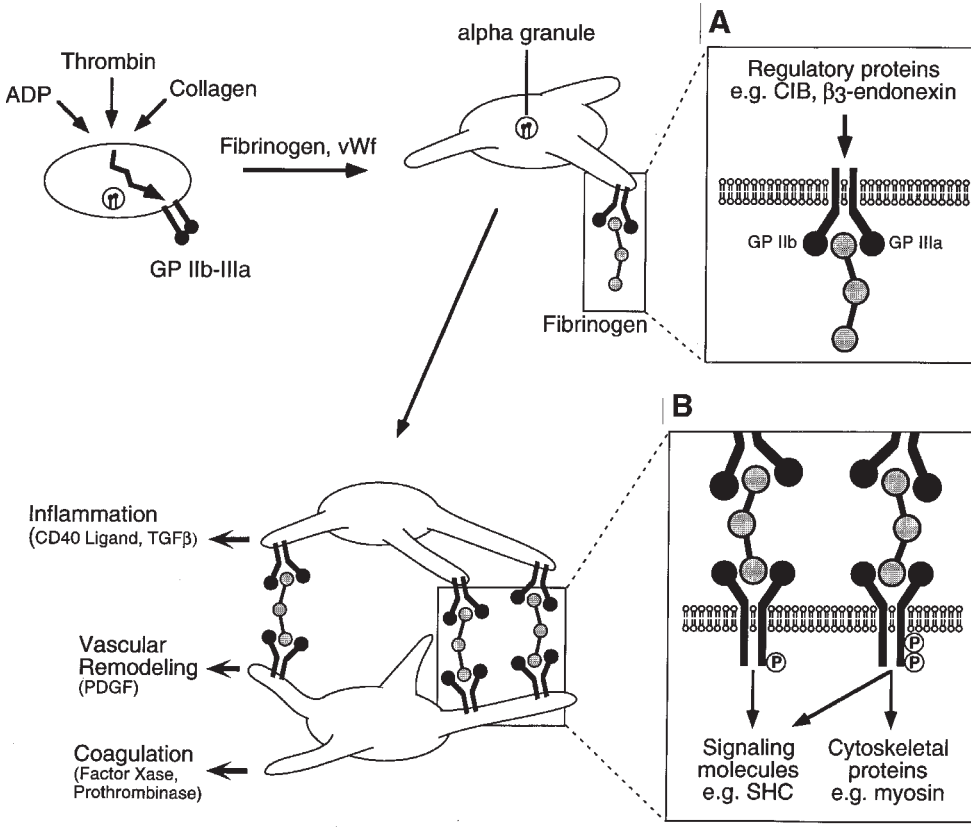

Fig. 4. Model for signaling events involving the GP IIb-IIIa cytoplasmic domains in platelet activation and aggregation. Platelet stimulation by agents such as collagen, thrombin and ADP leads to the activation of the receptor function of GP IIb-IIIa. It is postulated that the binding of soluble fibrinogen to GP IIb-IIIa results in the interaction of GP IIb-IIIa with regulatory proteins and/or the activation of these regulatory proteins (illustrated in **A**). Possible candidates for such proteins include CIB and β_3-endonexin *(106,107)*. Upon platelet aggregation, mediated in part by bivalent fibrinogen crosslinking two GP IIb-IIIa molecules on adjacent, activated platelets, the cytoplasmic domain of GP IIIa becomes phosphorylated on tyrosine residues 747 and/or 759. This leads to the recruitment of phosphotyrosine-binding intracellular signaling molecules to the membrane and to the association of phospho-GP IIIa with the cytoskeletal protein myosin *(119,147)* (illustrated in **B**). Alpha granules containing intracellular GP IIb-IIIa are present in both resting and stimulated platelets. The contents of the alpha granules are released as a consequence of outside-in signaling resulting in the secretion of factors involved in inflammation, vascular remodeling, and coagulation.

mediate the arrest and adhesion of neutrophils *(18)*. Third, fibrinogen is one of the proteins bound to artificial surfaces, including extracorporeal devices such as in-dwelling catheters. The immobilization of fibrinogen on these surfaces, leading to the generation of an active conformation of fibrinogen capable of activating GP IIb-IIIa, may also account for the thrombotic potential of artificial surfaces when exposed to plasma proteins *(10)*. This idea is supported by observations showing that GP IIb-IIIa antagonists reduce platelet loss in animal models of cardiopulmonary bypass *(114)*.

OUTSIDE-IN GP IIb-IIIa SIGNAL TRANSDUCTION

The binding of adhesive ligands to GP IIb-IIIa, together with the resultant clustering of the integrin following adhesion or aggregation, initiates one or more signal transduction pathways within the platelet leading to secretion of the contents of alpha granules and dense bodies as well as the activation of the remaining GP IIb-IIIa molecules. These outside-in signals, although required for maximal platelet aggregation, adhesion, and clot retraction are, in and of themselves, insufficient to induce these responses in that low concentrations of other agonists (e.g., ADP) are also required as costimuli. However, since low concentrations of other platelet agonists are normally present at sites of vascular injury such as would occur with plaque rupture or following precutaneous intervention, it can be anticipated that platelet activation via outside-in GP IIb-IIIa signaling occurs in these settings and that GP IIb-IIIa antagonists will have effects on platelet functions like secretion, GP IIb-IIIa activation, and enhancement of coagulation that are normally attributed to the classical platelet agonists such as ADP, thrombin, and collagen. In support of this conclusion, Carroll et al. *(115)* showed that the GP IIb-IIIa antagonist Ro 44–9883 inhibited alpha granule secretion when platelet aggregation was induced by ADP or low doses of TRAP or collagen. Observations claiming that GP IIb-IIIa antagonists do not affect these reactions *(116)* apparently were performed under conditions where aggregation and outside-in GP IIb-IIIa signaling were not involved. The requirement for low levels of other agonists may also allow for a further level of regulation of platelet aggregation by confluent endothelium, which expresses CD39, an ecto-ADPase that acts by metabolizing the ADP released by platelets *(117),* and thrombomodulin *(118),* which neutralizes thrombin. Lack of these two activities at sites of disrupted endothelium would facilitate platelet activation through outside-in GP IIb-IIIa signaling.

The mechanism by which GP IIb-III propagates outside-in signaling is of extreme importance in understanding GP IIb-IIIa function. Analysis of mutations of GP IIb-IIIa, either by expressing mutant proteins in heterologous systems or by examination of naturally occurring mutations identified in patients with Glanzmann's thrombasthenia, have shown that two domains of GP IIb-IIIa are functionally important in these events: the extracellular adhesive protein binding pocket, discussed earlier; and, the cytoplasmic domains, which now appear to act as scaffolds for the recruitment of signaling proteins to the cell membrane and as anchor sites for cytoskeletal proteins. Recent work has identified a postaggregation modification of the cytoplasmic domain of GP IIIa that may act to recruit cytoplasmic signaling proteins to propagate outside-in signaling through GP IIb-IIIa *(119).* Although stimulation of platelets by any of the classical platelet agonists does not produce any apparent covalent modification of GP IIb or GP IIIa, subsequent aggregation of these platelets now has been found to induce GP IIIa tyrosine phosphorylation. To determine whether this phosphorylation event is involved in GP IIb-IIIa signaling, peptides corresponding to the carboxyterminal half of the GP IIIa cytoplasmic domain (residues 740–762), which contained both tyrosines (Y747 and Y759), were synthesized and examined for their interactions with platelet signaling proteins. Two proteins, SHC and GRB2, bound to the peptide in which the two tyrosine residues were phosphorylated whereas neither protein bound to the unposphorylated peptide. Both SHC and GRB2 are adapter proteins that have been shown to interact with several phosphorylated receptor complexes, including platelet-derived growth factor

(PDGF) receptor and the B-cell receptor, and link these transmembrane proteins to the Ras pathway via the guanine nucleotide exchange factor SOS *(120,121)*. Thus, it is attractive to hypothesize that outside-in signaling through GP IIb-IIIa is initiated by fibrinogen (or vWf) binding to GP IIb-IIIa and that activation of intracellular signaling during subsequent aggregation and/or adhesion is mediated by tyrosine phosphorylation of the GP IIIa cytoplasmic domain followed by the recruitment of phosphotyrosine-binding signaling molecules to the membrane (Fig. 4).

Two types of experiments also indicate the importance of the GP IIIa cytoplasmic tyrosine residues in GP IIb-IIIa signaling. First, Liu et al. *(122)* introduced peptides corresponding to the GP IIIa cytoplasmic domain into cell lines and found that they inhibited integrin-mediated cell adhesion to immobilized fibrinogen whereas peptides containing phenylalanine at either tyrosine 747 or tyrosine 759 were inactive. Second, expression of GP IIIa harboring cytoplasmic tyrosine-to-phenylalanine mutations in both CHO cells and K562 cells indicates that the GP IIIa tyrosine residues are required for the GP IIIa-dependent retraction of fibrin clots *(123,124)*.

The downstream signaling processes induced by outside-in GP IIb-IIIa signaling include protein tyrosine phosphorylation, as well as reorganization of the cytoskeletal/contractile apparatus within the platelet. Several of the proteins phosphorylated are tyrosine kinases, including multiple Src-family members (e.g., Src, Lyn, Fyn), Syk, and FAK *(125,126)*. The effects of tyrosine kinase inhibitors has highlighted the importance of tyrosine phosphorylation in platelet signaling reactions as a wide range of tyrosine kinase inhibitors including genistein, erbstatin, tyrphostins B42 and B46, and piceatannol have been found to inhibit platelet aggregation and secretion *(80,127–129)*. Both Syk and Src become phosphorylated and activated within seconds of fibrinogen binding to GP IIb-IIIa and translocate to the cytoskeleton on aggregation. Unlike the critical role of Syk kinase in collagen-induced platelet stimulation *(97),* gene targeting experiments have only indicated a partial role for this kinase in inside-out signaling and no role in adherence to immobilized fibrinogen *(130)*. Chemical crosslinking studies have demonstrated that Src is physically associated with GP IIb-IIIa within platelets as is another Src family member, Lyn *(131)*. The lack of any defect in platelet function in platelets from mice in which the gene for Src has been disrupted is probably due to the redundancy between members of the Src family of tyrosine kinases *(132)*.

In contrast to Syk and Src, the FAK tyrosine kinase only becomes phosphorylated and activated upon full spreading or aggregation of platelets and requires agonist costimulation in addition to integrin ligation *(133)*. FAK autophosphorylation at tyrosine 397 leads to its association with Src, which can phosphorylate other tyrosine residues within the protein to provide docking sites for other phosphotyrosine binding proteins including GRB2 *(134)*. Thus, the common theme emerging from the study of outside-in GP IIb-IIIa signaling involves the tyrosine phosphorylation of a series of proteins, including the phosphorylation and activation of tyrosine kinases, followed by the assembly of signaling complexes.

Other second messenger pathways have also been implicated in outside-in GP IIb-IIIa signal transduction events, one of which is the hydrolysis of phosphoinositides. As described earlier, platelet activation leads to activation of phospholipases leading to the production of inositol 1,4,5-trisphosphate. This polyphosphoinositide can be phosphorylated on the D3 position, subsequent to platelet activation, by at least two types of phosphoinositide 3-kinases (p85/PI3K and PI3Kγ) *(135)*. D3 phosphoinositides have

been implicated in actin assembly and filopodial extension in the platelet *(136)*. The activation of p85/PI3K and its translocation to the cytoskeleton are dependent on GP IIb-IIIa outside-in signaling and platelet aggregation *(137)*. Treatment of platelets with the PI3K inhibitor, wortmannin, results in the reversal of TRAP-induced platelet aggregation and affects aggregation induced by the LIBS6 antibody although platelet secretion remains unaffected. That wortmannin also inhibited the maintenance of GP IIb-IIIa in its high-affinity conformation suggests that PI3K may be involved in the formation of stable platelet aggregates possibly by sustaining GP IIb-IIIa in an active state *(138)*. Another protein involved in this signaling pathway, which is also phosphorylated and induced to translocate to the cytoskeleton upon GP IIb-IIIa-mediated platelet aggregation, is the inositol 5-phosphatase, SHIP. This 145 kD protein can dephosphorylate inositol 1,3,4,5-tetrakisphosphate and phosphatidyl inositol 3,4,5-trisphosphate leading to the further production of D3 phosphoinositides *(139)*.

The calcium activated protease calpain also becomes activated during platelet aggregation and the work of Du et al. *(140)* suggests that calpain may be yet another second messenger in GP IIb-IIIa signaling. These workers have shown that the cytoplasmic domain of GP IIIa can be cleaved by calpain during platelet aggregation and that calpain mediated hydrolysis lead to inhibition of fibrin clot retraction. However, since calpain cleaves several proteins in addition to GP IIIa, it is not clear which hydrolytic event is responsible for this effect *(141)*. The small GTPases including Ras, Rho, Rac, and CDC42 constitute a still further class of second messengers that appear to be involved in GP IIb-IIIa-mediated signaling. The Rho family (Rho, Rac, CDC42) are important in GP IIb-IIIa-mediated actin assembly *(142,143)*. The role that Ras plays in platelet integrin signaling remains to be established. Although in many cell types Ras is involved in activation of the MAP kinase cascade, it is not clear that this is its role in platelet biology. Indeed, Ras may even act to negatively regulate Rho since the Ras-associated protein GAP can associate with p190 Rho-GAP leading to the inactivation of Rho *(144)*.

GP IIb-IIIa CYTOSKELETAL INTERACTIONS

Many of the signaling events described above involve the translocation of proteins to the cytoskeleton leading to reorganization of the cytoskeleton/contractile apparatus so that it can consolidate a platelet aggregate and retract a clot, processes important to thrombus stability. Binding of fibrinogen and subsequent platelet aggregation result in an increased association of GP IIb-IIIa with cytoplasmic actin filaments and the formation of focal contact-like structures *(145,146)*. Important issues concern how outside-in signaling induces GP IIb-IIIa association with the cytoskeleton and the identity of the cytoskeletal protein used for integrin attachment. It is estimated that approximately 30% of GP IIb-IIIa becomes associated with the cytoskeleton following platelet aggregation. GP IIIa tyrosine phosphorylation is involved in this interaction, since tyrosine phosphorylated GP IIIa is retained preferentially with the cytoskeletal fraction from aggregated platelets *(123)*. Phosphorylated GP IIIa appears to bind to platelet myosin as phosphorylated GP IIIa cytoplasmic domain peptides, but not unphosphorylated peptides have been found to selectively interact with the heavy chain of platelet myosin *(147)*. This suggests a mechanism for regulation of the interaction of GP IIb-IIIa with the platelet cytoskeleton: the cytoplasmic domain of GP IIIa becomes tyrosine phosphorylated upon integrin ligation and platelet aggregation. In addition to the phospho-GP IIIa interacting

with signaling proteins (described above) it also translocates to the cytoskeleton where the phosphotyrosine residues may direct binding to the contractile protein myosin. GP IIb-IIIa on adherent platelets aligns with cytoplasmic actin filaments *(148)* and, with time, redistributes to the abluminal surface causing the platelet to become refractory for the recruitment of additional platelets *(149)*. The resultant loss, on the luminal surface, of accessible, activated GP IIb-IIIa, may serve to limit the recruitment of platelets to a growing thrombus *(149)*.

Clot retraction is GP IIb-IIIa-dependent, where the integrin serves as the transmembrane bridge between extracellular fibrin and the cytoplasmic contractile machinery. The direct binding of GP IIb-IIIa with several purified cytoskeletal proteins in addition to myosin has been demonstrated, e.g., the binding of purified GP IIb and GP IIIa to talin *(150),* the binding of GP IIb-IIIa incorporated into phospholipid vesicles with γ-actinin *(151),* and the binding of the GP IIIa cytoplasmic domain, in a yeast two-hybrid screen, to skelemin *(152)*. An unsolved problem is the identification of the cytoskeletal interactions that are responsible for the clot retraction process. In this regard, it is of interest that mutations in the cytoplasmic domains of GP IIb-IIIa that affect integrin signaling can also inhibit clot retraction *(153),* including those mutations involving GP IIIa tyrosine residues (as described above) *(123,124)*. Since tyrosine mutations would prevent GP IIIa phosphorylation, it is possible that one way such mutations can exert their effect on clot retraction is by preventing the association of phosphotyrosine-GP IIIa with myosin. In support of this idea, tyrosine kinase inhibitors can regulate the cytoskeletal attachment of GP IIb-IIIa and can inhibit the clot retraction process *(154)*.

GP IIb-IIIa GENETICS

Increased understanding of GP IIb-IIIa structure-function relationships has come from the study of patient's with Glanzmann's thrombasthenia. This hereditary disease, which results in a life-long bleeding diathesis, is due to a lack of GP IIb-IIIa function. More than 21 mutations in GP IIb and 19 in GP IIIa have been characterized at the genetic level *(155)*. The identified mutations cause either defective biosynthesis or normal or near normal expression of a defective protein due to a loss of function mutation. Mutations that lead to defective expression of GP IIb-IIIa include those that produce defective proteins due to early terminations, deletions or frameshifts, those that generate a GP IIb-IIIa complex that is sensitive to dissociation [e.g., an Arg214Trp point mutation in GP IIIa *(156)*], and those affecting mRNA stability [e.g., Arg53Stop in GP IIb *(157)*], or RNA processing [for example an extensive 3 kb insertion in GP IIIa *(158)*]. Loss of function mutations include those that affect the ligand binding and Ca^{2+} binding sites in GP IIb-IIIa and also those that affect GP IIb-IIIa signaling. Some of these latter mutations highlight the importance of the cytoplasmic domains in GP IIb-IIIa function. For example, a patient with a single point mutation in the cytoplasmic domain of GP IIIa where serine 752 is replaced by a proline (S752P) had platelets that expressed near normal amounts of GP IIb-IIIa but that failed to aggregate, to spread, or to undergo focal adhesion kinase phosphorylation in response to fibrinogen binding *(159)*. This mutation helped validate the Chinese hamster ovary (CHO) cell expression system as a model for studying GP IIb-IIIa. When transfected into CHO cells with GP IIb, GP IIIa protein bearing S752P also showed a loss of GP IIb-IIIa function with impaired integrin signaling observed *(153)*.

A recent mutagenesis study of GP IIb-IIIa in the CHO cell expression system has

identified several mutations in GP IIIa that affect integrin function. In this study, CHO cells expressing a constitutively active chimera containing the extracellular domains of GP IIb and GP IIIa were subjected to chemical mutagenesis and screened for those cells now failing to bind soluble fibrinogen *(160)*. This screen selected for several mutations previously identified in Glanzmann's patients as well as mutations that would be anticipated to interfere with ligand or cation binding sites. However, an additional three GP IIIa mutations (Glu312Lys, Gly331Glu, and Ser334Phe) were identified in the membrane-proximal extracellular region of GP IIIa, a region not thought to be involved in ligand binding. These mutations may indicate a region of GP IIIa that is important for generating the active conformation of GP IIb-IIIa possibly through an interaction with some cofactor required for integrin activation.

In addition to the mutations arising in GP IIb-IIIa that lead to Glanzmann's thrombasthenia, there are a number of naturally occurring allotypic variants of GP IIb-IIIa. One of these is the Pl^A alloantigen system that has frequently been implicated in syndromes of immune-mediated platelet destruction. The Pl^{A1} and Pl^{A2} isoforms arise because of a Leu33Pro amino acid polymorphism in GP IIIa *(161)*. Recently this polymorphism has generated considerable interest because it has been postulated that the $Pl^{A2/2}$ genotype is a possible risk factor for coronary thrombosis. A correlation between the $Pl^{A2/2}$ genotype (found in approximately 2% of the Caucasian population) and acute coronary thrombosis was observed in one study (71 patients, 68 controls) *(162)*. This correlation was strongest in patients who had had coronary events at a relatively young age (less than age 60). However, while a number of studies support this putative association between the $Pl^{A2/2}$ genotype and increased chance of coronary disease *(163,164),* it remains controversial since other investigators have failed to observe such an association *(165,166)*. It is of interest that Greenland Inuits, a population with a low incidence of thrombotic disease, have a lower frequency of the Pl^{A2} allele than does the general Caucasian population *(167)*. In addition, recent data has indicated a functional difference between Pl^{A1} and Pl^{A2}-bearing platelets with the Pl^{A2} platelets being associated with increased aggregability in response to ADP and epinephrine stimulation *(168)*. Although it remains to be established whether the $Pl^{A2/2}$ polymorphism is indeed a risk factor indicator for coronary disease, recent studies do suggest that platelet glycoprotein polymorphisms may provide a whole new array of thrombotic risk factors *(169)*.

GP IIb-IIIa IN COAGULATION, INFLAMMATION, AND VASCULAR CELL PROLIFERATION

It is well known that the presence of platelets in blood shortens the time required for clot formation. Recent studies *(170–172)* have shown that GP IIb-IIIa antagonists reduced tissue factor induced thrombin formation indicating that clot formation and GP IIb-IIIa may be linked. This finding raises the interesting possibility that GP IIb-IIIa antagonists may decrease thrombin formation at sites of vascular injury. In support of this suggestion, ex vivo samples have prolonged clot formation time when obtained from patients receiving GP IIb-IIIa antagonists *(173)*. Although unstimulated platelets have poor clot promoting activity, activated, adherent platelets assemble prothrombinase and Factor Xase on their surface to greatly enhance the rate of thrombin generation *(174)*. Since aggregation induces platelet activation through outside-in GP IIb-IIIa signaling, one possibility is that GP IIb-IIIa antagonists reduce thrombin formation by their ability

to reduce aggregation-induced platelet stimulation. An alternative hypothesis is derived from the experiments of Byzova and Plow *(11)* who showed that prothrombin binds to GP IIb-IIIa on unstimulated platelets. These authors speculate that prothrombin binding occurs via the RGD sequence in this proenzyme and found that RGD-containing peptides blocked this interaction. Since GP IIb-IIIa binding enhanced the rate of prothrombin activation, a reaction that was blocked by GP IIb-IIIa antagonists, these data suggest that GP IIb-IIIa antagonists may also inhibit clot formation because they displace prothrombin binding to the platelet surface. Either explanation could account for the long clotting times in the platelet rich plasma (PRP) from patients with Glanzmann's thrombasthenia *(175)*.

An additional way that GP IIb-IIIa may affect coagulation is derived from recent observations showing that the CD40 ligand is expressed on the platelet surface following alpha granule secretion *(176)*. CD40 ligand has been shown to upregulate the expression of tissue factor in macrophages *(177)*. These data suggest a third mechanism by which GP IIb-IIIa antagonists will reduce the ability of ruptured lesions to synthesize thrombin, i.e., by prevention of outside-in signaling through GP IIb-IIIa and the subsequent alpha granule secretion.

Inflammation is a complicating factor of cardiovascular disease and the fibrinogen binding activity of GP IIb-IIIa may contribute to this process in four ways: 1) by using the bound fibrinogen to bind the β2 integrin, Mac-1, and recruiting neutrophils into a growing thrombus *(18)*; 2) by enhancing, through aggregation, the release of inflammatory mediators such as TGFβ *(178)*; 3) by enhancing the production of inflammatory mediators from the coagulation cascade such as Factor Xa *(179)*; and 4) by mediating the binding of platelets to endothelial cells so that the CD40 ligand expressed on the platelet surface can effect the release of cytokines *(176)*. An untested hypothesis is that GP IIb-IIIa antagonists may reduce the pathological consequences of inflammation in acute coronary syndromes.

Platelets provide perhaps the most abundant source of PDGF, a growth factor implicated in atherosclerosis and restenosis *(180)*. PDGF is released with other alpha granule proteins during platelet stimulation and it has been speculated that sufficient PDGF is released during thrombosis to affect the vessel wall *(181)*. Tests of this idea using GP IIb-IIIa antagonists in the setting of angioplasty have proven negative in that the large, randomized clinical trials have not shown that the use of these drugs prevents the need for revascularization *(182-184)*. The exception was the EPIC trial using abciximab, which did show a decreased need for revascularization, a result attributed to the $\alpha_v\beta_3$ crossreactivity of abciximab *(185)*. However, since subsequent trials using this drug in the same indication did not reproduce this effect *(182),* it is now believed that the use of abciximab does not affect this clinical outcome *(2)*. Nonetheless, it remains to be determined whether other aspects of the mitogenic activity of PDGF such as matrix and metalloprotease production *(186,187)* are affected by the use of GP IIb-IIIa antagonists in other clinical indications and whether the need for revascularization is a true indication of a smooth muscle cell proliferative response.

CONCLUSIONS

The combined study of Glanzmann's thrombasthenia, of GP IIb-IIIa, and of GP IIb-IIIa antagonists in arterial thrombotic disease have now clearly established the pivotal

roles of GP IIb-IIIa in platelet aggregation and of platelet aggregation in arterial thrombosis. Glanzmann's thrombasthenia provided the first direct link between GP IIb-IIIa and aggregation and the characterization of the wide spectrum of loss of function mutations in GP IIb-IIIa that cause this disease has identified functional domains of this receptor. The study of GP IIb-IIIa helped to establish the integrin family of cell adhesion receptors. Further, it has characterized functional domains responsible for adhesive protein binding and for the cation-dependency of GP IIb-IIIa and has been instrumental in identifying the GP IIb-IIIa binding domains on ligands and other adhesive proteins that interact with integrins. Much of this information has proven essential in the past decade for the design and characterization of parenteral and orally available GP IIb-IIIa antagonists and in the development of strategies for pharmakodynamic monitoring of these drugs. It is now established that adhesion is only part of GP IIb-IIIa function in that its signaling in platelets facilitates a wide range of reactions such as platelet shape change, platelet secretion, consolidation of aggregates, and clot retraction. These functions of GP IIb-IIIa indicate that it is not only involved in thrombosis and hemostasis, but also links platelets and platelet aggregates to coagulation, inflammation, thrombolysis, atherosclerosis, and the proliferation of cells in the vessel wall in a dynamic way, more than would result from simple vessel occlusion. Use of GP IIb-IIIa antagonists is now a proven strategy for the reduction of arterial thrombosis to reduce morbidity and mortality in human disease.

Future directions on GP IIb-IIIa research can be expected to continue to focus on extracellular and intracellular domains, providing new insights into the molecular description of how GP IIb-IIIa interacts with its ligands and into how GP IIb-IIIa is involved in the various signal transduction pathways. One can anticipate that the next breakthroughs on the extracellular domain will come with the atomic level resolution of GP IIb-IIIa, which will provide a contour map of the GP IIb-IIIa surface and a precise description of the binding sites of this receptor. Such information will undoubtedly yield new information on the way GP IIb-IIIa interacts with adhesive proteins, and may also provide new insights into the design of therapeutic anatagonists that block the binding of adhesive proteins. Elucidation of the function of the intracellular domains of GP IIb-IIIa would appear to be less of a challenge due to their small size, but only recently have studies begun to delineate the signaling pathways that mediate GP IIb-IIIa-dependent process.

The pathways responsible for inside-out signaling are highly redundant, underscoring the therapeutic advantage of using GP IIb-IIIa antagonists to prevent arterial thrombosis as opposed to inhibitors of specific platelet agonists or of specific inside-out signaling pathways. Additional signaling cascades are involved in outside-in GP IIb-IIIa signaling, and although these are only just being elucidated, it is apparent that the pathways downstream of the integrin are far from unique in that they involve many of the same signal transduction proteins involved in the cascades initiated, for example, by the binding of soluble growth factors to their receptors. These considerations suggest that the signaling events that are unique to GP IIb-IIIa will undoubtedly be those most proximal to the integrin, i.e., those events directly involving the cytoplasmic domains of GP IIb-IIIa. Evidence, both from naturally occurring and experimentally induced mutations of GP IIb-IIIa, clearly indicates a role for the cytoplasmic domains of GP IIb and GP IIIa in the function of GP IIb-IIIa, both in linking the integrin to the platelet cytoskeleton and in associations with signaling proteins. Recent data also indicates that postaggregation

tyrosine phosphorylation of the cytoplasmic domain of GP IIIa may further influence GP IIb-IIIa interactions with signaling and cytoskeletal proteins. Since these events involve the interaction of protein(s) with the GP IIIa cytoplasmic domain, it is reasonable to speculate that they may be more specific to GP IIb-IIIa signaling. These proximal events in GP IIb-IIIa signaling may offer new targets for pharmacological intervention of GP IIb-IIIa in platelet aggregation and of platelet aggregation in arterial thrombosis.

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4 Glycoprotein IIb/IIIa Antagonists: Development of Abciximab and Pharmacology of Select Agents

Barry S. Coller, MD

CONTENTS

DEVELOPMENT OF ABCIXIMAB AS A GPIIb/IIIa ANTAGONIST

The rationale for developing platelet GPIIb/IIIa antagonists as antithrombotic agents for use in ischemic cardiovascular disease emerged from integrating data obtained in the early 1980s by many different investigators working in diverse fields. The data were, however, often controversial or fragmentary, or required extrapolation to human disease from in vitro systems or animals models. Thus, for those who are considering embarking on a similar adventure in drug development, it is important to emphasize that until efficacy was established in well-controlled, large clinical trials, many individuals remained skeptical about the potential benefits and safety of this approach.

Pathophysiology of Coronary Artery Occlusion

Despite strong evidence from postmortem examinations for a major role for thrombosis in the development of ischemic cardiovascular disease dating back to before 1950 *(1),* controversies regarding whether the thrombus occurred before or after myocardial infarction *(2)* and the role of coronary spasm *(3)* led to confusion as to the role of thrombosis in the etiology of ischemic damage. Moreover, a plaque hemorrhage model was favored by some investigators in which obstruction was primarily caused by a mechanical flap produced by hemorrhage-induced dissection of the coronary artery *(1)*. It was only with the advent of cardiac catheterization of patients with myocardial infarction in the early 1980s that there was general acceptance in the cardiologic community that thrombosis initiated most myocardial infarctions *(4)*. The success of thrombolytic therapy provided additional support for the importance of thrombosis in the pathogen-

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esis of myocardial infarction *(5,6)*. Thrombolytic therapy, however, fails to achieve rapid reperfusion in nearly 15–45% of patients *(7)*. The mechanism(s) of myocardial infarction in patients resistant to thrombolytic therapy remains a matter of speculation, but a number of the nonthrombotic theories mentioned above have been proposed *(7)*.

Even after it was established that thrombosis is a major contributor to acute ischemic syndromes, the relative contributions of platelets and fibrin to this process were unclear. The efficacy of thrombolytic therapy focused attention on the contribution of fibrin. These data logically led to attempts to develop strategies to decrease fibrin deposition by developing more effective inhibitors of thrombin generation and/or thrombin's action *(8)*. Detailed pathologic studies identified, however, that a white platelet "head" of variable size is usually identifiable at the site of atherosclerotic plaque rupture or erosion, indicating that platelets commonly initiate the thrombotic process *(1,9,10)*. In this formulation, the commonly identified large red fibrin "tail" reflects subsequent activation of the coagulation system (facilitated by thrombin generation on the surface of platelets) and entrapment of erythrocytes in the rapidly forming fibrin meshwork. Biochemical evidence of platelet activation during ischemic vascular events *(11)* provided additional evidence for a role for platelets, although it was not possible to exclude the possibility that platelet activation was an epiphenomenon. Animal models of thrombosis secondary to vascular injury provided compelling evidence that platelets make a major contribution to thrombus formation *(12–15),* but all of the animal models differ significantly from naturally occurring human cardiovascular disease in the mechanism of injury. Moreover, none of the animal models at that time involved arteries containing the complex types of atherosclerotic lesions on which virtually all human disease occurs.

Perhaps the most compelling evidence supporting an essential role for platelets in human ischemic cardiovascular events came from the Second International Study of Infarct Survival (ISIS-2), a double-blind randomized placebo-controlled study involving more than 17,000 patients with clinically diagnosed myocardial infarction *(6,16)*. This study demonstrated an 18.9% decrease in 35-day vascular mortality from taking aspirin alone (absolute reduction 2.5%), a reduction very similar to the 21.2% achieved with intravenous streptokinase alone *(6)*. Combining aspirin with streptokinase resulted in a 39.4% reduction in mortality, with this additive effect suggesting that the agents operated through different mechanisms of action. Data on the combined use of heparin and aspirin in ISIS-2 were especially important in conceptualizing the roles for antiplatelet and anticoagulant agents. Patients were not randomized with regard to heparin therapy, but the intention of each treating physician to either use or not use heparin was recorded. Aspirin improved the outcome of patients regardless of whether intravaneous, subcutaneous, or no heparin use was planned, providing further evidence that aspirin operates through a mechanism different from that of heparin *(6,16)*. Even the ISIS-2 data supporting an important role for platelets in myocardial infarction could be challenged, however, because it could be argued that aspirin's benefit may have been due to its effects on nonplatelet proteins or cells since many such effects have been described *(17)*.

The safety data in ISIS-2 also were extremely important in that they showed that aspirin alone did not increase the risk of hemorrhagic stroke and actually decreased the risk of any stroke by ~50% *(6,16)*. Aspirin's stroke benefit may result from several mechanisms linked to its antithrombotic action. These include: 1) decreasing the size of mural thrombi; 2) decreasing the size of the myocardial infarctions so that fewer are

transmural (the feature most predictive of developing a mural thrombus); 3) decreasing the extent of secondary thrombus formation on emboli that reach the cerebral circulation; and 4) decreasing microvascular thrombus formation that may result from even temporary interruption of the cerebral circulation. The stroke data from ISIS-2, when combined with those of the U.S. Physician's Health Study of primary prophylaxis of myocardial infarction with aspirin *(18),* which identified an increased risk of hemorrhagic stroke with aspirin treatment, lead to the interesting paradox that aspirin decreases the risk of stroke during and immediately after myocardial infarction, a period of high stroke risk, but increases the risk of hemorrhagic stroke in the normal population, in which the risk of stroke is very low. Since the protection from death from myocardial infarction afforded by aspirin, a relatively weak antiplatelet agent, extended to only a minority of patients in ISIS-2 and since aspirin's hemorrhagic toxicity during the first 30 days of treatment was minimal, it seemed logical to conclude that there was a potential role for more powerful antiplatelet agents in the treatment of ischemic cardiovascular events due to thrombosis.

Theories of the pathogenesis of acute reocclusion after percutaneous coronary interventions (PCI), including balloon angioplasty, atherectomy, and similar procedures, went through phases similar to those relating to the pathogenesis of myocardial infarction. Thus, in the 1980s, there was considerable controversy as to the relative roles of thrombosis versus mechanical dissection resulting in development of an obstructing intimal flap. Estimates that I obtained at that time from expert interventionalists for a key role for thrombosis ranged from 20–80%, with complementary estimates of 80–20% for mechanical dissection. Data from at least one relatively small retrospective study indicated that aspirin and dipyridamole decreased the risk of abrupt occlusion *(19),* but the uncertainty about pathogenesis made it unclear as to whether more potent antiplatelet agents would further decrease the risk of abrupt occlusion after PCI.

Glanzmann Thrombasthenia

Although first described in 1918, it was not until the 1960s that Glanzmann thrombasthenia was categorized as an autosomal recessive inherited disorder characterized by mucocutaneous hemorrhage, marked prolongation of the bleeding time, abnormal clot retraction, and the hallmark failure of platelets to aggregate in response to all of the agonists thought to operate in vivo, including ADP, epinephrine, collagen, and thrombin *(20,21).* Subsequently, it was observed that the platelets of patients with Glanzmann thrombasthenia are deficient in two different glycoproteins, identified as GPIIb and GPIIIa based on their migration in polyacrylamide gels after treatment with sodium dodecyl sulfate *(22,23).* Soon thereafter, it was established that these proteins exist as a calcium-dependent complex *(24).*

The nature and severity of the clinical hemorrhagic syndrome of Glanzmann thrombasthenia were important factors in the decision to consider GPIIb/IIIa as a target for antithrombotic therapy since the patients' symptoms could be considered the likely equivalent of maximal GPIIb/IIIa blockade by an antagonist. The spectrum of severity is wide among patients *(20,21,25),* but easy bruising, menorrhagia, epistaxis, and variable gingival bleeding (depending largely on dental hygiene and tooth repair) are most common. Severe gastrointestinal bleeding and bleeding in other organs occurs, but it usually is episodic and infrequent. Of particular note, unlike the bleeding in hemophilia A and B (factors VIII and IX deficiencies), spontaneous central nervous system bleeding

is not common in Glanzmann thrombasthenia *(20),* highlighting the variability of bleeding syndromes that occur with defects in different components of the hemostatic system. Thus, since it was known that complete absence of GPIIb/IIIa receptors is not incompatible with life, there was reason to hope that even high-grade GPIIb/IIIa blockade could be sustained for at least a short period of time with acceptable toxicity. The ability to extrapolate these data to the potential use of GPIIb/IIIa antagonists to treat thrombotic cardiovascular disease was limited, however, by the lack of knowledge about the impact of using a GPIIb/IIIa antagonist in combination with heparin, aspirin, a thrombolytic agent, or perhaps all of them together, since these agents are scrupulously avoided in thrombasthenia patients. Moreover, the invasive instrumentation that is required for PCI and frequently occurs during the management of myocardial infarction would add a serious hemostatic challenge. Thus, the safety considerations that could be deduced from analysis of patients with Glanzmann thrombasthenia were complex; GPIIb/IIIa antagonists were likely to cause a significant, but perhaps not unacceptable risk of hemorrhage; the use of aspirin, heparin, and thrombolytic agents would likely increase the risk significantly; and there would likely be a need for fastidious attention to the vascular sites of entry of invasive devices.

Observations on patients with Glanzmann thrombasthenia had little to offer with regard to potential efficacy of GPIIb/IIIa antagonists. Since it is such a rare disorder, few patients who have Glanzmann thrombasthenia are old enough to have thrombotic ischemic vascular disease. Currently, however, with Dr. Uri Seligsohn and his colleagues in Israel, we are embarking on a study in Glanzmann thrombasthenia patients and the much larger number of Glanzmann thrombasthenia carriers whom we have identified by DNA analysis *(26)* and whose platelets have ~50–60% of the normal number of receptors *(27),* to assess whether partial or complete loss of GPIIb/IIIa receptors affords protection from clinical or subclinical cardiovascular disease.

Platelet Physiology

The development of the platelet aggregometer in the 1960s *(28)* opened the modern era of platelet function analysis, with the rapid characterization of the aggregation response to ADP, epinephrine, collagen, and thrombin, among others *(29).* It also allowed for the appreciation of the complex interrelation of platelet aggregation and the release reaction *(30).* It further permitted the characterization of antiplatelet agents, most notably aspirin, which gave a characteristic pattern of inhibition involving the second wave of aggregation*(31,32)* associated with the release reaction, and later identified as being due to thromboxane A_2 production *(33,34).*

Platelet aggregation induced by ADP, epinephrine, collagen, and thrombin (the agonists that fail to aggregate platelets from patients with Glanzmann thrombasthenia) was established in the late 1970s to result from the binding of the bivalent adhesive glycoprotein fibrinogen to the platelet surface *(35,36),* followed by bridging by the fibrinogen molecule of receptors on two different platelets. Of course, the GPIIb/IIIa receptor became the logical choice to be the fibrinogen receptor, but even here there was controversy as to whether Glanzmann patients lacked the receptor or lacked the ability to activate the receptor *(37).* Although several lines of evidence supported the conclusion that the GPIIb/IIIa receptor was, in fact, the receptor that bound fibrinogen *(38),* it was this lingering controversy that led us in the early 1980s to try to develop monoclonal antibodies, which were coming into general use by investigators at that time, to help

resolve this issue. We wanted to focus on antibodies that would affect platelet function and thus our screening assay built on our earlier studies *(39)* and was based on the agglutination of fibrinogen-coated beads by platelets. We produced antibodies, 10E5 and 7E3, that blocked the agglutination and then discovered that they bound to the GPIIb/IIIa complex, providing very strong evidence for GPIIb/IIIa being responsible for the binding of fibrinogen *(40,41)*.

At approximately the same time, additional molecular biological data from investigators studying adhesion phenomena in many different tissues provided evidence that GPIIb/IIIa was a member of a large family of receptors with similar structures *(42)*. Hynes termed these receptors "integrins" to emphasize that they have binding domains on both the exterior of the cell and the cytoplasmic face; the former interact with adhesive ligands whereas the latter interact with cytoskeletal proteins and proteins involved in both receiving and sending signals *(42)*. GPIIb/IIIa is a prototypic integrin, being a heterodimer of an α-subunit (GPIIb, α_{IIb}) and a β-subunit (GPIIIa, β_3) *(43,44)*. Monoclonal antibody studies demonstrated that GPIIb/IIIa is essentially specific for platelets and megakaryocytes and is expressed at very high density on platelets (~80,000 receptors per platelet *(45)*) making it one of the densest adhesion/aggregation receptors in any biologic system. There is also an internal pool of GPIIb/IIIa receptors associated with α granule membranes and perhaps other structures *(46,47)*. At least some GPIIb/IIIa receptors probably cycle between the platelet surface and the internal pools *(47)*. The β_3-subunit of GPIIb/IIIa can also pair with another α-subunit, termed α_v to form the $\alpha_v\beta_3$ receptor, commonly called the vitronectin receptor *(48)*. This is present on platelets at extremely low levels (~50–100 molecules per platelet *(49)*) and is also present on many other cell types, including endothelial cells, osteoclasts, smooth muscle cells, and activated lymphocytes *(50,51)*.

Investigators studying the $\alpha_5\beta_1$ integrin receptor in the early 1980s made the remarkable discovery that a very small region of the large fibrinectin molecule was crucial for binding *(52)*. Ultimately they localized this to a three amino acid motif, arginine-glycine-aspartic acid [single letter code RGD *(52)*]. They went on to show that small peptides containing this sequence could actually block the binding of ligands to the $\alpha_5\beta_1$ receptor *(53)*. At approximately the same time, aided by the availability of monoclonal antibodies, it was discovered that the GPIIb/IIIa receptor could bind not only fibrinogen, but also von Willebrand factor (vWf), fibronectin, vitronectin, and other adhesive glycoproteins *(54)*. With only one exception, as these proteins were cloned and their amino sequences determined, it became clear that the regions responsible for binding to platelets contained RGD sequences *(55)*. Moreover, the binding of all of these ligands could be inhibited by RGD-containing peptides *(56,57)*. The one exception, ironically, was fibrinogen itself, where binding to GPIIb/IIIa was mediated by the C-terminal dodecapeptide of the γ chain, which contains a glycine-aspartic acid sequence and a crucial lysine (which, like arginine, is positively charged) two amino acids upstream *(58,59)*. It appears that the RGD and γ-chain peptides bind to the same or nearby sites in GPIIb/IIIa.

The $\alpha_v\beta_3$ receptor binds many of the same ligands as GPIIb/IIIa and it is also blocked by RGD-peptides *(60)*. It appears to have preference for vitronectin *(49),* however, and unlike GPIIb/IIIa, it binds to osteopontin when activated *(61)*. Moreover, it appears to bind fibrinogen via the latter's C-terminal RGD sequence in the Aα chain, not the γ-chain dodecapeptide *(58,59,62)*.

The multiplicity of ligands that can bind to GPIIb/IIIa adds complexity to its biology. Shear conditions may be important in determining the preferred ligand since vWf appears to be the most important ligand under high shear conditions, whereas fibrinogen is favored at low shear *(63)*. One unique feature of the GPIIb/IIIa-fibrinogen interaction is that GPIIb/IIIa activation is required for platelets to bind fibrinogen in fluid phase *(64),* but platelet activation is not required for GPIIb/IIIa to mediate adhesion to immobilized fibrinogen *(39,65)*.

The recognition that RGD-containing peptides could inhibit the GPIIb/IIIa receptor opened up exciting new opportunities to synthesize low molecular weight peptides, peptidomimetics, and nonpeptides that would have favorable pharmacologic features. This led to the production of literally thousands of compounds, including orally active compounds, that have high affinity for GPIIb/IIIa, and high specificity for GPIIb/IIIa compared to $\alpha_v\beta_3$ and other integrin receptors (see below) *(66,67)*.

Platelet Adhesion and Aggregation

Our current view is that occlusive thrombus formation in coronary arteries probably begins with deposition of platelets on a ruptured or eroded athersclerotic plaque, primarily mediated by constitutively active receptors on the platelet surface interacting with subendothelial proteins [including, but not limited to, GPIb/IX(vWf); GPIIb/IIIa (immobilized fibrinogen); GPIa/IIa ($\alpha_2\beta_1$) (collagen); GPIc*/IIa ($\alpha_5\beta_1$) (fibronectin); GPIc/IIa ($\alpha_6\beta_1$) (laminin); GPVI (collagen); and perhaps $\alpha_v\beta_3$ (fibrinogen, vitronectin, vWf, osteopontin); and GPIV (thrombospondin, collagen) *(30)*]. The adhesive proteins may be exposed by the initial injury (e.g., collagen and vWf), deposited from plasma or released by platelets onto subendothelial proteins (e.g., binding of vWf to collagen) *(68),* or deposited from plasma or released from platelets onto newly formed fibrin (e.g., vWf) *(69)*. Data demonstrating tissue factor in the lipid-rich core of atherosclerotic plaques, however, raise the possibility that the generation of at least small amounts of thrombin and local fibrin formation may be early events *(70)*. The adhesion process is likely to be much more complex after the release or generation of platelet agonists at the site of vascular injury since these agents may be able to cause nearby circulating platelets to expose P-selectin and activate GPIIb/IIIa to a high affinity ligand-binding state. Inflammatory cytokines may also affect the process, with platelets rolling or skipping along the surface before settling into stable interactions *(68,71)*. Under the relatively high shear rates found in coronary arteries, the GPIb/IX-vWf interaction appears to play a crucial role *(68)*.

The first layer of adherent platelets probably has little effect on blood flow. It is the recruitment of additional layers of platelets, mediated primarily or exclusively by the GPIIb/IIIa receptor, which poses the greatest risk of platelet thrombus formation and resulting vasoocclusion. The activated platelets in the thrombus, as well as microparticles released from platelets and adherent to the thrombus *(72,73),* furnish highly effective catalytic surfaces on which thrombin can be generated, leading to the initiation of fibrin deposition and further platelet activation and adhesion.

Thus, the rationale for targeting the GPIIb/IIIa receptor focused on its pivotal role in mediating the platelet–platelet interactions crucial for platelet thrombus formation, regardless of the agonist responsible for platelet activation. As such, it represents the final common pathway for platelet aggregation. This model predicts that GPIIb/IIIa receptor blockade would have little effect on platelet adhesion, which is largely mediated

by other receptors, and this was not considered a disadvantage since the first layer of platelets may contribute to maintaining hemostasis without compromising the inhibition of platelet thrombus function.

Animal Models

The goal of the early animal studies was to assess the role of the GPIIb/IIIa receptor in well-established models of thrombosis, in particular models where aspirin offered less than complete protection *(74)*. We chose antibody 7E3 for these studies because it reacted with human, primate, and dog platelets. The binding characteristics of 7E3 are discussed below in the section, Pharmacokinetics. To avoid the possibility that platelets coated with 7E3 would be cleared by dog splenic macrophages containing immunoglobulin Fc receptors, the experiments were conducted with antibodies digested with pepsin to cleave the Fc region from the immunoglobulin, leaving the $F(ab')_2$ *(74)*. In order to quantify the number of platelet GPIIb/IIIa receptors blocked, we developed an assay using radiolabeled 7E3 *(74)*.

In vivo dose-response experiments with 7E3-$F(ab')_2$ demonstrated that aggregation was not inhibited or minimally inhibited at ≤50% receptor blockade, partially inhibited from 50–80% receptor blockade, and essentially eliminated at >80% receptor blockade *(74,75)*. Thus, a dose of 7E3-$F(ab')_2$ that just achieves 80% receptor blockade will completely abolish platelet aggregation, but only produce a modest effect on the bleeding time *(75)*. And abolition of platelet aggregation cannot be equated with near 100% receptor blockade, since 80% receptor blockade will achieve this endpoint.

In the dog model developed by Dr. John Folts, a partially occluded and damaged coronary artery undergoes cyclical flow reduction as platelets deposit on the blood vessel wall, aggregate into massive platelet thrombi, and then abruptly embolize distally *(12,13)*. This model is designed to simulate human unstable angina. Early studies by Dr. Folts demonstrated that aspirin could preserve patency of the vessel and prevent the cycles, but cycles were restored by epinephrine infusion or increasing the stenosis *(12,76)*. Dr. Willerson and his colleagues used this same model to define the roles of serotonin, thromboxane A_2, and thrombin in platelet thrombus formation *(77,78)*. Most importantly, they demonstrated that a similar phenomenon occurs in some patients after coronary angioplasty, providing support for the relevance of the model for human disease *(79,80)*.

7E3-$F(ab')_2$ was the most potent antiplatelet agent Dr. Folts tested in this model, preventing platelet-mediated cyclical flow reductions despite infusing epinephrine, increasing the vascular stenosis, increasing the vascular damage, and even passing electric current through the cylinder used to create the vascular stenosis *(74,81)*. Similar results were obtained when the model was conducted on the carotid artery of nonhuman primates *(75)*. Dose-response studies demonstrated that the antithrombotic effect could be achieved with ~60–80% GPIIb/IIIa receptor blockade, which produced only a modest effect on the bleeding time *(74,75)*. Later studies by Anderson et al. demonstrated that 7E3 could abolish the cyclical flow reductions that occurred in some patients after coronary artery balloon angioplasty *(79,80)*.

Dr. Chip Gold and his colleagues had developed a dog model of reocclusion after thrombolysis by producing a severe fixed stenosis of a coronary artery, placing a whole blood thrombus adjacent to the constriction, and then administering t-PA in varying doses and regimens *(15)*. The coronary arteries of most animals were reperfused by the

t-PA, but reocclusion occurred nearly always, usually within minutes, and sometimes the reocclusion was followed by cyclical flow reductions similar to those observed in the Folts model *(15)*. Aspirin had only a minimal effect in this model. Pretreating dogs with 7E3-F(ab′)$_2$ shortened the time required to achieve reperfusion and completely protected from reocclusion, even when the dose of t-PA was reduced by as much as 75% *(15,74,82,83)*. A minority of animals achieved reperfusion with 7E3-F(ab′)$_2$ treatment alone, something virtually never observed in the controls. Later studies by Dr. Gold and his colleagues demonstrated similar results in a subset of patients with acute myocardial infarctions treated with abciximab *(84)*. In a variation of this model designed to elicit platelet-rich thrombi, t-PA alone did not produce reperfusion, whereas t-PA + 7E3-F(ab′)$_2$ was able to achieve reperfusion *(85)*.

Independent animal studies conducted by other investigators using 7E3- F(ab′)$_2$ produced very similar results in other animal models of thrombosis, including models that lasted as long as 5 days *(83,86–90)*.

Chimeric 7E3 Fab

In order to minimize the likelihood that humans treated with 7E3 would develop an immune response to the murine antibody, murine 7E3(41) was redesigned as a half-murine, half-human chimeric Fab fragment using recombinant techniques *(91,92)*. The resulting c7E3 Fab (abciximab; ReoPro™) contains the heavy- and light-chain variable regions from the murine antibody attached to the constant regions of human IgG_1 and kappa chains, respectively. c7E3 Fab is prepared by papain digestion of the intact antibody *(92)*.

The antithrombotic effect of c7E3 Fab was tested in a primate carotid artery model in which injury was induced by electrolytic injury *(93)*. c7E3 Fab treatment produced dose-response inhibition of thrombus formation; reduced the frequency of, or abolished the development of, occlusive thrombi; prolonged the time to occlusion; and decreased thrombus weight. In a baboon femoral artery thrombosis model, c7E3 Fab facilitated t-PA-induced thrombolysis, much like murine 7E3-F(ab′)$_2$ did in the comparable dog coronary artery model *(83,94)*.

Toxicology studies in nonhuman primates with c7E3 Fab given either as a bolus or as a bolus + continuous infusion for up to 96 h demonstrated that the agent produced transient mucocutaneous bleeding (gingival bleeding, epistaxis, and bruising) primarily related to sites of blood collection or restraints *(92)*. Similar results were obtained when c7E3 Fab was combined with aspirin, heparin, and either t-PA or streptokinase *(92)*.

Mechanisms of Action

Although the predominant mechanism by which GPIIb/IIIa antagonists prevent ischemic damage is by inhibiting thrombus formation by interfering with platelet aggregation, other potential effects of GPIIb/IIIa receptor blockade may also be important. Since activated platelets can facilitate thrombin generation by releasing factor V(a), binding prothrombin *(95),* shedding procoagulant microparticles, and providing a highly efficient catalytic surface for the reactions involved in thrombin generation *(72)*, decreasing the number of platelets in a thrombus may decrease thrombin generation. Moreover, in vitro, blockade of GPIIb/IIIa receptors appears to decrease the ability of platelets to undergo the release reaction, shed microparticles, bind prothrombin, and support thrombin generation in response to tissue factor stimulation *(72)*. Clinical stud-

ies with abciximab and eptifibatide also support the ability of GPIIb/IIIa receptor blockade to decrease thrombin generation in response to contact activation, as the activated clotting times (ACTs) of heparinized patients treated with these agents were longer than those of untreated heparinized patients *(96,97)*.

The enhanced thrombolysis observed with the combination of GPIIb/IIIa receptor blockade and a thrombolytic agent may result from a number of mechanisms, including: 1) inhibition of clot retraction *(98,99),* which limits the diffusion of thrombolytic agents; 2) inhibition of the release of fibrinolytic inhibitors from platelets, including plasminogen activator inhibitor-1 and α_2-plasmin inhibitor *(100,101)*; 3) inhibition of factor XIIIa binding to platelets and local release of platelet factor XIII, thus preventing fibrin crosslinking and crosslinking of fibrinolytic inhibitors to fibrin *(102)*; and 4) inhibition of the generation of the thrombin-activatable fibrinolysis inhibitor (TAFI) due to decreased thrombin generation *(101)*.

GPIIb/IIIa blockade may also prevent the platelet activation induced by thrombolytic agents *(103–105),* thus allowing the thrombolytic agents to act unopposed by the increased deposition of platelets into the thrombus.

GPIIb/IIIa receptor blockade may also decrease the release of agents from platelet granules *(72)* that have been implicated in producing intimal hyperplasia, one of the components of the restenosis process. These include PDGF, ADP, and serotonin. At present, however, there is little evidence that GPIIb/IIIa antagonists decrease restenosis with the dosing regimens currently used *(106,107)*.

Abciximab, but not the low molecular weight antagonists, crossreacts with the $\alpha_v\beta_3$ receptor *(49,108)*. It is unclear whether any of abciximab's effects are because of this crossreactivity. There are only a very few $\alpha_v\beta_3$ receptors on platelets *(49),* but they appear to contribute to the activation process that increases the catalytic efficiency of platelets in thrombin generation *(72),* and activated $\alpha_v\beta_3$ receptors appear to uniquely support binding of platelets to osteopontin *(61)*. Inhibiting $\alpha_v\beta_3$ produces apoptosis of cells that require $\alpha_v\beta_3$ for adhesion, most likely as a result of the decrease in the production of "survival" signals from the cytoplasmic protein complexes that are created at focal adhesions where integrin receptors localize and cluster *(50,109,110)*. Thus, it is possible that $\alpha_v\beta_3$ blockade can decrease smooth muscle cell proliferation or migration, processes that may be important in intimal hyperplasia, and a number of experimental animal models support this possibility *(111–114)*. Although the EPIC study suggested an effect of abciximab treatment on clinical restenosis, this was not confirmed in the EPILOG, CAPTURE, or ERASER studies *(107)*. The time of treatment with abciximab was only 12 to 24 h, however, in these studies and animal data indicate that $\alpha_v\beta_3$ upregulation after vascular injury persists for several weeks *(114)*.

PHARMACOKINETICS OF SELECT GPIIb/IIIa ANTAGONISTS

The theoretical considerations that led to the current strategy for the dose and duration of therapy for GPIIb/IIIa antagonists are the need for sufficiently high grade receptor blockade to prevent platelet thrombus formation despite extraordinary provocation, as may exist after vascular injury in the presence of low flow; and the need for the high grade receptor blockade to persist beyond the time necessary for the blood vessel to return to a state of low platelet reactivity ("passivation"). Animal model data in the Gold model *(82)* and a few anecdotal observations *(115)* suggested that ≥80% receptor blockade

would be required, and data from the EPIC study comparing the bolus alone group to the group receiving a bolus + 12 h infusion provided compelling support for this level of receptor blockade, because as soon as the level of receptor blockade in the bolus only group dropped below 80, there was a marked increase in the onset of new ischemic events *(116)*.

The duration of therapy depends on the passivation process, about which little is known. In animal models of vascular injury of normal blood vessels it appears to take approximately 6–8 h *(117,118)*. Analysis of the time to repeat urgent percutaneous coronary interventions (PCI) in the control group in EPIC, however, indicates that the period of high risk after PCI of human vascular lesions is 2 days and that the period of lower risk extends from days 2–8.

Abciximab (c7E3 Fab: ReoPro™)

Murine 7E3 and abciximab bind with high affinity to human platelet GPIIb/IIIa (nanomolar K_D) *(41,45,119–121)*. Murine 7E3 binds more rapidly to activated than unactivated platelets, probably because activation results in freer access to its binding site *(119)*. Smaller fragments of murine 7E3 [$F(ab')_2$ and Fab′] show less of a differential in on-rates, presumably reflecting easier access to the binding site due to their smaller size *(119,120)*. The dissociation rate of abciximab was estimated as ~40–45 min in one study *(120),* but in two other studies it required ~120 min and ~180 min to displace 50% of a subsaturating concentration of radiolabeled abciximab from the surface of GPIIb/IIIa-containing HEL cells and platelets, respectively *(45)*. Platelets are able to internalize at least some 7E3 after it binds to the surface *(122),* an observation consistent with data demonstrating that GPIIb/IIIa receptors cycle from the plasma membrane to α granule membranes and back *(47)*; the significance of this internalization is unknown.

Antibody 7E3 also blocks ligand binding to the $\alpha_v\beta_3$ receptor *(49,74,108)* and may crossreact with an activated form of the $\alpha_M\beta_1$ receptor (Mac-1) (CD11b/18) *(123,124)*. The importance of these cross reactivities is unclear.

The epitope(s) on GPIIb/IIIa and the other integrins that 7E3 binds to are unknown. Since 7E3 reacts with two different GPIIIa (β_3)-containing receptors, there is reason to speculate that its major binding site is on GPIIIa (β_3). It does not, however, react with isolated GPIIIa and so the epitope may only be exposed on GPIIIa when GPIIIa complexes with an α-subunit (GPIIb or α_v). Alternatively, the α-subunits may indeed contribute directly to the epitope.

After bolus administration of abciximab at the recommended dose of 0.25 mg/kg, approximately 65% of the injected antibody becomes attached within minutes to the GPIIb/IIIa receptors on the platelets in the peripheral circulation and spleen *(92,125)*. Based on unpublished toxicology studies *(126),* it is likely that a small amount also binds to mature megakaryocytes. In most patients, the initial bolus achieves ≥80% GPIIb/IIIa receptor blockade, ≥80% inhibition of ADP-induced platelet aggregation, and marked prolongation of the bleeding time *(92,125,127)*. Since the total number of molecules of abciximab that are injected exceeds the number of GPIIb/IIIa receptors normally present by only a factor of 1.5–2.0 *(92),* the standard dose may not achieve ≥80% receptor blockade in patients with either severe thrombocytosis *(128)* or marked splenomegaly with splenic pooling of platelets. The free plasma concentrations of abciximab drop very rapidly after injection of a bolus dose or when an infusion is terminated, with an initial half-life of ~30 min *(92,125)*. Within an hour or so the level is below the K_D of the

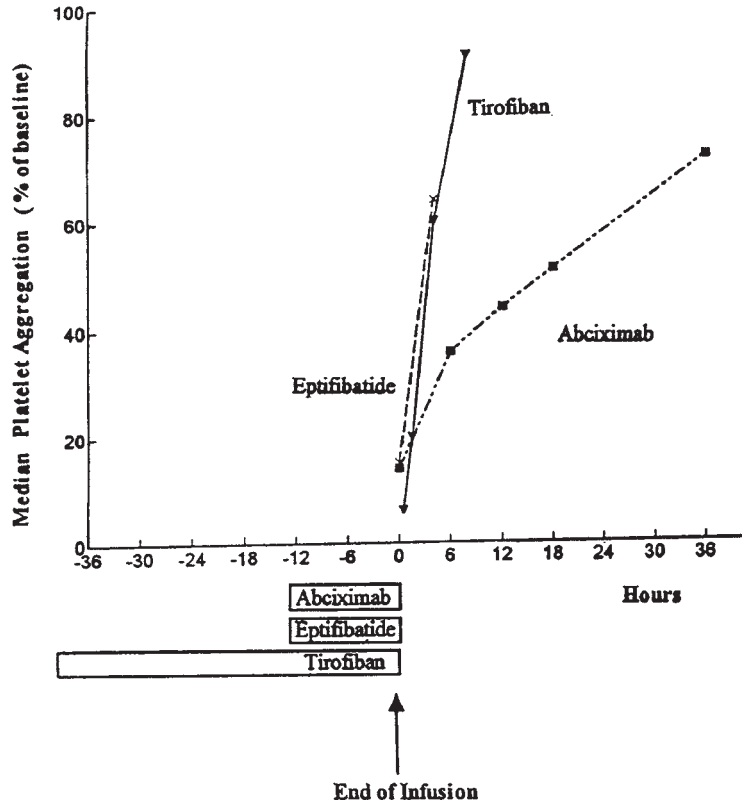

Fig. 1. Recovery of the platelet aggregation response after discontinuing tirofiban, eptifibatide, and abciximab. Aggregation was initiated by 20 μm ADP for the eptifibatide and abciximab studies, and 5 μm ADP for the tirofiban study. Tirofiban was given as a 10 μg/kg bolus + 0.15 μg/kg infusion for 36 h, eptifibatide was given as 135 μg/kg bolus + 0.5 μg/kg/min infusion for 12 h and abciximab was given as a 0.25 mg/kg bolus + 10 μg/min for 12 h. Data compiled by Dr. Robert Jordan, Centocor, Malvern, PA.

antibody. The platelet-inhibiting effects of the bolus injection of abciximab can be sustained by administering a continuous infusion of 10 μg/min or 0.125 μg/kg/min *(92,125,127)*. Very little abciximab can be detected in the urine, making it most likely that its major catabolic route involves digestion by the cells that remove platelets from the circulation *(126)*.

Currently the indicated duration of abciximab treatment is 12 h when the bolus dose is given immediately before PCI *(116,129)*. Bleeding time returns to normal within 12 h after the end of the infusion in most patients *(125)*. Platelet aggregation in response to ADP (20 μM) returns to ≥50% of baseline within 1 day in most patients and within 2 days in virtually all patients (Fig. 1). Platelet aggregation in response to a thrombin receptor activating peptide is less inhibited by bolus abciximab treatment than is platelet aggregation induced by ADP, most likely because activation of the thrombin receptor induces the release reaction and recruits internal GPIIb/IIIa receptors to the platelet surface *(130)*.

Abciximab can be detected at low levels on the platelets of patients for as long as 14 or more days after the infusion is ended, most likely reflecting the ability of abciximab

to redistribute from one platelet to another within minutes to hours *(92,131)*. Thus abciximab binding to platelets is reversible *(92,131)*. Platelet transfusion can rapidly reverse the platelet inhibition produced by abciximab *(132)*. This is probably partly because of the ability of the uncoated platelets to function in hemostasis before they have accumulated a significant amount of abciximab on their surface. Ultimately, however, when abciximab redistribution is complete, the transfer of abciximab from the heavily coated to uncoated platelets results in a decrease in the average GPIIb/IIIa receptor blockade. Decreasing the average blockade to <50% results in considerable return of platelet function.

Tirofiban (MK-383; Aggrastat™)

Tirofiban is a nonpeptide derivative of tyrosine (L-tyrosine, N-(n-butlysulfonyl)-*O*-[4-butyl(4-peperidinyl)], monohydrochloride, monohydrate) that is highly selective for GPIIb/IIIa compared with $\alpha_v\beta_3$ *(133,134)*. It inhibits platelet aggregation of gel-filtered platelets induced by ADP (10 μM) with an IC_{50} of 9 nM. Infusion of tirofiban at 10 μg/min into dogs produced nearly complete inhibition of ADP-induced platelet aggregation; aggregation returned to ~70% of normal within 30 min of ending the infusion *(134)*.

In humans, 0.15 μg/kg/min of tirofiban for 4 h produced a 2.5 ± 1.1-fold increase in bleeding time and 97 ± 5% inhibition of ADP (3.4 μM)-induced platelet aggregation *(135,136)*. Plasma clearance was 329 mL/min and the plasma half-life was 1.6 h. The bleeding time returned to normal and platelet aggregation returned to >80% of the pretreatment value (Fig. 1) 4 h after stopping tirofiban. Aspirin coadministration resulted in enhanced bleeding-time prolongation (4.1 ± 1.5-fold increase), which was not because of an effect on tirofiban plasma levels. The plasma concentration of tirofiban needed to inhibit platelet aggregation by 50% decreased from ~12 ng/mL to ~9 ng/mL when patients also received aspirin. Peak plasma concentration with the 0.15 μg/kg/min infusion was ~40 ng/mL, and 6 h after stopping the therapy, plasma levels were <3 ng/mL.

A pilot dose-ranging study conducted in patients undergoing coronary artery angioplasty who were simultaneously treated with aspirin and heparin demonstrated that: 1) ADP (5 μM)-induced platelet aggregation was inhibited by ≥93% within 5 min of administering 10 μg/kg; 2) bleeding time at 2 h was >30 min when a 10 μg/kg bolus was followed by either a 0.1 or 0.15 μg/kg/min infusion; 3) at the end of the infusions (16–24 h), platelet aggregation was inhibited by 87 and 95%, respectively; and 4) after terminating the infusions, platelet aggregation began to return toward normal in 1.5 h, and by 4 h platelet aggregation was greater than 50% of normal *(137)*.

Eptifibatide (Integrilin™)

Although the precise structure of eptifibatide has not been disclosed, it is reported to be a synthetic disulfide-linked cyclic heptapeptide patterned after the KGD sequence found in the snake venom disintegrin from *Sistrurus m. barbouri (138,139)*. It is highly specific for GPIIb/IIIa inhibition compared with $\alpha_v\beta_3$ inhibition. Animal studies suggested that eptifibatide produced less prolongation of the bleeding time than other GPIIb/IIIa inhibitors at doses producing comparable inhibition of platelet aggregation.

More recently it was reported that eptifibatide's inhibition of platelet aggregation is significantly augmented when blood is anticoagulated with citrate, the anticoagulant used in the animal studies *(140)*. Thus, the extent of platelet aggregation inhibition was probably overestimated in those early studies. When the citrate effect is considered,

administration of eptifibatide and other GPIIb/IIIa antagonists at comparable platelet aggregation inhibition doses probably produces comparable levels of bleeding time prolongation.

Clearance of eptifibatide does not appear to depend on drug metabolism and thus renal clearance is probably most important. As with abciximab, treatment with eptifibatide prolongs the ACT of heparinized patients, suggesting an inhibitory effect on thrombin generation stimulated by contact activation *(96,141)*.

In a pilot study of 21 patients undergoing elective PCI who were also treated with aspirin and heparin (10,000 U bolus + additional doses to maintain ACT at 300–500 s), a bolus dose of 90 μg/kg of eptifibatide followed by a 1 μg/kg/min infusion for 4 or 12 h, resulted in a decrease in platelet aggregation response using citrated blood to ADP (20 μM) from ~80% of a full scale change before eptifibatide treatment to ~15% at 1 h after the bolus dose and at the end of the infusion *(96)*. There was significant interindividual variability in response, with the 95% confidence limits extending from 0–30% and 0–40%, respectively, at the two time points. The average aggregation response was ~55% 4 h after stopping the infusion, but there was marked interindividual variation (95% confidence limits ~10–90%).

Bleeding times were prolonged from ~6 min before treatment to ~26 min after 1 h and at the end of the infusion. Bleeding times rapidly returned toward normal after stopping treatment, being ~12 min 1 h after the infusion was stopped.

A subsequent study *(142)* tested 4 different bolus + 18–24 h infusion regimens in 54 patients undergoing PCI who were also treated with aspirin and heparin (180 μg/kg bolus + 1 μg/kg/min; 135 μg/kg + 0.5 μg/kg/min; 90 μg/kg + 0.75 μg/kg/min; 135 μg/kg + 0.75 μg/kg/min). The 180 μg/kg bolus dose produced high-grade inhibition of ADP-induced platelet aggregation using citrated blood, and this was consistently sustained by the 1 μg/kg/min infusion. There was some return toward normal platelet aggregation in patients treated with the regimens that included the 0.75 μg/kg/min infusion. After stopping treatment for 4 h, platelet aggregation returned to more than 50% of the pre-treatment value (Fig. 1). Bleeding time prolongation was found with all regimens (median values of 22,12,12, and 17 min, respectively, compared to control of 7–8 min) and 1 h after stopping the infusion, there was a return toward the control values (9, 10, 9, and 11 min, respectively).

Lamifiban (Ro 44-9883)

Lamifiban is a nonpeptide of the structure ([[1-[N-(*p*-amidinobenzoyl)-L-tyrosyl)-L-tyrosyl]-4-peperidinyl]oxy acetic acid). It inhibits aggregation of citrated platelet-rich plasma induced by ADP (10 μM) with an IC_{50} of 25 μM and binds to gel-filtered platelets with a K_D of 5 nM *(143,144)*. It has little or no effect on $\alpha_v\beta_3$ function. Binding of lamifiban to GPIIb/IIIa does not induce the ligand binding conformation of the receptor as does the binding of some peptides and peptidomimetics *(145),* nor does it expose ligand-induced binding sites (LIBS) on GPIIb/IIIa *(146)*.

When infused in humans, a plasma level of 6.1 nM produced 50% inhibition of ADP-induced platelet aggregation *(147)*. Doses that nearly completely inhibited platelet aggregation caused doubling of the bleeding time. Based on a one compartment pharmacokinetic model, the volume of distribution for lamifiban was 22 L; free drug $T_{1/2}$ was 40 min; bound drug $T_{1/2}$ was 9.5 h, and plasma clearance was 417 mL/min.

In a pilot dose-ranging study conducted in patients with unstable angina, patients were

treated with bolus + 72–120 h infusions of 150 μg + 1 μg/min; 300 μg + 2 μg/min; 600 μg + 4 μg/min; and 750 μg + 5 μg/min (infusions were adjusted downward for patients weighing ≤70 kg) *(148)*. At steady state, the 4 and 5 μg/min infusion regimens caused almost 100% inhibition of ADP (10 μM)- and thrombin receptor activating peptide (100 μM)-induced aggregation in citrated platelet-rich plasma. Lower doses produced less inhibition, especially of thrombin receptor activating peptide-induced platelet aggregation. Bleeding times were also prolonged in a dose-dependent fashion, with the 5 μg/min infusion dose increasing the bleeding time to ~23 min.

Fradafiban (BIBU 52) and Lefradafiban (BIBU 104)

The structures of the nonpeptide RGD mimetics fradafiban and lefradafiban are given in Fig. 2 *(149)*. Fradafiban is highly selective for GPIIb/IIIa compared with $\alpha_v\beta_3$ and binds to GPIIb/IIIa with K_D 148 nM. Approximately 54,000–89,000 molecules bind per platelet. Fradafiban has limited oral activity due to poor absorption. Esterification of the carboxyl group and acylation of the amino group produced a less polar prodrug, lefradafiban, which is better absorbed; plasma esterases convert lefradafiban to fradafiban in vivo.

In normal volunteers, infusion of 1 mg of fradafiban intravenously over 30 min produced 50% receptor blockade (as measured by ^{3}H-fradafiban binding), ~35% inhibition of ADP (20 μM)-induced platelet aggregation, and a plasma level of ~40 μg/mL. Half-maximal inhibition of ADP-induced platelet aggregation occurred at ~54 μg/mL fradafiban. At doses of 5 mg or greater, ADP-induced platelet aggregation was completely inhibited. ADP-induced aggregation was ~40% of control and ~65% of the receptors remained blocked 2 h after the 5 mg infusion.

A 50-mg oral dose of lefradafiban inhibited ADP-induced platelet aggregation by 90 ± 5% at 2 h, 59 ± 14% at 8 h, and <10% at 24 h after administration. Higher doses produced greater inhibition, but aggregation returned to pretreatment values at 32 h even with a 150-mg dose. It required 2.3-fold higher plasma levels to half-maximally inhibit platelet aggregation in citrated whole blood compared to platelet-rich plasma.

When administered every 8 h to normal volunteers, the 25-mg dose of lefradafiban maintained inhibition of ADP-induced platelet aggregation between 30–75%, whereas the 50-mg dose maintained it between 50–95%. There was no evidence for desensitization during a 7-d course. Minor bleeding resulted in discontinuation of the 100-mg dose in this study. Interindividual variations in plasma concentrations were <20%. Absorption was estimated at ~25%, and >90% of the quantifiable drug in plasma was fradafiban. Fradafiban has low protein binding capacity and its catabolism is estimated as ~65% renal and ~35% biliary. The dominant half-life was ~12 h, suggesting that the ultimate plasma level is likely to ~1.8-fold the initial peak level with an every-8-h dosing regimen.

Xemilofiban (SC-54684A)

Xemilofiban is the ethyl carbolic acid ester prodrug of the GPIIb/IIIa antagonist SC-54701A *(150)*. The latter inhibits ADP (20 μM)-induced fibrinogen binding to washed human platelets with an IC_{50} of ~10 nM. In platelet-rich plasma, the IC_{50}'s of SC-54701A for collagen and ADP-induced platelet aggregation are ~70 and 35 nM, respectively. In contrast, the IC_{50} of the prodrug xemilofiban was at least 100-fold greater. SC-54701A is highly selective for GPIIb/IIIa compared with $\alpha_v\beta_3$.

In dogs, increasing doses of SC-54701A produced progressive inhibition of platelet

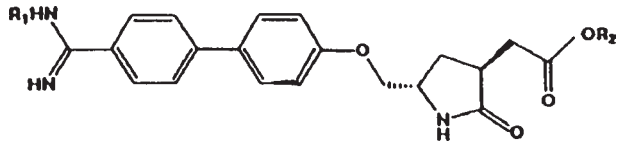

Fig. 2. Structure of fradafiban and lefradafiban.

aggregation and bleeding times, with 35% inhibition of collagen-induced aggregation and an ~2-fold increase in bleeding time at 0.1 μg/kg/min; 80% inhibition of aggregation and an ~2.5-fold increase in bleeding time at 0.2 μg/kg/min; and >85% inhibition of aggregation and a 5-fold increase in bleeding time at 0.3 μg/kg/min. The oral systemic activity of xemilofiban was ~19% when compared to the intravenous activity of SC-54701A in dogs. When SC-54701A was administered intravenously, the half-life of the β phase for elimination (two-compartment open model) was 6.5 h, total plasma clearance was 0.3 L/h/kg and the volume of distribution was 2.8 L/kg. The compound was excreted unchanged in the urine, with 75% recovery after 4 d. When xemilofiban was given orally, most of the dose was rapidly transformed into the free acid, and ~29% of the administered dose was excreted in the urine. The systemic availability of the active metabolite, SC-54701A, was ~21%.

In humans, a single-blind placebo-controlled dose-ranging study in 170 patients undergoing intracoronary stent deployment tested xemilofiban at 5-, 10-, 15-, and 20-mg oral doses administered twice a day *(151)*. All patients received aspirin before the procedure. Oral xemilofiban therapy was initiated on the morning after the procedure and continued for 14 d. Thirty of the 170 patients received abciximab as a bolus + 12-h infusion and in these patients xemilofiban was begun 8–18 h after stopping abciximab. In patients not treated with abciximab, the 20-mg bid dose produced ~70% decrease in ADP (20 μM)-induced platelet aggregation 4 h after taking the drug, with near return to baseline values at 12 h. The responses remained similar after 1 and 2 wk of therapy. The slope of the dose response was similar in patients who were and were not treated with abciximab.

In a randomized single-blind placebo-controlled pilot study, 30 patients with unstable angina undergoing PCI were treated with a loading dose of xemilofiban (35 mg) or placebo 3 h before the procedure; 4–6 h after the procedure, xemilofiban at a dose of 20 or 25 mg 3 times per day was begun and continued for 30 d *(152)*. All patients were heparinized during the procedure and received aspirin throughout the study. The study was stopped prematurely because of excessive bleeding, at which time 16 patients had been treated with the 25-mg dose and 4 received the 20-mg dose 3 times per day. Seven of the 20 patients were withdrawn from the study because of bleeding (4), stent placement (2), and cancelled procedure (1). Two patients had severe bleeding complications requiring 22 and 54 units of packed red blood cells, and one died. This last patient developed acute renal insufficiency and this resulted in marked prolongation of the clearance of SC-54701A. After the loading dose, ADP-induced platelet aggregation was inhibited ≥80% in 83% of the patients at 2 h, 85% at 4 h, and 100% at 6 h. Later in the study, 64% of patients had <80% inhibition of ADP-induced platelet aggregation before their morning dose of xemilofiban. Plasma levels of the active agent SC-54701A showed considerable interindividual variability.

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II Glycoprotein IIb/IIIa Blockade During Coronary Intervention

5 Abciximab During Percutaneous Coronary Intervention—The EPIC, EPILOG, and EPISTENT Trials

A. Michael Lincoff, MD

Contents

INTRODUCTION

Blockade of the platelet glycoprotein (GP) IIb/IIIa receptor as a therapy for ischemic heart disease was introduced into clinical practice by demonstration that the first of these agents, abciximab, reduces periprocedural complications among patients undergoing high-risk coronary angioplasty. From this initial "proof of concept," subsequent clinical evaluation of this agent has progressed through a series of large-scale trials, establishing the broad applicability, marked clinical efficacy, and acceptable safety profile of this therapeutic strategy in the setting of percutaneous coronary revascularization. Ongoing studies are now addressing the potential role of abciximab as medical therapy among patients with the acute ischemic syndromes of unstable angina and myocardial infarction. This chapter will review the clinical experience with this agent as a pharmacologic adjunct to elective or urgent coronary intervention.

PHARMACOLOGY OF ABCIXIMAB

Preclinical Data

The development and pharmacology of abciximab are reviewed in detail in Chapter 4 by Dr. Coller. In brief, abciximab (ReoPro™, Centocor, Malvern, PA) is a chimeric Fab monoclonal antibody fragment that binds to the platelet GP IIb/IIIa receptor. A murine antibody (7E3) was produced by immunization of mice with human platelets and iso-

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lated from hybridoma supernatants by its inhibition of the interaction between platelets and fibrinogen-covered beads. To limit the risk of thrombocytopenia because of clearance of 7E3-coated platelets by binding of the *Fc* region of the IgG molecule to the reticuloendothelial system, 7E3 was cleaved with pepsin into $F(ab')_2$ fragments. Animal experiments and initial human studies were carried out using this $F(ab')_2$ form. Because of continued concerns regarding the potential for thrombocytopenia by crosslinking of platelets with the bivalent $F(ab')_2$ antibody, an Fab antibody fragment was produced by papain digestion of m7E3 and subsequently evaluated. Finally, to reduce the risk of immunogenicity to murine protein, genetic reconstruction was used to produce the chimeric c7E3 Fab antibody fragment currently in clinical use (abciximab), which consists of human constant regions and murine variable regions of the IgG antibody.

In preclinical canine and primate animal models (*see* Chapter 4), 7E3 $F(ab')_2$ or c7E3 Fab were shown to markedly diminish thrombus formation and platelet-mediated cyclic flow reductions in injured and stenosed coronary arteries and to facilitate t-PA-induced thrombolysis and abolish reocclusion. These antithrombotic effects were observed at doses that achieved blockade of >60–80% of GP IIb/IIIa receptors.

Clinical Studies

Murine 7E3 and abciximab bind with high affinity to both activated and unactivated platelet GP IIb/IIIa, with inhibition of platelet aggregation observed at levels of receptor blockade >50% and nearly complete abrogation of aggregability at >80% receptor occupancy. Although binding is reversible, dissociation of the agent from the receptor is slow, and normalization of platelet aggregation does not occur until 24–36 h following discontinuation of abciximab infusion *(1,2)*. By flow cytometry, decreasing but measurable levels of platelet-bound abciximab are present for as long as 15 d, beyond the normal circulating platelet lifespan, indicating redistribution of abciximab to new platelets entering the circulation *(3)*.

Phase II clinical studies confirmed dose-related inhibition of platelet aggregation and prolongation of bleeding time by 7E3 in the setting of percutaneous coronary intervention. In a pilot study of 23 patients undergoing elective angioplasty, escalating bolus doses ranging from 0.15 to 0.35 mg/kg of m7E3 Fab produced levels of receptor blockade ranging from ~50–90% *(4)*. Doses of ≥0.20 mg/kg led to >70–80% inhibition of platelet aggregation, with partial recovery of platelet function noted by 6 h. Marked prolongation of bleeding times were observed at all doses, with normalization by 24 h.

Chimeric 7E3 Fab (abciximab) was evaluated in a multicenter open-label dose-escalation study of 56 patients undergoing elective percutaneous transluminal coronary angioplasty *(2)*. Levels of receptor blockade and inhibition of platelet aggregation following bolus doses of 0.15, 0.20, and 0.25 mg/kg are detailed in Table 1; although wide variability was observed among patients, particularly at the lowest dose, platelet aggregation was consistently suppressed to the theoretical target of ≤20% of baseline by the abciximab bolus dose of 0.25 mg/kg. In the second phase of this study, GP IIb/IIIa receptor blockade, platelet aggregation, and bleeding times were measured following a 0.25 mg/kg bolus dose, with and without a subsequent 12-h infusion at a rate of 10 μg/min (Fig. 1). With a bolus only (Fig. 1A), peak receptor blockade and inhibition of platelet aggregation to ≤20% occurred by the first (2 h) measurement, with prolongation of bleeding time to >30 min. Bleeding time returned nearly to normal by 12 h, although recovery of platelet aggregability occurred more gradually, remaining ~40% and ~30% inhibited by 12 and 24 h, respectively. Addition of an infusion of abciximab (10 μg/min)

Table 1
GP IIb/IIIa Receptor Blockade and Inhibition of Platelet Aggregation After Abciximab Bolus Doses[a]

Bolus dose (*mg/kg body weight*)	*GP IIb/IIIa receptor blockade* (*% Blocked*) *median (interquartile range)*	*Platelet aggregation to 20 mM ADP* (*% of baseline*) *median (interquartile range)*
0.15	.54 (15–94)	46 (34–80)
0.20	80 (67–89)	45 (19–71)
0.25	87 (62–96)	18 (9–25)

[a]Data from Tcheng et al. *(2)*.

following the bolus dose produced consistent levels of receptor occupancy of ~80%, with inhibition of platelet aggregation to ~20% and prolongation of bleeding time to >30 min during the 12-h infusion period (Fig. 1B). Following discontinuation of the infusion, recovery of these parameters occurred at rates similar to those following administration of the bolus only. The effectiveness of the 12-h abciximab infusion (10 μg/min or 0.125 μg/kg-min) in maintaining receptor blockade >80% and platelet aggregability <20% in the majority of treated patients was confirmed in a subsequent study of 41 normal volunteers *(3)*.

Kleiman et al. evaluated the influence of different agonists on measured inhibition of platelet aggregation by abciximab among 32 patients undergoing coronary angioplasty *(5)*. Receptor blockade was ~80% at 2 h following the single abciximab bolus of 0.25 mg/kg, declining to ~50% by 24 h. This level of receptor occupancy was associated with nearly complete inhibition of platelet aggregation in response to the traditional agonists ADP (20 and 5 μ*M* concentrations) and collagen (mean 76%, 88%, and 85% inhibition at 2 h, respectively). The circulating plasma half-life of abciximab was very short, calculated at <26 min. Inhibition of platelet aggregation at various time points in response to ADP and collagen was significantly correlated with the extent of receptor blockade by abciximab, but was not at all related to circulating abciximab plasma concentrations (which were very low by 2 h following the drug bolus). Platelet aggregation in response to stimulation with thrombin receptor-activating peptides (TRAP), an agonist which mimics platelet activation by thrombin, was significantly less inhibited 2 h following abciximab than was aggregation in response to ADP or collagen. Addition of exogenous abciximab to the platelet rich plasma, however, led to more complete inhibition of aggregation in response to TRAP. These investigators postulated that the continued ability of platelets to aggregate in response to TRAP 2 h following the bolus of abciximab may have been related to the "release reaction" induced by thrombin stimulation, in which GP IIb/IIIa receptors in the alpha granule membranes and perhaps other sites of the platelet are externalized to the platelet membrane. Given the brief circulating half-life of abciximab, plasma levels of this agent would be insufficient to bind such newly exposed receptors shortly after the bolus is administered. These findings suggested that a continued infusion of abciximab following a bolus would be required to provide complete inhibition of platelet aggregation in the setting of intense stimulation by agonists such as thrombin, where additional unbound GP IIb/IIIa receptors are externalized to the platelet membrane.

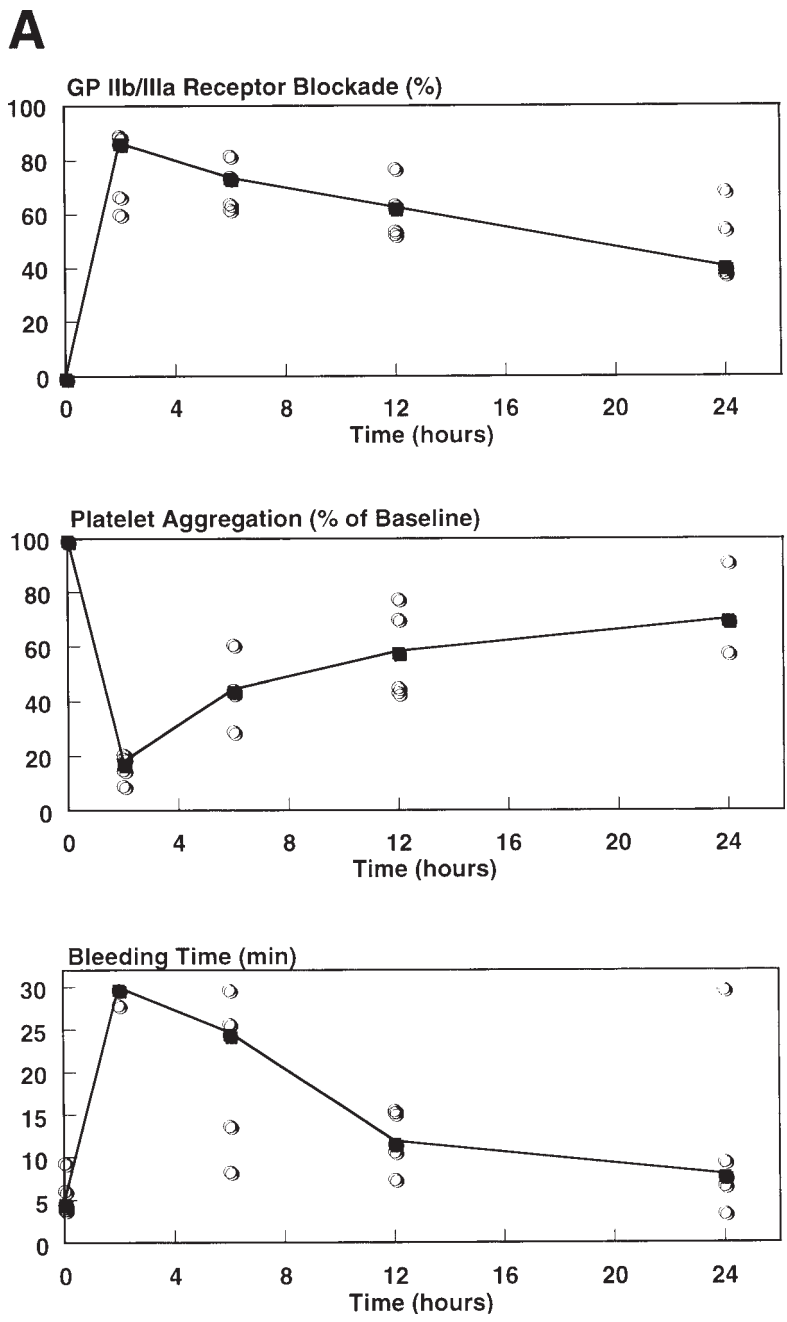

Fig. 1A. Pharmacodynamics of a 0.25-mg/kg bolus dose only of abciximab. The bolus was given at time 0. Effects were followed for 24 h. Data points for each patient are shown in open circles; median values are in solid squares, lines join the median values. For each panel: top graph—percentage of GP IIb/IIIa receptors blocked by abciximab; middle graph—inhibition of platelet aggregation, expressed as percentage of baseline aggregation in response to 20 μmol/L ADP; bottom graph—bleeing time. From Tcheng et al. *(2)* (with permission).

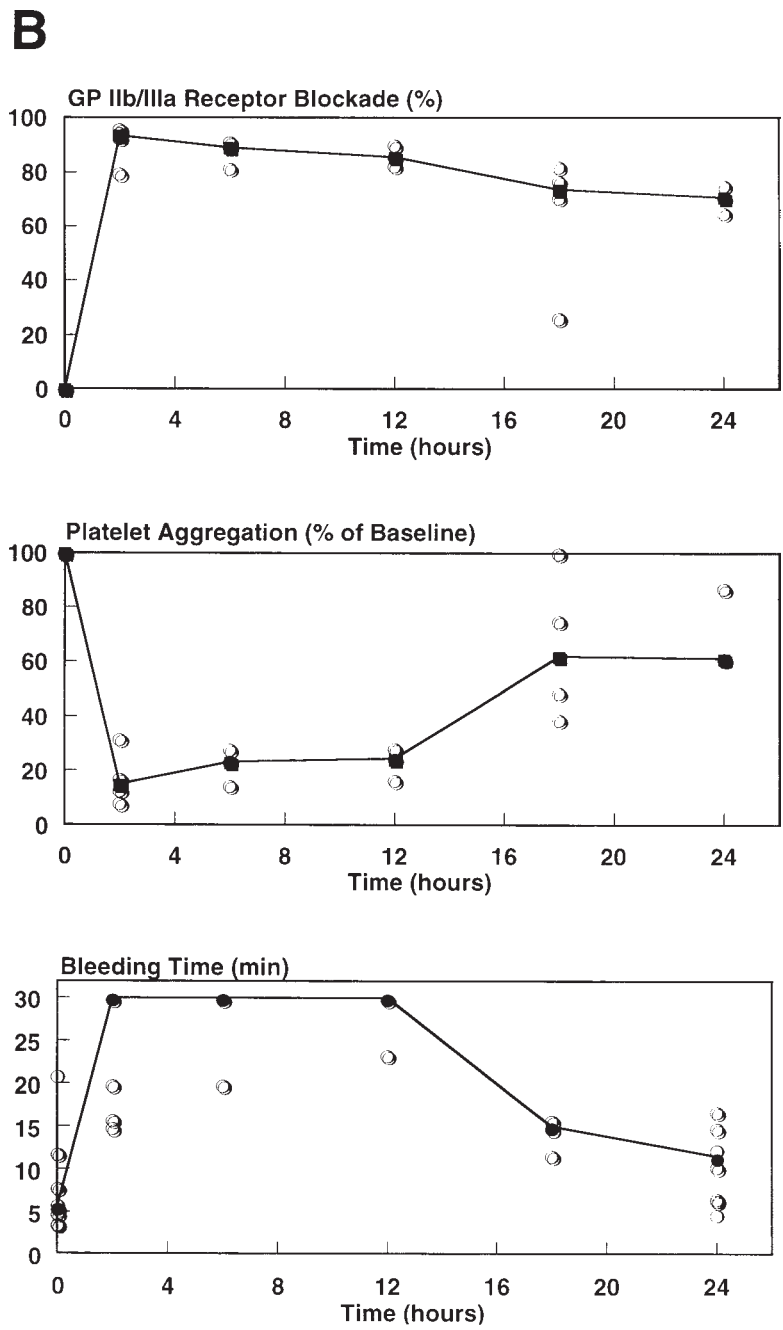

Fig. 1B. Pharmacodynamics of a 0.25-mg/kg bolus dose followed by a 10-μg/min infusion for 12 h of abciximab. The bolus was given at time 0, with the infusion between time 0 and 12 h. Effects were followed for 24 h. See **Fig. 1A** legend for explanation of data points and abbreviations.

PIVOTAL TRIALS OF ABCIXIMAB DURING CORONARY INTERVENTION

The role of abciximab during percutaneous coronary revascularization has been assessed in three large-scale placebo-controlled randomized trials, enrolling in total more than 7000 patients. The first of these trials, EPIC, focused on patients considered to be at high risk for thrombotic complications. EPILOG extended the findings of EPIC to the broad spectrum of patients undergoing coronary balloon angioplasty or atherectomy. EPISTENT investigated the complementarity of abciximab therapy with the recent dominant revascularization technique in interventional cardiology—elective coronary stenting.

The three abciximab trials all shared common design and analysis features, which allow for some degree of comparison and contrast among the studies. All trials were blinded, except for the asymmetric balloon angioplasty plus abciximab arm of EPISTENT. All were carried out according to the "intention-to-treat" principle, wherein patients were randomized before initiation of the interventional procedure and all patients were included in the efficacy analysis, regardless of whether or not they actually received study drug or underwent revascularization. Endpoints were adjudicated by independent, blinded Clinical Events Committees convened for each trial; these committees utilized data obtained systematically by protocol and identified events that may or may not have been determined by investigators at the individual clinical sites. Endpoint myocardial infarctions (MI) were defined by electrocardiographic and enzymatic criteria, which were similar in the three trials (in general, infarction was identified by new Q-waves or CK-MB elevations ≥3 times the control values) with CK-MB values obtained according to a protocolized schedule in all patients, even among those in whom infarction was not clinically suspected. Similarly, hemoglobin values were obtained prior to hospital discharge for assessment of bleeding complications in all patients, regardless of whether or not bleeding events were observed. Bleeding events were classified according to the criteria of the Thrombolysis in Myocardial Infarction (TIMI) Study Group *(6)*. Major bleeding was defined as intracranial hemorrhage or blood loss resulting in a decrease in hemoglobin by >5 g/dL; minor bleeding was defined by spontaneous gross hematuria or hematemesis, a decrease in hemoglobin by >3 g/dL in association with observed bleeding, or a decrease in hemoglobin by >4 g/dL if a site of blood loss could not be identified. Observed decreases in hemoglobin were adjusted for the influence of red blood cell transfusions *(7)*.

The EPIC Trial

Proof of concept that GP IIb/IIIa inhibition would diminish ischemic complications of percutaneous coronary revascularization was provided by the first Phase III study of this class of agents, the EPIC (Evaluation of c7E3 Fab for Prevention of Ischemic Complications) trial, leading to the marketing approval of abciximab. This trial evaluated the efficacy of two dosing strategies of abciximab versus placebo among patients considered to be at high risk for coronary intervention on the basis of acute ischemic syndromes or clinical and morphologic characteristics *(8)*.

Patient Population and Study Design

A total of 2099 patients scheduled to undergo balloon angioplasty or directional atherectomy were enrolled between November 1991 and November 1992. Criteria con-

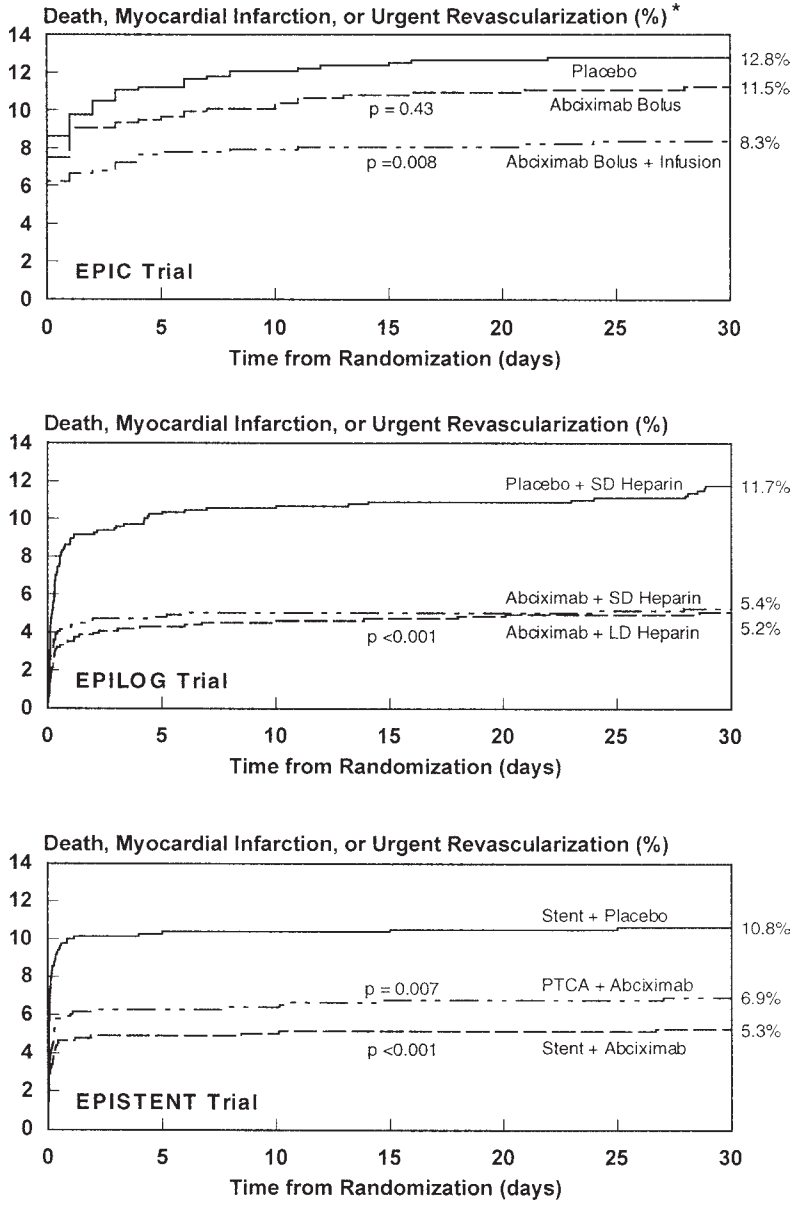

Fig. 2. Kaplan-Meier estimate of the percentage of patients with the composite endpoint of death, MI, or urgent repeat revascularization within 30 d of randomization, according to treatment assignment in the EPIC (top), EPILOG (middle), and EPISTENT (bottom) trials. PTCA = balloon coronary angioplasty. *The EPIC composite endpoint shown in top panel also includes stent or intraaortic balloon pump placement.

Abbreviations: LD = low-dose weight-adjusted; PTCA = balloon angioplasty; SD = standard-dose weight-adjusted.

stituting high-risk status for entry into the trial included acute or recent MI, unstable angina, complex target lesion angiographic morphology (modified ACC/AHA lesion score B2 or C), or a moderately complex target lesion (ACC/AHA score B1) in associa-

tion with advanced age, female gender, or diabetes mellitus. Patients were excluded from enrollment if more than 80 years of age or for known bleeding diatheses, major surgery within 6 wk, or stroke within the prior 2 yr.

The enrolled patient population had a median age of 72 years, 83% were male, 24% diabetic, and 57% had a history of prior MI. Entry criteria included unstable ischemic syndromes (acute infarction, prior infarction, or unstable angina) in 43% of patients, whereas 57% were enrolled with high-risk morphologic/clinical criteria.

All patients received aspirin and heparin to achieve and maintain an activated clotting time (ACT) >300–350 s. The initial heparin bolus was 10–12,000 U, followed by additional 3000 U boluses to a maximum of 20,000 U. Randomization was in a double-blind fashion to: placebo, abciximab 0.25 mg/kg bolus, or abciximab 0.25 mg/kg bolus followed by abciximab 10 μg/min infusion for 12 h. Heparin infusion was continued, to a target aPTT of 1.5–2.5 times the control value, and vascular access sheaths remained in place for the 12-h duration of study drug. Balloon angioplasty or directional atherectomy were carried out according to institutional practice, with stent implantation reserved for manifest or threatened vessel closure.

Clinical Efficacy

The primary efficacy endpoint was a composite of death, MI, urgent repeat revascularization, or stent or balloon-pump placement by 30 days following randomization. This composite event rate was reduced from 12.8% among patients receiving placebo to 11.4% among patients receiving the abciximab bolus (10% relative risk reduction, $P = 0.43$) and to 8.3% among patients receiving the abciximab bolus and 12-h infusion (35% relative risk reduction, $P = 0.008$) (Fig. 2, top). The incidences of MI and urgent revascularization were significantly reduced by the bolus and infusion of abciximab; the bolus only regimen resulted in a very modest and statistically insignificant event reduction (Table 2). Mortality rates were not influenced by abciximab according to the intention-to-treat analysis, but three patients who died in the abciximab bolus and infusion arm did so after randomization but before receiving study drug. The treatment effect of abciximab was present in all subgroups analyzed; a trend was observed toward less clinical benefit from abciximab in lighter weight patients, a finding that may have been related to the excess risk of bleeding in this subgroup (see below). Angiographic criteria, including the presence of thrombus, did not predict the extent of clinical benefit derived from abciximab therapy *(9)*.

The clinical efficacy of abciximab in EPIC was maintained at 6-mo and 3-yr clinical follow-up, during which time investigators remained blinded to patient treatment randomization allocation *(10,11)*. By 6 mo, the incidence of death, MI, or any revascularization (urgent or elective) was 35.1% in the placebo group, 32.6% in the abciximab bolus only group, and 27.0% in the abciximab bolus and infusion group (23% relative risk reduction, $P = 0.004$) *(10)*. Moreover, use of abciximab was associated with reduction in the need for target vessel revascularization procedures by 6 mo, from 22.3% among patients receiving placebo to 16.5% among those receiving the bolus and infusion of abciximab (26% relative risk reduction, $P = 0.007$). Most of the reduction in target vessel revascularization procedures occurred during the first 30 d of follow-up; when only patients who were free of events by 30 d were considered, target vessel revascularization rates were reduced during the period between 1 and 6 mo from 16.9 to 14.4% in the placebo and abciximab bolus and infusion groups, respectively. Although

Table 2
Components of 30-d Composite Endpoint in EPIC, EPILOG, and EPISTENT Trials

	EPIC			*EPILOG*			*EPISTENT*		
Endpoint (% of patients)	*Placebo* n = *696*	*Abcix bolus* n = *695*	*Abcix bolus + infusion* n = *708*	*Placebo* n = *939*	*Abcix + LD heparin* n = *935*	*Abcix + SD heparin* n = *918*	*Stent + placebo* n = *809*	*Stent + Abcix* n = *794*	*PTCA + Abcix* n = *796*
Death	1.7	1.3	1.7	0.8	0.3	0.4	0.6	0.3	0.8
MI	8.6	6.2	5.2	8.7	3.7	3.8	9.6	4.5	5.3
Q-wave	2.3	1.0	0.8	0.8	0.4	0.5	1.4	0.9	1.5
Large non-Q wave (CK ≥ 5X control)	4.0	2.7	3.0	5.6	2.0	2.5	5.8	2.0	2.6
Small non-Q wave (CK 3-< 5X control)	2.3	2.4	1.4	1.9	1.2	0.9	2.2	1.5	1.1
Urgent revascularization	7.8	6.4	4.0	5.2	1.6	2.3	2.1	1.3	1.9
Urgent PCI	4.5	3.6	0.8	3.8	1.2	1.5	1.2	0.6	1.3
Urgent CABG	3.6	2.3	2.4	1.7	0.4	0.9	1.1	0.8	0.6

Abcix = abciximab; CABG = coronary artery bypass graft surgery; LD = low-dose weight-adjusted; MI = myocardial infarction; PCI = percutaneous coronary intervention; PTCA = coronary balloon angioplasty; SD = standard-dose weight-adjusted

patients did not undergo routine angiographic follow-up in this trial, the finding in EPIC of a reduced need for repeat revascularization procedures over 6 mo led to speculation that this agent may reduce restenosis following coronary intervention.

By 3 yr following randomization, patients treated with abciximab had a sustained reduction in the composite endpoint of death, MI, or any revascularization: 47.2% in the placebo group versus 41.1% in the bolus and infusion group ($P = 0.009$) *(11)*. Patients receiving the bolus only of abciximab had no long-term clinical benefit (composite event rate = 47.4% at 3 yr). Survival curves diverged during the first year of follow-up, and remained largely parallel from 1 to 3 yr. There was a trend toward decreased mortality by abciximab over 3 yr in the overall cohort, with a more marked 60% reduction by abciximab among the 555 highest risk patients who had been enrolled with unstable angina or acute MI (12.7 vs 5.1% in the placebo and abciximab bolus and infusion groups, respectively, $P = 0.01$).

Safety

The reduction in ischemic events by abciximab in EPIC was associated with a significant increased risk of bleeding complications, a finding which raised concerns regarding the potential clinical utility of this form of therapy. Compared with placebo, the bolus and infusion of abciximab resulted in a doubling in the rates of major bleeding (7 vs 14%, $P = 0.001$) and red blood cell transfusions (7 vs 15%, $P < 0.001$) *(8)*. Most of the excess bleeding events associated with abciximab occurred at sites of vascular access, although

retroperitoneal or spontaneous gastrointestinal bleeding risk was increased as well. No differences among treatment groups in the incidence of intracranial hemorrhage were observed. At least two lines of evidence suggested that concurrent heparin therapy may have played a key role in the pathogenesis of bleeding among patients receiving abciximab in EPIC *(12)*. First, heparin dosages in the trial were not weight-adjusted, and a strong relationship was observed between the risk of bleeding and lighter body weight (and hence, relative "overdosage" of heparin on a per-weight basis). Second, major bleeding rates were strongly correlated with total heparin dose and the intensity of anticoagulation (as measured by peak ACT) during the interventional procedure.

Based upon the data from EPIC linking bleeding complications to heparin dosing, it was postulated that hemorrhagic risk associated with abciximab might be diminished by reduction in concomitant intraprocedural heparin dose and by removal of vascular access site sheaths during the 12-h abciximab infusion, thereby eliminating the need for postprocedural heparin therapy. These strategies were tested in a subsequent pilot study, PROLOG *(13)*. A total of 103 patients undergoing coronary intervention with abciximab were randomized in a 2-by-2 factorial design to receive standard-dose weight-adjusted heparin (100 U/kg initial bolus) or low-dose weight-adjusted heparin (70 U/kg bolus) and to undergo late vascular sheath removal (after discontinuation of postprocedural 12-h abciximab and heparin infusion as in the EPIC trial) or early sheath removal (no postprocedural heparin, vascular sheath removal during abciximab infusion). Efficacy of abciximab in preventing ischemic complications did not appear to be influenced by heparin dose or vascular sheath removal, as ischemic event rates were similar in all treatment groups. Bleeding rates, however, were attenuated independently and in an additive fashion by reduced-dose weight-adjusted heparin dosing and by early removal of the vascular sheath (during infusion of abciximab). Composite bleeding rates (TIMI major or minor bleeding, blood transfusion, or vascular access site hematoma formation of >5 cm diameter) among patients randomized to standard heparin/late sheath removal, standard heparin/early sheath removal, low-dose heparin/late sheath removal, and low-dose heparin/early sheath removal were 32, 11, 12, and 4%, respectively.

Apart from bleeding, abciximab therapy in EPIC was not associated with other major safety risks. Thrombocytopenia occurred somewhat more frequently among patients receiving abciximab (platelet count <100,000 per mm^3 in 3.4% and 5.2% and platelet count <50,000 per mm^3 in 0.7% and 1.6% of patients in the placebo and bolus plus infusion groups, respectively), but was transient and resolved following discontinuation of abciximab with or without platelet transfusions. No patient exhibited an allergic or hypersensitivity response to abciximab, although human antichimeric antibody (HACA) IgG titers were measurable in 6.5% of patients by 30 d after receiving this agent.

Subgroup Findings

Although the treatment effect of abciximab was present in all patients in the EPIC trial, patients enrolled with unstable ischemic syndromes appeared to derive enhanced benefit *(14,15)*. Most notable among these were patients enrolled with the diagnosis of unstable angina. Unstable angina was defined by the presence of early (within 7 d) postinfarction angina, at least two episodes of angina occurring at rest, or angina occurring despite heparin and nitrate therapy; transient electrocardiographic ST-T wave changes accompanying these clinical presentations were required to confirm the diag-

nosis. Among 470 patients with unstable angina who received study drug and underwent intervention, the 30-d composite efficacy endpoint rate was decreased by 71% with the abciximab bolus and infusion, from 13.1 to 3.8% ($P = 0.004$) *(14)*. The most serious endpoints of death or MI were reduced by 94% at 30 d (11.1 vs 0.6% in the placebo and abciximab bolus and infusion groups, respectively, $P < 0.001$) and by 88% at 6 mo (16.6 vs 2.0% in the placebo and bolus plus infusion groups, respectively, $P < 0.001$). The magnitude of this reduction by abciximab in the risk of death and MI was significantly greater among patients with unstable angina than among those enrolled without unstable angina (interaction: $P = 0.004$ at 30 d and $P = 0.003$ at 6 mo). Rates of urgent repeat revascularization among patients with unstable angina were halved by abciximab at 30 d (5.9 vs 2.6% in placebo and bolus plus infusion arms, respectively, $P = 0.145$), but total revascularization rates (urgent or elective) by 6 mo were not suppressed (16.6 vs 17.1%).

A small subgroup of 64 patients in EPIC was enrolled while undergoing angioplasty within 12 h of onset of acute MI (42 in the setting of direct angioplasty as primary reperfusion therapy and 22 during "rescue" angioplasty for failed thrombolysis). Of the 23 patients randomized to placebo, 6 (26.1%) suffered a primary composite endpoint event of reinfarction or urgent repeat revascularization. In contrast, the only endpoint event among the 22 patients receiving the bolus and infusion of abciximab was 1 death (4.5% rate) within 24 h of randomization of a patient who was in cardiogenic shock before the procedure *(16)*. Over the 6-mo follow-up, 11 of 23 patients in the placebo group experienced a composite endpoint event (death, reinfarction, or any revascularization), whereas no patient in the abciximab bolus and infusion group, aside from the 1 who had died acutely, suffered a subsequent event (47.8% versus 4.5% composite endpoint event rate in the placebo and bolus plus infusion groups, respectively, $P = 0.002$). Major bleeding occurred in 13, 32, and 18% and transfusions were required in 9, 32, and 23% of patients in the placebo, bolus only, and bolus plus infusion groups, respectively. Of the 13 major bleeding episodes, 9 (including 1 intracranial hemorrhage) occurred among rescue PTCA patients who had received thrombolytic therapy within the prior 12 h.

Patients treated by directional atherectomy also appeared to derive enhanced benefit from abciximab therapy in EPIC *(17)*. Of the 2099 patients in the trial, 197 underwent atherectomy at the discretion of the interventional physician. Patients treated by atherectomy had significantly higher rates of endpoint non-Q-wave MI than did those undergoing balloon angioplasty (9.6 vs 4.9% by 30 d, respectively, $P = 0.006$), despite fewer baseline risk factors. Randomization to the bolus and infusion of abciximab was associated with a 71% reduction in the risk of non-Q-wave MI in atherectomy patients (15.4% for placebo vs 4.5% for the bolus and infusion, $P = 0.046$), reducing the infarction risk to that among patient treated by balloon angioplasty. The composite endpoint of death, MI, or urgent revascularization following atherectomy was similarly reduced from 18.5 to 7.5% by the abciximab bolus and infusion.

The influence of therapy with abciximab on outcomes during and following saphenous vein graft interventions was evaluated in an analysis of the 101 patients in EPIC undergoing revascularization of 126 vein graft lesions *(18)*. Mean graft age was 8 yr. This analysis was limited by the small sample size, but the bolus and infusion of abciximab appeared to be associated with a significant reduction in the incidence of

distal embolization compared with placebo (2 vs 18%, $P = 0.017$) and a trend toward less-frequent large non-Q-wave infarction (2 vs 12%).

The EPILOG Trial

The EPILOG (Evaluation in PTCA to Improve Long-term Outcome with abciximab GP IIb/IIIa blockade) trial was designed to explore the potential role of abciximab therapy in the broad population of patients undergoing coronary intervention and to address the excessive hemorrhagic risk observed in the EPIC trial. The first objective of the trial was therefore to determine if the clinical benefits of abciximab therapy could be extended to all patients undergoing coronary intervention, regardless of their risk of ischemic complications. Moreover, based upon the data from the pilot PROLOG trial, the second key objective was to evaluate whether the incidence of hemorrhagic complications associated with this agent could be reduced without loss of efficacy by weight-adjusting or reducing the heparin dose.

Patient Population and Study Design

Patients undergoing elective or urgent percutaneous revascularization with an FDA-approved device were enrolled between February and December 1995 *(19)*. As the EPIC trial suggested that abciximab provided substantial clinical benefit among patients with acute ischemic syndromes *(14),* patients with unstable angina and associated electrocardiographic changes meeting the EPIC criteria during the previous 24 h were excluded. Patients undergoing primary angioplasty for acute MI were under evaluation in a separate dedicated trial (RAPPORT, *see* Chapter 11), and were therefore also excluded from EPILOG. Other exclusion criteria included planned stent implantation (based upon uncertainties regarding the optimal anticoagulation regimen during the time EPILOG was carried out) or rotational atherectomy (due to the frequent occurrence of CK elevations following this procedure which may have confounded assessment of the primary endpoint), percutaneous coronary intervention performed within the prior three months, or conditions that would be associated with excessive bleeding risk.

Patients representing a broad spectrum of risk strata and clinical indications for revascularization were enrolled in the trial. Median age was 60 years, 72% were male, 23% were diabetic, and 49% a history of prior MI. Balloon angioplasty was performed in 95% of cases, directional atherectomy in 5%, and unplanned ("bailout") stenting in 12%. Despite exclusion of patients meeting EPIC criteria for unstable angina, 47% of patients enrolled in EPILOG were considered to have other clinical criteria for unstable angina (without, for example, documented electrocardiographic changes or with symptoms occurring more than 24 h before randomization). Other indications for revascularization were recent infarction in 21% and stable ischemia in 32%.

Patients were given aspirin and randomized in a double-blind fashion to one of three treatment groups: placebo with standard dose, weight-adjusted heparin; abciximab with standard dose, weight-adjusted heparin; or abciximab with low-dose weight-adjusted heparin. For those receiving abciximab, a 0.25 mg/kg bolus was administered prior to balloon inflation or device activation, followed by a 0.125 μg/kg/min (maximum 10 μg/min) infusion for 12 h. The standard-dose weight-adjusted heparin regimen consisted of an initial heparin bolus prior to the interventional procedure of 100 U/kg (maximum 10,000 U), with additional weight-adjusted boluses according to an algorithm intended to achieve and maintain an activated clotting time ≥300 s. The low-dose weight-adjusted

heparin group received an initial bolus of 70 U/kg (maximum 7000 U), with additional boluses as necessary to achieve and maintain an activated clotting time ≥200 s. Postprocedural heparin was discouraged, and vascular sheaths were to be removed within 2–6 h (during the abciximab infusion). Specific guidelines or algorithms were provided for management of vascular access sites, uncontrolled bleeding, urgent coronary artery bypass surgery, thrombocytopenia, and blood transfusions.

Clinical Efficacy

Planned enrollment was 4800 patients, but the trial was terminated on the recommendation of the independent Data and Safety Monitoring Committee after entry of 2792 patients when an unexpectedly strong clinical benefit was observed at the first interim analysis. This interim analysis revealed that the incidence of death or MI at 30 d was reduced from 8.2% among patients in the placebo group to 2.6% or 3.6% among patients treated with abciximab and low-dose or standard-dose heparin, respectively ($P < 0.0001$), meeting the prespecified stopping rule.

The incidence of the primary composite endpoint of death, MI, or urgent revascularization at 30 d was 11.7% in the placebo group, 5.2% in the abciximab with low-dose heparin group (56% relative risk reduction, $P < 0.0001$), and 5.4% in the abciximab with standard-dose heparin group (54% relative reduction, $P < 0.0001$) (Fig. 2, middle). The magnitude of risk reduction by abciximab was similar for each of the components of the composite endpoint (Table 2), and the treatment effect of abciximab with either heparin regimen was homogeneous across all patient groups. Proportional hazards regression identified no significant interactions between baseline variables and the efficacy of abciximab therapy.

The early suppression of ischemic events by abciximab in EPILOG was maintained at 6 mo and 1 yr follow-up. In the placebo group, the timing of acute ischemic complications was clustered early in the course following coronary intervention: 72% of the primary composite endpoint events had accrued during the first 30 d (54% within 24 h), with an additional 19% occurring between 30 d and 6 mo and 9% between 6 mo and 1 yr. Therapy with abciximab resulted in a marked reduction in the risk of complications during the first 30-d period, after which time, incremental event rates were essentially equivalent among the three treatment arms. The composite endpoint (death, MI, or urgent revascularization) event rates were 14.7, 8.4, and 8.3% at 6 mo and 16.1, 9.6, and 9.5% at 1 yr in the placebo, abciximab with low-dose heparin, and abciximab with standard-dose heparin groups, respectively. Thus, the treatment effect achieved by abciximab early (at 30 d) was maintained without attenuation throughout the 1-yr follow-up period: the absolute reduction in events (number of events prevented per 100 patients treated) in the combined abciximab groups vs placebo was 6.40 at 30 d, 6.35 at 6 mo, and 6.55 at 1 yr. In contrast to the findings of EPIC, however, rates of repeat target vessel revascularization converged after 30 d. There were no significant differences in rates of target vessel revascularization among the three treatment groups at 6 mo and 1 yr, indicating that abciximab had no effect on the incidence of "clinical restenosis." An angiographic substudy had been planned, in which 900 patients (300 in each treatment group) would return for 6-mo angiographic follow-up to assess the influence of abciximab on angiographic restenosis; because of the early termination of the trial, however, an insufficient number of patients were enrolled in the Substudy to allow meaningful analysis of this secondary endpoint to be performed.

Safety

Hemorrhagic complications in EPILOG occurred infrequently and were not increased by abciximab therapy. Major bleeding occurred in 3.1, 2.0, and 3.5% and red cell transfusions were required in 3.9, 1.9, and 3.3% of patients randomized to placebo, abciximab with low-dose heparin, and abciximab with standard-dose heparin, respectively. One patient in each abciximab group, but no patient in the placebo group, suffered a hemorrhagic stroke; two patients receiving abciximab with standard-dose heparin experienced other intracranial bleeding or nonhemorrhagic stroke. When compared with the experience in EPIC, bleeding in EPILOG was reduced in both placebo and abciximab groups, likely as a consequence of weight-adjustment and reduction of heparin dosing and early vascular sheath removal. The treatment effect of abciximab in reducing ischemic complications was enhanced in EPILOG compared with EPIC (56 and 35% relative reductions in the risk of the primary 30-d efficacy endpoint, respectively), suggesting that elimination of excess bleeding may permit the full potential benefit of this form of therapy to be realized.

Thrombocytopenia in association with abciximab appeared to occur less frequently in EPILOG than in EPIC, particularly among patients receiving the low-dose heparin regimen. Severe thrombocytopenia (nadir platelet count <50,000 per mm^3) occurred in 4 (0.4%), 4 (0.4%), and 8 (0.9%, $P = 0.292$) patients in the placebo, abciximab and low-dose heparin, and abciximab and standard-dose heparin groups, respectively.

Subgroup Findings

As in the EPIC trial, limited subgroup analyses from EPILOG suggested that patients at elevated baseline (placebo group) risk for periprocedural complications had enhanced benefit from abciximab therapy. Notably among these were patients requiring unplanned stent deployment. Although elective stent use was prohibited by protocol, 326 patients (12%) underwent stent placement to optimize patency for >40% residual stenosis, extensive dissection, or abrupt or threatened closure *(20)*. Unplanned stent deployment occurred significantly less frequently among patients who had been randomized to abciximab with low-dose heparin than the other treatment regimens (13.7, 13.6, and 9.0% among patients in the placebo, abciximab with standard-dose heparin, and abciximab with low-dose heparin arms, respectively, $P = 0.001$). With placebo treatment, the patients who required bailout stents experienced a primary composite endpoint event by 30 d more than twice as frequently as did their counterparts in whom stents were not used (22.6 vs 10.0%, respectively) (Table 3). The relative risk reduction for this ischemic endpoint among stented patients (61%) was similar to that among nonstented patients (55%); because of their excess baseline (placebo group) risk, however, treatment with abciximab among patients with bailout stents resulted in a substantially enhanced absolute risk reduction (13.4 vs 5.5 events prevented per 100 patients treated among the bailout stent vs no bailout stent patients, respectively). This absolute treatment effect was preserved through 6-mo follow-up (Table 3). Among patients requiring stents, bleeding complications were not increased by abciximab relative to placebo therapy.

Another patient subgroup of particular interest in EPILOG were those with diabetes mellitus, given their increased risk for ischemic events following percutaneous coronary revascularization. Of the 2792 patients in the trial, 638 (23%) were diabetic *(21)*. Dia-

Table 3
30-d and 6-mo Clinical Outcome Among Patients With and Without Unplanned Stents in the EPILOG Trial

	Placebo (%)	*Abciximab + LD heparin (%)*	*Abciximab + SD heparin (%)*
30-d death, MI, or urgent revascularization			
Stent (n = 326)	22.6	8.6	9.9
No stent (n = 2369)	10.0	4.4	4.7
6-mo death, MI, or urgent revascularization			
Stent (n = 326)	24.2	11.1	12.5
No stent (n = 2369)	13.4	7.8	7.9

LD = low-dose weight-adjusted; MI = myocardial infarction; SD = standard-dose weight-adjusted
Data from Kereiakes et al. *(20).*

betic patients more frequently had adverse baseline clinical characteristics than did their nondiabetic counterparts, although angiographic target lesion morphology was similar in the two groups. By 30 d, the primary composite event rate occurred slightly more frequently among diabetic than nondiabetic patients randomized to placebo (12.6 vs 11.5%, respectively), but the excess risk in diabetic patients was completely neutralized by abciximab with low-dose heparin (5.7 and 5.0% event rates in patients with and without diabetes, respectively) or with standard-dose heparin (2.5 and 6.2% event rates, respectively). By 6 mo, death and MI rates were substantially higher in diabetic patients than in nondiabetic patients randomized to placebo (14.8 vs 10.0%, respectively), but the early suppression of these ischemic events by abciximab was preserved in both groups. Among diabetics, 6-mo death or MI was reduced from 14.8% to 7.1% or 4.1% in the placebo, abciximab plus low-dose heparin, and abciximab plus standard-dose heparin groups, respectively; in nondiabetics, the event rate was reduced from 10.0% to 5.4% or 6.9%, respectively. In contrast, there appeared to be a differential effect of abciximab on late target vessel revascularization rates among patients with and without diabetes; by 6 mo, target vessel revascularization rates were reduced by abciximab in nondiabetic (hazard ratio 0.79, 95% confidence interval 0.63–0.96) but not in diabetic patients (hazard ratio 1.40, 95% confidence interval 0.94–2.08).

The EPISTENT Trial

The introduction of GP IIb/IIIa receptor blockade into clinical interventional practice was paralleled by the widespread adoption of elective coronary stenting as the predominant means of percutaneous coronary intervention, based upon the efficacy of these devices in reducing repeat revascularization rates *(22,23)*. The initial pivotal studies of GP IIb/IIIa blockade had excluded enrollment of patients undergoing elective stent implantation, reserving the use of stents for "bailout" indications. In the high-risk setting of unplanned coronary stenting, the value of abciximab therapy in reducing ischemic complications was unequivocal (*see* Subgroup Findings), but the question of the role of enhanced antiplatelet therapy with GP IIb/IIIa inhibitors among patients *electively* undergoing "optimal" revascularization by stents remained to be answered. The

EPISTENT (Evaluation of Platelet Inhibition in STENTing) trial was designed to evaluate the clinical benefit of abciximab therapy in reducing ischemic complications among patients undergoing elective stent implantation, as well as to assess the clinical efficacy of abciximab (with balloon angioplasty) relative to stenting.

Patient Population and Study Design

A total of 2399 patients undergoing elective or urgent percutaneous coronary revascularization were enrolled between July 1996 and September 1997 *(24)*. Patients were eligible for inclusion if they had at least one target lesion suitable for allocation to either stenting or balloon angioplasty and were not undergoing primary intervention in the setting of acute MI. Exclusions for excessive bleeding risk were similar to those in the EPIC and EPILOG trials; patients were also excluded if a stent had previously been placed in the target lesion, any intervention had been performed in the prior 3 mo, or rotational atherectomy was planned.

EPISTENT enrolled a broad spectrum of patients, representing "real world" coronary stenting rather than the ideal or narrow subgroups assessed in previous stent vs balloon angioplasty trials. Mean age was 59 years, 75% were male, 20% were diabetic, and 34% had a history of MI. Indications for revascularization were (not mutually exclusive) recent infarction (within 7 d) in 16%, unstable angina within the prior 48 h in 36%, unstable ischemic symptoms within the prior 6 mo in 57%, and stable ischemia in 43% of patients.

Patients were treated with aspirin and randomized to one of three treatment groups: stent plus placebo, stent plus abciximab (0.25 mg/kg bolus and 0.125 μg/kg-min infusion for 12 h), or balloon angioplasty (PTCA) plus abciximab. Randomization to abciximab or placebo was blinded in the stented patients. Stenting was to be carried out using the Johnson and Johnson design unless this stent could not be deployed. Stent implantation in the PTCA plus abciximab group was to be reserved for clear "bailout" indications, specified by protocol, rather than suboptimal results. Patients randomized to treatment with abciximab received adjunctive heparin according to the EPILOG low-dose weight-adjusted regimen (70 U/kg bolus, ACT ≥200 s), whereas those randomized to placebo received the standard-dose weight-adjusted regimen (100 U/kg bolus, ACT ≥300 s). Vascular sheaths were to be removed early without postprocedural heparin infusion. Ticlopidine was administered following stent placement. As in the EPILOG trial, management guidelines and algorithms were provided for management of bleeding, emergency bypass surgery, blood transfusions, vascular access site care, and thrombocytopenia.

To evaluate the influence of abciximab therapy on restenosis following percutaneous coronary intervention, an Angiographic Substudy was performed. Approximately 900 patients—300 in each treatment group—were enrolled consecutively at participating clinical centers to return for systematic angiography and quantitative measurements of target lesion coronary luminal dimensions 6 mo following their index procedure.

Clinical Efficacy

Stents were successfully deployed in 96% of patients for whom they were assigned; 27% of these patients required two or more stents. Crossover to unplanned stenting occurred in only 19.3% of patients in the PTCA plus abciximab group, attesting to the compliance of clinical investigators in reserving stents in this group for clear "bailout"

indications. Angiographic complications of abrupt closure, transient closure, or side-branch closure occurred infrequently, but were observed in fewer patients receiving abciximab than placebo.

The primary efficacy composite endpoint of death, MI, or urgent repeat revascularization by 30 d following randomization occurred in 10.8% of patients in the stent plus placebo arm, 6.9% of patients in the PTCA plus abciximab arm (36% relative risk reduction, $P = 0.007$), and 5.3% of patients in the stent plus abciximab arm (51% relative risk reduction, $P < 0.001$) (Fig. 2, bottom). Most ischemic events occurred within the first 12 h following the procedure. Compared with stenting alone, adjunctive use of abciximab with stenting reduced rates of death, MI, and urgent repeat revascularization, with a similar magnitude of treatment effect for each of the components of the composite endpoint (Table 2). Compared with stenting alone, abciximab with PTCA was associated with equivalent rates of death and urgent repeat revascularization, but greater safety with regard to lower rates of MI (Table 2). The predominant influence of abciximab on MI (86%) was reduction in Q-wave or large non-Q-wave infarction (defined prospectively by CK-MB ≥ 5X normal); for the secondary composite endpoint of death or large MI (Q-wave or large non-Q-wave infarction), event rates were reduced from 7.8% in the stent plus placebo group to 4.7% in the PTCA plus abciximab group (40% relative risk reduction, $P = 0.009$) and 3.0% in the stent plus abciximab group (62% relative risk reduction, $P < 0.001$). The treatment effect of abciximab was homogeneous across all patient subgroups, as defined by their baseline characteristics or clinical indications for revascularization.

Long-term clinical follow-up was performed at 6 mo and 1 yr after randomization. Two principal endpoints were assessed at 60 mo: (1) a composite of death or myocardial infarction, and (2) the incidence of repeat target vessel revascularization. The rate of death of myocardial infarction was 11.4% in the stent plus placebo group, 5.6% in the stent plus abciximab group ($P < 0.001$), and 7.8% in the PTCA plus abciximab group ($P = 0.013$). The absolute reduction in this composite endpoint (number of events prevented per 100 patients treated) compared with stenting alone was 5.5 at 30 d and 5.8 at 6 mo in the stent plus abciximab group and 4.4 at 30 d and 3.6 at 6 mo in the PTCA plus abciximab group. EPISTENT ws the first trial of percutaneous coronary intervention to demonstrate a mortality benefit of a new therapy. At 6 mo, mortality was significantly reduced by stenting compared with PTCA among patients receiving abciximab (0.5% vs 1.8%, $P = 0.018$). By 1 yr, the combined treatment resulted in a lower mortality rate than either stenting or abciximab alone (stent + placebo = 2.4%, PTCA + abciximab = 2.1%, stent + abciximab = 1.0%, $P = 0.037$).

Rates of repeat target vessel revascularization (percutaneous or surgical) were 10.6% in the stent plus placebo group, 8.7% in the stent plus abciximab group ($P = 0.216$), and 15.4% in the PTCA plus abciximab group ($P = 0.005$) by 6 mo. Among stented patients, treatment with abciximab rather than placebo was associated with a nonsignificant trend toward reduced rates of target vessel revascularization (18% relative risk reduction, $P = 0.215$). Among the subgroup of patients with diabetes, repeat target vessel revascularization rates following stent implantation were significantly reduced by abciximab. Stenting alone (with placebo) did not reduce the incidence of subsequent target vessel revascularization procedures compared with PTCA among diabetes, whereas the rate of this endpoint was halved by the combination of abciximab and stenting.

An angiographic substudy was performed of the first 899 patients in the trial. Minimal luminal diameters (MLD) postintervention and early gain were significantly better among patients undergoing stenting rather than PTCA. Among stented patients, net gain in MLD was significantly better with abciximab than placebo (0.86 vs 0.73 mm, P = 0.025), with a trend toward greater follow-up MLD. Among diabetes, net gain and follow-up MLD were substantially greater among stented patients receiving abciximab than placebo.

Safety

Bleeding complications occurred infrequently in EPISTENT and were not increased by abciximab therapy. Major bleeding occurred in 2.2, 1.5, and 1.4% and transfusions (red cells or platelets, including those related to coronary bypass surgery) were required in 2.2, 2.8, and 3.1% of patients in the stent plus placebo, stent plus abciximab, and PTCA plus abciximab groups, respectively (all P = N.S.) No patient in the trial suffered an intracranial hemorrhage within 30 d postrandomization. Severe thrombocytopenia (platelet count <50,000 per mm^3) occurred in 0% of placebo-treated patients and 0.9–1.1% of abciximab-treated patients.

Comparison of EPILOG and EPISTENT

Comparison of outcomes in the EPILOG and EPISTENT trials allows assessment of the relative contributions of stenting and abciximab to the safety of percutaneous revascularization procedures. Such comparisons are somewhat limited by slight differences in entry criteria (including exclusion of patients with unstable angina and reversible electrocardiographic changes from EPILOG) and interventional practice (from 1995 to 1997) between the trials. Within these constraints, however, it is illustrative to analyze the influence of these two major advances in interventional cardiology on the clinical endpoints of death, MI, or repeat revascularization (Fig. 3).

Despite the widespread belief that stenting improves the safety of percutaneous revascularization, it is apparent from EPILOG and EPISTENT that stenting has no beneficial influence relative to angioplasty on the important ischemic endpoints of death or MI (Fig. 3, top). Rates of death or infarction were numerically higher in the stent-placebo compared with PTCA-placebo groups of the two trials (9.1% for angioplasty in EPILOG and 10.2% for stenting in EPISTENT). This finding is concordant with other randomized trials of stenting vs balloon angioplasty, in which death and MI rates were slightly higher with stenting *(22,23)*. In contrast, abciximab therapy (with stents or balloon angioplasty) consistently diminished the risk of death or MI by 50–60%, with no evidence of an interaction or complementarity with the technique of revascularization.

Revascularization rates within the first 30 d of the index procedure (Fig. 3, bottom), however, did appear to be diminished by stenting compared with balloon angioplasty (7.5% with angioplasty plus placebo in EPILOG versus 4.1% with stenting plus placebo in EPISTENT); this observation is in accordance with the clinical experience of most practicing interventional cardiologists. The somewhat unexpected finding of these two trials, however, is that a similar magnitude of suppression of early repeat revascularization rates can be achieved by treatment with abciximab or by stent implantation (4.7% with abciximab and balloon angioplasty in both EPILOG and EPISTENT vs 4.1% with stenting and placebo). Moreover, the combination of abciximab with stenting appears to provide additive beneficial effect (2.6% rate of repeat revascularization with

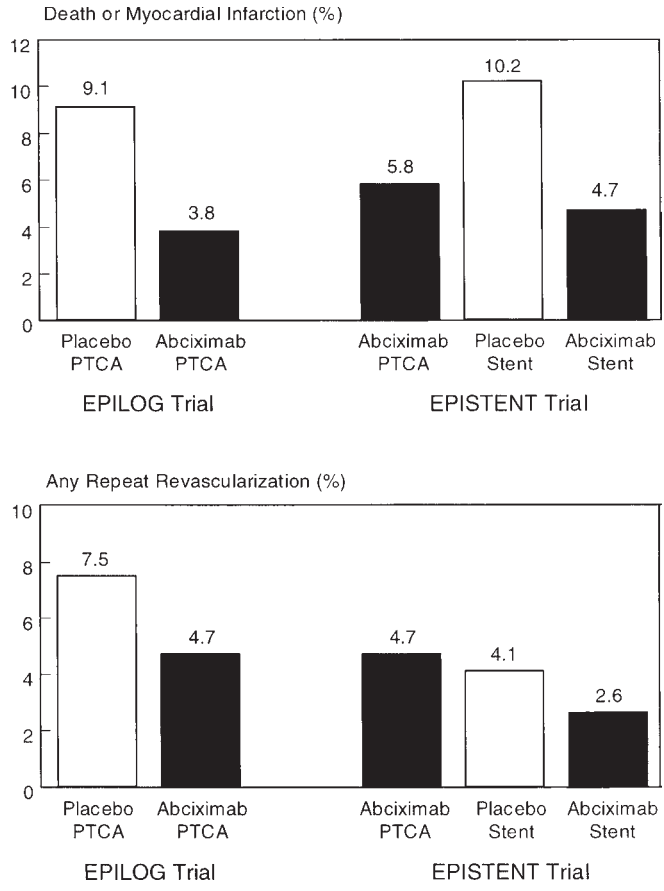

Fig. 3. Comparison of 30-d clinical outcomes in the EPILOG and EPISTENT trials among patients treated with stents or PTCA (balloon angioplasty) and abciximab or placebo. Top: end point of death or MI. Bottom: endpoint of any repeat revascularization (urgent or elective). The two abciximab arms (low-dose weight-adjusted heparin and standard-dose weight-adjusted heparin) of EPILOG are combined for analysis.

abciximab plus stenting in EPISTENT). A mortality benefit by one year is conferred by the combination of abciximab plus stenting relative to either therapy alone. Late (elective) revascularization rates are reduced independently by stenting rather than balloon angioplasty, providing an important complementarity between stenting and abciximab in improving overall patient outcome. The findings of EPISTENT also suggested that abciximab may reduce the neointimal hyperplastic component of restenosis, particularly among a group of patients (diabetics) who have been consistently shown to be at increased risk for this late complication *(25,26)*.

CONCLUSIONS

The EPIC, EPILOG, and EPISTENT randomized placebo-controlled trials provide a compelling body of evidence among over 7000 patients of the unequivocal and profound efficacy of abciximab in reducing the ischemic complications of death, MI, or urgent

repeat revascularization associated with percutaneous coronary interventional procedures. This clinical benefit is sustained for at least 3 yr of follow-up and appears to extend to all patients treated, regardless of their underlying risk profile or the modality chosen for percutaneous revascularization. Specific issues regarding optimization of efficacy and safety in the use of this and other agents of its class will be addressed in Chapter 9.

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6 Eptifibatide in Coronary Intervention—The IMPACT Trials

Conor O'Shea, MD and James E. Tcheng, MD

CONTENTS

INTRODUCTION

While percutaneous transluminal coronary intervention has revolutionized the management of patients with coronary artery disease, this technological advance is neither innocuous nor a panacea. In addition to the technical limitations of the procedure, it is now well recognized that serious vascular injury is caused by the treatment device that creates a perfect milieu for coronary thrombosis *(1–3)*. Clinical trials of the monoclonal antibody abciximab (c7E3 Fab; ReoPro™, Eli Lilly and Company [Indianapolis, IN]/Centocor [Malvern, PA]) directed at the platelet glycoprotein (GP) IIb/IIIa integrin have clearly documented that inhibition of this receptor during coronary intervention reduces thrombotic complications and improves clinical outcomes *(4–8)*. These positive clinical trial results, coupled with a clearer understanding of platelet physiology, have stimulated the search and encouraged the development of other parenteral inhibitors of the GP IIb/IIIa receptor.

Eptifibatide (Integrilin™, COR Therapeutics, South San Francisco, CA), a peptide inhibitor of GP IIb/IIIa, is one of a new class of parenteral agents designed and synthesized using the techniques of molecular engineering. This chapter will briefly outline the biopharmacology of eptifibatide and then focus on the experience to date with this novel compound in the setting of coronary intervention.

PLATELET PHYSIOLOGY AND EPTIFIBATIDE

As reviewed in other sections, the central role of the platelet in mediating vascular thrombosis is now clear. The final act among the processes leading to thrombus forma-

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tion is mediated by the binding of dimeric adhesive proteins, such as fibrinogen and von Willebrand factor (vWf) to activated GP IIb/IIIa *(9,10)*. The GP IIb/IIIa receptor recognizes an Arg-Gly-Asp (RGD) sequence as well as a Lys-Gln-Ala-Gly-Asp-Val sequence on the fibrinogen molecule *(11)*. These two areas serve as the linkage sites for GP IIb/IIIa receptor binding with fibrinogen *(12)*.

The path leading to the synthesis of eptifibatide has its roots in the search for GP IIb/IIIa inhibitors among the naturally occurring proteins. In these surveys, RGD-containing peptides isolated from pit viper venoms were discovered to bind the glycoprotein IIb/IIIa receptor and inhibit its function *(13)*. One in particular, barbourin, a protein isolated from the venom of the Southeastern Pygmy rattlesnake *Sistrurus m barbouri,* was found to have a higher specificity for GP IIb/IIIa than the rest. Analysis of the differences in amino acid sequences suggested that the reason for the increased specificity of Barbourin was that it differed by a single amino acid substitution of lysine (K) for arginine *(14,15)*.

Eptifibatide was created based on the Lys-Gly-Asp (KGD) sequence found in barbourin. It is a cyclic, constrained heptapeptide with a terminal half-life of approximately 1.5–2.5 h. In addition to the specificity for GP IIb/IIIa conferred by the KGD sequence, eptifibatide was engineered with a ring structure to impart resistance to proteolysis. Biological activity is concentration dependent, and the agent is cleared from the body largely via excretion in the urine of intact compound and break down products *(16)*.

IMPACT

Two phase II trials of eptifibatide as an adjunct to percutaneous coronary revascularization were conducted in the early 1990s to establish the pharmacodynamic and (preliminary) safety profiles of the agent. The first, the Integrelin to Minimize Platelet Aggregation and Coronary Thrombosis (IMPACT) study, was a randomized, placebo-controlled trial of 150 patients undergoing elective percutaneous coronary intervention *(17)*. Patients were allocated to one of three treatment approaches: placebo; a 90 μg/kg bolus before the initiation of the coronary intervention followed by a 1.0 μg/kg-min infusion of eptifibatide for 4 h after the bolus; or the same 90 μg/kg bolus followed by a 1.0 μg/kg-min infusion of eptifibatide for 12 h. Patients were followed for 30 d after the procedure; 101 patients were assigned to eptifibatide and 49 received placebo. In blood collected in citrate, the 90 μg/kg bolus produced an 86% inhibition of platelet aggregation to stimulation with 20 μ*M* adenosine diphosphate. There was a trend towards lower composite adverse clinical event rates with the longer infusions (12.2% for placebo, 9.6% for the 4-h infusion, and 4.1% for the 12-h infusion, P = NS). Major bleeding event rates were 5% with either eptifibatide treatment compared with 8% with placebo. Minor bleeding, primarily at the vascular access site, occurred in 40% vs 14%, respectively. Because the bleeding profile appeared acceptable, it was suspected that higher dosing of eptifibatide (and potentially better clinical efficacy) might still be possible. This led to the second phase II study in angioplasty, IMPACT Hi/Low.

IMPACT HI/LOW

The IMPACT Hi/Low study was the second phase II, dose-ranging study of eptifibatide as an adjunct to coronary intervention. The IMPACT Hi/Low study was a placebo-controlled randomized dose-escalation trial that measured ex vivo platelet

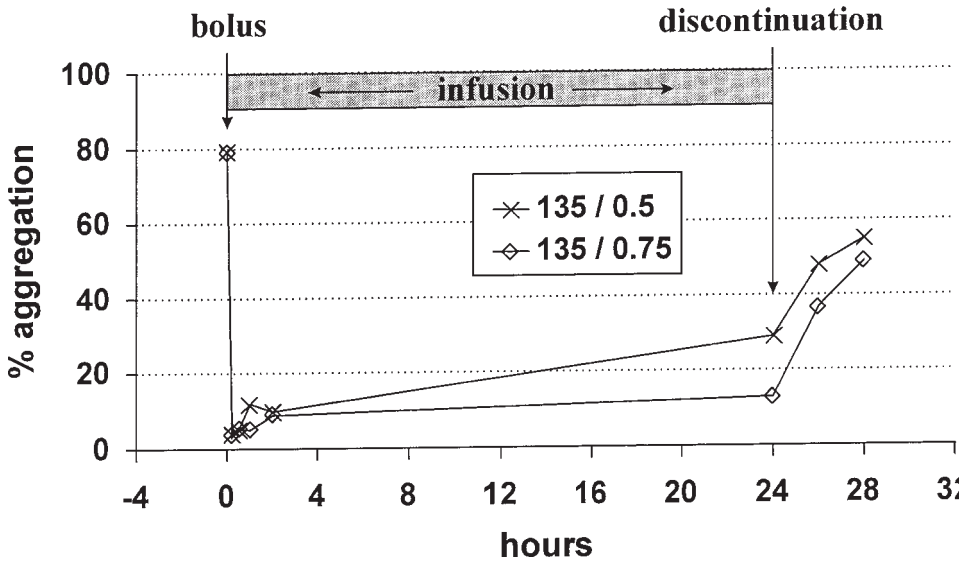

Fig. 1. Inhibition of platelet aggregation with eptifibatide—the IMPACT Hi/Low trial. Among the four different doses of Integrilin evaluated in the IMPACT Hi/Low trial, the 135 μg/kg bolus plus 0.75 μg/kg-min infusion produced a sustained inhibition of platelet aggregation below 20% of baseline, whereas the 135 μg/kg bolus plus 0.5 μg/kg-min infusion permitted some recovery of platelet function by the end of the 24-h infusion. The pharmacodynamic profiles of both regimens were nearly identical in the first 4 h. Data are raw (nonnormalized) results; assays were conducted on blood suspended in citrate.

aggregation, bleeding time, and plasma eptifibatide concentration in 73 patients *(18)*. Four different bolus plus infusion combinations were evaluated. A bolus dose of eptifibatide of 180 μg/kg followed by a 1.0 μg/kg-min infusion produced almost complete inhibition (>95%) of platelet aggregation (in whole blood anticoagulated with citrate). Problematically, increasing rates of both minor and major bleeding were observed with increasing doses of eptifibatide. Given the available pharmacodynamic profiles coupled with concerns about bleeding, dosing regimens lower than the maximal doses tested in the IMPACT Hi/Low trial (Fig. 1) were ultimately selected for testing in the phase III study of eptifibatide, the IMPACT II study.

IMPACT II

The IMPACT II study was designed to be the pivotal, phase III study of eptifibatide as an adjunct to coronary intervention. IMPACT II was a multicenter parallel-group double-blind randomized, controlled clinical trial that began patient recruitment in November 1993. It was carried out at 82 centers in the United States, with enrollment closed in November 1994 after 4010 patients had been entered *(19)*. The study specifically included a representative cross-section of all patients undergoing percutaneous revascularization.

Patients were assigned one of three treatment regimens: a bolus of 135 μg/kg of eptifibatide initiated just before coronary intervention followed by an infusion of 0.5 μg/kg-min of eptifibatide for 20–24 h; a 135 μg/kg bolus followed by a 0.75 μg/kg-min infusion for 20–24 h; or placebo bolus and placebo infusion. All patients received 325 mg of aspirin by mouth before (and continued thereafter) the coronary intervention. Heparin was given

as a 100 U/kg bolus before the intervention, with additional heparin as needed during the procedure, to attain and maintain an activated clotting time between 300 and 350 s.

The primary clinical endpoint was the composite occurrence within 30 d of death, myocardial infarction (periprocedural MB-CK ≥3 times the upper limit of normal), urgent or emergency repeat coronary intervention, urgent or emergency coronary artery bypass surgery, or placement of an intracoronary stent during the index procedure for the management of true abrupt closure. The principal safety endpoints were major bleeding, blood transfusion requirements, and stroke. Major bleeding was defined as intracranial hemorrhage or overt bleeding associated with a decrease in hemoglobin of more than 5 g/dL or a decrease in packed cell volume of 15% or more from baseline.

Primary Composite Clinical Efficacy

In IMPACT II, the clinical efficacy was determined with analyses based on the intention to treat principle. Two different approaches were used. The first, the "treated as randomized" analysis, was of the 3871 (96.5%) of patients who received any study drug (whether or not they underwent angioplasty). This approach was used because the delay between randomization allocation (which occurred before arrival of the patient in the catheterization laboratory) and actual treatment administration (which occurred in the catheterization laboratory just before the coronary intervention) resulted in dropout of patients unrelated to either randomization or treatment allocation. Unless otherwise indicated, data presented in this chapter are derived from the "treated-as-randomized" analyses. The second approach, the "all-randomized" analysis, included all patients enrolled in the study, whether or not the patient received any study drug and/or underwent coronary intervention. All endpoint events were adjudicated by a blinded endpoint committee.

The "treated-as-randomized" analysis (Table 1) demonstrated a statistically significant 22% reduction in the primary composite clinical endpoint at 30 d with the 135/0.5 treatment approach compared to placebo (9.1% vs 11.6%, $P = 0.035$; odds ratio 0.76 [0.59–0.98]). A trend towards improved outcomes was observed with the 135/0.75 dosing approach (10.0% vs 11.6%, $P = 0.18$). In absolute terms, treatment with the 135/0.5 regimen prevented 25 events per 1000 patients in the first 30 d. The "all-randomized" analysis showed only strong trends favoring a treatment effect. The composite primary endpoint occurred in 151 (11.4%) patients in the placebo group compared with 124 (9.2%) in the 135/0.5 treatment group ($P = 0.063$) and 132 (9.9%) in the 135/0.75 treatment group ($P = 0.22$).

Also included in Table 1 are the primary composite endpoint rates as determined by the principal investigators; in other words, events as recorded on the case report forms before adjudication. Several comments can be made about these data. Events documented by the site probably reflect those that were the most clinically apparent (in contrast to those picked up through the meticulous adjudication process). Also, a greater relative difference was reported by the principal investigators than by the adjudication committee; this would suggest that the rigorous adjudication process may produce a conservative underestimate of the actual benefits of drug treatment.

A consistent, similar degree of benefit was imparted to all patients regardless of risk profile. Efficacy was realized regardless of baseline demographic characteristics, medical history, procedures performed, or angiographic features. Representative data are included in Table 2.

Table 1
Composite Clinical Efficacy Results: Patients Receiving Any Study Drug (Treated as Randomized Analyses)

	Placebo (n=*1285*)	*Eptifibatide 135/0.5* (n=*1300*)	*Eptifibatide 135/0.75* (n=*1286*)
24-h Composite endpoint, *n* (%; 95% CI)	123 (9.6; 8.0–11.2)	86 (6.6; 5.3–8.0)	89 (6.9; 5.5–8.3)
Significance vs placebo	—	*P* = 0.006	*P* = 0.014
Odds ratio vs placebo (95% CI for OR)	—	0.67 (0.50–0.89)	0.70 (0.53–0.93)
30-d Composite endpoint, *n* (%; 95% CI)	149 (11.6; 9.8–13.3)	118 (9.1; 7.5–10.6)	128 (10.0; 8.3–11.6)
Significance vs placebo	—	*P* = 0.035	*P* = 0.18
Odds ratio vs placebo (95% CI for OR)	—	0.76 (0.59–0.98)	0.84 (0.66–1.08)
24-h Composite endpoint as determined by the Principal Investigators, *n* (%; 95% CI)	82 (6.4; 5.0–7.7)	49 (3.8; 2.7–4.8)	50 (3.9; 2.8–4.9)
Significance vs placebo	—	*P* = 0.002	*P* = 0.004
Odds ratio vs placebo (95% CI for OR)	—	0.57 (0.40–0.83)	0.59 (0.41–0.85)
30-d Composite endpoint as determined by the Principal Investigators, *n* (%; 95% CI)	103 (8.0; 6.5–9.5)	74 (5.7; 4.4–7.0)	84 (6.5; 5.2–7.9)
Significance vs placebo	—	*P* = 0.018	*P* = 0.142
Odds ratio vs placebo (95% CI for OR)	—	0.69 (0.51–0.94)	0.80 (0.59–1.08)
In-laboratory abrupt closure, *n* (%; 95% CI)	66 (5.1; 3.9–6.3)	36 (2.8; 1.9–3.7)	48 (3.7; 2.7–4.8)
Significance vs placebo	—	*P* = 0.002	*P* = 0.081
Odds ratio vs placebo (95% CI for OR)	—	0.53 (0.35–0.80)	0.72 (0.49–1.05)

Table 2
30 Day Composite Clinical Efficacy Results vs Placebo by Baseline Characteristics (Treated as Randomized Analyses). Point Estimates with 95% Confidence Intervals for the Odds Ratios

	Eptifibatide 135/0.5	*Eptifibatide 135/0.75*
Age ≤65	0.687 (0.493, 0.958)	0.816 (0.596, 1.117)
Age >65	0.875 (0.586, 1.306)	0.897 (0.593, 1.356)
Male	0.706 (0.523, 0.954)	0.834 (0.626, 1.111)
Female	0.919 (0.565, 1.495)	0.870 (0.524, 1.447)
Lowest weight tertile (<77 kg)	0.847 (0.557, 1.288)	0.951 (0.629, 1.439)
Middle weight tertile (77–90 kg)	0.781 (0.507, 1.202)	0.846 (0.549, 1.304)
Highest weight tertile (>90 kg)	0.630 (0.388, 1.025)	0.729 (0.462, 1.153)
Diabetes	0.827 (0.472, 1.450)	0.725 (0.406, 1.295)
No diabetes	0.743 (0.558, 0.989)	0.872 (0.661, 1.151)
Previous intervention	0.979 (0.593, 1.618)	1.108 (0.685, 1.791)
Previous CABG	0.685 (0.373, 1.259)	0.969 (0.553, 1.697)
High-risk stratum	1.005 (0.680, 1.484)	0.885 (0.593, 1.320)
Elective stratum	0.620 (0.442, 0.871)	0.817 (0.593, 1.125)
Balloon angioplasty	0.752 (0.577, 0.980)	0.851 (0.658, 1.100)
Rotablator	0.551 (0.275, 1.104)	0.577 (0.288, 1.158)
Stent implantation	0.377 (0.181, 0.783)	0.473 (0.233, 0.959)

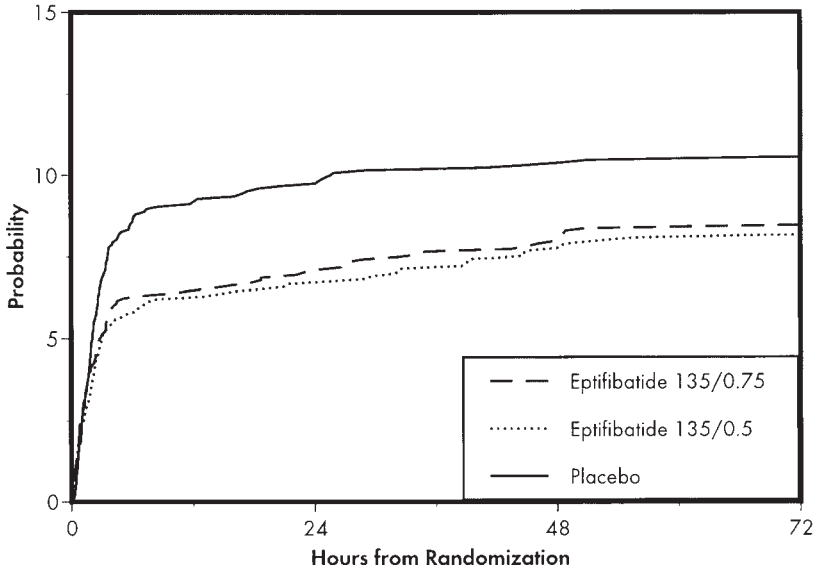

Fig. 2. Kaplan-Meier plot of probability of endpoint events to 72 h. Components of the composite clinical efficacy endpoint were death, myocardial infarction, or urgent revascularization. The majority of events occurred within 6 h of coronary intervention. Both eptifibatide regimens reduced events to essentially the same degree.

Time to Events

As might be expected from a short-acting parenteral agent, separation and differentiation of the treatment-event curves from placebo occurred early, within the first 24 h (the period of drug infusion) (Fig. 2). The differences appear most likely because of a reduction in thrombosis, as manifest by a reduction in angiographically apparent true-abrupt closure (Table 1). The figures included in Fig. 3, the Kaplan-Meier event curves to 6 mo illustrates three points. First, the two dosing approaches studied in IMPACT II are indistinguishable in clinical effect; second, the absolute difference achieved during treatment remains constant and durable without degradation over time; and third, the majority of the benefit of eptifibatide treatment was the result of reducing the incidence of myocardial infarction or death. In particular, Fig. 3B points out that eptifibatide treatment had no effect on the need for subsequent (clinically driven) revascularization.

With regards to angiographic restenosis, in the 900 patient IMPACT II Angiographic Substudy (in which all patients were required to return for 6-mo follow-up angiography), no differences in rates of angiographic restenosis among the treatment groups were observed *(20)*. Finally, all of the figures illustrate that coronary intervention itself induces adverse clinical events. Of end-point events to 30 d, over 75% of events occur within the first 24 h of the procedure.

Components of the Composite Endpoint

The event rates for the individual components of the primary efficacy endpoint were reduced with eptifibatide treatment consistent with the overall treatment effect seen in IMPACT II (Table 3). The majority of the treatment effect was in reducing the incidence of periprocedural myocardial infarction, with lesser absolute contributions from the other ele-

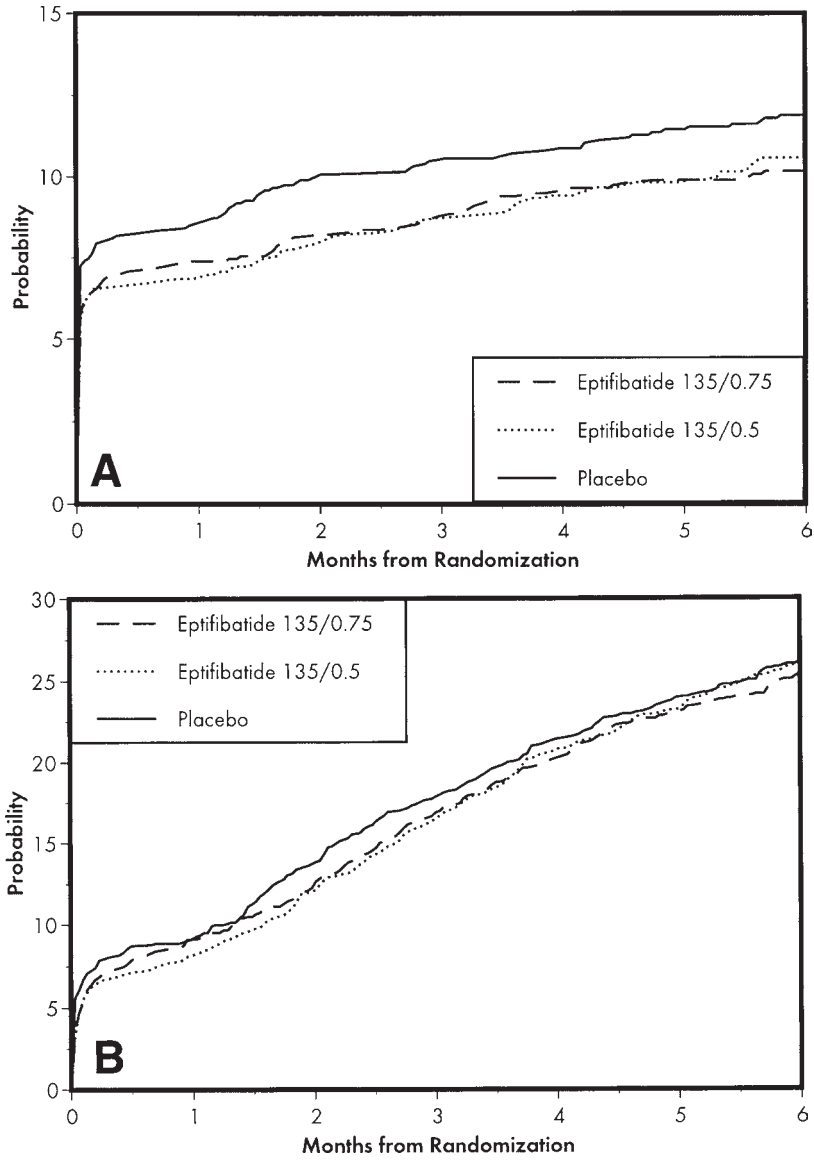

Fig. 3. Kaplan-Meier plots of probability of endpoint events to 6 mo. **(A)** is a plot of death or myocardial infarction; **(B)** is a plot of all revascularization (percutaneous coronary intervention or coronary artery bypass surgery). Part **(A)** illustrates that the benefit achieved early is maintained for the entire observation period; absolute differences established early remained constant. Almost all of the benefit is because of a reduction in rates of myocardial infarction; no differences in death were realized (data not shown). Part **(B)** indicates that eptifibatide treatment did not influence subsequent need for revascularization.

ments tracked. Stents were required for abrupt closure in 18 (1.4%) patients in the placebo group compared to 7 (0.5%) in each of the eptifibatide treatment groups. This finding paralleled the greater overall use of stent implantation in the placebo group at the initial coronary intervention (5.0% vs 2.7% in the 135/0.5 group, $P = 0.002$). Other statistics concerning the individual components of the composite endpoint are listed in Table 3.

Table 3
Primary Composite Efficacy Outcomes and Components: Patients Receiving Any Study Drug (Treated as Randomized Analyses)

	Placebo (n=*1285*)	*Eptifibatide 135/0.5* (n=*1300*)	*Eptifibatide 135/0.75* (n=*1286*)
30-d Composite endpoint, *n* (%; 95% CI)	149 (11.6; 9.8–13.3)	118 (9.1; 7.5–10.6)	128 (10.0; 8.3–11.6)
Significance vs placebo	—	*P* = 0.035	*P* = 0.18
Odds ratio vs placebo (95% CI for OR)	—	0.76 (0.59–0.98)	0.84 (0.66–1.08)
Endpoint components, *n* (%; 95% CI)			
Death	14 (1.1; 0.5–1.7)	6 (0.5; 0.1–0.8)	11 (0.9; 0.4–1.4)
Myocardial infarction	106 (8.2; 6.7–9.8)	86 (6.6; 5.3–8.0)	90 (7.0; 5.6–8.4)
CK-MB ≥3× upper normal limit	72 (5.6; 4.3–6.9)	54 (4.2; 3.1–5.2)	46 (3.6; 2.6–4.6)
CK-MB ≥5× upper normal limit	51 (4.0; 2.9–5.0)	38 (2.9; 2.0–3.8)	28 (2.2; 1.4–3.0)
Q waves	20 (1.6; 0.9–2.2)	12 (0.9; 0.4–1.4)	15 (1.2; 0.6–1.8)
Q waves or CK-MB ≥5× upper normal limit	59 (4.6; 3.4–5.7)	47 (3.6; 2.6–4.6)	39 (3.0; 2.1–4.0)
Death or CK-MB ≥3× upper normal limit	82 (6.4; 5.0–7.7)	57 (4.4; 3.3–5.5)	54 (4.2; 3.1–5.3)
Significance vs placebo	—	0.024	0.013
Odds ratio vs placebo (95% CI for OR)	—	0.67 (0.48, 0.95)	0.64 (0.45, 0.92)
Urgent or emergency bypass surgery	36 (2.8; 1.9–3.7)	19 (1.5; 0.8–2.1)	26 (2.0; 1.3–2.8)
Urgent or emergency repeat percutaneous revascularization	37 (2.9; 2.0–3.8)	35 (2.7; 1.8–3.6)	36 (2.8; 1.9–3.7)
Stent for abrupt closure	18 (1.4; 0.8–2.0)	7 (0.5; 0.1–0.9)	7 (0.5; 0.1–0.9)

CK denotes creatine kinase; CI, confidence interval; and OR, odds ratio.

Adverse Events

There was no increase in the frequency of major bleeding, blood transfusion, or other morbidity associated with eptifibatide administration. Rates of major bleeding were 4.8% vs 5.1% vs 5.2% for the placebo, 135/0.5, and 135/0.75 groups, respectively. Transfusion rates likewise were comparable. The majority (~60%) of all bleeding was attributable to the vascular access site. Four patients sustained an intracranial hemorrhage, one each in the placebo and 135/0.5 groups and two in the 135/0.75 treatment group. Rates of thrombocytopenia were low overall and indistinguishable among groups, with no patients developing acute profound thrombocytopenia. Finally, no patients developed anti-Integrilin antibodies.

ISSUES, QUESTIONS, AND DIRECTIONS

The IMPACT II study adds to the body of evidence focusing on the central role of the platelet in mediating the ischemic complications of coronary intervention. Following a unanimous recommendation for approval for commercial distribution by the United States FDA Cardiac and Renal Drugs Advisory Committee in January 1998, eptifibatide is now available as an adjunct to percutaneous coronary intervention. Based on the data from IMPACT II, a salutary benefit in the range of a 20–25% in adverse clinical events can be expected, with benefit achieved without a concomitant safety penalty. With

consideration of the third element (cost) of the treatment decision triad, eptifibatide has a very favorable overall profile with respect to other standard therapeutics.

Despite what has been accomplished, however, key issues remain. Perhaps the most daunting is that the treatment effect was less than expected, particularly in the context of the results achieved in the EPIC trial with the monoclonal antibody fragment abciximab *(4)*. Whether greater clinical efficacy can be realized, perhaps by increasing eptifibatide dosing and/or treatment duration, remains to be discovered. Recent investigations (performed well after the IMPACT II trial had been completed) lend support to the notion that higher doses of eptifibatide might provide better clinical efficacy. It has recently been elucidated that the pharmacodynamic effects of eptifibatide were overestimated in the phase II dose-finding studies. This overestimation was secondary to the use of the anticoagulant sodium citrate to suspend the blood samples; since citrate chelates calcium, and since calcium normally occupies the ligand binding site within GP IIb/IIIa, ex vivo ADP-induced platelet aggregation was artificially enhanced vis á vis the in vivo clinical effect *(21)*. The best current estimate is that the doses used in IMPACT II achieved only 30–50% of maximal platelet GP IIb/IIIa integrin blockade *(22)*.

Two subsequent studies have direct relevance to the issue of eptifibatide dosing and the potential for more robust clinical efficacy. In the PRIDE (Platelet Aggregation and Receptor Occupancy with Integrilin—a Dynamic Evaluation) study, the pharmacodynamic effects of three different dosing regimens of Integrilin during coronary intervention were intensively investigated *(23)*. Also incorporated into the PRIDE trial was a lower dose strategy for heparin anticoagulation, using a 70 U/kg heparin bolus to achieve an activated clotting time of 200–300 s. Inhibition of platelet aggregation was determined with blood suspended in a calcium chelator (sodium citrate) and in PPACK (D-Phe-Pro-Arg-CH_2Cl, an anticoagulant that does not chelate calcium) using 20 μ*M* adenosine diphosphate to stimulate platelet aggregation. Representative data from the 135 μg/kg bolus plus 0.75 μg/kg-min infusion and the 180 μg/kg bolus plus 2.0 μg/kg-min infusion regimens are depicted in Fig. 4. Two findings are illustrated. First, the ex vivo determinations of inhibition of platelet aggregation are quite dependent on the anticoagulant used to suspend the blood sample. Second, only the higher dose (180/2.0) regimen consistently inhibited platelet aggregation to less than 20% of baseline throughout the duration of treatment. No increase in bleeding was seen with the higher doses of eptifibatide.

The second trial with direct relevance to the issue of eptifibatide dosing during coronary intervention is the 10,948 patient PURSUIT trial of eptifibatide versus placebo as an adjunct to the medical management of patients presenting with an acute coronary syndrome *(24)*. In PURSUIT, the dosing of eptifibatide was a bolus of 180 μg/kg followed by an infusion at 2.0 μg/kg-min. Among the 1228 patients in PURSUIT who underwent coronary intervention during study drug infusion, the relative reduction in the composite endpoint of death or myocardial infarction at 30 d was 30% with eptifibatide treatment (16.8% vs 11.8%, $P = 0.01$). In summary, the body of evidence suggests that a higher dose than the 135/0.5 regimen studied in IMPACT II will be needed for maximal efficacy during coronary intervention; from a pharmacodynamic perspective, the 180 μg/kg bolus plus 2.0 μg/kg-min infusion (continued for 24 h after the procedure) appears to be the most logical dosing choice in this setting.

A second issue relates to the inverse dose-response results at 30 d between the lower

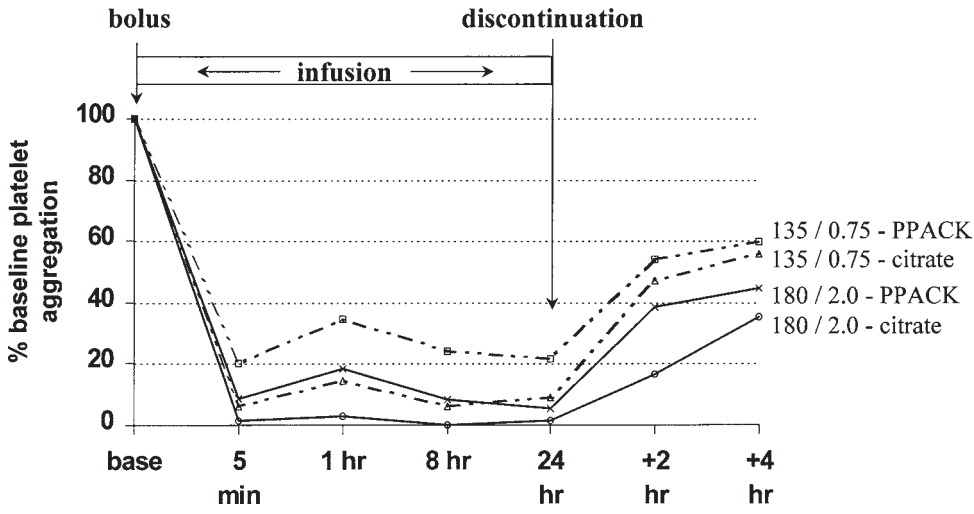

Fig. 4. Inhibition of platelet aggregation by eptifibatide—the PRIDE trial. Two dosing regimens from the PRIDE trial are shown, the 135 μg/kg bolus plus 0.75 μg/kg-min infusion and the 180 μg/kg bolus plus 2.0 μg/kg-min infusion. Blood samples were suspended in either sodium citrate anticoagulant or PPACK anticoagulant. Assays of blood in citrate reported higher degrees of platelet inhibition than blood in PPACK at the same concentration of eptifibatide. In PPACK, the higher dose (180/2.0) regimen suppressed platelet aggregation below 20% of baseline, whereas the lower dose (135/0.75) regimen did not. Data are normalized to 100% aggregation at baseline. PPACK: D-Phe-Pro-Arg-CH_2Cl.

dose (135/0.5) and higher dose (135/0.75) regimens in IMPACT II. Curiously, the 30-d (primary endpoint) mark coincided with the greatest separation of effect between the two dosing approaches; as noted above, the 6-mo event curves for the two dosing approaches became virtually identical. These findings appear to be attributable to a number of factors. First, the efficacy of eptifibatide in IMPACT II was largely secondary to the (identical) 135 μg/kg bolus used in both arms, not the continuous infusion. Second, neither of the infusion regimens adequately inhibited platelet function; if anything, the regimens were more similar than different in biological activity. Finally, the role of statistical chance cannot be discounted; in fact, the differences between the primary endpoint rates at 30 d is statistically negligible.

Another issue, particularly in light of the PROLOG *(7)* and EPILOG *(6)* trials of abciximab that report even lower rates of major bleeding with reduced heparin dosing and early sheath removal, is whether the safety profile of eptifibatide can be further improved beyond what has already been achieved. Finally, the efficacy of eptifibatide when combined with elective intracoronary stent implantation has not been delineated. Although extrapolation of the results of the abciximab trials would suggest that these approaches make sense, definitive clinical trials have not as of yet been conducted.

In conclusion, the results of the IMPACT II trial affirm the glycoprotein IIb/IIIa hypothesis. Eptifibatide inhibits coronary thrombosis; in IMPACT II, at the end of the 24-h treatment period, there was a highly significant relative reduction of 30–35% in the composite endpoint of death, myocardial infarction, urgent or emergency repeat coronary intervention or coronary bypass graft surgery, and stent placement for abrupt clo-

sure with both eptifibatide dosing strategies. The absolute magnitude of this benefit is maintained out to 6 mo. These findings, combined with those of the trials of the monoclonal antibody abciximab *(4,6–8)* and the nonpeptide mimetic tirofiban *(25),* support the routine use of glycoprotein IIb/IIIa inhibitors in general, and eptifibatide in particular, in the setting of percutaneous coronary intervention to reduce morbidity and mortality and improve clinical outcomes.

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7 Tirofiban in Coronary Intervention—The RESTORE Trial

Mahomed Y. Salame, MD, Spencer B. King III, MD, and Nicolas A. Chronos, MD

CONTENTS

INTRODUCTION

Atheromatous plaque rupture, platelet activation with consequent thrombus formation and impairment of coronary arterial blood flow is a common theme in acute coronary syndromes (ACS) *(1–4)*. The importance of antiplatelet therapy in the treatment of acute

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myocardial infarction (MI) was amply demonstrated in the second International Study of Infarct Survival (ISIS-2). At present, aspirin and to a lesser extent heparin, are used in nearly all patients with ACS. In spite of the improvements in prognosis that these treatments have brought, the incidence of adverse events in patients with ACS is still significant *(5–8)* and demonstrates the need for further improvement. There has been a rapid expansion of data from large multicenter trials on the use of IIb/IIIa receptor antagonists in the full spectrum of ACS. This is in part related to a recognition of the limitations of other antiplatelet agents, a better understanding of the mechanisms of platelet activation and aggregation. The realization that the GPIIb/IIIa platelet receptor is the final common pathway through which all the platelet agonists exhibit their effects on platelet aggregation make this receptor a promising target for antiplatelet therapy *(9)*.

LIMITATIONS OF OTHER CLASSES OF ANTIPLATELET AGENTS

Aspirin is a relatively weak antiplatelet agent. It works by acetylating and thus inactivating prostaglandin synthetase/cyclooxygenase *(10)* and results in the decreased formation of thromboxane A_2 *(11),* a potent agonist of platelet aggregation *(12)*. However, platelets are able to undergo aggregation by a number of thromboxane A_2-independent pathways (via platelet activators such as thrombin, ADP, epinephrine, and subendothelial collagen) *(13–15)* thus limiting the antithrombotic effect of aspirin. In addition, cyclooxygenase is also needed for the synthesis of a number of antithrombotic prostaglandins by endothelial cells including prostacyclin *(16),* a potent platelet inhibitor. Apart from its pharmacodynamic limitations, aspirin causes the side effect of gastritis, which results in a significant proportion of patients being noncompliant and stopping the medication.

Ticlopidine inhibits the ADP-induction of platelet aggregation *(17)*. Its use in cardiology is presently restricted to patients receiving coronary stent implantation. Its major limitation is the significant side effect of neutropenia *(18)*. Recently, clopidogrel, a ticlopidine analog, has been shown to decrease ischemic events in patients with atherosclerotic vascular disease *(19)*.

THE PLATELET GLYCOPROTEIN IIb/IIIa RECEPTOR AND ITS INHIBITORS

Whereas there are a number of receptors involved in platelet activation and adhesion to the plaque surface, the GP IIb/IIIa platelet receptor is one that mediates platelet aggregation. The GP IIb/IIIa receptor belongs to the integrin family of adhesion molecules and binds the bivalent molecule fibrinogen, as well as fibrinogen, von Willebrand factor, and vitronectin, and is therefore able to form crossbridges between adjacent platelets. Platelet activation results in the expression of approximately 70,000 copies of the GP IIb/IIIa receptor on the cell surface.

The GP IIb/IIIa inhibitors are a relatively new class of compounds that offers several potential advantages to other currently available therapies. These compounds are specific to platelets; they inhibit platelet aggregation induced by all platelet agonists, whereas at the same time they do not affect platelet adhesion.

Coller et al. developed the 7E3 monoclonal antibody to the GP IIb/IIIa receptor *(20),* which was further refined to a chimeric antibody (abciximab) in order to minimize the immune reaction to the foreign protein *(21)*. Likewise, a number of peptide and

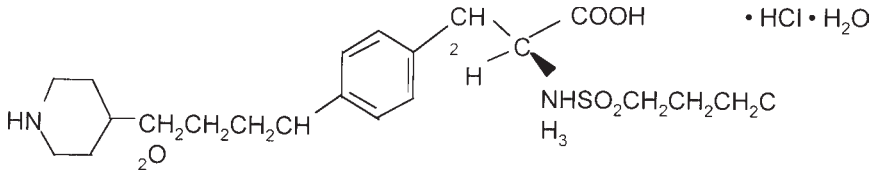

Fig. 1. Structure of tirofiban.

nonpeptides that either contain or mimic the arginine-glycine-asparagine (RGD) amino acid sequence necessary for ligand binding to the GP IIb/IIIa receptor have been developed. The small molecule (either peptidic or nonpeptidic) GP IIb/IIIa inhibitors are designed to avoid antibody-induced disadvantages such as immunogenicity and to have rapid onset of action and rapid off-rate with cessation of drug delivery.

TIROFIBAN: DEVELOPMENT AND INITIAL STUDIES

Tirofiban (Aggrastat®, Merck & Co., Inc., Rahway, NJ) is a synthetic, short-acting, highly selective nonpeptide tyrosine derivative (N-(butylsulfonyl)-O-[4-(4-piperidinyl)butyl]-L-tyrosine monohydrochloride monohydrate) that inhibits fibrinogen binding to the platelet GP IIb/IIIa receptor. Its structural formula is shown in Fig. 1. Structure-function studies on peptides derived from the venom of vipers led to the development of RGD and KGD (Lys-Gly-Asp)-containing synthetic peptide GP IIb/IIIa inhibitors. NMR studies on one of the viper's disintegrins, echistatin, revealed the characteristics of the RGD unit *(22)*. A directed search for compounds to mimic the RGD stereochemistry, followed by a potency-optimization strategy *(23),* resulted in the development of the nonpeptide tyrosine analog, tirofiban, a molecule that inhibits the binding of fibrinogen to GP IIb/IIIa in a concentration-related manner with a IC_{50} of 10 ng/mL and a K_I of 2.1 nM in vitro *(24)*. Whereas abciximab acts like a cap over the whole of the GP IIb/IIIa integrin and the peptidic analogs bind competitively to the RGD recognition site, tirofiban mimics the geometric, stereotactic and charge characteristics of the RGD sequence. Following the promising in vitro results with tirofiban, the anti-aggregatory and antithrombotic efficacy with tirofiban was demonstrated in canine models of coronary artery injury *(25)*. Subsequently, the safety, tolerability, pharmacokinetics, and pharmacodynamics were assessed in placebo-controlled trials in healthy volunteers *(26–28)* and then in patients with coronary artery disease *(29,30)*. Dose ranging studies of adjunctive tirofiban were performed in patients with unstable angina treated with heparin and aspirin *(31,32)* or with heparin, aspirin, and angioplasty *(33)*. Based on these studies, three large multicenter clinical trials [RESTORE *(34),* PRISM *(35),* and PRISM-PLUS *(36)*] were conducted in patients with ACS. The Randomized Efficacy Study of Tirofiban for Outcomes and Restenosis (RESTORE) trial differed from the other two tirofiban trials (PRISM and PRISM-PLUS) in that it was designed for drug evaluation in patients undergoing coronary intervention. RESTORE will therefore be discussed more in relation to the other similarly designed trials EPIC *(37),* EPILOG *(38),* CAPTURE *(39),* and IMPACT-II *(40)*.

The potential advantages of tirofiban are that it is a highly selective inhibitor of fibrinogen binding to platelet GP IIb/IIIa, it has an early onset of action with a rapid reversal of antiplatelet activity after discontinuation, it is suitable for repeat administrations, and, being a small molecule, it is relatively inexpensive to manufacture.

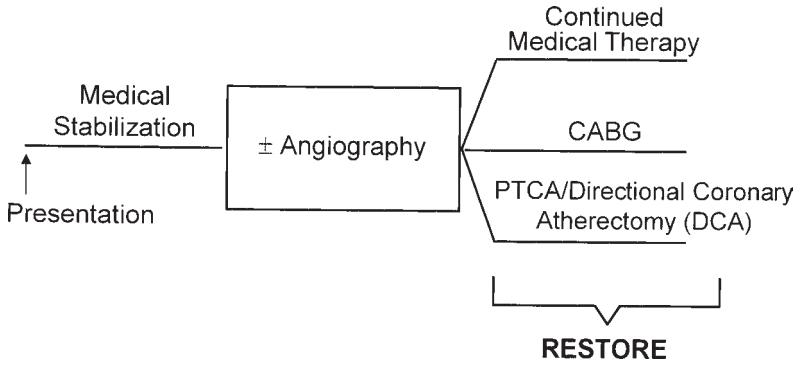

Fig. 2. RESTORE trial in management of ACS. CABG = coronary artery bypass graft surgery; PTCA = coronary angioplasty.

THE RESTORE TRIAL: STUDY DESIGN FOR EVALUATION OF TIROFIBAN IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTIONS

The prognostic benefit of using antiplatelet therapy in patients with ACS has been known for some time *(41)*. However, in spite of the use of aspirin, the morbidity and mortality of patients in this group remain a significant problem *(42,43)*. The hope that an incremental prognostic benefit in patients with unstable angina/non-Q-wave MI could be obtained with the use of thrombolytic therapy *(44,45)* or coronary intervention *(45–47)* has not (yet) been realized. A contributing cause for the lack of prognostic benefit in using fibrinolytic therapy for unstable angina/non-Q-wave MI could have been the lack of sufficient antiplatelet efficacy of the therapeutic regimen, as platelets are able to undergo aggregation by a number of thromboxane A2-independent pathways, thus limiting the antithrombotic effect of aspirin.

The RESTORE trial was started in January 1995 and completed enrollment in December 1995. RESTORE was a randomized, double-blind, placebo-controlled trial designed to evaluate the efficacy and safety of tirofiban in approximately 2100 patients receiving aspirin and heparin and undergoing coronary interventions using balloon angioplasty or directional atherectomy within 72 h of presentation with an episode of unstable angina or acute MI (see Figs. 2 and 3 for design).

Unstable angina was defined as typical anginal pain at rest or with minimal effort and either 1) electrographic changes; 2) hemodynamic changes suggestive of myocardial ischemia; or 3) angiographic evidence of thrombus in the target vessel immediately before percutaneous transluminal coronary angioplasty (PTCA) or directional coronary atherectomy (DCA) characterized by a stenosis of >70% with a hazy appearance, intraluminal filling defect, overhanging edge, high degree of eccentricity or reduced thrombolysis in myocardial infarction (TIMI) flow grade. Acute MI was defined as typical ischemic pain lasting >20 min with ST-T-wave changes or pathologic Q-waves and a serum creatine kinase elevation greater than twice the upper limit of normal or an elevated creatine kinase-MB fraction value.

Patients received 300 to 325 mg of aspirin orally within 12 h of PTCA or DCA.

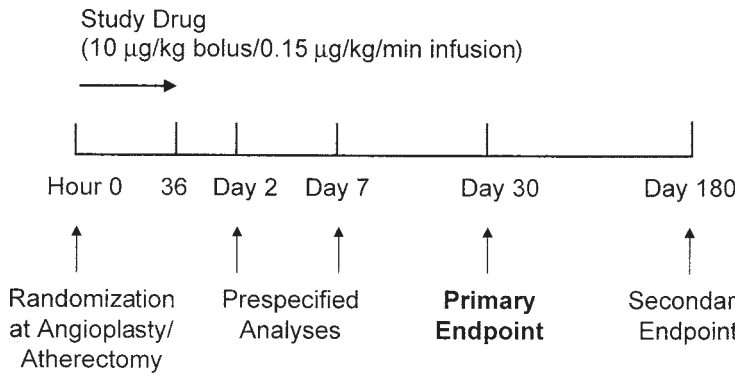

Fig. 3. RESTORE trial design.

Guidelines for heparin administration during PTCA were a maximal initial bolus of 10,000 U before the procedure (weight adjusted to 150 U/kg for patients <70 kg), and intraprocedural heparin administered as required to maintain an activated clotting time (ACT) of 300 to 400 s. After the lesion was crossed with the guide wire, the patient was randomized to receive either a bolus of tirofiban (10 μg/kg body weight) or placebo intravenously over 3 min. Each patient then received a corresponding intravenous infusion of tirofiban (0.15 μg/kg per min) or placebo for 36 h. Operators were urged to place intracoronary stents only in urgent "bail-out" situations such as actual or threatened abrupt closure. The choice between DCA or PTCA was left to the discretion of the operator. Investigators were advised to discontinue heparin administration at the conclusion of the PTCA or DCA procedure and to remove sheaths when the ACT was <180 s.

The clinical endpoints of the study were death from any cause; new MI; CABG for angioplasty failure or recurrent ischemia; repeat target vessel revascularization for recurrent ischemia; implantation of an intracoronary stent because of actual or threatened abrupt closure of the target vessel; and a composite endpoint, which was the occurrence of any of these events *(34)*. Endpoints were evaluated at 2, 7, and 30 days, and at 6 mo. The prespecified primary hypothesis of the study was that tirofiban would result in a reduction in the 30-d composite endpoint compared with placebo *(34)*. The statistical significance of differences between treatment groups with respect to the composite endpoint and its components was assessed using logistic regression analysis. All tests were two-sided, and statistical significance was declared if $P \leq 0.05$.

Selected study sites enrolled all consecutive patients in the angiographic substudy until a total of approximately 500 patients had been enrolled. Follow-up angiography was performed as close to 6 mo after the index procedure as possible; however, angiography performed between 17 and 30 wk after the initial PTCA or DCA was accepted. If repeat cardiac catheterization was necessary before the end of 16 wk and there was evidence of restenosis, this early angiogram was used as the follow-up angiogram and a repeat 6-mo follow-up angiogram was not necessary. However, if there was no evidence of restenosis, angiography was repeated between 17 and 30 wk. All follow-up angiography was completed before the end of study week 30. Patients who underwent intracoronary stent placement at the time of the initial procedure were not required to return for coronary arteriography.

All physicians and technicians in the angiographic core laboratory were blinded to treatment group assignment, the investigative center's interpretation of the angiogram and the clinical outcome of the patient. A previously described and validated automated edge detection algorithm was utilized for quantitative angiographic analysis *(48)*. Restenosis in the target culprit lesion was prospectively defined as follows: 1) ≥50% diameter stenosis at the time of follow-up angiography in those patients who had a <50% stenosis after the initial intervention; 2) late loss in minimal luminal diameter ≥0.72 mm *(49)*; 3) late loss ≥50% of the initial gain in minimal luminal diameter. Flow before and after PTCA was assessed according to both the conventional TIMI flow grade classification scheme *(50)* and the corrected TIMI frame count *(51)*. Thrombus grade was assessed using the standard TIMI definitions *(46)*.

THE RESTORE TRIAL: EFFICACY OF ADJUVANT TIROFIBAN IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

A total of 2141 patients received study drug infusion and were included in the efficacy and safety analyses as prespecified in the protocol *(34)*. The baseline characteristics of all patients included in the RESTORE trial were similar between patients treated with tirofiban or placebo. For patients in the 6-mo angiographic substudy, baseline clinical characteristics again did not differ between placebo and tirofiban groups and were similar to those of patients in the overall trial. Fifty-nine percent of substudy patients in the placebo group and 50.2% in the tirofiban group had one diseased vessel, 25.9% and 32.7% had two diseased vessels, and 11.2% and 15.2% had three diseased vessels, respectively (P = NS for treatment group differences). Unstable angina pectoris was the most common inclusion criterion in the placebo (67.3%) and tirofiban (66.8%) groups. The intervention was performed during acute MI (i.e., as primary angioplasty) in 2.0% of the placebo group and 2.4% of the tirofiban group. The intervention was performed as a nonprimary procedure within three days of acute MI in 30.7% of the placebo group and 30.8% of the tirofiban group. The initial procedure performed was most frequently conventional PTCA (92.2% in the placebo group, 91.9% in the tirofiban group), whereas DCA was performed in the remainder of patients.

During and immediately following the period of drug administration the composite endpoint and its components, excluding death, were substantially reduced. At two days, for placebo and tirofiban groups, respectively, the frequency of death was 0.2% and 0.2%, MI 4.4% and 2.7% (relative risk reduction—39%), repeat PTCA 3.2% and 1.1% (relative risk reduction—66%), CABG 1.4% and 0.9% (relative risk reduction—36%), and stenting for acute closure 2.5% and 1.5% (relative risk reduction—40%). At two and seven days postintervention, there were 38% (8.7 vs 5.4%; P = 0.005) and 27% (10.4 vs 7.6%; P = 0.022) reductions in the composite endpoints, respectively, reflecting the reductions in nonfatal MI and repeat angioplasty. However at 30 d, the primary composite endpoint showed a relative reduction that was half that seen at 24 h, being 12.2 vs 10.3% in the placebo and tirofiban groups, respectively, a 16.2% relative reduction (P = 0.169, *see* Fig. 4). The effect, however, was consistent across all the components of the composite endpoints (except death, which was a low-frequency event, 0.7% for placebo vs 0.8% for tirofiban). The relative benefit was comparable for all subgroups of patients treated. Likewise, at the 6-mo follow-up, the composite endpoint of death from

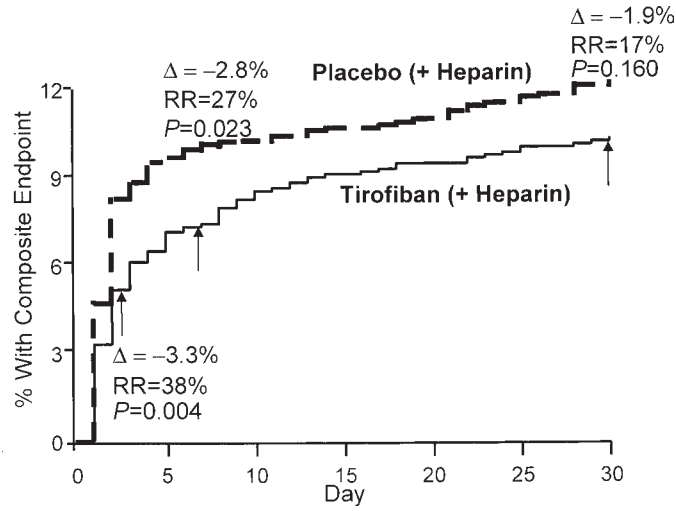

Fig. 4. Kaplan-Meier curve of 30-d composite endpoint of death, MI, or repeat target vessel revascularization.

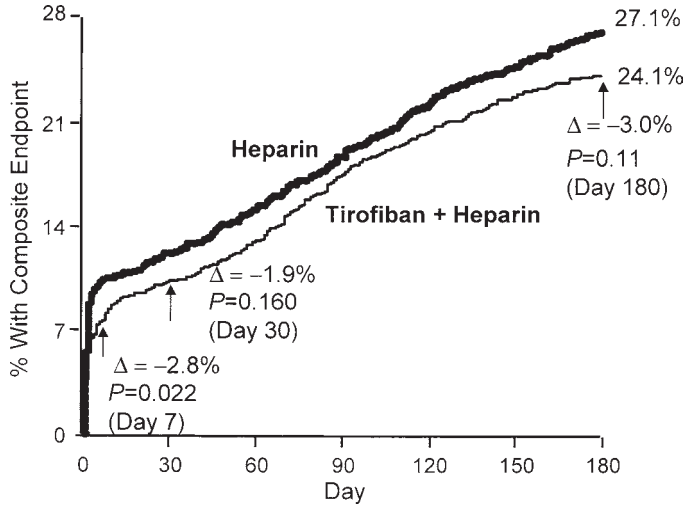

Fig. 5. Kaplan-Meier curve of 6-mo composite endpoint of death, MI, or target vessel revascularization.

any cause, new MI or target vessel revascularization was 24.1% in the tirofiban arm vs 27.1% in the placebo group, $P = 0.11$ (Fig. 5). It is important to note that the absolute reduction in events was therefore 3%, similar to the absolute reduction at 2, 7, and 30 d, and also comparable to results of the EPILOG study at 6 mo. These same trends were observed in patients treated both with conventional PTCA (267 [26.8%] of 997 vs 237 [24.1%] of 985) and those treated with DCA (23 [31.5%] of 73 vs 21 [24.4%] of 86.) Except for death (which rarely occurred), all differences in the incidence of endpoint components were in the same direction, favoring tirofiban, but none was statistically significant.

Of the total of 2141 patients in the RESTORE trial, 619 (315 for the placebo group,

Table 1
6-mo Follow-Up: RESTORE Angiographic Substudy

	Placebo group (n = *205)*	*Tirofiban group* (n = *211)*	P *Value*
≥50% loss of gain	103 (50%)	105 (50%)	0.99
% DS ≥50%[a]	110 (57%)	100 (51%)	0.26
Loss ≥ 0.72 (mm)	90 (44%)	88 (42%)	0.69

[a]Requires initial postcoronary angioplasty stenosis < 50% (193 for placebo, 196 for tirofiban). Data presented are number (%) of patients; DS = diameter stenosis.

314 for the tirofiban group) were enrolled in the 6-mo angiographic restenosis substudy. Paired serial angiograms were available for 416 (67.2%) of these 619 patients (205 for the placebo group, 211 for the tirofiban group). Among tirofiban-treated patients, 1057 (99%) of 1071 were available for 6-mo follow-up, and among placebo patients, 1051 (98%) of 1070 were available. Coronary angiography at the 6-mo follow-up showed that there was no difference between the placebo and tirofiban groups in either the preinterventional corrected TIMI frame count (CTFC: 47.8 ± 33.0 vs 52.0 ± 34.5, respectively, P = NS) or the TIMI flow grade distribution. After PTCA or DCA there was no significant difference in the CTFC between the two groups (18.2 ± 11.3 for placebo vs 20.2 ± 12.5 for tirofiban). Moreover, tirofiban at 6-mo follow-up did not reduce the incidence of restenosis as prospectively defined (Table 1) *(48)*.

COMPARATIVE EFFICACY OF ADJUNCTIVE TIROFIBAN VS OTHER GP IIb/IIIa INHIBITORS IN PATIENTS UNDERGOING CORONARY INTERVENTIONS

Tables 2 and 3 outline the differences in the patient population and treatment protocols for the trials evaluating adjunctive GP IIb/IIIa inhibitors in patients with coronary artery disease undergoing percutaneous intervention. The efficacy of GP IIb/IIIa inhibitors in these trials was measured in terms of the frequency of endpoints (single or composite). A comparison of efficacy between the different agents must therefore take into account any difference in the way the endpoints were defined. The endpoint definitions in RESTORE differed from those of the other trials in this group in that all ischemia-related repeat target vessel percutaneous interventions were included, not just urgent/emergent ones. In contrast, the 30-d analysis of the other trials (EPIC, EPILOG, CAPTURE, and IMPACT-II) included *only* those repeat revascularization procedures (CABG and repeat angioplasty) that were considered to have been performed on an urgent/emergent basis. In order to better evaluate the RESTORE data in comparison with these other trials, the Endpoint Committee blindly readjudicated the 30-d revascularization endpoints to determine whether or not they were performed on an emergent basis. The RESTORE event rates were then recalculated, including only those cases deemed emergent revascularizations (*see* Table 4 and Fig. 6).

EFFICACY RESULTS FROM RESTORE IN CONTEXT

The efficacy results at 30 d for the adjunctive GP IIb/IIIa trials of coronary intervention in ACS showed a significant drug-related benefit over placebo in EPIC, EPILOG,

Table 2
Trials of Adjunctive GP IIb/IIIa Inhibitors in Patients with Coronary Artery Disease Undergoing Percutaneous Coronary Intervention

Trial	N	*Phase*	*Drug*	*Start*	*Finished*	*Published*
EPIC	2099	Phase III	Abciximab	Nov 1991	Nov 1992	Apr 1994 *(37)*
6-month follow-up						Apr 1994 *(54)*
IMPACT II	4010	Phase III	Eptifibatide	Nov 1993	Nov 1994	May 1997 *(40)*
CAPTURE	1265	Phase III	Abciximab	May 1993	Dec 1995	May 1997 *(39)*
EPILOG	2792	Phase III	Abciximab	Feb 1995	Dec 1995	June 1997 *(38)*
RESTORE	2141	Phase III	Tirofiban	Jan 1995	Dec 1995	Sept 1997 *(34)*
6 month-follow-up						July 1998 *(55)*

Table 3
Differences in Patient Populations and Treatment Protocols between the Trials of GP IIb/IIIa Inhibitors in Patients with Coronary Artery Disease Undergoing Percutaneous Coronary Intervention

	Patient population	*Treatment protocol*
EPIC	High-risk group including acute MI, unstable angina on ECG criteria, high-risk clinical or angiographic characteristics	Abciximab bolus, abciximab bolus + infusion, placebo + infusion. All patients had *nonweight adjusted* heparin 10,000–12,000U bolus; ACT goal 300–350 s
EPILOG	Elective or urgent percutaneous coronary intervention; excluded AMI and unstable angina with ECG changes	Abciximab + infusion, heparin at 70 U/kg or 100 U/kg
CAPTURE	Unstable angina with ECG changes refractory to intravenous heparin and nitrates	Abciximab bolus and infusion, heparin bolus at 100 U/kg max or 10,000 U
RESTORE	Within 72 h of an episode of ACS	Tirofiban + weight adjusted heparin (150 U/Kg), ACT maintained at 300–400 s
IMPACT-II	Elective, urgent or emergent percutaneous coronary intervention	Eptifibatide bolus of 135 mg/kg with infusion of 0.5 μg/kg/min or 0.75 μg/kg/min, + heparin 100 U/kg heparin, ACT kept at 300–350 s

CAPTURE, and IMPACT-II. When the RESTORE 30-d data were analyzed on the same basis as the 30-d data from EPIC, EPILOG, CAPTURE, and IMPACT-II trials, i.e., defining the 30-d endpoint of revascularization as *urgent* revascularization, there was a 24% relative reduction (2.5% absolute reduction) in composite endpoint, $P = 0.052$, indicating that, unlike initial impressions suggesting a lack of benefit from tirofiban at 30 d, there was indeed a preserved late benefit. When the 2- and 7-d data were similarly reanalyzed by the adjudication committee, there was a 40% ($P = 0.002$) at 2 d and 30% ($P = 0.015$) at 7 d relative reduction in the frequency of the composite endpoint, similar to the primary analysis and clearly demonstrating an early effect. These reductions in

Table 4
Efficacy Data for the Use of Adjunctive GP IIb/IIIa Inhibitors in Patients with Coronary Artery Disease Treated with Percutaneous Coronary Intervention

	Composite end point				
Trial	*Treatment*	*Placebo*	*Absolute reduction*	*Relative reduction*	P *Value*[a]
RESTORE 30 d	10.3%	12.2%	1.9%	16%	0.169
RESTORE readjudicated 30 d	8.0%	10.5%	2.5%	24%	0.052
RESTORE 6 mo	24.1%	27.1%	3.0%	11%	0.11
EPIC 30 d	8.3%	12.8%	4.5%	35%	0.008
EPIC 6 mo	27%	35.1%	8.1%	23%	0.001
EPILOG 30 d					
Low-dose heparin	5.2%		6.5%	56%	<0.001
Standard-dose heparin	5.4%	11.7%	6.3%	54%	<0.001
EPILOG 6 mo					
Low dose heparin (death, MI, or *urgent* revascularization)	8.4%		6.3%	43%	<0.001
Standard-dose heparin	8.3%	14.7%	6.4%	44%	<0.001
EPILOG 6 mo					
Low dose heparin (death, MI, or *any* revascularization)	22.8%		3.0%	43%	0.07
Standard-dose heparin	22.3%	25.8%	3.5%	14%	0.04
CAPTURE 30 d	11.3%	15.9%	4.6%	23%	0.012
CAPTURE 6 mo	30.6%	30.4%	–0.2%	~0%	NS
IMPACT-II 30 d					
135/0.5 eptifibatide	9.2%	11.4%	2.2%	19%	0.063
135/0.75 eptifibatide	9.9%	11.4%	1.5%	13%	0.22

[a]All *P* values computed based on intention to treat.

adverse outcomes were larger than those obtained in the initial analysis that included all revascularizations and more consistent with both the EPIC trial, in which the relative reduction was 35%, and IMPACT-II trial, in which the corresponding relative reductions were 13% (high-dose eptifibatide) and 19% (low dose eptifibatide). At 6 mo, the EPIC data suggested a late added benefit of abciximab on repeat revascularization, which was not seen in the EPILOG or CAPTURE trials. The reasons for the disparate effects on nonurgent-revascularization rates between the EPIC trial and the EPILOG and CAPTURE trials are not known. In common with EPILOG, CAPTURE, and IMPACT-II, the RESTORE trial did not show a significant benefit at 6 mo, thus it also did not support the role of thrombus as the sole, or prime mediator underlying restenosis.

Another confounding problem in comparing the results of the RESTORE trial with others is the sampling protocol for creatinine kinase (CK). Whereas EPIC, EPILOG, and IMPACT II trials used sampling at multiple time points, the RESTORE investigators obtained CK levels only at the end of the 36-h infusion of the drug and at the discretion of the operator. Therefore one cannot be sure that the lower rate of MI seen in the RESTORE trial for both the placebo and tirofiban arms is not entirely because this

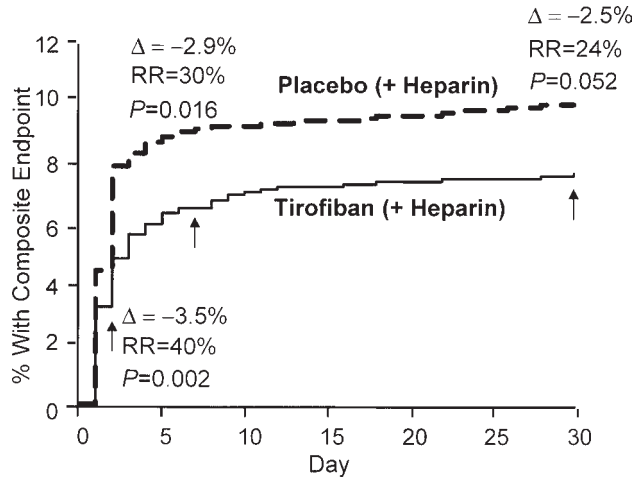

Fig. 6. Kaplan-Meier curve of 30-d readjudicated composite end point of death, MI, or urgent repeat revascularization.

less frequent CK sampling methodology. A lower reporting of elevated CK values could have reduced the power to detect differences in treatment effect on CK-defined acute MI.

It is possible that the lack of concordance in the 6-mo data between the EPIC trial on the one hand and RESTORE and IMPACT-II trials on the other may not be related simply to differences in trial design or methods of endpoint adjudication, but to differences in pharmacokinetics or pharmacodynamics of the agents used. It may be pertinent that the drug used in RESTORE (tirofiban) has a short biological half-life compared to abciximab. Although the pharmacokinetics of abciximab are complex, it is clear that the drug avidly binds to platelets, and that by flow cytometry platelet-bound drug is still detectable in low levels two weeks after administration. Since platelet survival remains normal, this implies that abciximab is transferred to newly released platelets. This redistribution phenomenon could prolong the biologic effects of abciximab if significant clinical activity is associated with a platelet receptor occupancy well below the >80% occupancy necessary for "complete" inhibition of platelet aggregation. Whether longer treatment periods for the peptide and nonpeptide GP IIb/IIIa inhibitors or an adjunctive course of an orally active agent (e.g., ticlopidine, clopidogrel, or a future oral GP IIb/IIIa inhibitor) may yield added benefit remains speculative.

Another major pharmacodynamic difference between abciximab and the smaller peptide and nonpeptide inhibitors lies in the selectivity of these agents for the GP IIb/IIIa receptor. Abciximab is able to bind to other receptors including the $\alpha_v\beta_3$ (vitronectin) receptor present on platelets, endothelial cells and vascular smooth muscle cells. Furthermore, platelet-leukocyte and leukocyte-endothelial interactions may be inhibited by the interference of abciximab with the CD11/CD18 complex. The clinical importance of these binding characteristics of abciximab is presently unknown although inhibition of the vitronectin receptor has been shown to reduce neointimal hyperplasia in a balloon injury model in baboons.

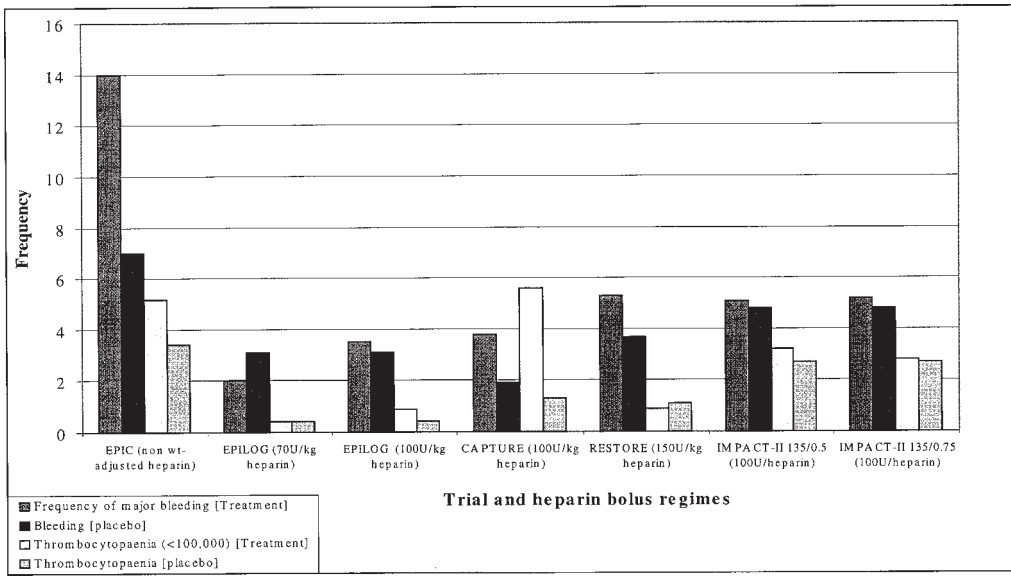

Fig. 7. Frequency of major bleeding and thrombocytopaenia in the trials of adjunctive GP IIb/IIIa inhibitors in patients undergoing percutaneous coronary intervention.

SAFETY ISSUES CONCERNING THE USE OF GP IIb/IIIa INHIBITORS IN PATIENTS WITH ACUTE CORONARY SYNDROMES UNDERGOING CORONARY INTERVENTION

Although the EPIC study amply demonstrated the effectiveness of abciximab in decreasing ischemic complications in high-risk angioplasty or atherectomy, it also demonstrated a significant increase in bleeding complications (Fig. 7). All patients were treated with 325 mg aspirin and heparin. The heparin was given as a bolus dose (10,000–12,000 U) at the start of the procedure, and further boluses of 3000 U were given to keep the ACT at 300–500 s during the procedure. Heparin was then continued after the procedure as an infusion for 12 h. The arterial sheath was removed 4 h after the end of the heparin infusion. The group given bolus plus infusion of abciximab had a substantial incidence of major bleeding (14%) (Fig. 7) compared to the no abciximab group (7%) and abciximab bolus only group (11%). It also was noted that the risk of bleeding increased in lighter patients, a finding that prompted the design of the PROLOG and EPILOG trials to incorporate weight-adjusted heparin regimens. With a weight-adjusted regimen, in the EPILOG trial, there were no significant differences in the incidence of major hemorrhage between the three arms, with the incidence of bleeding in the abciximab arms being similar to that of placebo. However, the incidence of minor bleeding was significantly increased in the abciximab plus standard heparin arm compared to the other two arms. Nevertheless, the EPILOG study demonstrated that the benefits of abciximab could be achieved without necessarily incurring a substantial penalty with serious hemorrhage.

SAFETY ISSUES IN RESTORE

In the RESTORE study, initial heparin boluses administered prior to PTCA procedures were weight-adjusted and given at 150 U/kg to an initial maximum bolus of

10,000 U, with supplemental heparin boluses administered during procedures to maintain ACT at 300-400 seconds. Heparin was stopped at the end of PTCA procedures and arterial sheaths removed early, i.e., when ACT was <180 s or at 4 h after stopping heparin. The incidence of major bleeding—defined as a decrease in hemoglobin level by > 5 g/dL, transfusion of > 2 U of blood, or corrective surgery or a retroperitoneal or intracranial bleed—was statistically not significantly increased in the tirofiban group but was nevertheless relatively high at 5.3% (compared to 3.7% in the placebo group). This may be a reflection of the heparin bolus being as high as 150 U/kg, a dose higher than that used in the EPILOG study. This in part may be explained by the timing of the trial protocol development in relation to the data from EPIC and EPILOG that together revealed advantages of a weight-adjusted heparin regimen. The incidence of major bleeding in the RESTORE study as defined by the TIMI criteria (decrease in hemoglobin level by > 5 g/dL, or intracranial hemorrhage or cardiac tamponade) was less impressive and was not different between the tirofiban and placebo groups at 2.2 and 1.6%, respectively ($P = 0.344$). The primary sites of bleeding in patients with protocol-defined major bleeding were: catheterization site (65% of tirofiban vs 63% of placebo); hematuria (23% of tirofiban vs 25% of placebo); and gastrointestinal (16% of tirofiban vs 8% of placebo).

CONCLUSION

There is now a large body of evidence that the GP IIb/IIIa inhibitors reduce the risk of adverse ischemic events in both high-risk and low-risk patients undergoing percutaneous coronary intervention (PCI). The RESTORE trial was the first large-scale randomized trial of a nonantibody-based GP IIb/IIIa inhibitor that demonstrated the clinically beneficial effects of potent antiplatelet therapy in reducing the acute thrombotic complications of PCI. The source of the apparent differences in efficacy results between the trials of the clinically available agents remain controversial. The readjudication of the RESTORE trial endpoints demonstrates the difficulties and pitfalls of comparing different trials with similar but not identical endpoint definitions.

In addition, the methodology used in determining the endpoints of MI may also be relevant. In the abciximab trials, the protocols mandated the frequent sampling of CK-MB, thus increasing the chance of detecting myonecrosis. The higher incidence of MI in the placebo arms of the abciximab trials may be a reflection of this. The RESTORE study on the other hand mandated only a single CK-MB measurement—which occurred relatively late—at 36 h after the procedure. This is likely to have underestimated the incidence of asymptomatic MI in RESTORE and may have potentially reduced the statistical power to detect a treatment effect associated with this endpoint.

An important unresolved issue concerning GP IIb/IIIa blockade is the optimal duration and degree of treatment effect both before and after intervention. The CAPTURE trial demonstrated the potential for pre-PCI "vessel wall passivation." The TACTICS (Treat Angina with Aggrastat® and determine Cost of Therapy with Invasive or Conservative Strategy) study will in part shed some light on tirofiban's ability to pacify the vessel wall by preintervention therapy. The EPIC study suggested that the use of abciximab resulted in reduced restenosis and target vessel revascularization beyond 6 mo; however, this finding has not been confirmed in subsequent abciximab trials. Indeed, a large body of evidence now suggests that late restenosis is not importantly affected by acute GP IIb/IIIa therapy.

Another ongoing challenge facing these agents is their cost effectiveness in the setting of decreasing budgets and managed care. Should all interventions be covered with GP IIb/IIIa blockade? There appears compelling evidence from RESTORE, EPIC, IMPACT II and EPILOG that patients presenting with both ACS as well as chronic stable angina benefit from this form of therapy. However, whether the benefit is worth the high cost, especially that associated with abciximab, merits consideration. It should be acknowledged that the predominant influence of these agents is in reducing non-Q-wave MI and CK-MB leaks. The clinical significance of the non-Q-wave infarction and CK-MB leak remains uncertain. It is probably fair to say that myonecrosis cannot be considered to be a good outcome of intervention, but a reduction in asymptomatic non-Q-wave infarcts through the indiscriminant use of expensive GP IIb/IIIa antagonists could potentially be an expensive burden to the health care system. In this regard, the results of a cost substudy analysis of 818 patients of the RESTORE trial are particularly relevant. The cost substudy demonstrated a trend toward lowering of mean hospital costs with tirofiban in stented and unstented patients (stented: $13,863 ± 5221 tirofiban, $14,339 ± 6156 placebo; unstented: $9,759 ± 5508 tirofiban, $10,141 ± 6366 placebo—where cost analysis in each case excludes tirofiban cost) *(52)*. Clinical, resource, and cost benefits of RESTORE results projected to the entire United States population also suggest benefit *(53)*. Nevertheless, further research is clearly needed to ascertain the significance of CK findings, relate them to other markers of myonecrosis, including the troponins, and define the medium and long-term outcome of patients who suffer such ischemic events. Once such data exists, it will be much easier to advocate the widespread use of these potent antiplatelet agents in all patient groups undergoing coronary intervention.

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8

The Use of Abciximab in Therapy Resistant Unstable Angina

Clinical and Angiographic Results of the CAPTURE Pilot and the CAPTURE Study

Marcel J. B. M. van den Brand, MD
and Maarten L. Simoons, MD

CONTENTS

INTRODUCTION

In 1989, Braunwald provided a classification for patients with unstable angina, based on the clinical circumstances under which the syndrome occurred and the severity of the symptoms *(1)*. A further subdivision took the intensity of medical treatment and the presence or absence of reversible electrocardiographic changes during anginal attacks into consideration. Outcome of patients with unstable angina could be predicted using this classification *(2)*.

The underlying cause of unstable angina is thought to be rupture and ulceration of a preexistent atherosclerotic plaque, leading to platelet adhesion and aggregation, and thrombus formation *(3)*. In most patients, the syndrome can be stabilized with bed rest and anti-ischemic and antithrombotic therapy *(4)*. However, in a minority of patients ischemic symptoms continue in spite of intensive medical therapy. Such patients with unstable angina refractory to medical treatment are usually referred for urgent angioplasty or bypass operation *(2),* even though interventions are associated with a higher complication rate compared to stable or stabilized unstable angina patients *(5,6)*. Prevention and or resolution of platelet aggregates and thrombi may help to diminish ongoing ischemic attacks as well as angioplasty complications.

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The chimeric 7E3 (c7E3) monoclonal antibody Fab fragment (abciximab) is a potent inhibitor of platelet aggregation. It blocks the glycoprotein IIb/IIIa fibrinogen receptor on the platelet surface, and by preventing the binding of fibrinogen to the platelet surface, platelet aggregation, and platelet thrombus formation are inhibited *(7)*.

In a double-blind, randomized, placebo-controlled CAPTURE pilot study, the safety and preliminary efficacy of c7E3 Fab treatment were studied in patients with refractory unstable angina undergoing percutaneous coronary angioplasty (PTCA). It was hypothesized that c7E3 Fab in combination with nitrates, heparin, and aspirin would facilitate stabilization of the culprit lesion and thus reduce recurrent ischemia prior to PTCA and reduce the complication rate during and after the PTCA procedure. The effect of c7E3 Fab on the severity of the culprit lesion was assessed by qualitative and quantitative angiographic analysis. After this pilot study, yielding favorable results, a larger trial was designed in patients presenting with the same refractory unstable angina syndrome. The doses of abciximab and concomitant medication, as well as the duration of administration in relation to the angioplasty procedure, were identical to the study outline of the CAPTURE pilot trial.

All angiograms were revised centrally by the Angiographic Committee, to assess qualitative lesional aspects, and the outcome of the angioplasty procedure. The CAPTURE (c7E3 Fab antiplatelet therapy in refractory unstable angina) study was discontinued on the recommendation of the Safety and Efficacy Monitoring Committee after interim analysis of 1050 patients (1400 patients planned).

PATIENTS AND METHODS

Sixty patients were enrolled in the pre-CAPTURE trial from September 1991 to July 1992 in seven hospitals in the Netherlands, Belgium, Germany, and the United Kingdom. The CAPTURE trial recruited 1265 patients from 69 hospitals in 12 countries between May 1993 and December 1995. Patients were eligible for the pilot study as well as for the main study if they had refractory unstable angina defined as chest pain at rest with concomitant electrocardiographic (ECG) abnormalities compatible with myocardial ischemia (ST-segment depression, ST-segment elevation, or abnormal T-waves), and one or more episodes of typical chest pain, ECG abnormalities, or both, compatible with myocardial ischemia during therapy with intravenous heparin and glyceryl trinitrate, started at least 2 h previously. The latest episode of ischemia should have occurred within 48 h before enrollment, corresponding to Braunwald class III "acute" unstable angina *(1,2)*.

All patients had undergone angiography and had significant coronary artery disease with a culprit lesion suitable for angioplasty. Patients were enrolled within 24 h of angiography, and angioplasty was scheduled 18–24 h after the start of study medication. In the pilot trial, diagnostic angiography was performed within 12 h of the most recent episode of coronary ischemia. After this angiogram, enrollment and start of the trial medication had to be accomplished within 4 h. If necessary because of recurrent ischemia, angioplasty could be done earlier, at the discretion of the investigator. Reasons for exclusion from both studies were: recent myocardial infarction (MI), unless creatine kinase values had returned to below two times the upper limit of normal (in pre-CAPTURE patients were excluded if they experienced a Q-wave MI within 7 days in the region subtended by the culprit artery); features of persisting ischemia that would require

immediate intervention; a greater than 50% occlusion of the left main coronary artery or a culprit lesion located in a bypass graft. Total occluded vessels were only acceptable in the pretrial if the occlusion was suspected to be of recent origin by the presence of thrombi or contrast staining at the site of the total occlusion. In CAPTURE all total occluded vessels could be included, if these were considered as culprit arteries.

Re PTCA of the same segment was excluded in the pilot trial, but not in the CAPTURE trial. Other exclusions included bleeding risk factors such as surgery, gastrointestinal, or genitourinary bleeding during the 6 wk before enrollment, or a cerebrovascular accident within the previous 2 yr; planned administration of oral anticoagulants, intravenous Dextran, or a thrombolytic agent before or during PTCA; underlying medical conditions such as persistent hypertension despite treatment; history of hemorrhagic diathesis; history of autoimmune disease, or a platelet count below 100×10^9/L. After enrollment, patients received aspirin at a daily dose of 50 mg. In patients not previously on aspirin, the first dose was at least 250 mg. Heparin was administered from before randomization until at least 1 h after the PTCA procedure, and adjusted to achieve an activated partial thromboplastin time between 2–0 and 2–5 times normal. The protocol recommended that the initial bolus dose before PTCA should not exceed 100 U/kg or 10,000 U, whichever was lower. Subsequent heparin boluses were given during PTCA after the clotting time had been checked. The recommended anticoagulation target was an activated clotting time of 300 s or an activated partial thromboplastin time of 70 s.

Heparin was administered until at least 1 h after PTCA. All patients received intravenous glyceryl trinitrate; beta-blockers, calcium-channel blockers, and other cardiovascular drugs were allowed. In addition, patients were randomly assigned abciximab (0.25 mg/kg bolus followed by a continuous infusion of 10 μg/min) or matching placebo. Randomization was obtained by telephone call to an independent service organized by the Department of Clinical Epidemiology of the University of Amsterdam. The randomization treatment was started within 2 h of allocation and given during the 18–24 h before angioplasty and for 1 h after completion of the procedure.

Arterial sheaths were kept in place after the diagnostic angiogram, during administration of study drug, and were exchanged before angioplasty. Balloon angioplasty was done by standard techniques. The use of stents was not encouraged, unless required to maintain immediate patency of the dilated segment. Sheaths remained in place from the time of the qualifying angiogram until 4–6 h after discontinuation of heparin and study drug. Special care was given to obtain complete hemostasis at the site of arterial access. During the hospital stay in the pilot trial and also during 30-d follow-up in the CAPTURE trial all events and medication were recorded, with special attention to bleeding complications and recurrent ischemic symptoms.

The primary endpoint in the trials was the occurrence of death (from any cause), myocardial infarction, or an urgent intervention for treatment of recurrent ischemia (angioplasty, coronary artery bypass surgery, intracoronary stent placement, intra-aortic balloon pump) during the initial hospital stay for the pilot trial, and within 30 d in the CAPTURE trial.

ECGs were obtained before enrollment and both during and after episodes of chest pain. Additional ECGs were recorded at enrollment; 6, 12, and 18 h after enrollment; just before PTCA; 1, 6, and 24 h after PTCA; at discharge; and whenever patients experienced recurrent chest pain. Myocardial infarction during the index hospital stay was defined as values of creatine kinase or its MB isoenzyme more than three times the upper

limit of normal in at least two samples and increased by 50% over the previous value, or an ECG with new significant Q-waves in two or more contiguous leads. MI after discharge was defined as concentrations of creatinine kinase or its MB isoenzyme above two times the upper limit of normal, or new significant Q-waves in two or more contiguous ECG leads. For the CAPTURE trial the following additional measures were taken to ensure independent reporting of data.

A Clinical Endpoint Committee reviewed all case-report forms, ECG's, and supporting documents for confirmation that patients met the study entry criteria for refractory unstable angina; the occurrence of endpoints; the frequency of recurrent ischemia; and important adverse events (bleeding, thrombocytopenia, and stroke).

Bleeding was classified as major, minor, or insignificant, by previously published criteria *(8)*. Major bleedings were defined as intracranial bleeding or episodes associated with a decrease in hemoglobin of more than 3.5 mmol/L (5 g/L). Bleeding was defined as minor if it was spontaneous and observed as gross hematuria or hematemesis, or if blood loss (spontaneous or not) was observed with a decrease in hemoglobin of more than 2.1 mmol/L, or if there was a decrease in hemoglobin of more than 2.8 mmol/L with no significant bleeding site identified.

Blood loss insufficient to meet criteria for minor bleeding was classified as insignificant. To account for transfusion, packed-cell volume and hemoglobin measurements were adjusted for any transfusion of packed red blood cells or whole blood within the 48 h before measurement by the method of Landefeld and colleagues *(9)*. Thrombocytopenia was defined as an acute fall in platelet count during or after administration of the study agent to below 100×10^9/L or a decrease of 25% or more from baseline. The protocol recommended that blood transfusion should be given according to the guidelines of the American College of Physicians *(10)*. These guidelines state that normovolaemic anaemia is acceptable for patients without symptoms and that those with symptoms should receive transfusions on a unit-by-unit basis to relieve symptoms.

A Safety and Efficacy Monitoring Committee was established to monitor safety data continuously, and to carry out interim analyses after enrollment of 350 and 700 patients. After the second interim analysis, the Committee recommended a third interim analysis after 1050 patients.

The protocol specified that the trial would be stopped if the difference in the rate of the primary endpoint between the abciximab and placebo groups was significant with a probability value of 0.0001, 0.001, or 0.0072 at the first (350 patients), second (700 patients), or third interim analysis, respectively. The study design was group sequential, with plans for accrual of up to 1400 patients. This sample would allow detection of a reduction in the primary endpoint from 15 to 10% with $\alpha = 0.05$ and power = 0.80.

The Lan-DeMets method was used to assign *P* values for interim analysis *(11)*. A log-rank test was done at interim and final analysis to test for differences in the rates of occurrence of the primary endpoint in the abciximab and placebo groups. Event rates were calculated for patients in each treatment group by the Kaplan-Meier method. Fisher's exact test was used to make pairwise comparisons between the groups for binary measurements. Logistic regression analysis was used to verify the association between bleeding complications, bodyweight, and heparin dose; analyses were by intention to treat.

Coronary Arteriography and Angioplasty

Coronary arteriography and left ventricular angiography were performed as soon as possible after the qualifying anginal attack using the Judkins technique. Heparin 2500–5000 IU was administered at the beginning of the procedure. A second angiogram was performed within 24 h after the start of study medication followed by angioplasty. The coronary artery responsible for the ischemia was identified through lesion characteristics, electrocardiographic location of reversible ST-T segment changes and left ventricular wall contraction abnormalities. For quantitative analysis of angiograms the following additional measures were taken in the pilot trial: At least two orthogonal projections were made of the culprit coronary segment, after injection of 1–3 mg of isosorbide dinitrate. During the first and second angiogram, the same projections and X-ray gantry settings were employed to compare lesion severity. Low osmolar contrast medium was used for all angiograms.

Qualitative and Quantitative Assessment of Coronary Angiograms

In the pilot trial both a qualitative and quantitative assessment of all angiograms was performed by the Core Laboratory at Cardialysis, Rotterdam.

The following items were visually scored after the first contrast injection: thrombolysis in myocardial infarction (TIMI) flow grade *(12)* of the culprit artery; stenosis severity, as visually assessed in multiple projections, and presence of intracorornary thrombus, defined as an intraluminal filling defect, visible during at least one complete cine-run, and surrounded on three sides by contrast medium *(13)*. A totally occluded coronary artery could contain a filling defect, but was not automatically scored as containing such a defect. In addition, all angiograms were analysed quantitatively, using the computer-assisted cardiovascular angiography analysis system *(14,15)*. Any area sized 6.9 × 6.9 mm in a selected cineframe (overall dimensions 18 × 24 mm) encompassing the desired arterial segment was digitized by a high-resolution CCD camera with a resolution of 512 × 512 pixels and 8 bits of gray level. Vessel contours were determined automatically, based on the weighted sum of the first and second derivative functions applied to the digitized information along scanlines perpendicular to the local centerline directions of an arterial segment. A computer-derived estimation of the original arterial dimensions at the site of the obstruction was used to define the interpolated reference diameter. This technique is based on a computer-derived estimation of the original diameter values over the analyzed region (assuming there was no disease present) according to the diameter function. The absolute diameter of the stenosis as well as the reference diameter were measured, using the known guiding catheter diameter as a calibration factor. All contour positions of the catheter and arterial segments were corrected for pincushion distortion. "Plaque area" is the difference in area in mm^2 between the reference and the detected contours over the length of the lesion *(16)*.

In the CAPTURE trial, no quantitative measurements were performed on the coronary arteriograms. The qualitative assessment was more extensive than in the pilot trial, and was carried out by an Angiographic Committee, consisting of six experienced interventional cardiologists. From the angiograms at baseline and before angioplasty the ischemia related artery (IRA), severity of all lesions present, collateral flow to the IRA, TIMI flow *(12)* in the IRA, as well as all AHA/ACC lesion characteristics *(17)* were scored individually from multiple projections.

These lesion characteristics included length (< 10 mm; 10–20 mm; >20 mm), eccentricity or concentricity, ostial or nonostial location, smoothness or irregularity, angulated or nonangulated, easy or difficult accessibility, presence or absence of thrombus, none, moderate or heavy calcification, and involvement or non involvement of a side branch at the lesion site. *TIMI flow* was scored visually as TIMI 0 if no contrast penetrated distal to the entire IRA for the duration of the filming sequence, TIMI 1 if contrast penetrated the site of the lesion without complete filling of the distal IRA for the duration of the cinerun, TIMI 2 if contrast medium filled completely the IRA, but rate of inflow or clearing of contrast was slower than in comparable areas, TIMI 3 if both inflow and clearance of contrast were at the same speed in the IRA compared with other vessels. *Thrombus* was scored as present if a filling defect without calcification could be visualized near the lesion, or an embolus in the distal territory of the IRA. A lesion was classified as *angulated* if the artery at the site of the lesion exhibited an angle of ≥45 degrees. *Lesion length* was estimated taking the length of the inflated balloon as a reference and defined as that segment of the artery with a narrowing of ≥50% of the reference diameter. A *bifurcation* was scored as being present at the lesion site, when the inflated balloon covered the ostium of a side branch with a minimal estimated diameter of 1.5 mm. A lesion was scored as *ostial* if the inception of the lesion started within 10 mm of the origin of the left anterior descending or right coronary artery. *Eccentricity* was scored if the remaining connection between the proximal and distal part of the IRA at the site of the lesion was not situated in the middle of the artery. The stenotic site is judged to be irregular if its luminal edge is irregular or has a sawtooth component. *Accessibility* is scored as easy if the lesion is distal to maximal one bend of ≥45°, as moderate tortuous if the stenosis is distal to two bends of ≥45° and as excessive tortuous if the lesion is distal to three or more bends of ≥45°. *Calcification* was scored if moderate or heavy radio densities were noted with fluoroscopy or cinearteriography at the site of the target lesion.

If one of the characteristics could only be verified in one angiographic projection, this was considered enough evidence for the existence of the pertinent item. The definitions of TIMI flow, lesion severity, and AHA/ACC characteristics were discussed with the members of the Angiographic Committee before angiograms were reviewed. The actual viewing was performed by one cardiologist and an angiographic technician from Cardialysis and agreement reached after deliberation. In case of disagreement consensus was reached by a verdict of a second cardiologist. After angioplasty the procedure was scored as angiographically successful if the TIMI flow in the IRA was 3 (normal), and the remaining stenosis occupied less than half the diameter of the vessel, compared with the adjacent segment.

The use of stent(s) if visible on the angiogram was also noted, as were side branch occlusions if the side branch was at least 1.5 mm in diameter. Dissections after the procedure were categorized according to modified National Heart Lung and Blood Institute criteria as A through F *(18)*. Dilatation of other significant stenoses outside the IRA during the same procedure was discouraged, but if deemed necessary by the investigator the result of dilatation of the additional lesions was also evaluated.

Statistical Analysis

Differences between groups are analyzed by intention to treat. Categorical variables are summarized by count and/or percentages. Continuous variables are summarized by

Table 1
Baseline Clinical, Electrocardiographic, and Angiographic Characteristics of Patient Groups

Group	*c7E3Fab*	*Placebo*
Male/female, *n*	20/10	24/6
Age (years), (median, range)	61, 38–73	60, 38–73
Previous infarct	9	16
within 7 days	6	5
Previous CABG	0	2
Previous PTCA	4	5
Medication prior to qualifying ischemic attack		
Heparin	25	27
Aspirin	21	24
Nitrates		
Intravenous	24	27
Oral	4	2
β-Blocker	22	24
Calcium channel blocker	15	22
Ischemia-related vessel		
Left anterior descending artery	16	14
Left circumflex	8	6
Right coronary artery	6	10
Multivessel disease	6*	15*

CABG = coronary artery bypass grafting.
PTCA = percutaneous transluminal coronary angioplasty.
*P = <0.05.

means and standard deviations. Fisher's exact test is used to assess differences in categorical variables with respect to treatment.

Multivariable logistics modeling is used to examine relationships between primary endpoint and individual lesion characteristics, age, gender and treatment and between bleeding complications, body weight and heparin dose. Variable selection is done in a stepwise fashion. The selected variables are tested for interactions with all other variables. For the modeling the continuous variable age is dichotomized. Odds ratios with their 95% confidence intervals are calculated from the logistic model. When an odds ratio needs to be calculated from more than one regression coefficient, the relevant variance and covariance components from the variance–covariance matrix are used for calculation of the 95% confidence intervals for the subgroup odds ratios.

RESULTS

Pre-CAPTURE Trial

Baseline Characteristics

Between September 1991 and July 1992, 60 patients were enrolled in six different hospitals. Baseline characteristics are summarized in Table 1. The groups were balanced although more patients in the placebo group had sustained a previous infarct compared to the treatment group (P = ns), and more patients in the placebo group demonstrated multivessel disease, defined as a more than 50% diameter stenosis in one of the three

Table 2
Recurrent Ischemia

	c7E3	*Placebo*
n	30	30
Pain + ST-T changes	2	5
Pain – ST-T changes	1	2
Pain, no ECG available	1	0
Silent ST-T changes	5	12
Patients with pain or ST-T changes	9	16

Patients with recurrent ischemia between diagnostic and second angiography. Multiple episodes and different types of ischemia could occur in one patient. Episodes of ischemia during angiography or PTCA were not included.

main epicardial vessels. Medication at the time of the qualifying ischemic attack was intense and similar in both groups.

Recurrent Ischemia

During infusion of the study drug, ischemia occurred in nine patients treated with c7E3 Fab and in 16 placebo patients ($P = 0.06$) (Table 2). Most patients had multiple episodes of ischemia. The total number of episodes was not significantly different between patients receiving c7E3 Fab (33 episodes) or placebo (56 episodes, $P = 0.17$). In one placebo patient, an urgent intervention was performed because of recurrent ischemia before the scheduled PTCA.

Whereas in hospital, a total of 12 major ischemic events occurred in seven placebo patients: death ($n = 1$), myocardial infarction ($n = 4$), urgent intervention because of severe recurrent ischemia ($n = 7$). One event (a myocardial infarction) occurred in a patient treated with c7E3 Fab (Table 3; 1 vs 7 patients, $P = 0.03$) . Three placebo patients experienced more than one major event. Multivessel disease was present in five of the seven placebo patients who developed an event, whereas the c7E3 Fab-treated patient with an event had single-vessel disease. After correction for the imbalance in baseline characteristics, the difference between the two groups was not statistically significant in this pilot study ($P = 0.16$). One patient from the placebo group died 26 days after allocation after a complicated clinical course, including two urgent PTCA procedures, myocardial infarction, heart failure, intra-aortic balloon pump and severe bleeding.

Qualitative Evaluation of Coronary Angiograms

TIMI flow grade 3 in culprit arteries, assessed centrally by the Core Laboratory, was present in 57% and 63% of patients at the first angiogram in the placebo and treatment groups, respectively. A substantial improvement in coronary blood flow occurred after treatment in the c7E3 Fab patient group. In the placebo group, a mix of either improvement or deterioration was observed in TIMI flow score (Table 4). Extensive filling defects in the coronary arteries were rare. Most filling defects were small, although visible in more than one projection and located distally from the culprit lesion. The number of intracoronary filling defects, and total occlusions, in the pre- and posttreatment angiogram are presented in Fig. 1. In the placebo group, one new occlusion was

Table 3
Major Events

	c7E3 Fab (n = *30*)	*Placebo* (n = *30*)
Death	0	1
Myocardial infarction	1	4
Before PTCA	0	1
After PTCA	1	3
Urgent procedure	0	7
PTCA	0	3
CABG	0	3
Stent	0	1
Total events	1	12
Total number of patients with one or more major events	1	7

CABG = coronary artery bypass grafting.
PTCA = percutaneous transluminal coronary angioplasty.

Table 4
Qualitative Angiographic Data

	c7E3 Fab (n = *30*)		*Placebo* (n = *30*)	
	B	*A*	*B*	*A*
TIMI flow				
0	1	1	4	3
1	2	1	0	1
2	10	5	7	6
3	17	23	19	20
Improved		6		4
Worsened		0		3
Intracoronary filling defect	5	2	1	1

B = before study drug infusion.
A = after study drug infusion.

observed, in two cases patency was restored with in one of these two cases thrombotic remnants. Three out of five coronary clots resolved in the c7E3 group.

Quantitative Coronary Angiographic Analysis

Quantitative coronary angiographic data are summarized in Table 5. In one patient with a very proximal left anterior descending coronary artery lesion, calculation of percentage diameter stenosis was not possible because the reference diameter could not be ascertained. Plaque area and extent of obstruction could only be calculated in patent coronary arteries. A significant decrease in percentage diameter stenosis, extent of obstruction and plaque area was observed in the c7E3 Fab patients. In the placebo group, similar changes were observed to a lesser extent, except for the extent of obstruction,

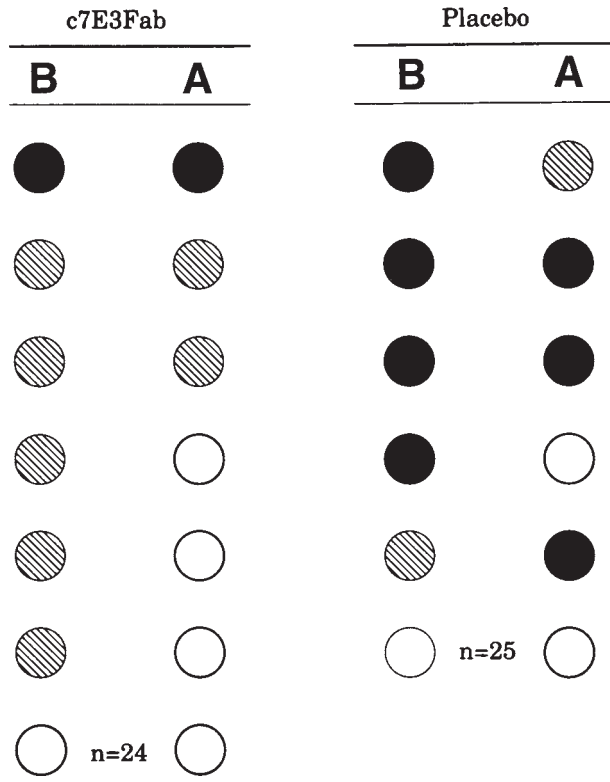

Fig. 1. Qualitative coronary angiographic data of the ischemia-related coronary artery before **(B)** and after **(A)** study drug infusion. ● = totally occluded coronary artery; ◍ = intracoronary filling defect; ❍ = patent coronary artery, without filling defect. Note: The number of patent vessels with no filling defect in either angiogram is depicted in the bottom line.

which increased in the placebo group patients. Differences between groups were not significant.

Angioplasty Procedure

Angioplasty was performed in all 60 patients. In one patient who received c7E3 Fab, PTCA was deferred because of a total occlusion due to a large intracoronary clot at the second angiogram. He was treated with intracoronary alteplase 50 mg infused over 30 min, followed by 50 mg intravenously over 2 h. The next day, the clots were partly resolved and PTCA was performed successfully. One other c7E3 Fab patient with a thrombocytosis of unknown origin received alteplase during PTCA because of persistent thrombus formation. PTCA was completed with success, and the subsequent clinical course was uncomplicated. The total success rate of the angioplasty procedure was 83% in the c7E3 Fab group and 70% in the placebo group (Table 6).

CAPTURE Trial

The CAPTURE trial was discontinued after the third interim analysis of 1050 patients. Complete data, fully reviewed by the Clinical Endpoint Committee, were available for 976 patients, and 74 patients had been reviewed partially. By that point, 87 (16.4%) of

Table 5
Quantitative Angiographic Data from the First Angiogram (I) and After Study Drug Infusion (II) and the Difference between Both Measurements (II-I)

Fab (n = *30)*	*Placebo* (n = *30)*			*c7E3*		
DS (%)	*n*			*n*		
I	30	65.7	(8.6)	29	67.7	(16.1)
II	30	62.3	(10.5)	29	65.6	(15.8)
II-I	30	–3.4	(6.7)*	29	–2.1	(12.4)
MLD (mm)						
I	30	0.9	(0.3)	30	0.9	(0.4)
II	30	1.0	(0.3)	30	0.9	(0.4)
II-I	30	0.1	(0.2)	30	0.0	(0.3)
Ext Ob (mm)						
I	29	7.3	(2.2)	26	7.2	(3.4)
II	29	6.9	(2.1)	26	7.3	(2.9)
II-I	29	–0.5	(1.2)*	26	0.3	(1.8)
Plq Area (mm^2)						
I	29	8.2	(3.4)	26	9.1	(6.8)
II	29	7.1	(2.5)	27	8.6	(4.8)
II-I	29	–1.1	(1.9)*	25	–0.5	(3.3)
AS (%)						
I	20	89.2	(6.7)	21	90.7	(7.9)
II	23	88.3	(8.4)	22	89.6	(10.2)
II-I	19	–1.8	(8.1)	19	–1.3	(6.4)

DS= diameter stenosis; MLD = minimal lumen diameter; Ext Ob = extent of the obstruction; Plq area = Plaque Area; AS = area stenosis.

*P ≤ 0.05 for paired comparison of angiogram I vs II in each patient group. The changes between patient groups (c7E3 fab vs placebo) did not reach statistical significance.

Table 6
Complications and Untoward Events During and After Coronary Angioplasty

n	*C7E3Fab* *30*	*Placebo* *30*
Mortality	0	1
Myocardial necrosis	1	3
Urgent CABG	0	3
Re-PTCA	0	2
Residual stenosis >50%	4	6
Number of patients with one or more untoward events	5	9
Procedural success (%)	83	70

CABG = coronary artery bypass grafting.
PTCA = percutaneous transluminal coronary angioplasty.

532 patients in the placebo group and 56 (10.8%) of 518 in the abciximab group had a primary endpoint (death, myocardial infarction, or urgent intervention within 30 days). Since the *P* value for the difference ($P = 0.0064$) was below the prespecified stopping criterion ($P = 0.0072$), and since the data were consistent among all subgroups analyzed,

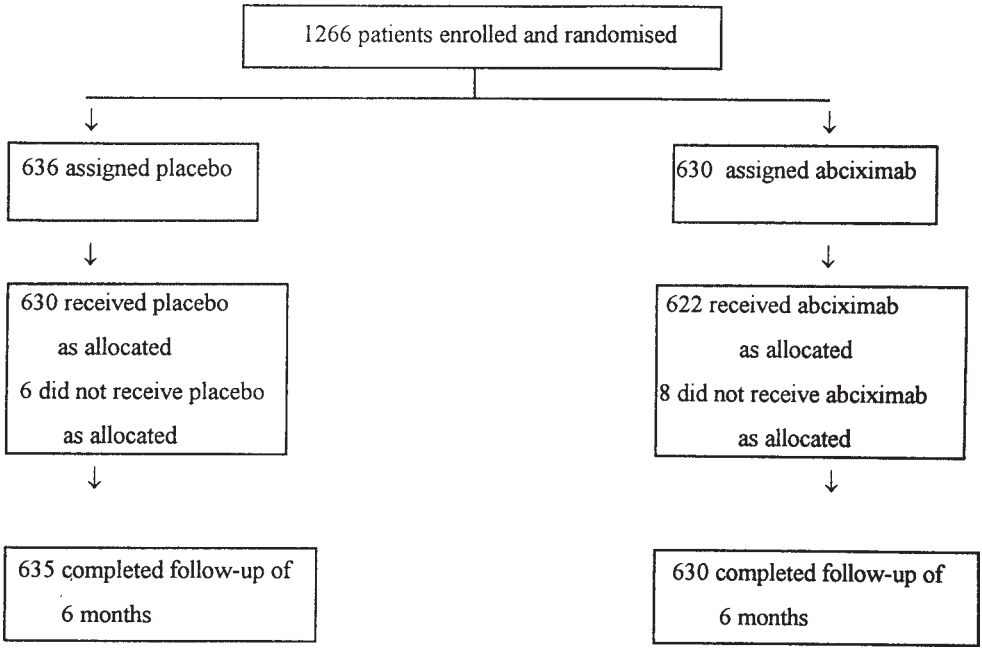

Fig. 2. Trial profile.

the Safety and Efficacy Monitoring Committee recommended that recruitment should cease. This recommendation was followed by the Steering Committee, after consultation with regulatory authorities. Figure 2 shows the flow of patients through the trial. There were 1266 patients enrolled, of 1400 scheduled. Follow-up data were complete for all but one patient (placebo) who withdrew consent after randomization.

Five other patients in the placebo group did not receive placebo (two refused, but allowed follow-up and three for logistic reasons). Eight patients did not receive abciximab (one received other therapy, five withdrew consent but allowed follow-up, two for logistic reasons).

The two treatment groups were similar in terms of baseline characteristics (Table 7): 73% were male, 50% had a history of angina, and 41% had had a previous myocardial infarction. 72% of patients were enrolled within 6 h of the first (diagnostic) angiogram, 60% had experienced myocardial ischemia within the 12 h before treatment, and 95% had an ischemic episode after a minimum of 2 h treatment with nitrates and intravenous heparin. Study drug was started in 1253 patients. It was discontinued early (before 30 min after PTCA) in 86 patients (45 placebo, 41 abciximab) for various reasons, including bleeding (one vs nine), bypass surgery (five vs one), and stent placement (eight vs three). Angioplasty was attempted in 1241 patients (98%). The procedure was done earlier than planned in 23 patients (1.8%), 14 of whom were in the placebo group (Table 8). According to the investigators, the procedure was not successful, with a residual stenosis greater than 50%, in 70 patients receiving placebo and in 37 receiving abciximab (11.2 vs 6.0%, $P = 0.001$). Treatment with abciximab also resulted in lower rates of urgent repeat PTCA, urgent stent placement, and bypass surgery (Table 9); however, these differences were not statistically significant.

Table 7
Baseline Data and Concomitant Medication

	Placebo (n = *635*)	*Abciximab* (n = *630*)
M/F	459/176	461/169
Mean (SD) age in years	61 (10)	61 (10)
Anthropometry: mean (SD)		
Weight (kg)	76 (12)	76 (12)
Height (cm)	170 (9)	170 (9)
Number of patients with		
Angina > 7 d previously*	322 (51.4%)	300 (48.6%)
Infarction within previous 7 d	78 (12.3%)	88 (14.0%)
Infarction 8–30 d	43 (6.8%)	53 (8.4%)
Infarction >30 d previously	116 (18.3%)	116 (18.4%)
Infarction, date not reported	6 (0.9%)	7 (1.1%)
PTCA	86 (13.5%)	84 (13.3%)
CABG	21 (3.3%)	11 (1.7%)
Risk factors		
Diabetes*	82 (13.0%)	95 (15.1%)
Hypertension*	261 (41.4%)	271 (43.4%)
Current smokers*	255 (40.8%)	235 (37.5%)
Medication within 7 d before enrollment		
Aspirin	582 (91.7%)	586 (93.0%)
Intravenous heparin	634 (99.8%)	627 (99.5%)
Nitrates	633 (99.7%)	627 (99.5%)
β-Blockers	392 (61.7%)	400 (63.5%)
Calcium antagonists	297 (46.8%)	286 (45.4%)
Medication after enrollment		
Aspirin	608 (95.7%)	604 (95.9%)
Ticlopidin	25 (3.9%)	25 (4.0%)
Intravenous heparin	616 (97.0%)	613 (97.3%)
β-Blockers	395 (62.2%)	412 (65.4%)
Nitrates	616 (97.0%)	613 (97.3%)
Calcium antagonists	314 (49.4%)	289 (45.9%)

*In a few patients no data were available for these items; percentages calculated for patients in whom these data were reported.

The primary endpoint (death, myocardial infarction, or urgent intervention within 30 days of enrollment) occurred in 101 (15.9%) patients in the placebo group and 71 (11.3%) in the abciximab group ($P = 0.012$; Table 9, Fig. 3). This difference was due mainly to a difference in the proportion with myocardial infarction (52 [8.2%] vs 26 [4.1%], $P = 0.002$; Table 9).

The findings were consistent in all subgroups studied and were independent of age, sex, ECG findings at enrollment, and the presence of diabetes, peripheral vascular disease, or renal dysfunction. Progression to myocardial infarction during the first 18–24 h after enrollment was rare, despite the inclusion of patients with acute, refractory, unstable angina. Even so, the frequency of myocardial infarction before PTCA was

Table 8
Angiography and PTCA Results

	Placebo (n = *635*)	*Abciximab* (n = *630*)
Artery with culprit lesion		
Left anterior descending	383 (60.3%)	385 (61.1%)
Left circumflex	104 (16.4%)	105 (16.7%)
Right coronary	144 (22.7%)	138 (21.9%)
PTCA timing		
Urgent (before planned)	14 (2.2%)	9 (1.4%)
18–26 h*	613 (96.5%)	597 (94.8%)
Delayed (>26 h)	8 (1.3%)	15 (2.4%)
No PTCA	11 (1.7%)	13 (2.1%)
PTCA result		
Attempted	624	617
Succeeded†	554 (88.8%)	580 (94.0%)
Failed†	70 (11.2%)	37 (6.0%)

*Prespecified time window was 18–24 h after enrollment; in 70 patients procedure was done between 24 and 26 h for logistic reasons.

†Percentages of those attempted.

significantly lower in patients receiving abciximab than in those receiving placebo (Table 9, $P = 0.029$). Most infarcts occurred during or within 24 h of PTCA ($P = 0.021$, Fig. 4), whereas infarction rates were low in both groups 2–30 days after PTCA (Table 9). The lower rate of myocardial infarction in patients receiving abciximab than in those receiving placebo was found for both Q-wave and non-Q-wave infarcts, and independently of the creatine kinase threshold used to define an infarct (Table 9).

Major bleeding complications occurred in only 3.8% of patients, although both major and minor bleeding events were more common during treatment with abciximab than during placebo treatment (Table 9). No excess strokes were observed with abciximab. In the placebo group, two patients had nonhemorrhagic stroke and one had an intracranial haemorrhage (1, 5, and 7 days after enrollment, respectively). Stroke occurred in a single patient treated with abciximab (15 days after enrollment), but the type of stroke could not be determined. Most bleeding complications occurred at arterial puncture sites. In both treatment groups, bleeding was more common in patients who received a high dose of heparin during PTCA, and in patients with low body weight. For patients receiving less than 100 IU/kg heparin, the bleeding rates were 1.2 and 4.4% in the placebo and abciximab groups, respectively. The corresponding rates were 2.7 and 6.6% in those receiving 100–149 IU/kg and 7.9% and 14.8% in patients receiving 150 IU/kg heparin or more. In logistic regression analysis both heparin dose per kg ($P = 0.0001$) and use of abciximab ($P = 0.0008$) were significantly related to bleeding risk. By contrast, the reduction in primary endpoint was related only to use of abciximab ($P = 0.016$) and not to heparin dose ($P = 0.70$).

Thrombocytopenia ($<100 \times 10^9$/L) occurred in 5.6% of the abciximab group and 1.3% of the placebo group. Ten patients receiving abciximab had platelet counts below 50×10^9/L within 24 h; no placebo recipient had this complication. None of these patients had

Table 9
Clinical Events, 30 Days' Follow-up

	Placebo (n = *635)*	*Abciximab* (n = *630)*	P*
Death, infarction, or urgent intervention	101 (15.9%)	71 (11.3%)	0.012
Death	8 (1.3%)	6 (1.0%)	>0.1
Myocardial infarction			
Before PTCA	13 (2.1%)	4 (0.6%)	0.029
During PTCA (< 24 h)	34 (5.5%)	16 (2.6%)	0.009
After PTCA (2–30 days)	5 (0.9%)	6 (1.0%)	>0.1
Non-Q-wave	36 (5.5%)†	19 (3.0%)	0.036
Q-wave	17 (2.7%)†	7 (1.1%)	0.067
Peak CK>5 × normal	21 (3.3%)	10 (1.6%)	0.067
Peak CK>10 × normal	15 (2.6%)	5 (0.8%)	0.040
All myocardial infarction	52 (8.2%)	26 (4.1%)	0.002
Myocardial infarction/death	57 (9.0%)	30 (4.8%)	0.003
Urgent intervention			
Urgent PTCA			
Before planned time	14 (2.2%)	9 (1.4%)	>0.1
Repeat PTCA	28 (4.4%)	19 (3.1%)	>0.1
Urgent CABG	11 (1.7%)	6 (1.0%)	>0.1
Urgent stent	42 (6.6%)	35 (5.6%)	>0.1
All urgent interventions	69 (10.9%)	49 (7.8%)	0.054
Nonurgent interventions			
Repeat PTCA	16 (2.6%)	21 (3.4%)	>0.1
CABG	9 (1.4%)	4 (0.6%)	>0.1
Stent	47 (7.4%)	49 (7.8%)	>0.1
Stroke	3 (0.5%)	1 (0.2%)	>0.1
Major bleeding‡			
Puncture site	9	19	>0.1
Retroperitoneal	0	2	>0.1
Pulmonary	0	1	>0.1
Gastrointestinal	0	3	>0.1
Urogenital	1	0	>0.1
All major bleeding	12 (1.9%)	24 (3.8%)	0.043
Minor bleeding‡	13 (2.0%)	30 (4.8%)	0.008
Transfusion‡	21 (3.4%)	44 (7.1%)	0.005

CK = creatine kinase; CABG = coronary artery bypass graft.
*P values (two-sided) <0.1 are reported.
†One patient had both.
‡Excluding those in patients who underwent CABG.

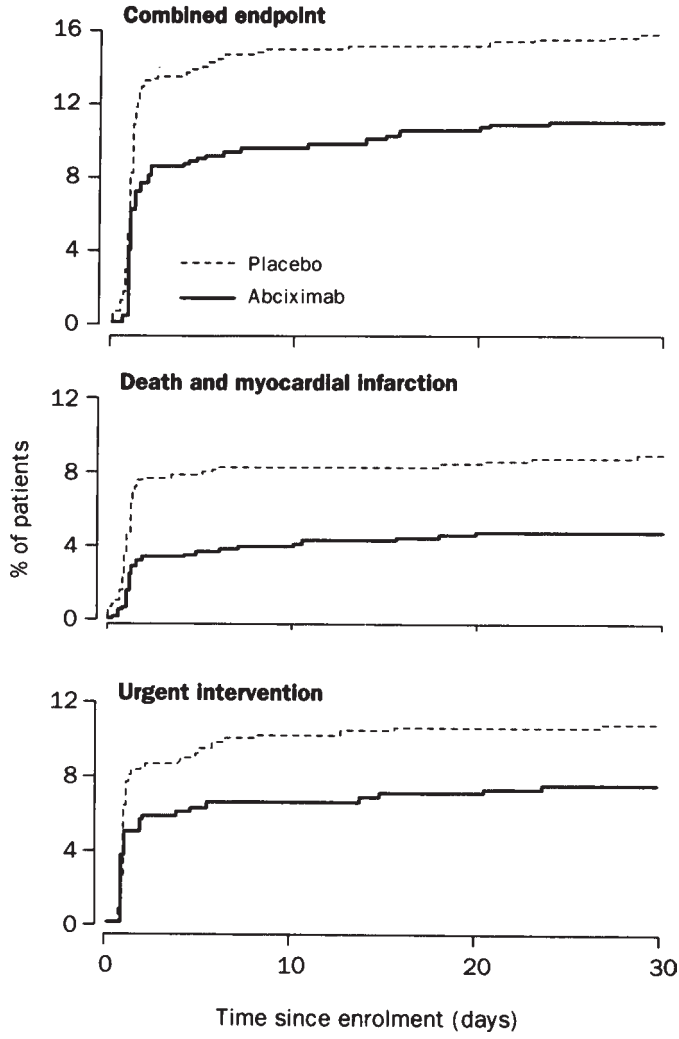

Fig. 3. Time course of combined primary endpoint and its major components.

bleeding complications. Two patients had platelet counts below 20×10^9/L. Treatment with study drug (abciximab) was discontinued in five patients, who all received platelet transfusions. Full recovery of platelet counts (to more than 100×10^9/L) occurred within 24 h in three patients, within 48 h in three, and within 5 d in three. Follow-up measurements were not available in one patient.

At follow-up 6 mo later, death or myocardial infarction had occurred in 56 (9.0%) abciximab-treated patients and 69 (10.9%) placebo recipients ($P = 0.19$, Fig. 5). Bypass surgery had been required by 33 (5.4%) and 44 (7.1%), respectively ($P = 0.20$). PTCA was needed for similar proportions of patients in both groups, mainly because of restenosis (Table 10). Also, medication up to 6 mo of follow-up was similar in the two groups (Table 11). At 6 mo, 242 events had occurred in 193 abciximab-treated patients compared with 274 events in 193 placebo recipients. Thus, the number of events per patient was lower after abciximab ($P = 0.067$).

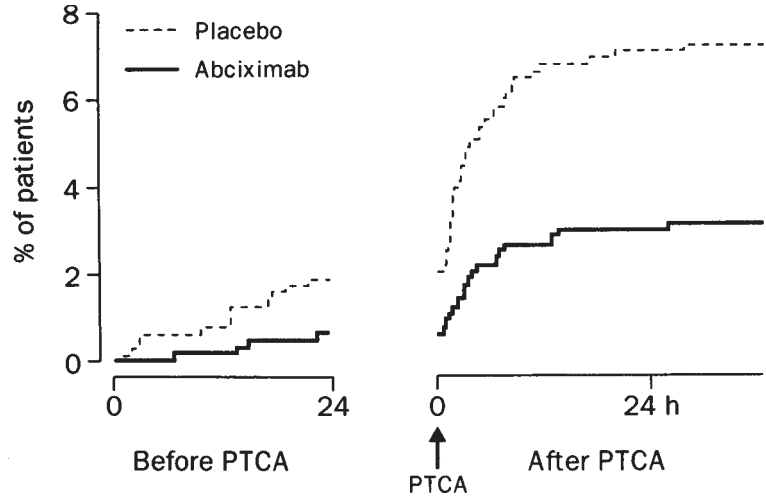

Fig. 4. Development of myocardial infarction during treatment with abciximab or placebo, before and in association with PTCA.

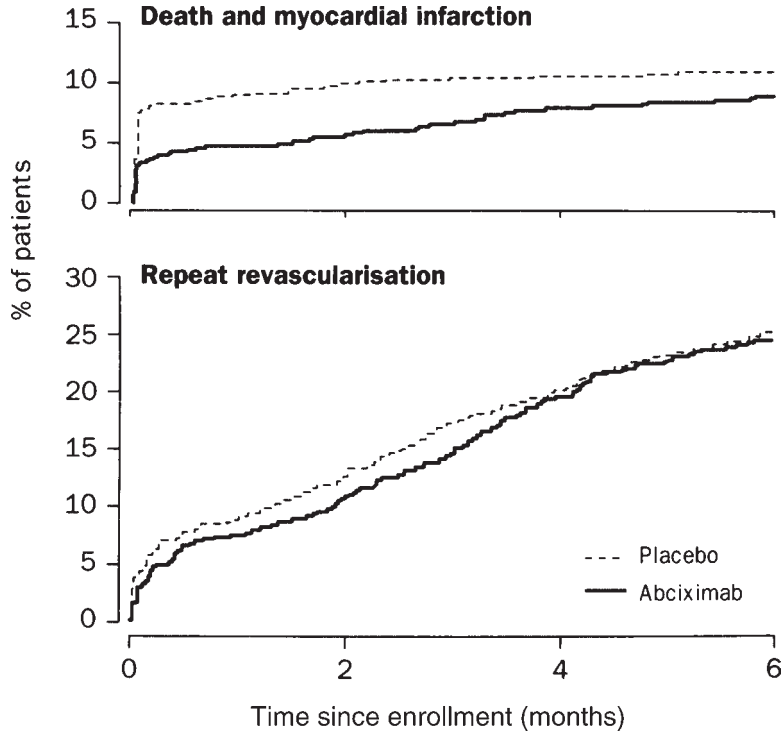

Fig. 5. Time course of death and myocardial infarction and repeat revascularization during a 6-mo follow-up.

Table 10
Clinical Events During 6-Mo Follow-up

	Placebo	*Abciximab*
Death, infarction, or intervention	193 (30.8%)	193 (31.0%)
Death	14 (2.2%)	17 (2.8%)
Myocardial infarction	59 (9.3%)	41 (6.6%)
Repeat intervention	154 (24.9%)	156 (25.4%)
Repeat PTCA	127 (20.7%)	131 (21.4%)
CABG	44 (7.1%)	33 (5.4%)

None of the differences was significant at $P < 0.01$ (two sided).

Table 11
Medication at Discharge and at 6-Mo Follow-up (% of patients)

	Discharge		*6 mo*	
	Placebo	*Abciximab*	*Placebo*	*Abciximab*
Aspirin	93.5	94.3	88.0	88.4
Other antiplatelet*	11.8	10.9	7.0	6.3
Coumadin	7.7	6.8	4.1	5.2
LMW heparin	3.3	3.3	—	—
β-blockers	59.8	60.2	54.5	53.8
Calcium antagonists	54.0	47.0	43.7	48.8
ACE inhibitors	16.4	17.9	20.0	19.3

LMW = low molecular weight; ACE = angiotensin-converting enzyme.
Data not available for 18 patients (placebo, abciximab) at discharge and 12 patients at 6 mo.
None of the differences was significant.
*Mostly ticlopidin.

Angiographic Results of the CAPTURE Trial

Of 1265 patients enrolled in the CAPTURE trial, 1233 underwent angioplasty after treatment with abciximab or placebo. Of these 1233 patients two angiograms were available for central review in 1197 patients and one angiogram in another six patients. So of 97.1% of all treated patients both angiograms were available for review. Patients without angio review had more previous angina and previous infarction than those with review of the angiograms (Table 12). A primary composite endpoint (death, MI, and urgent reintervention) was reached in 13.1% of the angio-available patients, 19.4% in the angio not-available group (P = n.s.) and 13.3% of all study patients. The incidence in reaching a primary composite endpoint at 30 days of the angio-available patients treated with placebo or abciximab was, respectively, 15.5 and 10.8% ($P = 0.017$), with a significant reduction in death and nonfatal myocardial infarction in the abciximab treated patients from 8.5 to 4.5% ($P = 0.007$) and a marginally significant reduction in urgent reinterventions from 10.8 to 7.2% ($P = 0.034$).

In the angio not-available patients, the primary end point was also lower after abciximab (26.3 vs 11.8 %, $P = 0.408$), although this was not statistically significant in this small group of patients.

Table 12
Baseline and Demographic Data from the Total Study Population and the Angio-Available and Angio Not-Available Group

	Total population†	*Angio available*	*Angio not available*
Number of pts	1233	1197	36
Males (%)	73.2	72.8	86.1
Age (mean, [SD])	61 (10)	61 (10)	60 (11)
Weight (kg, [SD])	76 (12)	76 (12)	79 (10.0)
Height (cm, [SD])	170 (9)	170 (9)	171 (6)
Previous angina (%)	50	50	58*
Previous infarct (%)	39	39	50*

*$P < 0.05$ Angio available vs not available.
(†) patients undergoing PTCA.

TIMI flow could be assessed in 1168 pairs of baseline and pre PTCA angiograms. No significant differences were present at baseline (Fig. 6). After infusion 88 abciximab patients improved the TIMI flow rate of the IRA with at least one class versus 81 placebo group patients (P = n.s.). Worsening of TIMI flow with at least one class was seen in 27 placebo- and 16 abciximab-treated patients (P = n.s.). Thrombus in any coronary segment was present in the first angiogram in 50 and 49 patients from the placebo- and abciximab-treated patients and resolved prior to angioplasty in 11 and 21 patients, respectively (22 vs 43% $P = 0.033$) (Fig. 7).

Lesion type according to the AHA/ACC criteria assessed in the first angiogram was not different between the two groups (Table 13). Missing data are due to total occluded vessels. In the placebo group more complications occurred and endpoints were reached in patients with more severe lesions (Table 13). Treatment with abciximab did not affect endpoints in patients with type A or B_1 lesions, but reduced events in patients with complex lesions.

By univariate analysis endpoints (death, myocardial infarction, or urgent reinterventions) were more frequent in patients with long lesions, angulated lesions, or bifurcation lesions (Table 14). By multivariable analysis only lesions at bifurcation were associated with an increased endpoint risk, particularly in younger patients.

The angioplasty procedure was angiographically successful in 88.0% of placebo patients and 94.1% of abciximab patients ($P < 0.001$). Placebo patients showed both a higher incidence of failure to cross (11 vs 2, $P = 0.02$) and failure to dilate (66 vs 34, $P = 0.001$) the lesion successfully. Of all patients with a failed procedure 33.3% of placebo-treated patients reached a primary endpoint, whereas this occurred in only 11.4% of abciximab-treated patients with a failed procedure ($P = 0.019$). If the procedure was successful the respective numbers were 13.0 and 10.7%. Stents were implanted in 56 placebo and 60 abciximab patients. All implants in the abciximab patients were successful, but implantation was not successful in nine placebo patients ($P = 0.003$).

After the procedure more abciximab patients showed a type A through C dissection of the dilated vessel than placebo-treated patients (31.5% vs 25.1%, $P = 0.014$). Higher grade dissections were equal in both groups (2.2% in abciximab vs 2.0% in placebo

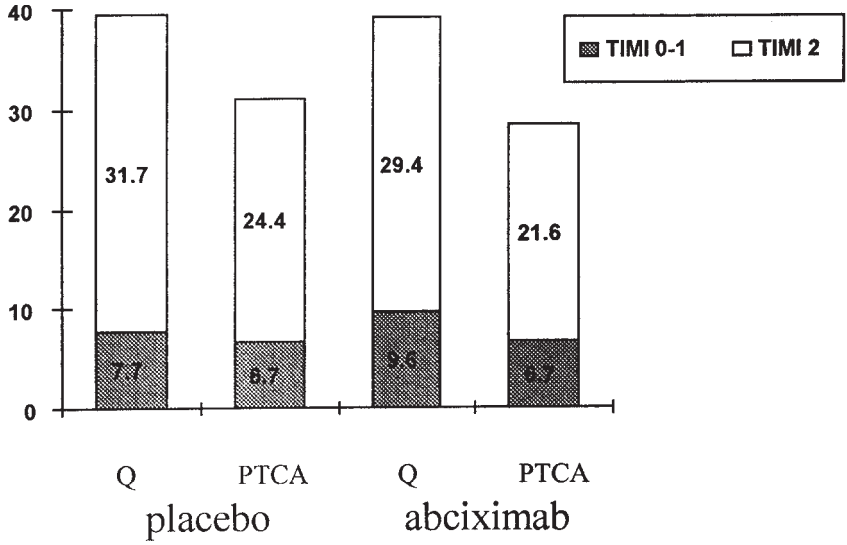

Fig. 6. TIMI flow grade of the IRA in abciximab-treated and placebo patients at the time of diagnostic angiography before treatment (Q) and 18–24 h later during treatment (PTCA). Neither differences between groups, nor the changes are statistically significant.

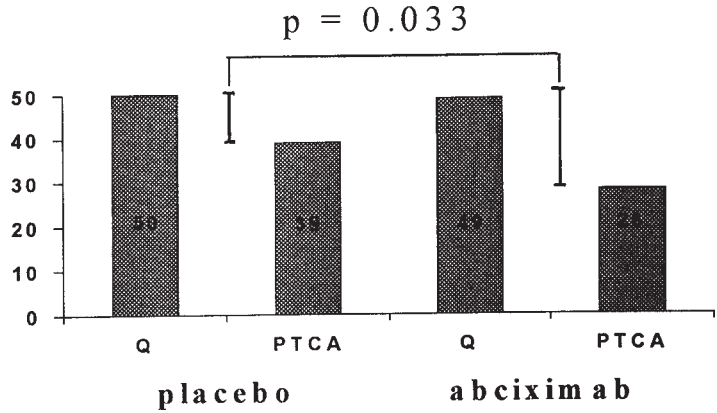

Fig. 7. Presence of any thrombus observed at the first (Q) and second angiogram (PTCA). There is a significant difference in thrombus resolution between patients treated with placebo or abciximab.

patients). Occlusion of a side branch of ≥1.5 mm diameter, originating from the dilatation site, occurred in 2.8% of placebo patients and 1.0% of abciximab patients ($P = 0.03$). No vessel perforation was visible on any angiogram. A single additional non-IRA lesion was dilated in 113 patients. Two or more additional lesions were treated in another 17 patients.

The results of these dilatations were similar to those of the IRA dilatation, with success rates in the abciximab treated patients of 92.5% and in the placebo-treated patients of 83.7%.

Table 13
Lesion Type According to AHA/ACC Criteria as Scored in the Baseline Angiogram, and Primary Composite Endpoint

Lesion type	*Placebo* 584	*Endpoint*	*Abciximab* 569	*Endpoint*	P *Value*
A	48 (8.2%)	21 (9.6%)	50 (8.8%)	22 (9.6%)	n.s.
B_1	170 (29.1%)		179 (31.5%)		
B_2	188 (32.2%)	32 (17.0%)	166 (29.2%)	20 (12.0%)	n.s
$> B_2$	166 (28.4%)		167 (29.3%)		
C	12 (2.1%)	34 (19.1%)	7 (1.2%)	20 (11.5%)	0.055

n.s. = not significant.

DISCUSSION

Both the pre-CAPTURE and the CAPTURE trial showed a significant reduction in the primary endpoint of death, myocardial infarction or urgent repeat intervention when patients were pretreated with abciximab, compared to placebo. The corresponding reduction in the rate of myocardial infarction was 50%. These findings accord with those of other clinical studies of abciximab *(19–21)* and studies with other inhibitors of the platelet glycoprotein IIb/IIIa receptor *(22,23)*. In the EPIC (Evaluation of c7e3 for the Prevention of Ischemic Complications) trial *(19),* administration of a bolus abciximab followed by 12 h infusion at the same dose as in our study reduced the rate of death or myocardial infarction from 9.6% (placebo) to 6.1% ($P = 0.015$) and reduced the need for urgent reintervention. A bolus-only regimen was less effective *(19)*. In our study, pretreatment with abciximab reduced the rates of these events both before and during and immediately after the interventions. In the EPILOG (Evaluation of PTCA to Improve Long-term Outcome by ReoPro GPIIb/IIIa receptor blockade) study *(21),* which was also stopped early by the Safety and Efficacy Monitoring Committee, similar results were obtained in patients undergoing elective PTCA. In all three studies with abciximab, the initial treatment effects were maintained for at least 30 days. Modest reductions in the same events, although not statistically significant, were observed in patients undergoing PTCA and treated with tirofiban ($P = 0.16$) *(22)* or eptifibatide ($P = 0.06$) *(23)*. These two agents differ from abciximab in that they are small molecules with short half-lives and with more reversible binding to the IIb/IIIa receptor. Nevertheless, these studies consistently support the efficacy of platelet glycoprotein IIb/IIIa receptor blockers in preventing thrombotic complications before and during coronary intervention.

In contrast with other studies, patients in CAPTURE with more severe, refractory, unstable angina were treated during the 18–24 h before planned PTCA. Abciximab resulted in a reduction of events during this period as well as of procedure-related events (Fig. 4). Apparently, some stabilization of the unstable plaque was achieved during this treatment period. Since most infarctions occurred during or after the intervention, further event reduction might have been achieved by a longer treatment period before PTCA. PTCA might even have been avoided in some of these patients after stabilization of the plaque had been achieved. Thus, further studies can be justified to investigate the

Table 14
Individual IRS Lesion Characteristics and Outcome in Patients Treated with Abciximab or Placebo

Lesion Characteristic	n	*Abciximab primary endpoint*	n	*Placebo primary endpoint*
Length				
<10 mm	418	46 (11.0%)	442	57 (12.9%)
>=10 mm, < 20 mm	143	16 (11.2%)	128	26 (20.3%)*
> =20 mm	0	0 (0.0%)	12	3 (25.0%)
Eccentricity				
Concentric	97	7 (7.2%)	93	15 (16.1%)
Eccentric	470	54 (11.5%)	486	71 (14.6%)
Angulation				
< 45 degree	528	57 (10.8%)	532	74 (13.9%)
> 45 degree	40	4 (10.0%)	44	11 (25.0%)*
Contour				
Smooth	451	46 (10.2%)	457	69 (15.1%)
Irregular	115	16 (13.9%)	123	17 (13.8%)
Calcification				
Yes	86	11 (12.8%)	89	15 (16.9%)
No	481	50 (10.4%)	486	69 (14.2%)
Bifurcation				
Yes	129	13 (10.1%)	153	36 (23.5%)*
No	437	49 (11.2%)	427	50 (11.7%)
Thrombus				
Yes	41	5 (12.2%)	40	9 (22.5%)
No	551	54 (10.6%)	558	76 (14.4%)
Ostial				
Yes	23	1 (4.3%)	25	4 (16.0%)
No	545	61 (11.2%)	558	82 (14.7%)
Accessibility				
Readily accessible	554	62 (11.2%)	564	84 (14.9%)
Moderate tortuosity	0	0 (0%)	14	2 (14.3%)

*$P < 0.05$ placebo vs abciximab.

efficacy of abciximab and related drugs in patients with unstable angina, but no planned coronary revascularisation procedure. As in the EPIC trial *(19,20)* patients treated with abciximab in CAPTURE had higher bleeding rates than those in the placebo group. However, the rate of major bleeding complications was much lower in CAPTURE than in the previous study (3.8 vs 10.6%), by the same definitions. Minor bleeding rates were also lower in our study (4.8 vs 18.8% in EPIC).

This reduction was achieved by reduction of the heparin dose, and greater attention to the site for vascular access. However, we also observed a significant relation between heparin use and bleeding risk. A further reduction of heparin doses and early sheath removal in the EPILOG study *(21)* avoided excess bleeding episodes in patients receiving abciximab. Heparin dose should be monitored closely in patients treated with abciximab. During PTCA, heparin dose should be restricted to 70 IU/kg. Complications during coronary angioplasty are of either mechanical or thrombotic origin. Balloon

angioplasty results in a disruption or dissection of the arterial wall, which lead to exposure of plaque contents, collagen, and other components of the vascular wall to the blood, resulting in platelet activation and thrombosis. Mechanical complications from large dissection flaps can now be treated by stents *(24,25)*. Stents may also reduce the area of exposure of thrombogenic components of the vascular wall. Many of the thrombotic complications and associated myocardial infarctions can be avoided when abciximab is given, whether for 18–24 h before the procedure as in CAPTURE, or for 10–30 min before and 12 h after intervention as in the other studies *(19–21)*.

Review of the angiograms of patients participating in the CAPTURE trial showed that more thrombi were resolved in the patients treated with abciximab, underscoring the thrombolytic potential of the drug *(26)*. This finding is in agreement with the qualitative angiographic data from the CAPTURE pilot trial where 3 out of 6 vessels being occluded or showing thrombi at the first angiogram, contained no intracoronary filling defects after treatment with abciximab, whereas this occurred in only one out of five patients pretreated with placebo. Similar results were obtained in a study with tirofiban (PRISM PLUS) in which angiograms were analyzed in 1168 patients who had been treated with this IIbIIIa receptor blocker or placebo during 65 ± 17 h. In these patients medium or large thrombi were reported in 20% of placebo patients and in 14% of patients receiving tirofiban *(27)*.

The angiographic success rate of the procedure was higher if patients were pretreated with abciximab (94.1 vs 88.0%; $P = <0.001$). Most of the failures of angioplasty were due to the inability to dilate the culprit lesion successfully. This is also true if a stent was implanted. Stent implantation resulted in a 100% angiographically successful procedure in abciximab treated patients, whereas success after stent implantation was only achieved in 86% of placebo-treated patients ($P = 0.003$). Abciximab thus improves the angiographic outcome both with and without stent implantation. Again, these data have recently been confirmed in other trials comparing patients receiving stents with or without abciximab *(28)*.

Apparently heparin and aspirin are unable to control for ongoing thrombus formation at the dilatation site, whereas abciximab is able to control this process, thus resulting in a successful procedure. Angiography might not be the most sensitive diagnostic tool to detect thrombi in coronary arteries *(18),* but it is tempting to ascribe the better angiographic outcome after abciximab to less thrombus formation at the site of the dilatation. In keeping with this finding is our observation of a significantly less occurrence of side branch occlusion after dilatation, if patients are pretreated with abciximab. Although this finding was rather rare in both groups, the absolute difference of 1.8% of side branch occlusion between both groups, might have contributed to the absolute difference of 3.6% in the number of infarctions between both groups, since most of these infarctions occurred in conjunction with the angioplasty procedure (Fig. 4).

It is remarkable that in this trial patients pretreated with abciximab and a failed angioplasty procedure did not exhibit a higher incidence of the primary endpoint at 30 days (11.4%) than the total group of patients treated with abciximab (10.8%). Yet 33% of patients in the placebo group with a failed angioplasty procedure reached a primary endpoint versus 13% of placebo patients with a successful angioplasty procedure.

This raises the question whether treatment with abciximab during a longer waiting period before angioplasty could further reduce the number of events, and even reduce the total number of revascularization procedures. This issue is addressed in the ongoing

GUSTO IV acute coronary syndromes trial. An improvement in TIMI flow of at least one grade between the first and the second angiogram was observed in equal numbers in both patient groups in the CAPTURE trial, as was already noted in the pilot CAPTURE trial.

Grading lesions according to the system devised by the AHA/ACC task force has led to the recognition of lesions more susceptible to angioplasty complications. In the group of patients treated with placebo or abciximab the number of primary end-points for A or B_1 lesions was 9.6 vs 9.6%; for B_2 lesions 17.0 vs 12.0% and for $>B_2$ or C lesions 19.1 vs 11.5%. When looking at individual lesion characteristics only a lesion at the site of a bifurcation led to a significant higher incidence of complications when patients were not pretreated with abciximab. The short course of abciximab treatment did not affect the rate of recurrent myocardial infarction after the first few days; such infarctions are probably due to new plaque rupture at the same or at another coronary segment.

Furthermore, there was no indication that abciximab influenced the restenosis process, since rates of repeat PTCA were the same in abciximab and placebo groups. These results contrast with those of the EPIC study, in which a consistently lower rate of target lesion revascularization was observed with abciximab up to 6 mo and 3 yr after enrollment *(20)*. This difference between CAPTURE and EPIC follow-up results may be a chance finding, or it may be due to the difference in treatment regimen. In CAPTURE, abciximab infusion was discontinued 1 h after PTCA, whereas the infusion continued for 12 h in EPIC. Higher plasma concentrations of abciximab after PTCA might result in binding of abciximab to the $\alpha_v\beta_3$ (vitronectin) receptor, which is exposed on vascular smooth-muscle cells after vessel injury. This receptor, to which abciximab binds with the same affinity as to the glycoprotein platelet IIb/IIIa receptor, is thought to be involved in migration and proliferation of smooth-muscle cells *(29)*.

This hypothesis has been studied in more detail in the ERASER trial. Instent restenosis rates did not differ at 6 mo after angioplasty, when patients were treated with placebo, or 12 or 24 h abciximab infusion *(30)*. Follow-up data from EPILOG *(21)* show results intermediate between those of CAPTURE and EPIC; there was sustained benefit of treatment with abciximab, with similar low event rates between 1 and 6 mo in the two treatment groups *(21)*.

The collective experience in large trials with more than 6000 patients has shown unequivocally that treatment with abciximab greatly reduces the rate of thrombotic complications in association with PTCA. Treatment with abciximab during and after the intervention can be recommended in all patients undergoing PTCA, if the drug costs are not prohibitive *(31)*. Patients with unstable angina are at particular risk of myocardial infarction and will benefit most from pretreatment with abciximab. A longer pretreatment period, for example, 2 or 3 days, may be even more beneficial, though there is not yet sufficient evidence. Continuation of treatment for at least 12 h after PTCA seems prudent in view of the long-term efficacy observed with that regimen *(20)*. Additional long-term benefit might be obtained by long-term treatment with related agents that can be taken orally.

In view of the costs of abciximab, some physicians may decide to use this drug only or mainly to treat thrombotic complications when these occur during an intervention. Such use may be effective, but it has not been tested rigorously in randomized trials. Currently available data indicate that pretreatment with abciximab is warranted in all patients undergoing PTCA, and particularly in patients with refractory unstable angina with more complex lesions as determined by angiography.

ACKNOWLEDGMENT

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9

Overview of the Glycoprotein IIb/IIIa Inhibitor Interventional Trials

A. Michael Lincoff, MD and Eric J. Topol, MD

CONTENTS

INTRODUCTION

Plaque rupture and vascular thrombosis are key initiating factors in the pathogenesis of ischemic complications of percutaneous coronary revascularization *(1,2)*. The central role of platelet activity in this setting is highlighted by the unequivocal benefit of aspirin in preventing death or myocardial infarction among patients undergoing coronary intervention *(3)*. Newer strategies for more potent inhibition of platelet activity at the injured coronary plaque focus on the integrin glycoprotein (GP) IIb/IIIa receptor on the platelet surface membrane, which binds circulating fibrinogen or von Willebrand factor and crosslinks adjacent platelets as the final common pathway to platelet aggregation *(4)*. Pharmacologic compounds directed against GP IIb/IIIa block this receptor, prevent binding of circulating adhesion molecules, and potently inhibit platelet aggregation.

The studies and trials evaluating the roles of specific GP IIb/IIIa inhibitors in the setting of percutaneous coronary intervention have been described in detail in Chapters 5–8. The RAPPORT trial, which evaluated the efficacy of abciximab during primary angioplasty for acute myocardial infarction (MI), is discussed in Chapter 11. This current chapter will review the body of clinical data regarding this class of agents during coronary revascularization and provide a perspective on the efficacy, safety, patient selection, and optimal use of this new therapeutic strategy.

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THE AGENTS

Three intravenous GP IIb/IIIa antagonists have undergone large-scale Phase III and Phase IV trial evaluation in the setting of percutaneous coronary revascularization, and all are currently approved for clinical use by the United States Food and Drug Administration. *Abciximab* (c7E3 Fab, ReoPro™, Centocor, Malvern, PA), the first agent of this class, is a human-murine chimeric monoclonal Fab antibody fragment that binds with high affinity and a slow dissociation rate to the GP IIb/IIIa receptor *(5,6)*. Abciximab is cleared rapidly from the plasma (half life ~25 min) *(7),* but remains bound to circulating platelets for as long as 21 d *(8)*. Binding of abciximab is not specific for the platelet GP IIb/IIIa receptor; this agent has equal affinity for the vitronectin receptor ($\alpha_v\beta_3$), which appears to play a role in cell adhesion, migration, and proliferation. *Eptifibatide* (Integrilin™, COR Therapeutics, South San Francisco, CA), a cyclic heptapeptide based upon the Lys-Gly-Asp (KGD) amino acid sequence, is a highly specific, competitive inhibitor of the GP IIb/IIIa complex. Blockade of the receptor by eptifibatide is rapidly reversible, with a plasma half-life in man of about 2.5 h *(9)*. *Tirofiban* (Aggrastat™, Merck, Whitehouse Station, NJ) is a tyrosine-derivative nonpeptide mimetic inhibitor of GP IIb/IIIa, which also specifically and competitively binds to the receptor in a rapidly reversible fashion *(10),* and has a short (~1.6 h) serum half-life. Platelet aggregation is inhibited by all of these agents in a dose-related manner, with nearly complete abolition of platelet thrombosis at levels of receptor occupancy >80% *(6)*. After discontinuation of abciximab, platelet aggregation returns toward baseline over the subsequent 12–36 h *(6),* whereas normalization of platelet function occurs much more quickly (over 30 min to 4 h) following discontinuation of the reversible eptifibatide or tirofiban *(9,10)*.

THE RANDOMIZED TRIALS

The role of GP IIb/IIIa inhibitors administered as periprocedural intravenous therapy in the setting of percutaneous coronary revascularization has been tested in seven large-scale, randomized, placebo-controlled trials enrolling in total over 15,000 patients. The monoclonal antibody fragment abciximab was systematically evaluated in five of these trials. The efficacy of this agent in reducing ischemic complications among patients considered to be at high risk, a broad spectrum of patients undergoing elective or urgent revascularization, and those patients suitable for stent implantation was assessed in EPIC, EPILOG, and EPISTENT, respectively. CAPTURE tested the role of abciximab as treatment prior to angioplasty among patients with refractory unstable angina, whereas RAPPORT focused on the high-risk group of patients undergoing coronary angioplasty as primary reperfusion therapy for acute myocardial infarction. The utility of the peptide inhibitor eptifibatide was evaluated among all patients undergoing coronary intervention in the largest of the GP IIb/IIIa intervention trials, IMPACT II. The RESTORE trial focused on high-risk patients with acute ischemic syndromes in its assessment of the nonpeptide inhibitor, tirofiban.

Trial Designs

Study algorithms are summarized in Fig. 1 and enrollment details provided in Table 1. Individual trial protocols are described, whereas efficacy and safety findings are detailed in subsequent sections.

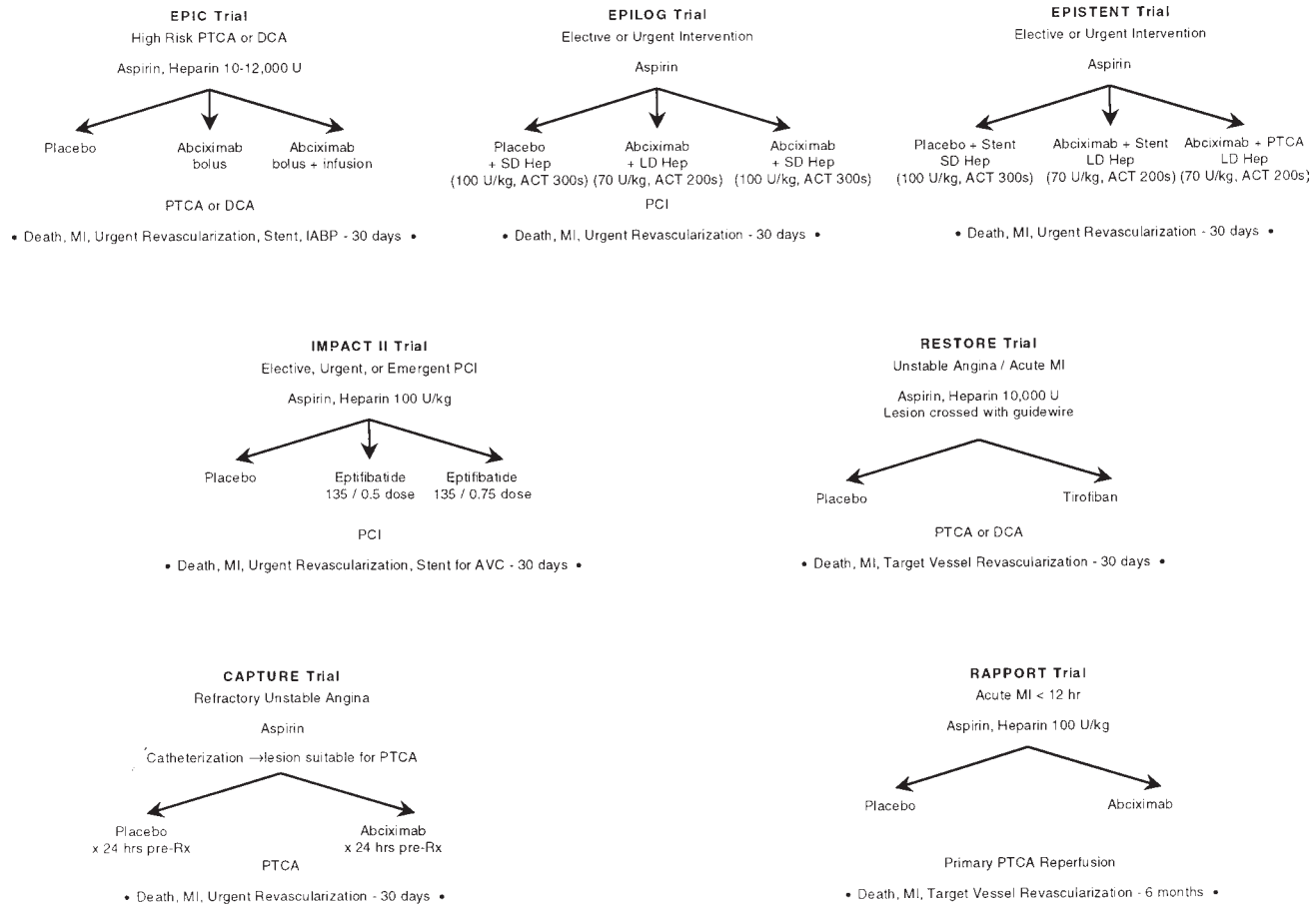

Fig. 1. Protocol outlines for the seven large-scale trials of GP IIb/IIIa blockade in interventional cardiology. Abbreviations: AVC = abrupt vessel closure; DCA = directional coronary atherectomy; LD Hep = low-dose, weight-adjusted heparin; PCI = percutaneous coronary intervention; PTCA = balloon angioplasty; pre-Rx = pretreatment; SD Hep = standard-dose, weight-adjusted heparin; 135/0.5 and 135/0.75 = eptifibatide doses (*see* text).

Table 1
Randomized Interventional Trials—Patient Populations

Trial	*Agent tested*	*Number of patients*	*Enrollment period*	*Entry criteria*
EPIC	Abciximab	2099	11/91–11/92	*High risk PTCA or DCA* Acute MI—within 12 h (direct or rescue PTCA) Recent MI—within prior 7 d Unstable angina—within 24 h of chest pain (rest or postinfarction angina with ischemic ECG changes) Clinical-morphologic criteria (ACC/AHA lesion score B2 or C, ACC/AHA score B1 with DM or with female of age >65)
EPILOG	Abciximab	2792	2/95–12/95	*Urgent or elective PTCA, DCA, TEC, or laser* Excluding unstable angina or acute MI (EPIC criteria) within prior 24 h, elective stents, rotational atherectomy
EPISTENT	Abciximab	2399	7/96–9/97	*Coronary intervention suitable for PTCA or elective stent* Excluding acute MI within 24 h, rotational atherectomy
IMPACT II	Eptifibatide	4010	11/93–11/94	*All clinical indications, all approved interventions*
RESTORE	Tirofiban	2139	1/95–12/95	*High risk PTCA or DCA* Acute MI—within 72 h (Q-wave or non-Q-wave) Unstable angina—within 72 h (chest pain at rest or on minimal effort with ECG changes, hemodynamic changes, or angiographic thrombus)
CAPTURE	Abciximab	1265	5/93–12/95	*Refractory unstable angina* Unstable angina—within 48 h (chest pain with ECG changes) despite IV heparin and nitroglycerin
RAPPORT	Abciximab	483	11/95–2/97	*Primary PTCA for Acute ST Elevation MI*—within 12 h

ACC/AHA = American College of Cardiology/American Heart Association modified lesion complexity score *(45)*.
DCA = directional coronary atherectomy.
DM = diabetes mellitus.
ECG = electrocardiographic.
MI = myocardial infarction.
PTCA = coronary balloon angioplasty.
TEC = transluminal extraction catheter atherectomy.

Trial Protocols

The EPIC Trial. EPIC is described in detail in Chapter 5. In brief, this trial provided the "proof of concept" of the efficacy of GP IIb/IIIa blockade in improving clinical outcome among patients undergoing coronary revascularization. A total of 2099 patients deemed to be at high risk for ischemic complications on the basis of acute ischemic syndromes (MI or unstable angina) or adverse clinical and lesion morphologic charac-

teristics were enrolled between 1992 and 1993 *(11)*. Given the prolonged duration of abciximab's binding to the GP IIb/IIIa receptor, this trial assessed whether a single bolus of the agent would be efficacious or if a more prolonged period of administration would be required. Patients were therefore randomized to treatment with placebo, an abciximab 0.25 mg/kg bolus administered immediately prior to initiation of the interventional procedure, or an abciximab bolus followed by a 10 μg/min infusion for 12 h. All patients received aspirin; concomitant heparin was administered as a preprocedural bolus of 10–12,000 U (with additional boluses as necessary to achieve and maintain an activated clotting time [ACT] of 300–350 s), followed by a postprocedural infusion for at least the 12 h duration of the abciximab study drug. Vascular access sheaths were left in place during the heparin and study drug infusions, and removed 6 h after the heparin was discontinued. Patients were followed for 30 d for the primary efficacy endpoint of death, MI, urgent repeat revascularization, or stent or intra-aortic balloon pump placement; double-blinding was maintained and clinical assessments were also performed at 6 mo and 3 yr following randomization.

The EPILOG Trial. EPILOG is described in detail in Chapter 5. This study was designed to extend the findings of the EPIC trial in two ways. First, in order to determine if the clinical benefits of abciximab therapy observed among high-risk patients in EPIC could be extrapolated to all patients treated by percutaneous coronary revascularization, regardless of their perceived risk for ischemic complications, a broad spectrum of patients undergoing elective or urgent intervention were enrolled. The second objective was to determine if the substantial increase in bleeding complications associated with abciximab therapy in EPIC (*see* Safety) could be attenuated by modification of conjunctive heparin dosing. The EPILOG trial design was based upon the findings of the pilot PROLOG study *(12),* which suggested that hemorrhagic complications of abciximab could be reduced without loss of clinical efficacy by weight-adjustment and reduction of heparin dose and by early sheath removal during the abciximab infusion (with no postprocedural heparin). Patients were randomized to placebo with standard-dose, weight-adjusted heparin; abciximab bolus plus infusion with standard-dose, weight-adjusted heparin; or abciximab bolus plus infusion with low-dose, weight-adjusted heparin (Table 2) *(13)*. Postprocedural heparin was not given, and vascular sheaths were removed 2–6 h after the procedure. Planned sample size was 4800 patients, but the trial was terminated early after the first interim analysis demonstrated an unexpectedly marked treatment efficacy of abciximab therapy; at that point, a total of 2792 patients had been enrolled between February and December 1995. The primary efficacy endpoint was the occurrence of death, myocardial infarction, or urgent repeat revascularization by 30 d; patients were also followed in a double-blinded fashion to 6 mo and 1 yr.

The EPISTENT Trial. EPISTENT is described in detail in Chapter 5. All previous trials of GP IIb/IIIa receptor blockade during coronary intervention had excluded patients undergoing elective stent implantation, due to rapid changes in the "optimal stenting" technique during the time periods that these trials were underway as well as uncertainties regarding the best conjunctive anticoagulation regimen. EPISTENT was therefore designed to evaluate the complementary and comparative roles of GP IIb/IIIa blockade with abciximab and stenting in reducing ischemic complications of percutaneous coronary revascularization. A total of 2399 patients undergoing urgent or elective percuta-

Table 2
Randomized Interventional Trials—Heparin Regimens and Vascular Sheath Removal

Trial	*Bolus**	*Heparin target ACT**	*Postprocedural infusion†*	*Sheath removal† (time after interventional procedure)*
EPIC	10–12,000 U initial 3000 U additional to maximum total 20,000 U	300–350 s	>12 h target aPTT 1.5–2.5 × control	>6 h after 12 h study drug infusion (>18 h total)
EPILOG	"Standard-dose"—100 U/kg "low-dose"—70 U/kg	"Standard-dose"—300 s "low-dose"—200 s	None	When ACT < 175 s (~2–6 h)
EPISTENT	Placebo—100 U/kg abciximab—70 U/kg	Placebo—300 s abciximab—200 s	None	When ACT < 175 s (~2–6 h)
IMPACT II	100 U/kg	300–350 sec	None	When aPTT < 45 s (4–6 h)
RESTORE	10,000 U 150 U/kg for weight <70 kg	300–400 s	None	When ACT < 180 s
CAPTURE	10,000 U	300 s	1 h after PTCA (all patients received infusion for 24 h before PTCA–target aPTT 2.0–2.5 × control)	4–6 h (>24–30 h total, including during pre-procedural study drug infusion)
RAPPORT	100 U/kg	300 s	None	When ACT < 175 s (4–6 hr)

* Prior to first balloon inflation or device activation of interventional procedure.
† Recommended by protocol.
ACT = activated clotting time.
aPTT = activated partial thromboplastin time.

neous revascularization of lesions suitable for balloon angioplasty (percutaneous transluminal coronary angioplasty or PTCA) or stenting were enrolled between 1996 and 1997 and randomized to stent plus placebo, stent plus abciximab (bolus and 12 h infusion), or PTCA plus abciximab *(14)*. "Bailout" or unplanned stents were utilized in only 19% of patients in the PTCA group. The primary efficacy endpoint of death, myocardial infarction, or urgent revascularization was assessed at 30 d, with clinical follow-up to 6 mo and 1 yr. Additionally, an angiographic substudy of 900 patients (300 in each treatment group) was carried out to evaluate the influence of abciximab on restenosis.

The IMPACT II Trial. IMPACT II is described in detail in Chapter 6. This "all comers" trial tested the clinical efficacy of eptifibatide among 4010 patients undergoing percutaneous intervention for any clinical indication between 1993–1994 *(15)*. Patients received aspirin and weight-adjusted heparin and were randomized to placebo, eptifibatide 135 μg/kg bolus followed by an infusion of 0.5 μg/kg-min for 20–24 h ("135/0.5 group"), or eptifibatide 135 μg/kg bolus followed by an infusion of 0.75 μg/kg-min for 20–24 h ("135/0.75 group"). Clinical outcome was assessed at 30 d and 6 mo. An angiographic substudy, enrolling approximately 900 patients, was performed to assess the influence of eptifibatide on angiographic restenosis.

The RESTORE Trial. RESTORE is described in detail in Chapter 7. The clinical efficacy of tirofiban was tested during 1995 among a total of 2139 patients considered to be at high risk for coronary revascularization owing to unstable angina or MI *(16)*. Patients were randomized after passage of a guidewire across a target lesion to placebo or tirofiban, administered as a 10 μg/kg bolus followed by a 0.15 μg/kg-min infusion for 36 h. The predefined primary endpoint of the study was the occurrence of death, MI, or *any* target vessel repeat revascularization by 30 d; clinical outcome was also assessed at 6 mo. A post hoc reclassification of revascularization events was performed to produce a composite endpoint that was comparable to other interventional trials by including only *urgent* revascularization procedures. An angiographic substudy of approximately 600 patients (300 in each group) evaluated the effect of tirofiban on restenosis.

The CAPTURE Trial. CAPTURE is described in detail in Chapter 8. This trial differed from the other interventional GP IIb/IIIa trials in that CAPTURE evaluated a strategy of *pretreatment* with abciximab prior to percutaneous revascularization among patients with refractory unstable angina. Patients qualified for enrollment if they had unstable angina with episodes of chest pain and ischemic electrocardiographic changes, despite therapy with intravenous heparin and nitroglycerin, and had been demonstrated on angiography to have a lesion suitable for coronary angioplasty *(17)*. All patients received aspirin and were randomized to placebo or abciximab for 18–24 h prior to angioplasty and continued for 1 h after completion of the procedure. Heparin was administered and vascular sheaths remained in place from enrollment throughout the pretreatment phase and until at least 1 h after angioplasty. Planned sample size was 1400 patients, but the trial was stopped on the basis of efficacy at the third interim analysis with 1265 patients enrolled between 1993 and 1995. The primary endpoint of death, M1, or urgent revascularization was assessed at 30 d, with continued clinical follow-up through 6 mo.

The RAPPORT Trial. RAPPORT is described in detail in Chapter 11. The role of abciximab therapy among patients treated by direct ("primary") angioplasty for acute myocardial infarction was evaluated in this trial. A total of 483 patients undergoing intervention within 12 h of onset of symptoms were randomized to receive placebo or abciximab (bolus and 12 h infusion) in addition to aspirin and heparin *(18)*. The primary endpoint was the occurrence of death, recurrent myocardial infarction, or any (elective or urgent) repeat target vessel revascularization by *6 mo*. A composite endpoint of the acute ischemic events of death, reinfarction, or urgent repeat target vessel revascularization (comparable to the primary endpoints used in the other trials) was also assessed at 7 d, 30 d, and 6 mo.

Intertrial Comparisons

Pooled analyses of these seven large-scale trials of GP IIb/IIIa blockade allow general principles regarding the efficacy and safety of this treatment strategy to be evaluated. Important differences among the trials must be recognized, however, with regard to patient populations, study drug regimens, adjunctive medical therapies, and endpoint assessment.

Entry Criteria. As a general rule, therapies that are effective will often exhibit the most clinical efficacy among patients who are at heightened risk for the events to be prevented. For this reason, initial trials of GP IIb/IIIa blockade during coronary interven-

tion often focused on patients deemed to be at high risk for periprocedural ischemic complications. The first trial, EPIC, designated high risk status by either the unequivocal unstable ischemic syndromes or complex angiographic target lesion morphology in combination with clinical criteria. The RESTORE entry criteria were even more narrow than those of EPIC, including only patients with unstable ischemic syndromes, the subgroup that had been found in EPIC to have experienced the most clinical benefit from GP IIb/IIIa blockade (*see* Study Drug Regimens) *(19)*. CAPTURE was a trial focused on the particular application of GP IIb/IIIa blockade as pretreatment among patients with refractory unstable angina, defined by ongoing ischemic symptoms and electrocardiographic changes despite optimal medical management. RAPPORT specifically evaluated patients with ST-segment elevation acute MI, undergoing direct angioplasty as primary reperfusion therapy.

In contrast, one of the primary objectives of the EPILOG trial was to assess whether the clinical efficacy of abciximab therapy could be extended to the low as well as high-risk patients undergoing coronary intervention; all patients were therefore to be included, except those with severe unstable angina (with symptoms and electrocardiographic changes within the previous 24 h) for whom the profound benefit of abciximab had already been demonstrated in EPIC *(19),* or those with acute MI, a setting under evaluation in the dedicated RAPPORT trial. Similarly, the philosophy behind design of IMPACT II was one of broad applicability, and patients representing various risk profiles were enrolled. Finally, EPISTENT sought to evaluate the role of abciximab in combination with or relative to elective stenting in the broad spectrum of patients considered for "real world" stenting, and therefore EPISTENT represents the most diverse PTCA versus stent trial thus far performed.

Study Drug Regimens. The study drug regimens utilized in these trials are depicted in Fig. 2. Aside from CAPTURE, all trials evaluated a strategy whereby study drug (GP IIb/IIIa inhibitor or placebo) was administered as a bolus immediately prior to coronary intervention, followed by infusions of varying durations. The EPIC trial was the only study to also test a bolus only regimen of a GP IIb/IIIa antagonist. Given that abciximab has the longest duration of GP IIb/IIIa blockade of all the agents under evaluation, and that the group in EPIC receiving the bolus of abciximab experienced only a small and statistically insignificant reduction in endpoints compared with placebo (*see* 30-Day Efficacy), it was concluded that post-bolus infusions would be required with all of these compounds in subsequent trials for optimal passivation of the arterial plaque and reduction in clinical ischemic events. The durations of study drug infusion were based upon pharmacodynamic differences among the agents: 24 h or 36 h infusions of eptifibatide and tirofiban were chosen for the IMPACT II and RESTORE trials, respectively, in order to achieve approximately the same duration of platelet inhibition as that obtained with the 12-h abciximab infusion in EPIC.

As previously described, CAPTURE utilized a pretreatment regimen of study drug. Abciximab was administered for only 1 h after the interventional procedure, owing partly to the fact that this trial was designed and initiated before the findings of EPIC regarding the importance of a 12-h postprocedural abciximab infusion were fully appreciated. This short postprocedural drug infusion may have adversely influenced the magnitude of treatment effect observed in this trial.

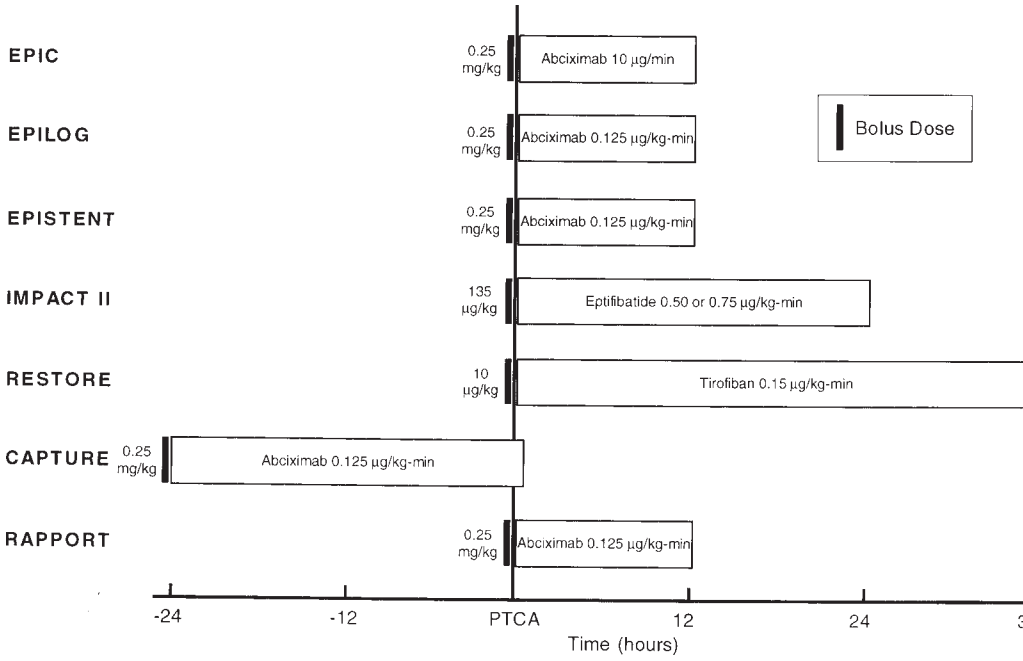

Fig. 2. Schematic of study drug infusion regimens for the GP IIb/IIIa interventional trials. All trials included a blinded matched placebo regimen.

Conjunctive Heparin and Vascular Access Site Management. Protocol guidelines for conjunctive heparin dosing and vascular sheath removal are summarized in Table 2. As described below, a major limitation of abciximab therapy in EPIC was a marked increase in the incidence of bleeding complications. This hemorrhagic risk appeared to be linked to excessive intraprocedural heparin dosing as well as the practice of leaving vascular access sheaths in place with ongoing heparin infusion for the entire 12 h study drug infusion period. A pilot study demonstrated that vascular sheaths could be removed and hemostasis achieved during abciximab infusion, if heparin had been discontinued, and that bleeding rates actually tended to be less with this strategy than with prolonged sheath dwell times *(12)*. Moreover, the risk of hemorrhagic complications was further diminished by reduction and weight-adjustment of the procedural heparin doses. Based upon these findings, the EPILOG trial explicitly tested low-dose versus standard-dose weight-adjusted heparin regimens with abciximab, and the other trials incorporated some form of protocol-directed limitation or weight-adjustment of heparin dosing. All trials after EPIC recommended that little or no heparin infusion be administered after the procedure and that vascular access sheaths be removed early (usually during study drug infusion). Of note, however, is that although these guidelines were provided in the RAPPORT study protocol, many investigators were reluctant to eliminate post-procedural heparin from their management of patients with acute MI, and median vascular access sheath dwell times were 17–19 h. Similarly, vascular sheaths remained in place with ongoing heparin infusion for the entire 24-h pretreatment period between qualifying angiography and percutaneous revascularization in CAPTURE, although sheaths were generally removed within a few hours thereafter.

Other Medications. All patients in these interventional trials, as well as other large-scale trials of intravenous GP IIb/IIIa blockade, were treated with aspirin before the procedure and indefinitely thereafter. Although it is unlikely that aspirin adds significant platelet antiaggregatory effect during ongoing administration of a GP IIb/IIIa antagonist, there may be an incremental benefit of its inhibition of platelet activation or anti-inflammatory properties. Moreover, long-term ischemic event rates are suppressed by chronic aspirin therapy, but would not be expected to be influenced by a brief periprocedural administration of a GP IIb/IIIa agent. Other anticoagulant drugs were generally restricted by protocol to the extent that they could exacerbate bleeding complications of the study agent. Patients on therapeutic warfarin therapy were excluded, as were those in whom administration of dextran was planned. Thrombolytic therapy as an adjunct to angioplasty was discouraged, with reduced doses suggested if administration of these drugs was considered imperative. Ticlopidine was permitted among patients receiving intracoronary stents. Antianginal and other medical therapies were not protocol-directed and were utilized at the discretion of physicians at the clinical sites.

Trial Conduct and Endpoints. The interventional trials shared many common design and analysis features. All trials were blinded, except for the asymmetric balloon angioplasty plus abciximab arm of EPISTENT. Endpoints were adjudicated by review of primary data by independent, blinded Clinical Events Committees. Myocardial infarction was in general identified by new Q-waves or CK-MB elevations ≥3 times the control value. Urgent repeat revascularization was defined by evidence of precipitating myocardial ischemia and rapid (within 24 h) performance of the revascularization procedure. Bleeding events were classified as "major" or "minor" based upon observed hemorrhage and changes in hemoglobin according to the criteria of the thrombolysis in myocardial infarction (TIMI) Study Group *(20)*.

Important differences existed, however, between the endpoint assessment of RESTORE and all of the other interventional trials. First, the abciximab and eptifibatide trials were carried out according to the "intention-to-treat" principle, wherein patients were randomized before initiation of the interventional procedure and all patients were included in the efficacy analysis, regardless of whether or not they actually received study drug or underwent revascularization. In contrast, patients were not randomized into RESTORE until they had their target lesions crossed with a guidewire and were included in the efficacy and safety analyses only if study drug was administered. Thus, the RESTORE endpoints are the result of a "treated-patient" rather than an "intention-to-treat" analysis. Second, the prespecified endpoint of RESTORE included any target vessel revascularization (urgent *or* elective), whereas the other trials included only urgent revascularization procedures (although of any lesion or vessel). As described above, this endpoint in RESTORE was redefined by a post hoc analysis to allow comparison with the other trials. Third, the Clinical Events Committees of the abciximab and eptifibatide trials reviewed CK enzyme and hematology laboratory values which had been obtained systematically by a protocol-driven schedule, and thereby identified MIs and bleeding events that may or may not have been noted by site investigators. In contrast, enzyme and hemoglobin values were obtained in RESTORE when ischemic or bleeding were suspected to have occurred, and Clinical Events Committee reviews were confined to adjudication of events which had been identified by the site investigators.

The RAPPORT trial also differed from the other interventional trials, in that the

Treatment Group	Placebo % (n)	GP IIb/IIIa % (n)	p-value
EPIC			
Abciximab B	12.8% (696)	11.4% (695)	0.430
Abciximab B+I	12.8% (696)	8.3% (708)	0.008
EPILOG			
Abciximab LDH	11.7% (939)	5.2% (935)	<0.001
Abciximab SDH	11.7% (939)	5.4% (918)	<0.001
EPISTENT			
Abciximab + Stent	10.8% (809)	5.3% (794)	<0.001
Abciximab + PTCA*	10.8% (809)	6.9% (796)	0.007
IMPACT II			
Eptifibatide 135/.5	11.4% (1328)	9.2% (1349)	0.063
Eptifibatide 135/.75	11.4% (1328)	9.9% (1333)	0.220
RESTORE			
Tirofiban	10.5% (1070)	8.0% (1071)	0.052
CAPTURE			
Abciximab	15.9% (635)	11.3% (630)	0.012
RAPPORT			
Abciximab	11.2% (242)	5.8% (241)	0.030

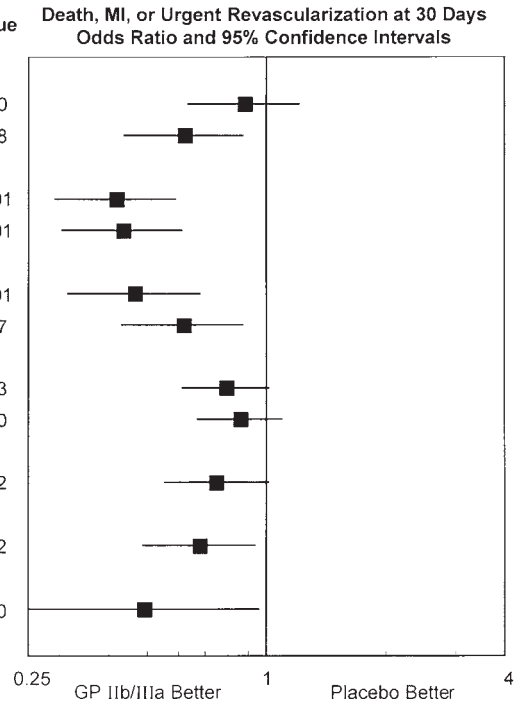

Fig. 3. Composite 30-d endpoint (death, myocardial infarction, or urgent repeat revascularization) event rates for the 7 GP IIb/IIIa interventional trials. RESTORE trial endpoints listed here are for the published post hoc analysis including only *urgent* repeat revascularization for consistency with the other trials (the prespecified primary composite endpoint of RESTORE included *urgent or elective* repeat revascularization). RESTORE trial endpoints listed here differ from those of the other trials in that only patients with successful crossing of the lesion with the guidewire were included in the efficacy analysis of RESTORE, providing a "treated patient" analysis rather than the "intention-to-treat" analysis utilized in the other studies. RAPPORT trial endpoints listed here are for secondary endpoint of death, MI, or urgent repeat *target vessel* revascularization.

*EPISTENT trial groups compared with reference group of Placebo + Stent; thus, the "placebo control" for the Abciximab + PTCA group underwent stenting rather than PTCA.

Abbreviations: B = bolus, B+I = bolus plus infusion, LDH = low-dose, weight-adjusted heparin, MI = myocardial infarction, SDH = standard-dose, weight-adjusted heparin, 135/0.5 and 135/0/75 = eptifibatide doses (*see* text).

primary endpoint was the occurrence of death, myocardial reinfarction, or target vessel revascularization (urgent or elective) by 6 mo rather than 30 d. This endpoint was chosen based upon the findings of EPIC suggesting a reduction in "clinical restenosis" (the need for elective target vessel revascularization) over 6 mo among patients with acute MI. A secondary endpoint of death, myocardial reinfarction, or urgent target vessel revascularization at 30 d was also assessed, however, allowing comparison with the other studies.

30-Day Efficacy

Event rates for the composite 30-d endpoint of death, MI, or urgent repeat revascularization in the various treatment groups of the seven interventional trials are

summarized in Fig. 3. Ischemic endpoint event rates were consistently diminished by GP IIb/IIIa blockade in all of the trials, although the magnitude of treatment effect was variable. In EPIC, this composite event rate was reduced from 12.8% among patients receiving placebo to 11.4% among patients randomized to the abciximab bolus (10% relative risk reduction, $P = 0.43$) and to 8.3% among patients randomized to the abciximab bolus and 12 h infusion (35% relative risk reduction, $P = 0.008$). The treatment effect of abciximab appeared to be amplified in the subsequent EPILOG trial, representing the greatest magnitude of clinical benefit observed with a GP IIb/IIIa inhibitor to date: the composite endpoint event rate at 30 d was 11.7% in the placebo group, 5.2% in the abciximab with low-dose heparin group (56% relative risk reduction, $P < 0.0001$), and 5.4% in the abciximab with standard-dose heparin group (54% relative reduction, $P < 0.0001$). A treatment effect of similar proportion was observed among patients receiving abciximab compared with placebo in EPISTENT; the primary efficacy composite endpoint occurred in 10.8% of patients in the stent plus placebo arm, 6.9% of patients in the balloon plus abciximab arm (36% relative risk reduction, $P = 0.007$), and 5.3% of patients in the stent plus abciximab arm (51% relative risk reduction, $P < 0.001$).

In contrast, although the IMPACT II and RESTORE trials provided evidence that eptifibatide and tirofiban diminish periprocedural ischemic events, the magnitude of treatment effect with these agents was less marked than in the abciximab trials and did not reach traditional levels of statistical significance. In IMPACT II, the composite endpoint occurred in 11.4% of patients in the placebo group, 9.2% of patients in the eptifibatide 135/0.5 group (19% relative risk reduction, $P = 0.063$), and 9.9% of patients in the eptifibatide 135/0.75 group (16% relative risk reduction, $P = 0.22$). No dose response was observed among the eptifibatide doses evaluated in this study. In RESTORE, the predefined primary endpoint of death, MI, or repeat target lesion revascularization occurred in 12.2% of patients in the placebo group and 10.3% of patients in the tirofiban group (16% relative risk reduction, $P = 0.16$). With the post hoc reclassification of revascularization events to allow comparison with the other trials, the 30-d composite of death, MI, or urgent revascularization was 10.5% in the placebo group and 8.0% in the tirofiban group (24% relative risk reduction, $P = 0.052$).

In the specific disease states assessed in CAPTURE (refractory unstable angina) and RAPPORT (acute MI), therapy with abciximab conferred a reduction in ischemic endpoints similar to that seen in EPIC, EPILOG, and EPISTENT. The primary composite endpoint in CAPTURE occurred in 15.9% of patients in the placebo group and 11.3% of patients in the abciximab group (29% relative risk reduction, $P = 0.012$). Clinical benefit of abciximab began to accrue during the pretreatment phase before the angioplasty procedure, with the preprocedural MI rate reduced from 2.1% among control patients to 0.6% among those treated with abciximab ($P = 0.029$). In RAPPORT, the predefined primary endpoint of death, recurrent myocardial infarction, or any (elective or urgent) repeat target vessel revascularization by *6 mo* was not different in the two treatment groups (28.1 and 28.2% among patients randomized to placebo and abciximab, respectively), owing to the absence of an effect of abciximab therapy on long-term revascularization procedures. The composite endpoint of death, reinfarction, or urgent repeat target vessel revascularization (comparable to the primary endpoint used in the other trials) was significantly reduced by abciximab, however, from 11.2 to 5.8% at 30 d (48% relative risk reduction, $P = 0.038$).

Table 3
Randomized Interventional Trials— 30-Day Efficacy Endpoint Events

	Death (%)	*MI (%)*	*Urgent PCI (%)*	*Urgent CABG (%)*
EPIC Trial				
Placebo	1.7	8.6	4.5	3.6
Abciximab bolus	1.3	6.2	3.6	2.3
Abciximab bolus + infusion	1.7	5.2	0.8	2.4
EPILOG Trial				
Placebo	0.8	8.7	3.8	1.7
Abciximab + reduced heparin	0.3	3.7	1.2	0.4
Abciximab + standard heparin	0.4	3.8	1.5	0.9
EPISTENT Trial				
Placebo + stent	0.6	9.6	1.2	1.1
Abciximab + stent	0.3	4.5	0.6	
Abciximab + PTCA	0.8	5.3	1.3	0.6
IMPACT II Trial				
Placebo	1.1	8.1	2.8	2.8
Eptifibatide 135/0.5 dose	0.5	6.6	2.6	1.6
Eptifibatide 135/0.75 dose	0.8	6.9	2.9	2.0
RESTORE Trial*				
Placebo	0.7	5.7	4.0	1.4
Tirofiban	0.8	4.2	2.3	1.1
CAPTURE Trial				
Placebo	1.3	8.2	4.4	1.7
Abciximab	1.0	4.1	3.1	1.0
RAPPORT Trial†				
Placebo	2.1	4.1	5.4	1.2
Abciximab	2.5	3.3	1.7	0

*RESTORE Trial endpoints listed here are for the published post hoc analysis including only *urgent* repeat revascularization for consistency with the other trials. The primary composite endpoint of RESTORE included *urgent or elective* repeat revascularization. RESTORE trial endpoints listed here differ from those of the other trials in that only patients in whom the lesion was successfully crossed with the guidewire were included in the efficacy analysis of RESTORE, providing a "treated patient" analysis rather than the "intention-to-treat" analysis utilized in the other studies.

†RAPPORT endpoints listed here are for the secondary composite of death, myocardial re-infarction, and urgent *target vessel* revascularization. This endpoint differs from those of the other trials in that it does not include urgent *nontarget vessel* revascularization procedures.

CABG = coronary artery bypass graft surgery; MI = myocardial infarction; PCI = percutaneous coronary intervention.

Components of 30-Day Endpoint

The breakdown of individual components of the 30-d composite endpoint is detailed in Table 3. In general, the treatment effect of GP IIb/IIIa blockade was similar for each of the components of the composite endpoint. Point estimates of event rates for infrequent complications such as death were imprecise, and although a trend toward diminished mortality was observed in EPILOG, EPISTENT, IMPACT II, and CAPTURE, these differences did not reach statistical significance. MI rates were consistently dimin-

ished by treatment with GP IIb/IIIa inhibitors compared with placebo; rates of urgent repeat revascularization were usually reduced as well. The EPISTENT trial requires particular explanation due to the asymmetry of the treatment groups (abciximab plus stent or abciximab plus PTCA compared with the reference arm of placebo plus stent). Compared with stenting alone, adjunctive use of abciximab with stenting reduced the rates of death, MI, and repeat revascularization. Compared with stenting alone, abciximab with balloon angioplasty was associated with equivalent rates of death and revascularization, but lower rates of myocardial infarction.

The most frequent ischemic event prevented in these GP IIb/IIIa interventional trials was myocardial infarction, predominantly non-Q-wave infarction. Although the clinical importance of such periprocedural non-Q-wave infarctions following percutaneous coronary revascularization was initially controversial, virtually every contemporary study that has examined the impact of periprocedural enzyme release over an adequate follow-up period has demonstrated that patients who experience MI during or after coronary intervention are at significantly greater risk for late cardiac death than those who do not *(21–27)*. Although an increased risk of late events has been observed in these studies even among patients with "small" CK-MB elevations (>1–1.5 times control) *(24–26),* the extent of mortality risk appears to be proportional to the degree of enzyme elevation. It is, therefore, notable that the effect of the GP IIb/IIIa inhibitors on reducing periprocedural myocardial infarction in these trials was primarily observed for large non-Q-wave infarctions (MB creatine kinase >5 times control), confirming that the ischemic events prevented were clinically relevant, not merely laboratory abnormalities.

Efficacy in Patient Subgroups

Although it might be anticipated that intense platelet inhibition with a GP IIb/IIIa inhibitor would provide substantial clinical benefit only among patients at high risk for thrombotic complications, a remarkable consistency of treatment effect has in fact been observed among all subgroups of patients enrolled in the trials. This finding was most apparent in EPILOG and EPISTENT, wherein the reduction in ischemic endpoints was of similar magnitude among patients undergoing intervention for unstable angina, recent MI, or stable ischemic symptoms; clinical benefit from GP IIb/IIIa blockade has been demonstrated in all patient subgroups in the other trials as well. Nevertheless, certain patients appeared to derived enhanced benefit. Most notably, among those with unstable angina and ischemic electrocardiographic changes in EPIC, the composite endpoint rate was decreased by 71% with abciximab and the most serious endpoints of death or MI were reduced by 94% (11.1 vs 0.6%; $P < 0.001$) *(19)*. Similarly, among the patients requiring "bailout" or unplanned stent placement in EPILOG, in whom high-risk status was evidenced by a doubling of the placebo group ischemic event rate compared with patients who did not require a stent (22.6 vs 10.0%, respectively), treatment with abciximab resulted in a marked reduction in the composite endpoint from 22.6 to 9.3% *(28)*.

Long-Term Efficacy

Patients were followed for at least 6 mo after randomization in all of the interventional trials for assessment of clinical endpoints. In EPIC, the earliest of these studies, double-blinding was maintained and clinical data were obtained for up to 3 yr.

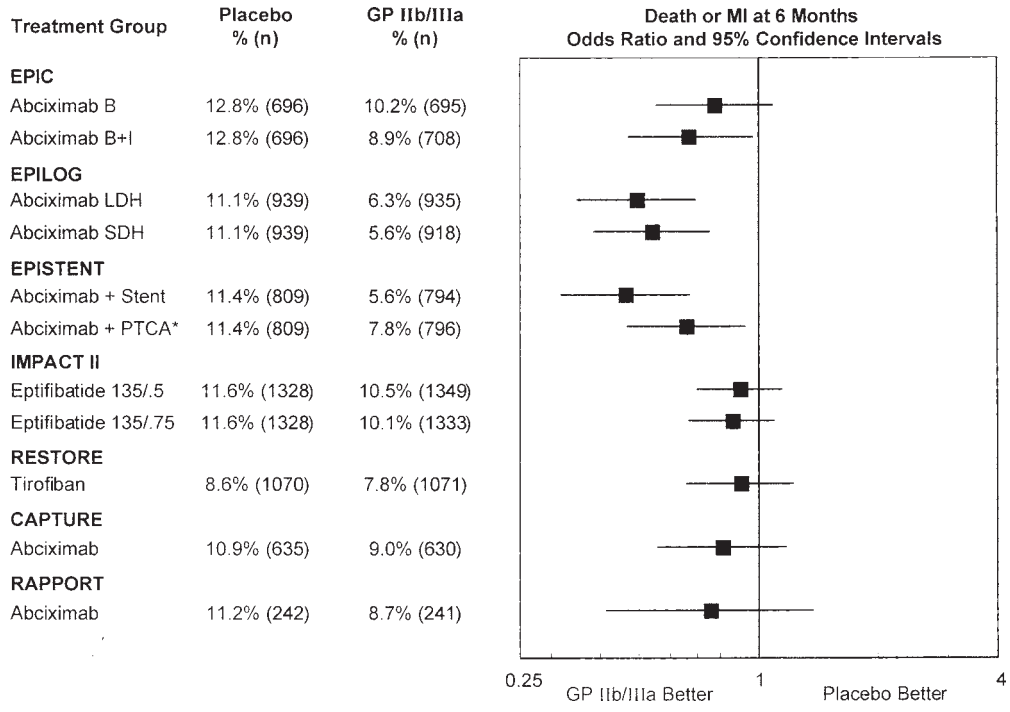

Fig. 4. Composite 6-mo endpoint of death or myocardial (re-) infarction event rates for the GPIIb/IIIa interventional trials. EPISTENT trial groups compared with reference group of Placebo + Stent. Abbreviations as for Fig. 3.

Acute Ischemic Endpoints

Rates of death or myocardial infarction at 6 mo in the seven trials are summarized in Fig. 4. In general, the treatment effect in reducing these acute ischemic events which was achieved early (by 30 d) by GP IIb/IIIa blockade was maintained without attenuation over the long term. The same observation held true for the suppression of urgent revascularization events in those trials where the urgency of revascularization procedures was adjudicated over long-term follow-up. In EPILOG, for example, the composite endpoint of death, MI, or urgent repeat revascularization was reduced in the combined abciximab groups compared with placebo from 11.7 to 5.3% at 30 d, from 14.7 to 8.4% at 6 mo, and from 16.1 to 9.6% at 1 yr; thus, the absolute reduction by abciximab in the composite endpoint (number of events prevented per 100 patients treated) was remarkably constant, 6.3 to 6.5, at each of these time points.

The long-term follow up at 6 mo and 1 yr after randomization in the EPISTENT trial provide compelling evidence of a complementary clinical benefit of abciximab and stenting. Against the background of the current optimal antiplatelet regimen of abciximab, mortality was reduced by more than 70% with stenting as compared with PTCA at 6 mo. By 1 yr, the combined treatment resulted in a lower mortality rate than either stenting or abciximab alone (stent + placebo = 2.4%, PTCA + abciximab = 2.1%, stent + abciximab = 1.0%, $P = 0.037$), establishing the combination of stenting and abciximab as the new standard of safety and efficacy during coronary intervention.

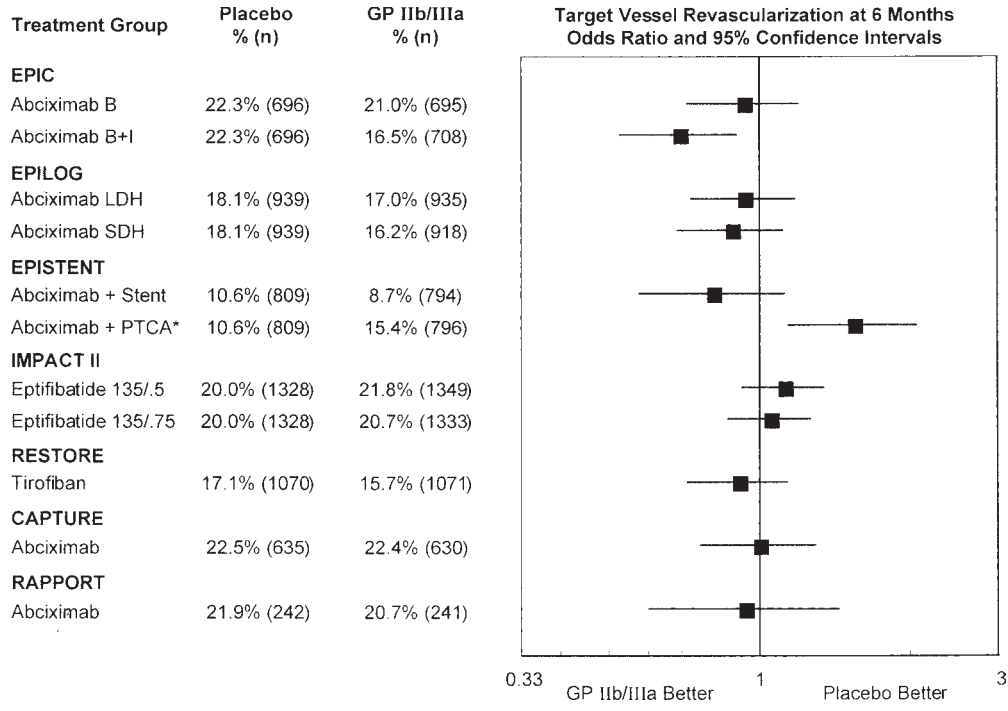

Fig. 5. Rates of 6-mo target vessel revascularization for the GP IIb/IIIa interventional trials. EPISTENT trial groups compared with reference group of Placebo + Stent. Abbreviations as for Fig. 3.

In the three-year EPIC follow-up, patients treated with abciximab had a sustained reduction in the composite endpoint of death, MI, or revascularization; survival curves diverged during the first year of follow-up and remained largely parallel thereafter. A trend toward decreased mortality with abciximab was observed over 3 yr in the overall cohort, with a more marked 60% reduction by abciximab among the 555 highest risk patients who had been enrolled with unstable angina or acute myocardial infarction (12.7 vs 5.1%; $P = 0.01$).

In the CAPTURE trial, the incidences of death or revascularization over 6 mo were not different among placebo- and abciximab-treated patients. The treatment effect of abciximab on myocardial infarction rates persisted by 6 months, but was somewhat attenuated (8.2 vs 4.1%, absolute 4.1% difference at 30 d; 9.3 vs 6.6%, absolute 2.7% difference at 6 mo). Such attenuation over long-term follow-up is in contradistinction to the experience in the other GP IIb/IIIa trials during coronary intervention, and may be related to the severity of the acute ischemic syndrome or to inadequacy of the one-hour postprocedural abciximab infusion in CAPTURE.

Target Vessel Revascularization

The 6-mo follow-up results of the EPIC trial demonstrated that therapy with abciximab was associated with a significant reduction in the need for target vessel revascularization procedures (elective or urgent) at 6 mo, from 22.3% among patients receiving placebo

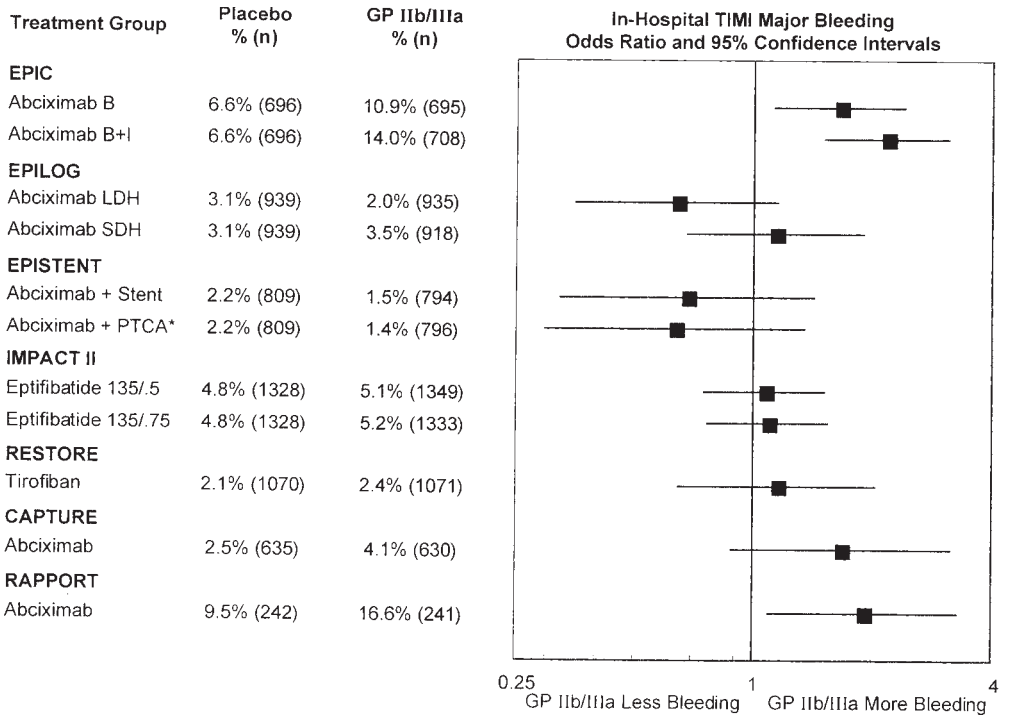

Fig. 6. Rates of in-hospital TIMI major bleeding *(20)* in the GP IIb/IIIa interventional trials. EPISTENT trial groups compared with reference group of Placebo + Stent. Abbreviations as for Fig. 3.

to 16.5% among those receiving the bolus and infusion of abciximab (26% relative risk reduction, $P = 0.007$), a finding that led to speculation that this agent may reduce restenosis following coronary intervention. Routine angiographic follow-up was not performed in this trial; thus, the influence of abciximab on angiographic restenosis could not be confirmed. A significant reduction in the need for late elective target vessel revascularization procedures by abciximab was not observed at 6 mo or 1 yr in the subsequent EPILOG trial, however, nor have the other interventional trials with GP IIb/IIIa blockade during balloon angioplasty demonstrated a significant decrease in "clinical restenosis" (Fig. 5). Angiographic substudies within the IMPACT II and RESTORE trials showed no differences among treatment groups in angiographic measurements of luminal dimensions or restenosis. Yet this question remains incompletely resolved. A trend toward reduction in long-term revascularization rates was consistently observed in the EPIC, EPILOG, and EPISTENT trials, with a pooled relative risk reduction of ~13%; such a difference is statistically insignificant, but may not be clinically irrelevant. Moreover, the 6-mo findings of EPISTENT suggest that abciximab may inhibit the neointimal hyperplastic component of in-stent restenosis. Target vessel revascularization rates and angiographic parameters of restenosis trended toward improvement among patients receiving abciximab rather than placebo with stenting. Strikingly, diabetic patients receiving stents, a group in whom increased neointimal hyperplasia has been

observed *(29,30),* had marked and statistically significant improvements in repeat target vessel revascularization rates and angiographic restenosis with abciximab.

Safety

The major limitation of abciximab therapy in the first interventional trial, EPIC, was a substantially increased risk of bleeding. The bolus and infusion regimen of abciximab was associated with a doubling in the incidence of major bleeding events and the need for red blood cell transfusions. During subsequent trials, in which heparin dosing was limited and vascular sheaths removed early, bleeding rates were diminished in all treatment groups and no significant increase in hemorrhagic risk was associated with GP IIb/IIIa therapy. It is remarkable to note that with increasing clinical experience with this class of agents, incremental bleeding risk associated with their use during coronary intervention has steadily declined (Fig. 6); moreover, a trend toward *lower* rates of bleeding in the abciximab groups compared with placebo has consistently been observed when the low-dose, weight-adjusted heparin regimen was used (in EPILOG and EPISTENT). The high rate of bleeding in RAPPORT is likely related to the long sheath dwell times.

Thrombocytopenia occurred infrequently, but was somewhat increased among patients receiving GP IIb/IIIa antagonists, particularly abciximab (Table 4). Rates of severe thrombocytopenia (platelet count <50,000 mm^{-3}) ranged as high as 1.0–1.8%.

Coronary Intervention in Trials of Unstable Angina

Four large-scale trials have assessed GP IIb/IIIa inhibition among patients with unstable angina or non-Q wave myocardial infarction in whom percutaneous revascularization was not mandated. In two of these trials, Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) and Platelet Receptor Inhibition in ischemic Syndrome Management in Patients Limited by Unstable Signs and symptoms (PRISM PLUS), a substantial number of patients underwent early coronary intervention while on study drug infusion, providing important information regarding the efficacy of these agents as preprocedural and postprocedural therapy. In PURSUIT, 9461 patients were randomized to receive placebo or eptifibatide (180 μg/kg bolus, then 2.0 μg/kg-min infusion for 72–96 h) in addition to heparin or aspirin; of these, 1228 underwent percutaneous revascularization within the first 72 h (while receiving study drug) *(31)*. Eptifibatide was associated with a significant reduction in the risk of myocardial infarction prior to the revascularization procedure (5.5 vs 1.8%, P <0.001), as well as a significant reduction in the composite endpoint of death or MI by 30 d (16.8 vs 11.8%, 30% relative risk reduction, P = 0.01). In PRISM PLUS, 1570 patients were treated with placebo or tirofiban (0.4 μg/kg-min for 30 min, then 0.1 μg/kg-min infusion for 48–96 h) in addition to aspirin and heparin *(32)*. Catheterization and percutaneous revascularization were encouraged, but to be deferred for 48 h, and 475 patients underwent coronary intervention while receiving study drug. During the first 48 h (before intervention), death or myocardial infarction rates were reduced from 2.6 to 0.9% by tirofiban in the overall 1570 patient cohort (P = 0.01); among the 475 patients undergoing intervention, 30-d rates of death or MI were 10.2 and 5.9% in the placebo and tirofiban groups, respectively (42% relative risk reduction, P = 0.12).

Table 4
Rates of Thrombocytopenia

	Agent	*Placebo (%)*	*GP IIb/IIIa Inhibitor (%)*
Moderate Thrombocytopenia (Platelet Count < 100,000 mm^{-3})			
EPIC	Abciximab	3.4	3.6 (bolus) 5.2 (bolus + infusion)
EPILOG	Abciximab	1.5	2.6 (std dose heparin) 2.5 (low dose heparin)
EPISTENT	Abciximab	0.6	3.1 (plus stent) 2.9 (plus PTCA)
CAPTURE	abciximab	1.3	5.6
RAPPORT	Abciximab	2.9	5.0
IMPACT II	Eptifibatide	2.7	3.2 (135/0.5 dose) 2.8 (135/0.75 dose)
RESTORE*	Tirofiban	0.9	1.1
Severe Thrombocytopenia (Platelet Count < 50,000 mm^{-3})†			
EPIC	Abciximab	0.7	0.4 (bolus) 1.6 (bolus + infusion)
EPILOG	Abciximab	0.4	0.9 (std dose heparin) 0.4 (low dose heparin)
EPISTENT	Abciximab	0	1.1 (plus stent) 0.9 (plus PTCA)
CAPTURE	Abciximab	0.3	1.8
IMPACT II	Eptifibatide	0.6	0.2 (135/0.5 dose) 0.4 (135/0.75 dose)
RESTORE	Tirofiban	0.1	0.2

*Reported in RESTORE for platelet count < 90,000 mm^{-3}.
†Data not available for severe thrombocytopenia in RAPPORT.
std = standard.

SYNTHESIS OF THE RANDOMIZED TRIALS

Efficacy

The consistent finding among over 15,000 patients enrolled in the trials of GP IIb/IIIa receptor blockade during coronary intervention has been that of reduction in the risk of important acute ischemic events by as much as 50–60%, unequivocally establishing the clinical efficacy of this class of therapy in this setting. This treatment effect extends to each of the components of the composite clinical endpoint (death, MI, and emergency revascularization), attesting to the common platelet-thrombus mediated pathophysiology of these events. The inhibition of acute ischemic events is achieved early, primarily in the first 12–48 h after the revascularization procedure, and is almost invariably maintained without attenuation over long-term (up to 3 yr) follow-up.

Improved outcome with this therapy has been apparent in every subgroup of patients

tested, and no demographic, clinical, angiographic, or procedural characteristic has been observed that will identify patients who do *not* benefit from GP IIb/IIIa blockade. Patients with acute ischemic syndromes such as unstable angina, however, appear to derive exceptional treatment effect from this class of therapy. Other subsets of patients who are at increased risk for acute ischemic events, such as those requiring bailout stenting *(28)* or with diabetes *(33),* also tended to experience an enhanced absolute treatment effect from GP IIb/IIIa inhibition.

Clinical benefit is derived from GP IIb/IIIa blockade irrespective of the technique or modality used for percutaneous coronary revascularization. The EPISTENT trial clearly demonstrated a magnitude of treatment effect with abciximab during elective stenting (absolute risk reduction 5.5%, relative risk reduction 51%) that was essentially identical to that obtained with abciximab during balloon angioplasty in EPILOG (absolute risk reduction 6.4%, relative risk reduction 56%). Similarly, subgroup analysis of patients in EPIC and EPILOG undergoing directional atherectomy confirmed previous findings that atherectomy patients are at substantially greater risk for ischemic complications than their counterparts treated with angioplasty, and that the treatment effect of abciximab during atherectomy tended, if anything, to be greater than that during balloon angioplasty *(34)*. The decision to utilize these agents during a revascularization procedure thus should not be made contingent upon whether balloon angioplasty, stent implantation, or atherectomy is planned.

An important influence of GP IIb/IIIa blockade on restenosis following balloon angioplasty is unlikely. Although a significant reduction by abciximab in long-term target vessel revascularization rates was observed in the EPIC trial, this treatment effect has not been definitively confirmed in any subsequent study of a GP IIb/IIIa inhibitor during nonstent interventions. Nor have angiographic substudies of IMPACT II and RESTORE found an influence of GP IIb/IIIa blockade on angiographic restenosis. In contrast, patients receiving stents (wherein the processes of recoil and remodeling are not operative and neointimal hyperplasia remains as the predominant mechanism of restenosis) trended toward better outcome with regard to both clinical and angiographic parameters of restenosis if treated with abciximab rather than placebo. In particular, the subgroup of patients with diabetes mellitus had significant and marked clinical and angiographic benefit (a halving of the rate of repeat revascularization following stenting), suggesting that abciximab may reduce in-stent restenosis, particularly among patients at elevated risk for this late complications.

Differences in Efficacy Among the Agents

Although all three of the agents tested in large-scale trials (abciximab, eptifibatide, and tirofiban) reduce ischemic risk, there does appear to be heterogeneity among the drugs with regard to the magnitude of treatment effect. The bolus followed by 12-h infusion regimen of abciximab was demonstrated to reduce 30-day endpoints by as much as 50–60% in the EPILOG, RAPPORT, and EPISTENT trials, whereas more modest risk reductions on the order of 15–25% were achieved with eptifibatide and tirofiban in IMPACT II and RESTORE. In the latter two trials, ischemic events were reduced by GP IIb/IIIa blockade by 30–40% at 24–48 h, but attenuation of treatment effect occurred over the subsequent 30 d. Definitive assessment of the magnitude of the differences among these agents is limited by the lack of direct comparative trials. Nevertheless, based upon indirect comparisons, the differences in treatment effects may be real and

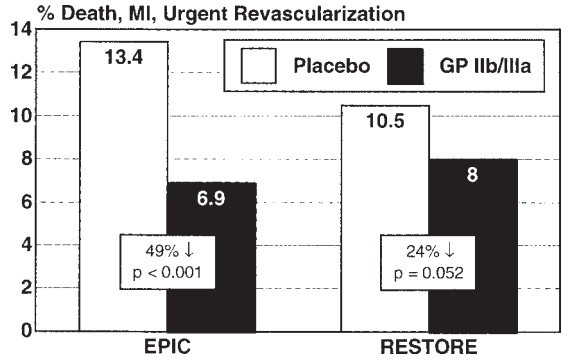

Fig. 7. Comparison of EPIC and RESTORE trial results for the 30-d composite endpoint of death, MI, and urgent repeat revascularization. The EPIC endpoints are recalculated to match the analysis of the RESTORE trial; only patients in whom the coronary lesion was successfully crossed with a guidewire and who received study drug are included ("treated patient" analysis).

clinically relevant. Variability among agents with regard to the magnitude of treatment effect is particularly apparent in the comparison between the EPIC and RESTORE trials, which enrolled similar patient populations. When the EPIC primary endpoint is adjusted to match the "treated patient" analysis used in RESTORE (by including only patients in whom the lesion was crossed with a guidewire and study drug administered), the relative risk reduction for the 30-d composite endpoint of death, myocardial infarction, or urgent revascularization was 49% with abciximab in EPIC and 24% for tirofiban in RESTORE (Fig. 7).

Variability among agents in efficacy may in part be due to the pharmacodynamics of receptor binding. Abciximab dissociates slowly from the GP IIb/IIIa receptor, thus providing gradually diminished inhibition of platelet aggregation for 36 h or more after termination of drug infusion *(6,8)*; in contrast, platelet aggregation following discontinuation of the rapidly reversible agents (eptifibatide and tirofiban) is normalized within 2–4 h *(9,10)*. Additionally, the non-specific blockade by abciximab of both the platelet GP IIb/IIIa receptor and the $\alpha_v\beta_3$ vitronectin receptor may provide an advantage over the specific agents, as ex vivo experimental studies have suggested that dual receptor blockade more completely suppresses platelet-mediated thrombin generation than does inhibition of either receptor alone *(35)*.

For eptifibatide, the relatively modest treatment effect in the IMPACT II trial was almost certainly due in part to inadequate dosing based upon dose-finding studies which used sodium citrate as an anticoagulant for platelet aggregation measurements. Subsequent to IMPACT II, it was observed that the binding of eptifibatide to GP IIb/IIIa was exaggerated in blood anticoagulated by citrate (which chelates calcium) relative to that which would occur at physiologic calcium concentrations *(36)*. Doses of eptifibatide used in IMPACT II thus likely achieved only 30–40% inhibition of platelet aggregation in vivo. The potential for higher doses of eptifibatide to more effectively reduce ischemic complications is suggested by the greater treatment effect among the subgroup of patients in the PURSUIT trial who underwent early percutaneous coronary revascularization (described previously). Importantly, however, patients undergoing coronary intervention with tirofiban in the PRISM PLUS trial also had a more substantial

treatment effect than was seen with the same drug (at a 50% higher dose) in RESTORE. Thus, a prolonged duration of treatment (72 h or more), perhaps with a period of preprocedural therapy, may be required for the rapidly reversible agents to have an optimal effect on reduction of ischemic endpoints. Alternatively, prolonged treatment with an oral GP IIb/IIIa inhibitor (*see* Chapter 14) after periprocedural therapy with eptifibatide or tirofiban may provide a greater durability of the early (24–48 h) treatment effect.

Safety

Bleeding

The major potential safety issue with this as well as other classes of agents directed against platelet function or coagulation is that of bleeding. The findings of the first trial of this class of therapy, EPIC, highlighted the potential for hemorrhagic risk with these agents. Most bleeding with GP IIb/IIIa antagonists in this and subsequent trials was at sites of vascular access, although spontaneous gastrointestinal and genitourinary bleeding also occurred; long-term sequelae were infrequent. Importantly, pooled analysis of the trials indicate that rates of intracranial hemorrhage do *not* appear to be increased. The remarkable improvement in the safety profile of these agents subsequent to the EPIC experience clearly shows that modification of conjunctive anticoagulant therapy with heparin by weight adjustment and dose reduction is the key intervention in abrogating excess bleeding risk. Early removal of vascular sheaths and meticulous care of the access site are also likely important means of avoiding hemorrhage. Bleeding may, nevertheless, be a concern in certain groups of patients, such as those who receive GP IIb/IIIa blockade as an unplanned or "bailout" intervention in the setting of full-dose heparinization or those undergoing "rescue" angioplasty for failed reperfusion after full-dose thrombolysis. Partial reversal of heparinization with protamine in the former situation and very careful heparin dose-reduction and ACT monitoring in the latter will likely improve the balance between risk and benefit in these patients.

For patients who develop refractory or life-threatening bleeding, the antiplatelet effect of the intravenous GP IIb/IIIa inhibitors may be reversed by platelet transfusions. Platelet transfusions are rarely necessary with the rapidly reversible agents eptifibatide and tirofiban, but may be an important intervention in patients who have received abciximab. Following transfusion, abciximab redistributes from old to new platelets, reducing the mean level of receptor blockade. In experimental studies, transfusion of the equivalent of 10 U of platelets in primates treated with abciximab led to prompt reduction in mean receptor blockade from 80 to 90% to less than 70%, near normalization of bleeding times, and partial (~30%) recovery of platelet aggregation *(37)*.

Emergency Coronary Bypass Surgery

There is often considerable concern on the part of cardiac surgeons regarding the risk of excessive perioperative bleeding among patients who require emergency coronary artery bypass surgery for failed angioplasty after administration of a GP IIb/IIIa inhibitor. In this regard, the rapidly reversible agents eptifibatide and tirofiban present little in the way of perioperative bleeding risk; platelet aggregation and bleeding times return to normal following discontinuation of both of these agents within a few hours, the time period required for coronary artery bypass to be performed. For patients treated with abciximab, transfusion of mixed donor platelets (following discontinuation of

abciximab infusion) ameliorates much of the hemorrhagic risk *(38)*. As some data suggest that GP IIb/IIIa blockade may actually be *protective* of platelets during cardiac surgery, with less thrombocytopenia following cardiopulmonary bypass *(39),* it seems reasonable to defer platelet transfusions if possible until after the cardiopulmonary bypass is completed.

Thrombocytopenia

Thrombocytopenia occurs infrequently following GP IIb/IIIa inhibition, but may be profound (platelet count < 20,000 mm^{-3}); the excess risk of profound thrombocytopenia associated with abciximab (~0.4–1%) appears to be higher than with eptifibatide or tirofiban. The mechanism of development of thrombocytopenia is unknown. Thrombocytopenia occurring after administration of a GP IIb/IIIa agent can usually be differentiated from that because of the heparin-induced thrombocytopenia syndrome by the early and precipitous onset, generally within 1 to 24 h after administration of the GP IIb/IIIa inhibitor *(40)*. There is little evidence of ongoing platelet clearance following discontinuation of the GP IIb/IIIa antagonist, and most patients experience an increase in platelet count of about 20–30,000 mm^{-3} per day (the rate of bone marrow production). Unlike heparin-induced thrombocytopenia, platelet transfusions are a safe and protective therapy for profound thrombocytopenia with or without serious bleeding induced by GP IIb/IIIa inhibitors. It is thus important that platelet counts be measured early (within the first 2–4 h) and again approximately 12 h after administering these agents in order to detect this rare, potentially life-threatening, but manageable complication.

Readministration

The development of a human antichimeric antibody (HACA) response in approximately 5–6% of patients within the first month after receiving abciximab raises the question of safety of readministration of this agent. No antibodies have been observed to develop in response to treatment with eptifibatide or tirofiban. A prospective abciximab readministration registry has found no instances of hypersensitivity or anaphylactic reactions following abciximab readministration, and efficacy of the agent in reducing ischemic complications appears to be similar with readministration as with first-time use *(41)*. Rates of thrombocytopenia following readministration were somewhat higher, however, than those seen with first-time administration, although the presence or absence of a positive HACA titer was not predictive of a lack of clinical effectiveness, development of thrombocytopenia, or other sequelae in patients undergoing readministration.

Economics

An important factor which appears to limit the widespread use of these agents during coronary intervention is that of cost. The average (weight-adjusted) dose (bolus plus 12 h infusion) of abciximab costs approximately $1400. Prices for eptifibatide and tirofiban are lower, approximately $300–400 per day, although the optimal duration of therapy for these latter drugs is not defined. The economic aspects of GP IIb/IIIa therapy are discussed in detail in Chapter 12. In general, though, it is important to recognize that drug price does not reflect the true economic cost of these therapies, as the prevention of ischemic events by GP IIb/IIIa blockade translates into cost savings. In both EPIC and EPILOG, for example, the prevention of ischemic events by abciximab was associated with $600–700 cost savings during the hospitalization period *(42)*. In EPIC, cost savings

from suppression of ischemic events were offset by the increased incidence of bleeding complications; in contrast, in EPILOG, where bleeding risk was not increased, the net hospitalization cost per patient for abciximab therapy was only ~$600. Among the highest risk patients undergoing revascularization for unstable angina in EPIC, abciximab therapy actually appeared to be *cost saving,* and thus the "dominant strategy" ("dominant strategy" defined as one that reduces both adverse events *and* cost) *(19)*.

RECOMMENDATIONS

Whom to Treat

From a scientific standpoint, the extensive body of randomized data with this class of agents indicates that virtually all patients undergoing percutaneous coronary revascularization should receive a GP IIb/IIIa receptor antagonist. In clinical practice, however, these agents are not universally employed, primarily owing to economic considerations. Despite these constraints, it seems imperative to at least provide the marked benefits of this class of therapy to patients who are at elevated risk for periprocedural complications–most importantly those with the acute ischemic syndromes of unstable angina or myocardial infarction, but also those with complex lesion morphology, extensive myocardium at jeopardy, multivessel or multilesion interventions, or unplanned "bailout" stent implantation or diabetes mellitus. As noted earlier, the elective use of stents should not be considered as a replacement for GP IIb/IIIa receptor blockade in high risk patients, and in fact, the combination of stenting and abciximab appears to confer long-term mortality benefit.

In many centers and countries, abciximab is utilized primarily in an unplanned or "bail out" fashion as a strategy to reduce overall costs for this agent. Although anecdotal data suggest that abciximab is useful in reversing thrombotic complications of coronary intervention *(43),* this approach has never been tested in a randomized fashion. It is apparent that "bailout" abciximab administration does not completely reverse the increased ischemic risk in these complicated patients and that bleeding complications are likely to be relatively high because of full-dose heparinization. Until systematically investigated, such a strategy must, therefore, be regarded as suboptimal relative to planned or prophylactic use of GP IIb/IIIa receptor blockade. The upcoming Comparison of Abciximab Complications with Hirulog (and Abciximab Backup) Ischemic Events Trial (CACHET) will evaluate the efficacy of provisional abciximab therapy superimposed upon optimal antithrombin coverage with hirulog instead of heparin.

Which Agent to Use

Aside from the economic considerations, the available data consistently suggest that the current efficacy standard for GP IIb/IIIa blockade during and after percutaneous coronary intervention is abciximab administered as a bolus followed by a 12 h infusion. A pretreatment regimen of abciximab, as was utilized in CAPTURE, is effective in stabilizing patients prior to coronary revascularization, but offers no clear advantage in stable patients or even in unstable patients for whom revascularization can be immediately performed. Regardless of whether or not a period of pretreatment with abciximab is used, the 12-h postprocedural infusion appears to be mandatory for optimal passivation of the revascularized lesion.

For patients presenting with unstable angina or non-Q wave myocardial infarction in

whom revascularization is not immediately planned, both eptifibatide and tirofiban have been unequivocally shown to improve clinical outcome prior to intervention, following intervention, or in patients treated conservatively without early intervention (*see* Chapter 10). Once a course of empiric therapy with eptifibatide or tirofiban has been initiated, it is unknown whether patients would experience substantial incremental benefit by conversion to abciximab during subsequent percutaneous coronary revascularization, as the treatment effects of eptifibatide and tirofiban in PURSUIT and PRISM PLUS were considerable in these patients. Moreover, pharmacodynamic data do not yet exist regarding the extent of receptor blockade (and resultant hemorrhagic risk) if an abciximab bolus were to be administered immediately after therapy with eptifibatide or tirofiban. Until such data becomes available and greater clinical experience is obtained with the newly available agents, the most prudent recommendation might be to switch to abciximab during the periprocedural period only in those patients who appear to be at highest risk for intervention on the basis of clinical course or angiographic morphology. When eptifibatide is employed during coronary intervention, the available pharmacodynamic and clinical data (see earlier) strongly suggest that the PURSUIT dose of 180 μg/kg bolus and 2.0 μg/kg-min infusion be utilized, rather than the lower doses employed in IMPACT II.

Conjunctive Heparin and Vascular Access Site Management

The low-dose, weight-adjusted heparin regimen, using an initial bolus of 70 U/kg (maximum 7000 U) adjusted to attain and maintain an ACT ≥200s, has clearly been shown to be the safest and most effective means of administering heparin when abciximab therapy is planned. There are no data to suggest that postprocedural heparin provides additional benefit in this setting, even in patients treated for acute ischemic syndromes. Vascular access sheaths can typically be removed 2–6 h after the procedure, during the abciximab infusion, once the ACT is <175 s or the aPTT is <50 s. Manual or mechanical groin compression should be maintained for at least 30 min, followed by strict bedrest with leg immobilization for 6–8 h after sheath removal. The use of femoral artery closure devices in patients treated with abciximab has not yet been extensively investigated, but appears to be promising. Other measures to help reduce bleeding risk at the vascular access site include anterior arterial puncture only (rather than the traditional Seldinger "through-and-through" technique), avoidance of routine venous sheath placement, and adequate patient sedation and immobilization during periods of strict bedrest.

Optimal heparin dosing during coronary intervention in patients treated with eptifibatide and tirofiban has not been investigated. The higher bleeding rates observed with eptifibatide in PURSUIT as compared with IMPACT II, as well as favorable preliminary experience with high-dose eptifibatide and reduced-dose heparin during coronary intervention in the ongoing Phase II PRIDE study, suggest that intraprocedural heparin doses should be weight-adjusted and reduced with eptifibatide and tirofiban in manner similar to that as with abciximab.

Finally, many laboratories routinely employ a "standard-dose" weight-adjusted heparin regimen (100 U/kg, maximum 10,000 U, initial bolus, adjusted to attain and maintain target ACT>300 s) among patients undergoing percutaneous coronary intervention for whom abciximab therapy is *not* planned. With this strategy, bleeding risk may be reduced if abciximab or other GP IIb/IIIa inhibitors are required on a "bailout" basis, as very high ACT levels (>400 s) are usually avoided.

Clinical Trials and "Real World" Practice

Given the broad spectrum of patients and revascularization techniques evaluated in the seven interventional trials of GP IIb/IIIa inhibitors, it would be reasonable to anticipate that the findings of these trials would reflect outcome in clinical practice. A recent report of 3758 patients treated by percutaneous revascularization over a two-and-a-half year period in a community hospital confirms that trial results with these agents can indeed be extrapolated to the "real world" of medical practice *(44)*. Despite having greater lesion complexity on average, patients treated with abciximab during PTCA or stenting experienced a marked reduction in adverse ischemic events compared with patients who did not receive GP IIb/IIIa blockade. The occurrence of a composite endpoint of in-hospital death, myocardial infarction, or repeat revascularization was reduced from 5.0 to 1.9% (relative risk reduction 62%) following PTCA and from 2.4 to 1.3% (relative risk reduction 46%) after stenting ($P < 0.01$). Bleeding rates were low (0.6–0.8%) and not influenced by abciximab therapy. These and other data validate the controlled results of clinical trials in unselected populations of patients undergoing coronary interventional procedures.

SUMMARY

Platelet GP IIb/IIIa receptor blockade represents one of the most significant advances in the practice of interventional cardiology. Large-scale randomized controlled trials have unequivocally demonstrated that these agents reduce the risk of periprocedural ischemic complications by up to 50–60% and are efficacious in a broad spectrum of patients undergoing revascularization irrespective of risk profile, clinical indication for revascularization, or interventional technique. This clinical benefit may be achieved without excess bleeding risk by modification of conjunctive heparin dosing. Issues for future study include critical evaluation of the medico-economic aspects of this therapy, the effectiveness of these agents when used in an unplanned or "bail out" fashion, the role of oral GP IIb/IIIa antagonists currently under evaluation in extending the clinical benefit achieved by periprocedural parenteral administration, and the potential for combining GP IIb/IIIa antagonists with novel inhibitors of thrombin or other components of the coagulation cascade.

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III Glycoprotein IIb/IIIa Blockade for Acute Ischemic Syndromes

10 Unstable Angina: PARAGON, PURSUIT, PRISM, and PRISM-PLUS

David J. Moliterno, MD
and Harvey D. White, MB, DSC

CONTENTS

INTRODUCTION

Unstable angina is part of the acute coronary syndrome (ACS) spectrum and is adjacent to non-Q-wave myocardial infarction (MI) and slightly more distantly related to Q-wave MI. The diversity of conditions leading to unstable angina, as well as the varying symptoms upon presentation, have made the definition and classification of unstable angina difficult *(1)*. The Global Unstable Angina Registry And Treatment Evaluation (GUARANTEE) study reported that one-third of hospitalized patients had new or accelerated symptoms associated with exertion, whereas two-thirds had rest angina *(2)*. This distinction is important because patients with exertional angina may have a gradual worsening of an underlying atherosclerotic coronary arterial narrowing as opposed to those with rest angina who have an abrupt reduction in myocardial perfusion because of a ruptured plaque (Chapter 1). Angina at rest is often associated with electrocardiographic changes of ischemia and shares a similar underlying pathophysiologic mechanism to threatened vessel closure following percutaneous coronary angioplasty, i.e., plaque disruption. Whether spontaneous or as a result of angioplasty, plaque rupture leads to exposure of the coronary arterial subendothelial components, platelet activation, and thrombus formation.

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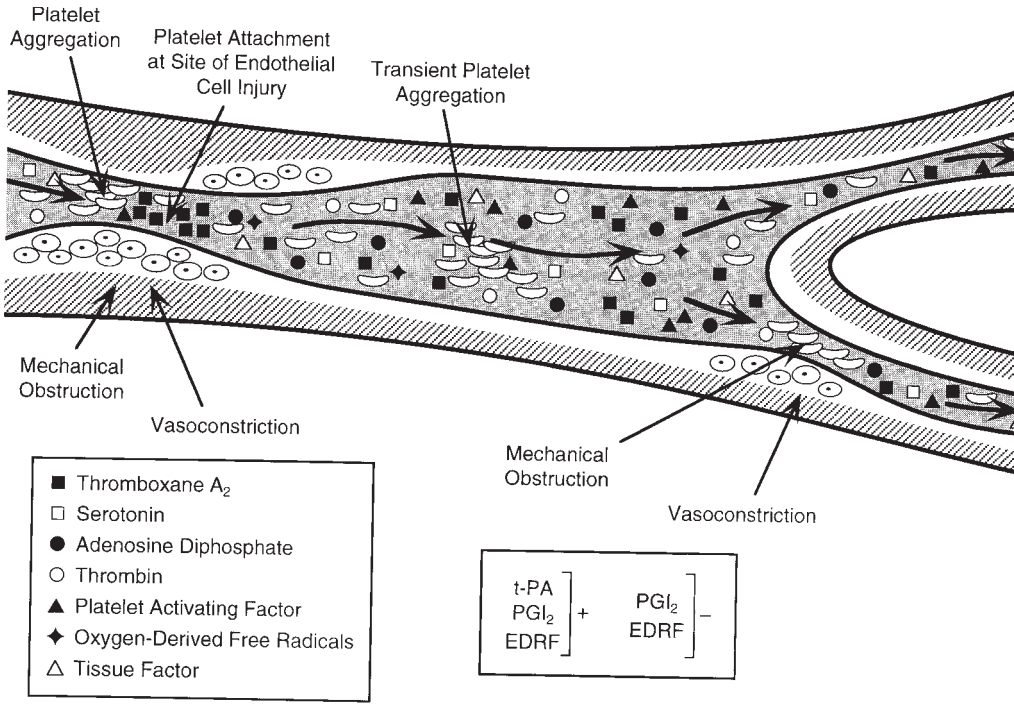

Fig. 1. Schematic diagram of mechanism underlying primary ACS. At the site of atherosclerotic plaque (anatomic obstruction) endothelial injury is present. This in combination with the release of vasoactive and platelet-activating substances such as thromboxane A_2, serotonin, thrombin, and adenosine diphosphate (ADP) causes a physiologic obstruction superimposed on the anatomic obstruction. Platelet activation and aggregation can occur as a result of these substances or in response to exposure of the subendothelial matrix following plaque fissuring or rupture. Platelets release additional vasoactive factors and fibrinogen which, in turn, leads to further vasoconstriction, platelet activation, thrombin formation, and potentially vessel obstruction. Adapted with permission from *(3)*.

The subendothelial constituents exposed following plaque rupture (e.g., collagen, von Willebrand factor, and fibronectin) are recognized by platelet surface receptors (primarily glycoprotein Ib) and platelet adhesion and activation occurs. During activation platelets secrete a host of substances from their alpha-granules, which lead to vasoconstriction, chemotaxis, mitogenesis, and activation of neighboring platelets *(3,4)* (Fig. 1). Platelet activation leads to the recruitment and "functionalization" of glycoprotein IIb/IIIa integrins or specialized surface receptors, which mediate aggregation (platelet-platelet binding). Aggregated platelets accelerate the production of thrombin by providing the surface for the binding of cofactors required for the conversion of prothrombin to thrombin. In a reciprocating fashion, thrombin is a potent agonist for further platelet activation, and it stabilizes the thrombus by converting fibrinogen to fibrin. Because the final common pathway leading to platelet aggregation is via fibrinogen binding between adjacent platelet IIb/IIIa receptors, blocking these receptors is a strategically ideal means to limiting ischemic events associated with unstable angina.

Despite the increased use of aspirin, an effective though weak antiplatelet agent,

Table 1
Randomized Trials of Aspirin in Patients with ACS

Study	*Patients*	*ASA duration (months)*	*Daily dose*	*MI or death*		Δ *Relative risk (%)*	P *Value*
				Control (%)	*Aspirin (%)*		
Unstable angina trials							
Lewis et al. *(9)*	1266	3	324 mg	10.1	5.0	–51	<0.001
Cairns et al. *(8)*	278	24	1300 mg	14.1	11.5	–20	0.008
Théroux et al. *(5)*	239	<1	650 mg	11.9	3.3	–72	0.012
Wallentin et al. *(6)*	288	3	75 mg	17.6	7.4	–58	0.004
Pooled	2071	~6		11.8	6.0	–49	<0.001
Acute MI trials							
Antiplatelet Trialists' Collaboration *(10)*	20,543	~1	~150–325 mg	14.4	10.6	–26	<0.001

unstable angina remains among the leading causes of adult hospitalizations in industrialized countries. In fact, the incidence of unstable angina continues to increase, and reportedly nearly one million hospitalizations each year in the United States are with a primary diagnosis of unstable angina. Thus, the testing and institution of platelet IIb/IIIa inhibitors into the medical armormentarium of the ACS is as necessary and clinically relevant as ever. This chapter will review the use of glycoprotein IIb/IIIa inhibitors in unstable angina and non-Q-wave MI, with particular emphasis on the phase III trials of intravenous IIb/IIIa inhibitors.

ANTIPLATELET THERAPIES

Aspirin

The benefit of aspirin therapy alone or in combination with heparin in the treatment of unstable angina has been proven in several randomized trials *(5–9)*. Aspirin, a cyclooxygenase and hydroperoxidase inhibitor, blocks synthesis of thromboxane A_2 and hinders platelet aggregation from some, but not all stimuli. In the study by Théroux and colleagues *(5)* entitled "Aspirin, Heparin, or Both to Treat Acute Unstable Angina," there was a significant reduction in cardiac death or MI from 11.9% in the placebo group, to 3.3% with aspirin alone, and 1.6% with the combination of aspirin and heparin ($P = 0.0042$). The Research Group on Instability in Coronary Artery Disease in Southeast Sweden (RISC) *(6)* demonstrated a 57% ($P = 0.033$) reduction in MI and death with aspirin therapy compared with placebo, whereas intermittent intravenous heparin showed no significant influence on these endpoints. One year follow-up of these patients continued to show nearly a 50% reduction ($P < 0.0001$) in death and MI in aspirin-treated patients compared with placebo *(7)*. Whereas the dose of aspirin and the duration of follow-up varied in each of these studies, a substantial reduction in relative risk of adverse cardiac events was consistently seen. Pooling data from more than 2000 patients *(5,7–9)* (Table 1), the occurrence of infarction or death was reduced from 11.8% (control) to 6.0% (aspirin). Similar results were reported by the Antiplatelet Trialists' Collaboration study for aspirin-treated patients with infarction *(10)* (Table 1).

Ticlopidine

Ticlopidine, a recently popularized antiplatelet agent because of its use following intracoronary stent implantation, inhibits adenosine diphosphate (ADP)-mediated platelet aggregation *(11)* and antagonizes the interaction of fibrinogen with the platelet's IIb/IIIa receptor *(12)*. Because these mechanisms are distinctly different from the actions of aspirin and require 48–72 h to become clinically manifest *(13)*, available data suggest ticlopidine to be similarly effective as aspirin in reducing adverse cardiac events in unstable angina although unlikely with a later onset of effect. Balsano et al. *(14)* reported the use of ticlopidine in a randomized study of 652 patients with unstable angina. Nonfatal infarction and vascular death were reported in 13.6% of control subjects and 7.3% of patients receiving ticlopidine, or a 46% risk reduction ($P = 0.009$). Considering available trial data with aspirin or ticlopidine compared with placebo, the Antiplatelet Trialists' Collaboration reported on seven trials including 4018 patients. The occurrence of adverse vascular events (MI, stroke, or vascular death) was reduced by 35% by antiplatelet therapy (14.1 vs 9.1%, $P < 0.001$) *(10)*.

Clopidogrel

Clopidogrel, an analog of ticlopidine, is also considered to antagonize the ADP-dependent activation of the platelet IIb/IIIa receptor by irreversibly and selectively blocking the binding of ADP to its receptor on the platelet surface. The largest comparative antiplatelet trial was a secondary prevention study testing aspirin vs clopidogrel among 19,185 patients with prior MI, ischemic stroke, or symptomatic peripheral arterial disease. The Clopidogrel vs Aspirin in Patients at Risk for further Ischemic Events (CAPRIE) trial enrolled patients between 1992 and 1995 in 16 countries *(15)*. At a mean follow-up of approximately 2 yr, there was a 9% relative reduction in the composite of ischemic stroke, myocardial (re)infarction, or vascular death with the use of clopidogrel. The annual occurrence of the primary endpoint was 5.83% of those treated with aspirin and 5.32% ($P = 0.043$) of those treated with clopidogrel. Among the subgroup enrolled following MI ($n = 6302$), no reduction in the primary endpoint was observed with clopidogrel, but rather a nonsignificant ($P = 0.66$) 3.7% relative increase in events compared with aspirin therapy.

IIb/IIIa RECEPTOR ANTAGONISTS

The newest, and most promising family of antiplatelet agents are the glycoprotein IIb/IIIa receptor inhibitors. As noted, platelet aggregation can be initiated by a number of pathways. However, the final common pathway of aggregation—irrespective of how it is initiated—involves the binding of the IIb/IIIa receptors of adjacent platelets by an interposing fibrinogen molecule. By blocking IIb/IIIa receptors, platelet aggregation can be effectively prevented. Many intravenous antagonists to the glycoprotein IIb/IIIa receptor have been recently developed; they are used during percutaneous coronary revascularization. Several clinical studies are ongoing for the use of oral IIb/IIIa inhibitors for in-hospital and postdischarge management of unstable angina and acute MI. Most recently, IIb/IIIa receptor antagonist have become approved for the primary treatment of unstable angina and non-Q-wave MI. Four such large clinical trials, the so-called "4 P's", were recently published: the Platelet IIb/IIIa Antagonism for the Reduction of Acute Coronary Syndrome Events in A Global Organization Network (PARAGON)

trial *(16)*; the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) study *(17)*; the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) study *(18)*; and the Platelet IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) trial *(19)*.

Abciximab

The first large-scale study with platelet IIb/IIIa inhibitors was with abciximab in the setting of percutaneous coronary revascularization *(20)* (Chapter 5). Subsequently, several large studies have been completed with this monoclonal antibody to the IIb/IIIa receptor. The Evaluation of c7E3 for the Prevention of Ischemic Events (EPIC) *(20)* trial enrolled high-risk angioplasty patients; whereas the Evaluation of PTCA to Improve Long-term Outcomes by c7E3 Glycoprotein Receptor Blockade (EPILOG) *(21)* trial enrolled "all comers" and excluded only those having an acute infarction or unstable angina with electrocardiographic changes at rest. Both trials included a large number of patients with unstable angina *(22,23)*. The Coronary Angioplasty for Refractory Unstable Angina (CAPTURE) enrolled only those angioplasty patients with medically refractory unstable angina *(24)* (Chapter 9). Whereas the overall cohort receiving abciximab (c7E3, ReoPro) in these three trials had a dramatic reduction in the composite occurrence of death, MI, or need for urgent revascularization following angioplasty or atherectomy, an even greater benefit was extended to those with unstable angina. Compared to placebo, those with unstable angina who received abciximab as bolus plus infusion had a 62–70% reduction in death, MI, or urgent revascularization in the first 30 d of follow-up (Fig. 2) *(22)*. Moreover, the EPIC investigators reported a 60% reduction in death at 3-yr follow-up among patients with unstable angina or evolving infarction receiving bolus plus infusion of abciximab during angioplasty *(23)*. Interestingly, this benefit became most evident after the first year of follow-up.

The indications for abciximab for patients with unstable angina have broadened following completion of the CAPTURE trial. The benefit of abciximab in reducing ischemia events during its adminstration in the 24 h before angioplasty demonstrates the effectivenesss of these agents in passivating the ruptured plaque of unstable angina. An arm of the fourth Global Utilization of Strategies to Open Occluded Arteries (GUSTO-IV) trial will be testing the use of abciximab as a primary therapy specifically among patients with unstable angina.

Lamifiban

Lamifiban (Hofmann-LaRoche, Basel, Switzerland) is a low-molecular-weight synthetic, nonpeptide, highly specific IIb/IIIa receptor antagonist. Lamifiban (then called Ro 44-9883) was first studied in unstable angina patients in The Canadian Lamifiban study *(25)*, a phase II, dose-exploring study of 365 patients. Individuals presenting with chest pain within 24 h and either electrocardiographic evidence of ischemia or known ischemic heart disease were randomized in a double-blind fashion to IIb/IIIa therapy or placebo *(25)*. Aspirin was given to all patients, and heparin was given to a minority (26%) of patients (at the discretion of the primary physician). Lamifiban was used as bolus plus infusion, with the bolus ranging from 150 g to 750 μg and infusion doses ranging from 1 to 5 μg/min for 72 h. Importantly, patients weighing < 70 kg had their infusion decreased 10–20%. In a dose-dependent fashion, platelet aggregation to 10 μmol ADP was inhib-

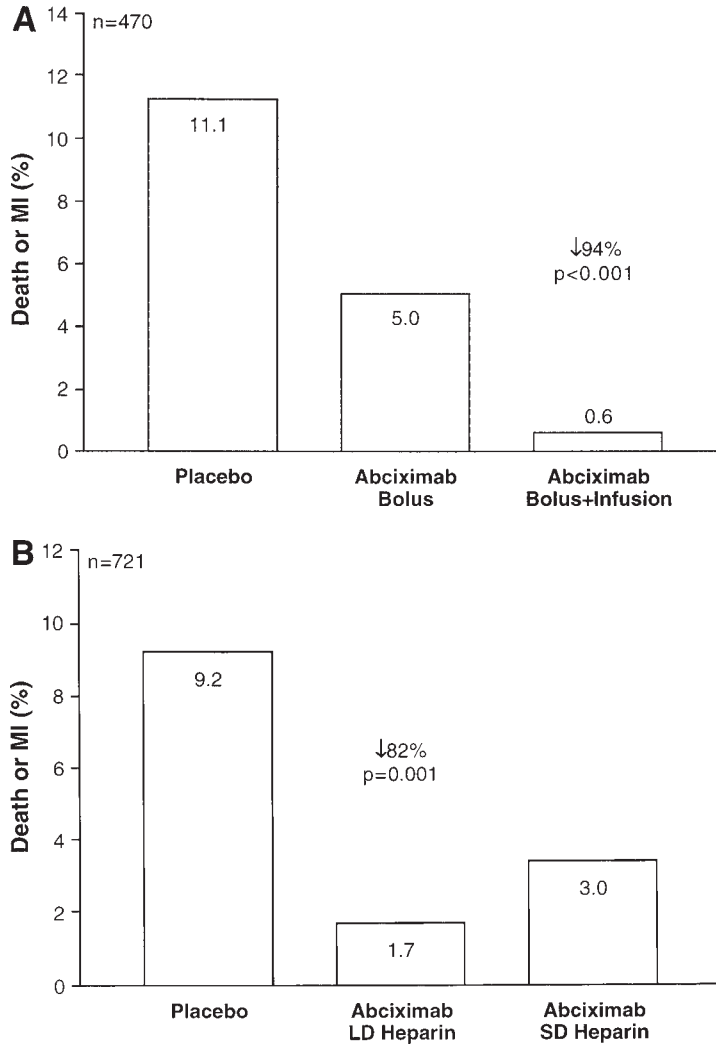

Fig. 2. The percent of unstable angina patients with death or myocardial infarction in the first 30 d of the EPIC trial **[panel A]** and the percent of patients with death or MI in the first 30 d of the EPILOG trials **[panel B]**. LD = low dose and SD = standard dose heparin In both studies, potent platelet inhibtion with abciximab produced a striking reduction in ischemic events among patients with unstable angina. Data from *(21,22)*.

ited 60 to >95%, and bleeding time was prolonged. At 30-d follow-up, compared with placebo, those assigned to low-dose lamifiban (1 or 2 μg/min) had a 23% reduction and those assigned to high-dose lamifiban (4 or 5μg/min) had a 70% reduction in the composite of death and MI (Fig. 3).

A phase IIIa study of lamifiban in unstable angina, the PARAGON trial, with 2282 patients was subsequently completed *(16)*. As previously described *(26)*, the objective of this trial was to assess several treatment strategies of glycoprotein IIb/IIIa inhibition (lamifiban), with the optimal strategy to be further tested versus standard therapy. Therefore, a partial factorial design was implemented with patients randomized to low-dose

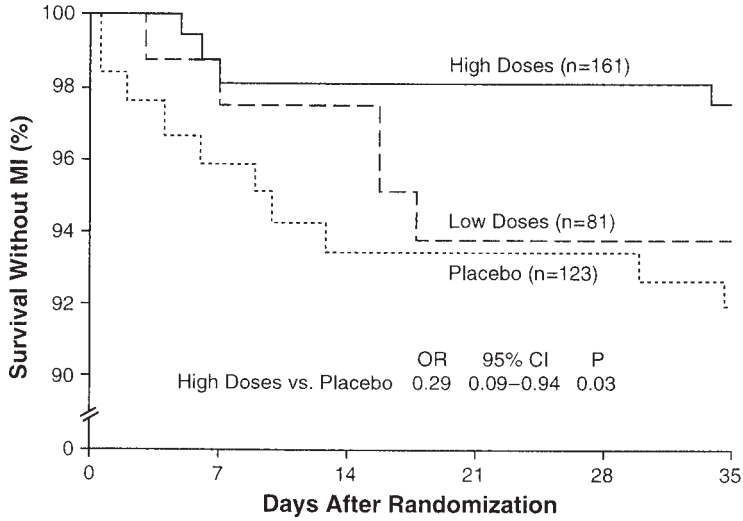

Fig. 3. Likelihood of infarction-free survival at 30 d among patients with unstable angina receiving placebo, or lamifiban infusion at low-dose (1 μg or 2 μg/min) or high-dose (4 μg or 5 g/min). Those assigned to a high dose of lamifiban had the best outcome. Reproduced with permission from *(25)*.

versus high-dose lamifiban and to heparin or no heparin therapy. The fifth possible group to be randomized to was the control group, who received placebo and heparin. Thus, each patient received lamifiban, unfractionated heparin, or both in addition to aspirin (Fig. 4). Patients assigned to low-dose lamifiban received a 300 μg bolus followed by a 1.0 μg/min infusion; those assigned to high-dose lamifiban received a 750 μg bolus followed by a 5.0 μg/min infusion. Patients enrolled into study had chest discomfort within the previous 12 h associated with transient or persistent electrocardiographic evidence of ischemia. Patients were excluded if they were already receiving oral anticoagulants, intravenous heparin, or recent thrombolytic therapy.

Lamifiban infusion was initiated and maintained according to initial treatment assignment; whereas, heparin was weight-adjusted and activated partial thromboplastic time (aPTT)-titrated. Systematic blinding of heparin administration and careful control of anticoagulation for all patients was achieved by use of a bedside aPTT device that produced encrypted results. A Hemochron® Jr. microcoagulation instrument automatically assayed whole blood collected and generated a multidigit code corresponding to the aPTT in seconds. Using the patient's study number and the coded aPTT result, the health care provider telephoned a central computer system, which deciphered the patient's aPTT and treatment assignment and then directed heparin or heparin-placebo infusion adjustments. The computer program titrated the heparin infusion according to a standardized nomogram to keep the aPTT to a laboratory equivalent of 60 to 85 s.

The primary endpoint was a composite of all-cause mortality and nonfatal MI (or reinfarction) in the first 30 d of follow-up. Secondary endpoints included death, myocardial (re)infarction, disabling stroke, major and intermediate bleeding at 30 d; death and MI at 6 mo; and death at 1-yr follow-up. Study groups sizes, drug administration, and in-hospital cardiac procedures are detailed in Table 2. Demographic data and

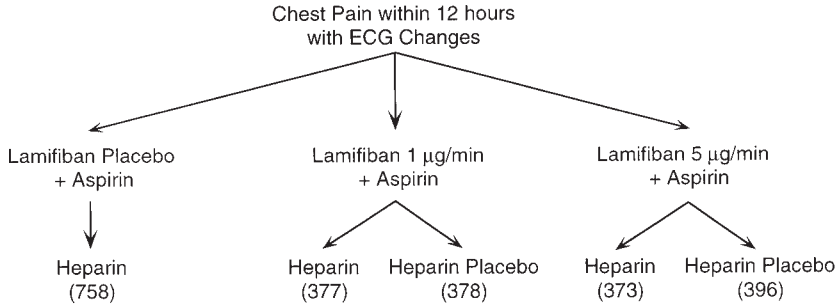

Fig. 4. Flow diagram summarizing the PARAGON study protocol, partial factorial design, and number of patients randomized. Reproduced from *(16)* with permission.

comparsions with the other 4-P trials are listed in Table 3. Principal safety and outcome data at 30 d and 6 mo are provided in Table 4. No difference in the composite of death or nonfatal MI was noted between the control group and any lamifiban group or the combination of all lamifiban-treated groups at 30 d. In contrast, at 6-mo follow-up, a substantial difference in the composite was present. Compared with the control group, three of the four lamifiban-treated groups had a lower event rate (Fig. 5). Specifically, death or nonfatal MI at 6 mo was lowered 24% by low-dose lamifiban with or without heparin (odds ratio, 0.72; 95% confidence interval, 0.54 to 0.96; $P = 0.027$) and 9% by high-dose lamifiban with or without heparin (odds ratio, 0.89; 95% confidence interval, 0.68 to 1.16; $P = 0.450$) compared with control. High-dose lamifiban with heparin patients had 30-d and 6-mo outcomes similar to those of the control group. In both of these groups approximately 12% of patients had reached the primary endpoint at 30 d and 18% had by 6 mo. All remaining lamifiban-treated groups had continued reduction in events compared with the control group over the first 180 d.

As can be seen in Figs. 5 and 6, the event rates diverged after 30 d. Between 30 and 180 d, the relative increase in myocardial (re)infarction was 36% for the control group, 15% for the low-dose lamifiban groups, and 15% for the high-dose lamifiban groups. Likewise, the relative increase in deaths during this time was 128% for the control group, 89% for the low-dose lamifiban groups, and 73% for the high-dose lamifiban groups. At 1 yr, all-cause mortality was 8.7, 7.3, and 8.9%, respectively ($P = 0.320$, Fig. 6). The overall incidence of bleeding complications and stroke are presented in Table 4. There were more bleeding-related events among those receiving high-dose lamifiban. The rates of major bleeding were 1.8, 0.6, and 0.8% for high-dose lamifiban groups, low-dose lamifiban groups, and control, respectively ($P = 0.058$).

Low-dose lamifiban coadministered with heparin had the greatest composite event rate reduction at both 30 d (12%) and 6 mo (30%) relative to standard therapy. This group also had the greatest reduction (20%) in all-cause mortality at 1 yr compared with control. With the minimal differences observed for clinical outcome with or without conjunctive heparin, the study was not adequately powered to draw clear conclusions regarding the benefit of heparin coadminstration. However, in combination with observations from Oler et al. *(27)*, PURSUIT, PRISM, and PRISM-PLUS, heparin likely adds a moderate benefit to weak antiplatelet agents and a more limited benefit to more potent (IIb/IIIa antagonists) agents.

Table 2
Study Designs and Enrollment Details

	PARAGON	*PRISM*	*PRISM-PLUS*	*PURSUIT*
Enrollment dates	8/95–5/96	3/94–10/96	11/94–9/96	11/95–1/97
Drug	Lamifiban	Tirofiban	Tirofiban	Eptifibatide
Patients enrolled total	2282	3232	1915	10,948
Treatment groups (bolus + infusion)	755 @ 300 μg + 1 μg/min 769 @ 750 μg + 5 μg/min 758 @ placebo	1616@ 0.6 μg/kg/min × 30 min + 0.15 μg/kg/min 1616 @ placebo	773 @ 0.4 μg/kg/min × 30 min + 0.1 μg/kg/min 345 @ 0.6 μg/kg/min × 30 min + 0.15 μg/kg/min 797 @ Placebo	1487 @ 180 μg/kg + 1.3 μg/kg/min 4722 @ 180μg/kg + 2.0 μg/kg/min 4739 @ placebo
Infusion duration	72 h	46 h	71 h	72 h
Heparin	Randomized, blinded aPTT 60–85 s	Randomized, blinded aPTT 1.5–2 × control	All patients aPTT 2 × control	Recommended (open-label) aPTT 50–70 s
Early drug discontinuation	19%	12%	14%	36%
PTCA during study	13%	21%	31%	24%
CABG during study	11%	17%	23%	14%
1° end point	Death or MI	Death, MI, or refractory ischemia composite	Death, MI, or refractory ischemia composite	Death or MI
Endpoint analysis	30 d	2 d	7 d	30 d

Table 3
Characteristics of Study Groups at Enrollment

Characteristic	*PARAGON*	*PRISM*	*PRISM-PLUS*	*PURSUIT*
Enrollment dates	8/95–5/96	3/94–10/96	11/94–9/96	11/95–1/97
Age, *y*	66	62	63	64
Female, %	35	32	33	35
Diabetes, %	18	21	23	22
Hypertension, %	49	54	54	55
Hypercholesterolemia, %	42	47	49	42
Current smoker, %	23	26	NA	28
Prior MI, %	35	47	43	32
Prior PTCA, %	11	15	10	13
Prior CABG, %	9	17	15	12
Presenting ECG				
ST-depression, %	52	32	58	50
ST-elevation, %	6	7	14	14
T-wave inversion, %	54	51	53	52
MI at enrollment, %	36	25	45	45

MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty; CABG = coronary artery bypass graft surgery; ECG = electrocardiogram

Tirofiban

Tirofiban (Aggrastat™, Merck, White House Station, NJ), a small molecule, nonpeptide IIb/IIIa receptor antagonist was initially studied in unstable angina in a phase II, heparin-controlled study of 71 patients *(28)*. Given for up to 48 h, tirofiban (then called MK-383) was well tolerated without significant bleeding. An encouraging reduction in the incidence of refractory angina was observed with tirofiban studied over a dose range of 0.075 to 0.15 μg/kg-min. These findings led to the designs of PRISM and PRISM-PLUS, two phase III tirofiban studies in unstable angina and non-Q-wave MI *(17,18)*. PRISM began enrollment in March 1994, and patients with accelerating or rest angina within 24 h and any clinical suspicion for ischemic heart disease were eligible. Suspicion for ischemia included: ST-segment or T-wave abnormalities consistent with ischemia; elevated cardiac enzymes; history of prior PTCA, CABG, or positive stress test; or a known coronary arterial narrowing ≥ 50%. In PRISM, 3232 patients were randomized to tirofiban (0.6 μg/kg-min "bolus" followed by a 0.15 μg/kg-min infusion) or heparin. The tirofiban loading dose was actually given as a 30-min infusion and the subsequent dose infusion was given for up to 47.5 h. The mean duration of infusion was 46 h with 12% of infusions being stopped early. All patients were to receive aspirin, and the target prolongation of aPTT among patients receiving heparin was twice the laboratory control value. Patient management was prespecified in that angiography and coronary intervention during the first 48 h were discouraged.

Dissimilar to other IIb/IIIa trials in unstable angina, the PRISM primary composite endpoint was at 48 h. The composite included death, MI, and refractory ischemia. The same endpoints plus rehospitalization for unstable angina were collected at 7 and 30 d. Refractory ischemia was defined as recurrent angina with either ST-T changes lasting ≥ 20 min within a 1-h period despite full medical therapy, or hemodynamic instability.

Table 4
Clinical Outcome

Characteristic	*PARAGON*			*PRISM*		*PRISM-PLUS*			*PURSUIT*		
Study group	*Placebo*	*Lamifiban 1 μg/min + Heparin*	*Lamifiban 5 μg/min + Heparin*	*Heparin*	*Tirofiban 0.15 μg/kg-min*	*Heparin*	*Heparin + Tirofiban 0.10 μg/kg-min*	*Tirofiban 0.15 μg/kg-min*	*Placebo*	*Eptifibatide 1.3 μg/kg-min*	*Epitifibatide 20 μg/kg-min*
n	758	755	769	1616	1616	797	773	345	4739	1487	4722
30-Day outcome											
Death, %	2.9	3.0	3.6	3.6	2.3	4.5	3.6	(6.1)	3.7	(3.4)	3.5
Nonfatal MI, %	10.6	9.4	10.9	4.3	4.1	9.2	6.6	(9.0)	13.5	(12.0)	12.6
Death or MI, %											
Overall	11.7	10.6	12.0	7.1	5.8	11.9	8.7	(13.6)	15.7	(13.4)	14.2
Relative reduction, %		9	–6		18		27				10
PTCA patients				9.1	7.2	10.2	5.9		16.8		11.8
Non-PTCA patients				6.2	3.6	7.8	3.6		15.7		14.6
6-Month outcome											
Death, %	6.6	5.2	6.8			7.0	6.9	(7.2)	6.2		17.8
Nonfatal MI, %						10.5	8.3	(10.1)	15.7		14.7
Death or MI, %						15.3	12.3	(15.9)	19.0		17.8
Relative reduction, %		23	8		NA		20				8
Major bleeding*, %	3.0	3.0	6.0	0.4	0.4	0.8	1.4		1.3		3.0
Intracranial hemorrhage, %	0	0	0.1	0.1	0.1	0	0		0.1		0.1
RBC transfusion†, %	4.4	4.4	8.7	1.4	2.4	2.8	4.0		1.8		4.4
Thrombocytopenia, %	1.1	1.5	1.3	0.1	0.4	0.3	0.5		0.4		0.6

MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty; RBC = red blood cells; Thrombocytopenia defined as platelet count ≤50,000/mm^3.

Numbers in parentheses () are from discontinued treatment arms and are not contemporaneous; these are listed only for completeness, not direct comparisons.

*Major bleeding as defined by intracranial hemorrhage or decrease in hemoglobin ≥5 g/dL not associated with coronary artery bypass grafting (CABG).

†Transfusions reported are not associated with CABG, except for PARAGON.

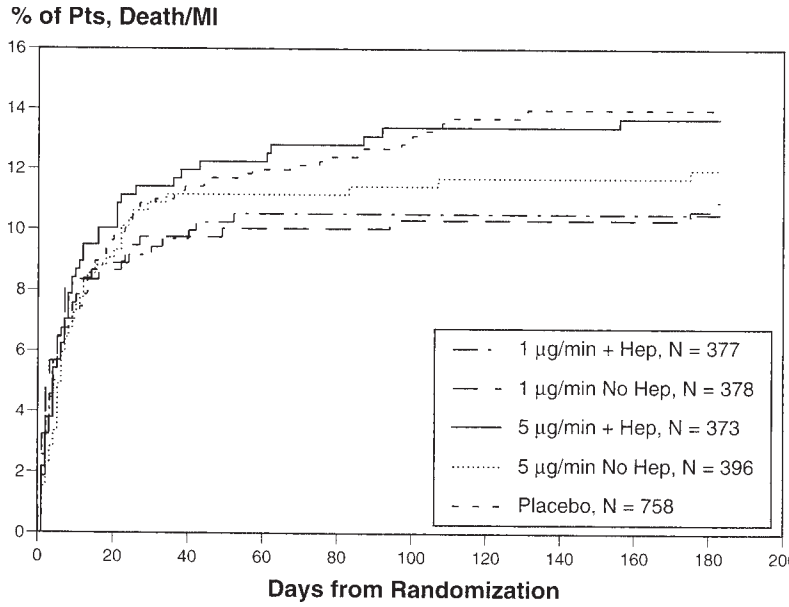

Fig. 5. Kaplan-Meier estimates of the probability of death or nonfatal myocardial (re)infarction during 6-mo follow-up separated according to treatment assignment in PARAGON. At 30 d, all groups had similar outcomes, whereas at 6 mo, the group receiving low-dose lamifiban with heparin had the fewest end point events compared with control ($P = 0.025$). Reproduced from *(16)* with permission.

The composite endpoint at 48 h was reduced 36% by tirofiban, with the greatest effect seen in the reduction of refractory ischemia events. At the time of endpoint ischemia, the majority of patients were receiving both nitrates and beta-blockers. At 7 d, the reduction in the composite endpoint by tirofiban was attenuated to a nonsignificant 8% lowering. Likewise, at 30-d follow-up, tirofiban still reduced the composite outcome, but only by 7% ($P = 0.34$).

The PRISM-PLUS trial started enrollment in November 1994, and randomized a total of 1915 patients. As compared with PRISM, patients in PRISM-PLUS were somewhat more unstable being required to have rest angina within 12 h plus electrocardiographic evidence of ischemia or serologic evidence of infarction. With these criteria, 45% of PRISM-PLUS patients had an infarction at enrollment as compared with 25% in PRISM. Patients in PRISM-PLUS were randomized to tirofiban alone (0.6 μg/kg-min loading followed by 0.15 μg/kg-min), heparin, or tirofiban (0.4 mg/kg-min followed by 0.1 mg/kg-min) plus heparin (Table 2). All patients were to receive aspirin, and the target prolongation of aPTT among patients receiving heparin was twice the laboratory control value. Patient management was prespecified in that angiography and coronary intervention before 48 h were discouraged; whereas they were encouraged to be performed between 48–96 h while continuing study drug infusion. Endpoint definitions were similar to those in PRISM, and the primary endpoint was a composite of death, MI, or refractory ischemia at 7 d.

An interim analysis revealed an increase in 7-d mortality among those randomized to high-dose tirofiban alone. Thus, after 1031 patients had been enrolled into the overall study, this treatment arm was discontinued. The 7-d mortality for the high-dose tirofiban,

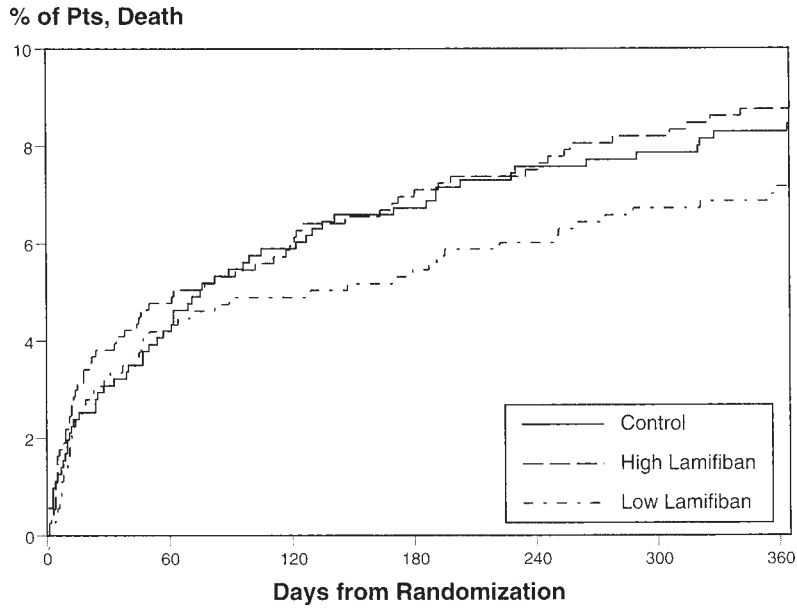

Fig. 6. Kaplan-Meier estimates of the probability of death during 1-yr follow-up in PARAGON with control compared to lamifiban patients grouped by low-dose and high-dose. All-cause mortality was 8.7, 7.3, and 8.9%, respectively ($P = 0.320$). Reproduced from *(16)* with permission.

heparin, and moderate-dose tirofiban plus heparin groups were 4.6, 1.1, and 1.5%, respectively. The odds ratio for mortality at 7 d with high-dose tirofiban as compared with heparin alone was 4.1 (95% CI, 1.4 to 12.3; $P = 0.012$). Whereas the 7-d, 30-d, and 6-mo rates of death, as well as the death or MI composite, were numerically highest for the high-dose tirofiban group, this difference was only statistically significant for 7-d mortality. Outcome information on this group is presented in Table 4 only for listing purposes and cannot be compared with the final data from the other two arms since it is not contemporaneous.

The primary endpoint (7-d death, MI, refractory ischemia) was reduced 28% by tirofiban plus heparin compared with heparin alone (12.9 vs 17.9%, respectively). Impressively, this reduction in ischemic events was extended similarly to nearly all subgroups (Fig. 7). Benefit from tirofiban was durable at 30 d as the composite endpoint including rehospitalization for unstable angina was reduced 17% (18.5 vs 22.3%, $P = 0.03$) (Fig. 8) . Considering the trend in event rate reduction provided by tirofiban, most of the benefit was present for preventing refractory ischemia and nonfatal MI. Even at 6-mo follow-up, both of these ischemic endpoints were reduced 21% in comparison to placebo.

Eptifibatide

Eptifibatide, (Integrilin™, COR Therapeutics, South San Francisco) a cyclic heptapeptide, competitive inhibitor of the IIb/IIIa receptor was studied in a phase II unstable angina trial published in 1996 *(29)*. This dose-finding trial randomized 227 patients to a low-dose of eptifibatide (45 μg/kg bolus, 0.5 μg/kg/min infusion), "high-dose" eptifiabtide (90 μg/kg bolus, 1.0 μg/kg/min infusion), or placebo. The study drug

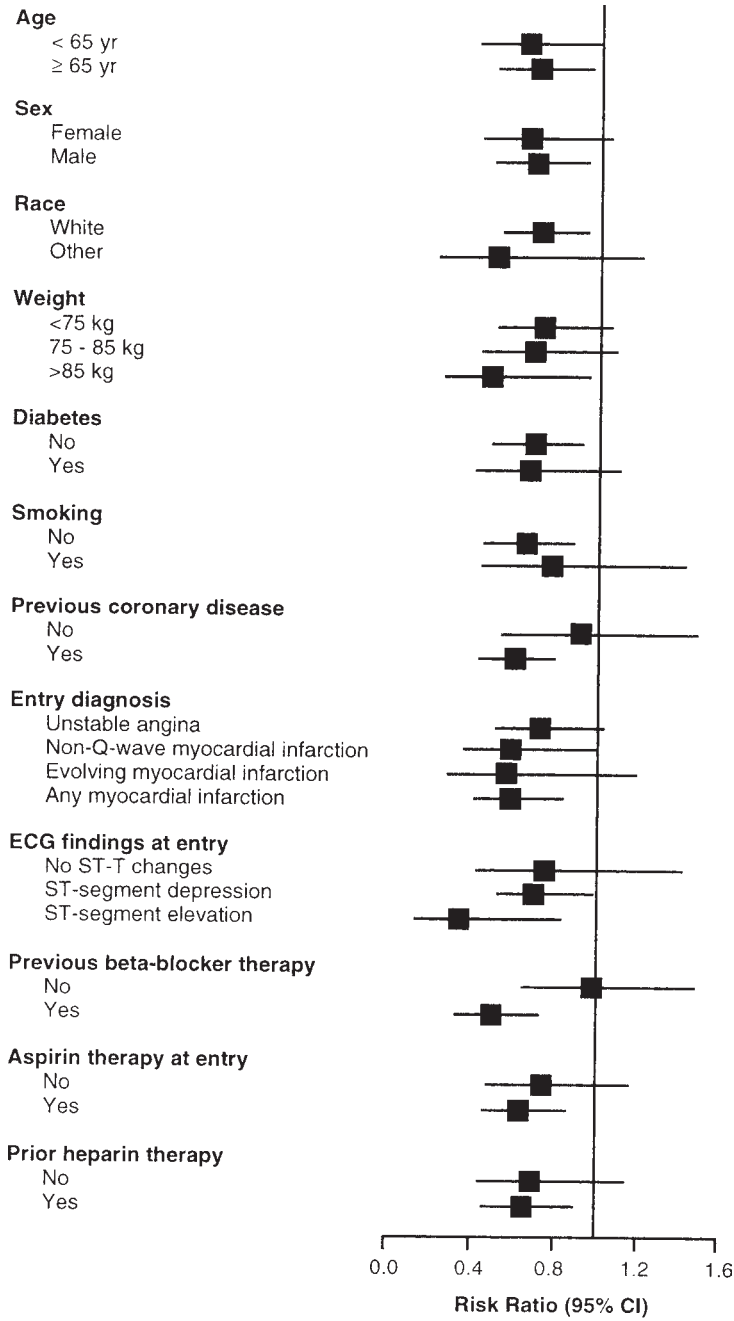

Fig. 7. Effect of treatment with heparin or tirofiban plus heparin on the composite primary endpoint at 7 d among various subgroups. Risk ratios <1 favor the subgroup receiving tirofiban plus heparin (horizontal lines represent the 95% confidence intervals [CI]). There were no statistically significant interactions between the assigned treatment and any of the other factors shown except that effects of tirofiban were particular marked among those already taking beta-blockers at enrollment. Reproduced from *(18)* with permission.

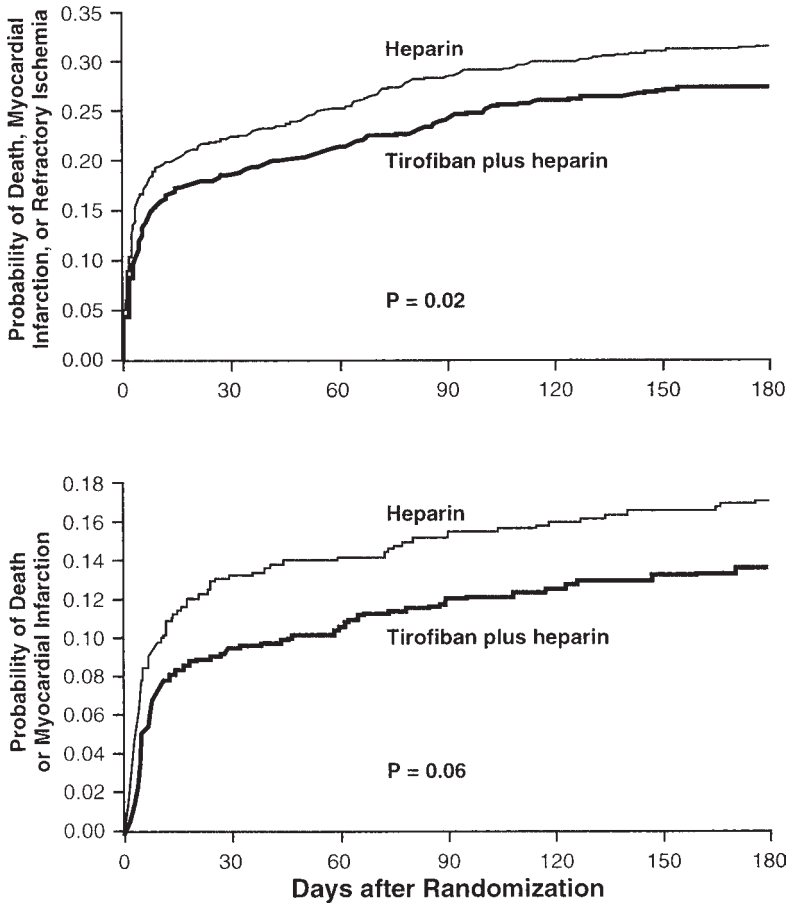

Fig. 8. Kaplan-Meier curves showing the cumulative incidence of events among patients randomly assigned to receive tirofiban plus heparin or tirofiban alone. The top panel is the composite of death, MI, refractory ischemia, or rehospitalization for unstable angina. The bottom panel is the composite of death or myocardial infarction. *P* values were computed by Cox regression analysis. Reproduced from *(18)* with permission.

was continued for 24–72 h, and all patients received heparin. The efficacy parameter was the frequency and duration of ischemic episodes as assessed by continuous electrocardiographic monitoring. A dose-dependent decrease in ischemic episodes was observed, though this only reached statistical significance in the high-dose group. During study drug infusion, no increase in bleeding events was noted with eptifibatide, and following drug cessation, no rebound phenomena occurred. Subsequent studies performed with eptifibatide during percutaneous coronary intervention used relatively higher doses of bolus plus infusion (135 μg/kg bolus and 0.5-0.75 μg/kg-min). Even these doses, however, were subsequently shown to only inhibit ADP-induced platelet aggregation by 40–60% *(30)*.

The largest unstable angina study ever performed and the largest study involving a IIb/IIIa inhibitor was the PURSUIT trial *(19)*. Between November 1995 and January 1997, 10,948 patients in 28 countries were randomized to one of two doses of eptifibatide or placebo. All patients randomized to eptifibatide received a 180 μg/kg bolus and either

a 1.3 μg/kg-min or 2.0 μg/kg-min infusion for 72 h. After 1487 patients received the moderate-dose infusion (1.3 μg/kg-min) of eptifiabtide, this arm was discontinued because the higher-dose arm had a similarly acceptable safety profile. The discontinued arm in PURSUIT was part a prespecified plan, involved dropping the lower-dose group, and was done because of acceptable safety. Except where noted, the following reported data are from the remaining 9461 patients equally randomized to placebo or high-dose eptifibatide. The study was designed to emulate, though not interfere with, the usual clinical treatment for patients with unstable angina. Hence, it was recommended, though not required, that all study patients receive oral aspirin and weight-adjusted intravenous heparin. Likewise, patients could have angiography and revascularization at anytime during the study to conform with the attending physician's preference.

The primary endpoint was a composite of all-cause mortality and nonfatal MI (or reinfarction) at 30 d. Secondary endpoints included death and myocardial (re)infarction at 30 d, the composite at 96 h and 7 d, and safety and efficacy outcome in patients undergoing percutaneous coronary interventions. Demographic data, study drug administration, and in-hospital cardiac procedures, are detailed in Tables 2 and 3. Principal safety and outcome data at 30 d and 6 mo are provided in Table 4. The outcome information for the discontinued treatment group is presented in Table 4 only for listing purposes and cannot be compared with the final data from the other two arms because it is not contemporaneous. At the time of enrollment, 45% of patients had a non-Q-wave MI. Importantly, aspirin was used in 93% of patients, and heparin was used in 90%. Coronary angiography was performed in 60% of patients, and percutaneous coronary revascularization was performed in 24%.

Based on the site investigators' reports, the primary 30-d endpoint was reached in 10% of control patients and 8% of those receiving eptifibatide. Following adjudication by the clinical events committee (CEC), the 12 to 28% relative reduction in events reported by sites among the four geographic regions was reduced to –7% to 22% (Fig. 9). Not surprisingly the death rate did not change with adjudication, but rather, the number of patients considered to have non-Q-wave MI during follow-up was substantially increased. The study's overall adverse event rate climbed from 9 to 15% with adjudication of non-Q-wave MIs by elevated CK-MB. The overall study remained positive at 30 d with a 10% relative reduction in death and nonfatal MI (15.7 vs 14.2%) (Fig. 10) with a 22% reduction seen in North America. These data were durable at 6 mo (Table 4).

Irrespective of site report or CEC, geographic region, or early vs late follow-up, there was approximately a 1.5% absolute reduction in death or nonfatal MI. This benefit was consistent among the subgroups except for women. Again, as seen in Fig. 11, some of this discrepancy in gender-related outcome may have been geographically influenced by specific practice patterns. For example, early diagnostic catheterization (≤72 h) was performed in between 5 and 66% of patients according to location (Eastern Europe vs North America). Not surprisingly the range of heparin usage (76 to 97%), early angioplasty (4 to 25%) and early bypass surgery (1 to 8%) was also associated with these regional differences.

CLINICAL OUTCOME: PARAGON, PRISM, PRISM-PLUS, PURSUIT (4 P'S)

The study designs of the "4 P's" have many similarities, but also several important distinctions (Table 2) with regard to endpoint definitions, percentage of patients with

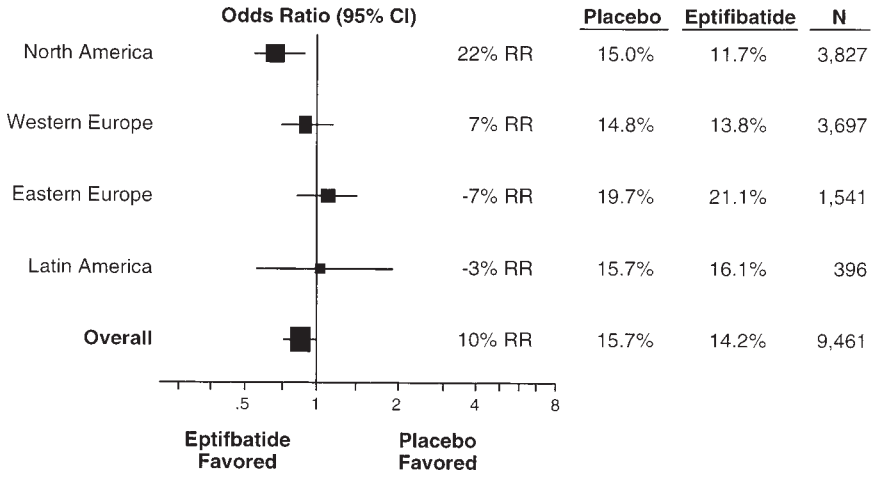

Fig. 9. Odds ratio for death or MI according to the clinical events committee adjudicated endpoints and separated according to geographic region in the PURSUIT trial. Data taken from *(19)*.

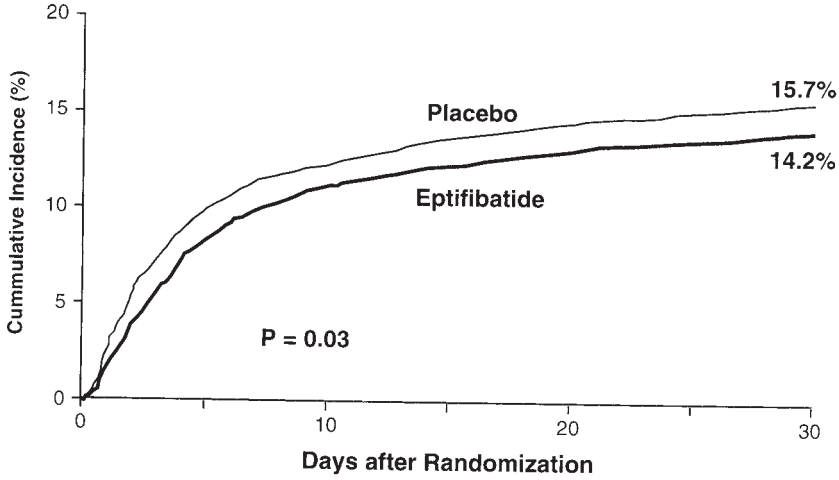

Fig. 10. Kaplan-Meier curves showing the incidence of death or nonfatal MI at 30 d. Events were based on endpoints adjudicated by the clinical events committee, and the *P* value is based on the log-rank test. Reproduced with permission from *(19)*.

infarction at enrollment, duration of therapy, concomitant use of heparin, use of percutaneous revascularization, and importantly, the timing of the primary endpoint analysis. Given these similarities and distinctions, several meta-analysis have already been considered *(31–33)*. Since most ACS trials have previously reported the treatment effect on death and nonfatal MI at 30 d as the primary endpoint, this may be the most appropriate point of comparison.

30-Day Mortality

Considering all patients enrolled into the 4-P trials (including those groups with discontinued enrollment), 10,467 were randomized to receive IIb/IIIa therapy. Overall 30-d mortality for these patients was 3.4% compared with 3.7% for the 7910 patients

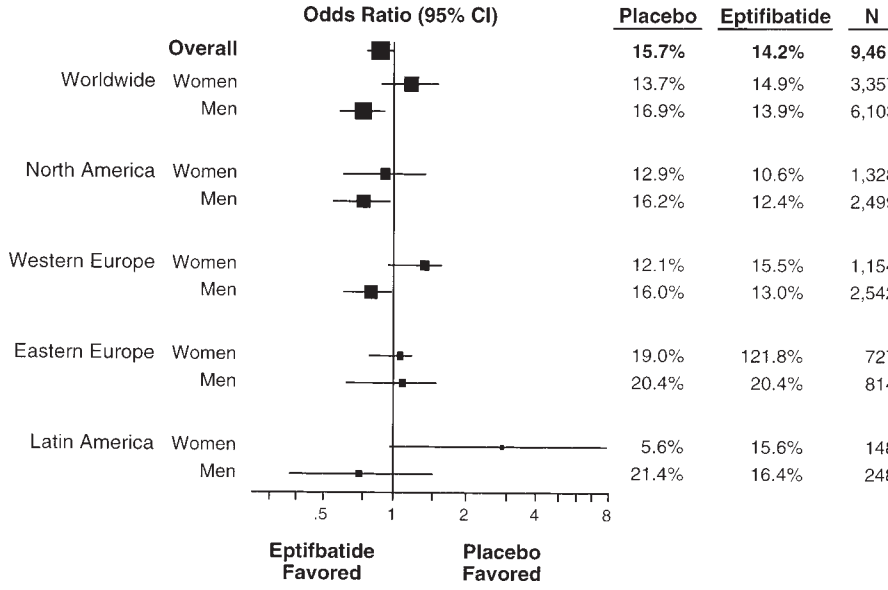

Fig. 11. Odds ratio for death or MI separated according to gender and geograpic region. Women overall showed no benefit from eptifibatide treatment though this subgroup finding was primarily limited to outside North America. Data taken from *(19)*.

assigned to receive placebo ($P = 0.27$). The lowest mortality was 2.3% for those receiving 0.15 μg/kg-min tirofiban in PRISM (36% relative reduction compared with placebo, $P = 0.02$) (Fig. 12). Ironically, the highest 30-d mortality among all studies' treatment groups was also for 0.15 μg/kg-min tirofiban but in PRISM-PLUS (6.1% among 345 patients). Thus, 30-d mortality was reduced with tirofiban in PRISM; however, this was not evident in any other study, suggesting any real treatment effect on mortality to be minor. Kong et al. *(33)* performing formal meta-analysis of the ACS trials testing IIb/IIIa receptor blockade found the odds ratio for mortality at an early (48–96 h) timepoint to be 0.71 (95% CI, 0.50–1.01) favoring IIb/IIIa therapy. The odds ratio at 30 d was 0.90 (95% CI, 0.76–1.06) and at 6 mo, 1.00 (95% CI, 0.77–1.15).

30-Day MI

Enzymatic (serologic) evidence of MI is present not uncommonly following percutaneous coronary intervention, especially among patients with an ACS. This must be kept in mind when interpreting unstable angina trials among patients also undergoing coronary interventions since it can be unclear whether the MI occurred as a result of the primary disease process, the coronary intervention, or both. Among all patients randomized to IIb/IIIa therapy in the 4-P trials, 10.3% had a nonfatal MI compared with 10.9% of the placebo cohort. Considering only the final treatment groups studied, MI occurred in 10.0% of patients randomized to IIb/IIIa therapy within 30 d (9% relative reduction compared with placebo, $P < 0.05$).

30-Day Death or MI

The composite rate of death or nonfatal MI among overall trial cohorts (i.e., treated and placebo groups) may be an indicator of the illness severity of the patients enrolled,

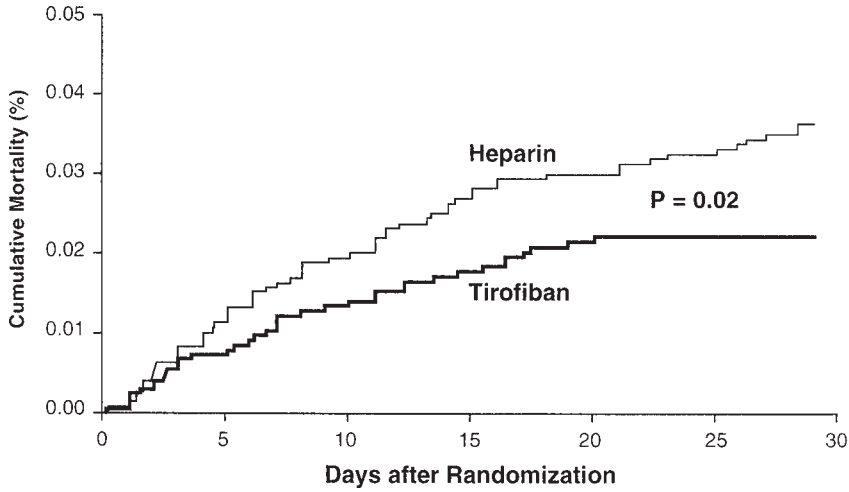

Fig. 12. Kaplan-Meier curves of mortality to 30 d from the PRISM trial. With a risk ratio of 0.62 favoring tirofiban, though wide confidence intervals PRISM was the only study to show a survial benefit with IIb/IIIa therapy. Reproduced with permission from *(17)*.

the background medical treatment given, or the level of endpoint adjudication. The highest overall rate of 30-d death or MI was in PURSUIT (15.7%), and the lowest was in PRISM (6.5%). Among all 4-P patients receiving IIb/IIIa therapy, the 30-d death or MI composite was 11.9% compared with 13.2% for those receiving placebo ($P < 0.01$). The 30-d odds ratio is 0.88 (95% CI, 0.81–0.97), or 13 fewer events per 1000 treated patients *(33)*. Among the final or best IIb/IIIa treatment groups studied, the composite occurred in 11.6% of patients for a 12% relative reduction. The greatest relative reduction (27% fewer events) was seen in PRISM-PLUS among patients receiving 0.10 μg/kg-min of tirofiban combined with heparin, but confidence intervals around this estimate are wide, and the magnitude of risk reduction is not statistically different from that seen in other trials.

Bleeding Events

An important side effect of IIb/IIIa inhibitors is bleeding, and hence an overall estimate of bleeding parameters from the unstable angina trials is helpful. There are two different bleeding classifications (i.e., the one developed by the TIMI study group and the one developed by the GUSTO study group) and two different patient groups (those undergoing vascular surgery, such as coronary artery bypass grafting [CABG], and those not undergoing surgery). Major bleeding events by the TIMI criteria include a >5 gm hemoglobin loss. This occurred in as few as 0.4% of IIb/IIIa-treated patients in PRISM and as many as 10% of IIb/IIIa-treated patients in PURSUIT when including bleeding related to bypass surgery and angioplasty. The highest bleeding among patients not undergoing CABG undertandably was among those receiving the highest doses of IIb/IIIa inhibitor, especially when combined with heparin. Severe bleeding according to the GUSTO classification (bleeding that is life threatening, leading to hemodynamic compromise, or an intracranial hemorrhage) occurred in ≤1% of most trials study groups and was highest in 2.0 μg/kg-min cohort of PURSUIT (1.5%).

Blood product transfusion (usually RBC's and classified as moderate bleeding)

occurred in 8.3% of patients randomized to IIb/IIIa therapy and 6.5% of those receving placebo. Most transfusions were related to surgery or angioplasty procedures. Important thrombocytopenia (≤ 50,000/μL) was infrequent (≤ 1%) in all study groups as were platelet transfusions. Fortunately, intracranial hemorrhage remains very rare with this class of drug with no events occurring in many study groups. The overall rate of intracranial hemorrhage is < 0.1% for both the placebo and treatment groups.

6-Month Death or MI

While information between 30-d and 6-mo follow-up are limited (i.e., there is no information for the number of patients having revascularization procedures or being maintained on beta-blockers or ACE inhibitors), most trials collect a composite rate of death and nonfatal MI by survey. Quite encouragingly, there is maintained treatment benefit at late follow-up. Even though there is an initial decay in benefit (between the first week and first month of follow-up), the benefit at 30 d is preserved at 6 mo. The 10% relative reduction of the death or MI present in the 30-d composite in PURSUIT was 8% at 6 mo. Likewise, the tirofiban plus heparin arm of PRISM-PLUS reported a 20% relative reduction in the composite at late follow-up (Fig. 8). Somewhat surprisingly, the 9% composite reduction at 30-d follow-up in PARAGON for low-dose lamifiban was improved to a 23% reduction in death or MI over control. These data collectively suggest that although there is an intial decay of acute benefit, passivation likely also occurs protecting against intermediate-term events. The 6-mo odds ratio for death or MI with IIb/IIIa treatement compared with placebo was 0.88 (95% CI, 0.79–0.97) which translates into 16 fewer events per 1000 treated patients (–0.016; 95% CI, –0.027 to –0.004) *(33)*.

CURRENT ISSUES

A number of important issues remain in the discussion and understanding of the 4-P trials and their application to contemporary medical practice. It is unarguable that IIb/IIIa receptor blockage provides an improved outcome during percutaneous coronary interventions, and that abciximab has become well established for this purpose. On the other hand, small molecules which are very specific for the IIb/IIIa receptor have several distinctions from the monoclonal antibody and among themselves. Questions therefore remain regarding the extent of inhibition needed with these compounds, the importance of comcomitant heparin, the duration of receptor blockage required, and the importance of underlying renal function. Because the small molecule IIb/IIIa receptor antagonists have an important plasma-phase (i.e., short half-life, need for continuous infusion, moderate dissociation constants), they are very sensitive to renal function and excretion. Finally, as many patients with ACS undergo percutaneous revascularization, it is important to separate primary treatment effects from those associated with angioplasty.

Primary Therapy vs PTCA Effect

In the CAPTURE trial *(24),* abciximab was given the day prior to angioplasty among patients with unstable angina. In this 24-h interim before PTCA, 2.1% of placebo patients had an MI compared with 0.6% of the abciximab group (13 vs 4 patients; 71% relative reduction). A larger number of MI events occurred during and immediately after the procedure, and these were reduced 58% by abciximab (Fig. 13). This early observation

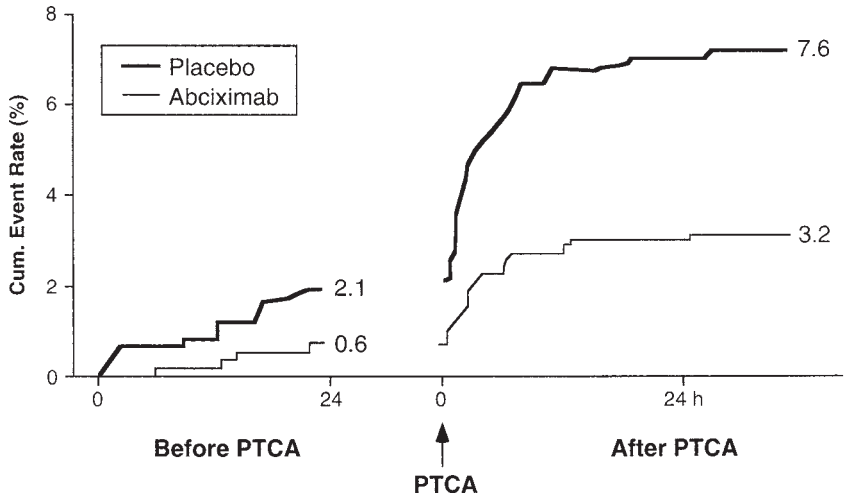

Fig. 13. Occurrence of pre-procedural and periprocedural myocardial infarction in the CAPTURE study. Abciximab provided both a primary (preprocedural) and angiplasty-related reduction in the incidence of infarction. Reproduced from *(24)* with permission.

suggested that IIb/IIIa inhibitors have both a primary and PTCA-related treatment effect. Therefore, in considering the results of PARAGON, PURSUIT, PRISM, and PRISM-PLUS, one must also consider separately the primary and PTCA-related effects.

By protocol design PARAGON did not allow PTCA during the first 48 h unless the patient had medically refractory angina. PRISM and PRISM-PLUS discouraged procedures before 48 h, and the PURSUIT design allowed for usual clinical practice. Between 48–96 h, angiography and if appropriate angioplasty, were encouraged in the PRISM-PLUS protocol. With these protocol differences the rate of PTCA among the studies varied from 13 to 31% (Table 2). Because only 13% of patients in PARAGON underwent angioplasty, and few of these patients were still receiving study drug, subgroup analyses are not powerful. Meaningful data, however, are available from the other three trials.

In PURSUIT, 24% of patients underwent percutaneous coronary revascularization. Roughly half of these patients (12.7% of total cohort) underwent intervention within 72 h of randomization and were therefore still receiving study drug. Among these 1228 patients, 609 were in the eptifibatide group, and the composite of death and MI at 30-d follow-up was reduced 30% compared with placebo (11.8 vs 16.8%, $P = 0.013$). Like in the CAPTURE trial, the rate of preprocedural MI was reduced by IIb/IIIa treatment (1.7 vs 5.5%, $P = 0.03$). Considering then the remaining 8149 patients not undergoing early percutaneous revascularization, the 30-d composite was reduced 7% with eptifibatide (14.6 vs 15.7%, $P = 0.185$). Finally, patients who underwent late intervention (i.e., after discontinuation of eptifibatide) had no reduction in 30-d adverse events.

Because angiography and revascularization were discouraged in the first 48 h of PRISM, few of the 21% of the study cohort who had percutaneous revascularization were receiving study drug (Fig. 14). The 30-d composite of death and MI was reduced 21% (7.2 vs 9.1%, P = NS) among those receiving prior or ongoing tirofiban. On the other hand, those treated without percutaneous or surgical revascularization (i.e., medical management only) the composite of death and MI was reduced 42% by tirofiban

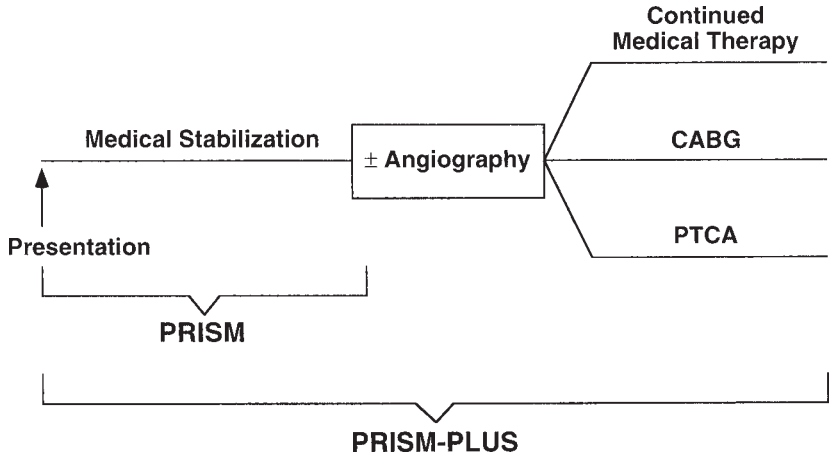

Fig. 14. Schematic of the study design timeline for the PRISM and PRISM-PLUS studies. In PRISM percutaneous coronary revascularization was not recommended during the first 48 h, but was allowed thereafter although study drug was completed. PRISM-PLUS also did not recommend angiography in the first 48 h, though did strongly encourage it to be performed along with angioplasty if possible during study drug infusion. Information collected from *(17,18)*.

(3.6 vs 6.2%, $P < 0.05$). Finally, in PRISM-PLUS where percutaneous revascularization was encouraged during study drug infusion, 31% of patients had angioplasty, and tirofiban reduced the 30-d death or MI composite by an impressive though nonsignificant 42% (5.9 vs 10.2%, P = NS) (Fig. 15). Among the 719 patients treated medically in PRISM-PLUS, tirofiban reduced the 30-d composite by 23% (7.8 vs 10.1%, P = NS). Overall, these data include 2357 patients undergoing PTCA, and prior or ongoing IIb/IIIa therapy reduced the 30-d composite of death or nonfatal MI by 30% (9.3 vs 13.3%). A smaller benefit was seen among the 10,867 patients treated without revascularization as IIb/IIIa therapy relatively reduced the 30-d composite by 11% (12.1 vs 13.6%).

The 11% relative reduction in death or MI at 30 d is similar to that reported for direct thrombin inhibitors or low-molecular-weight heparins tested against unfractionated heparin in acute coronary syndromes. Likewise, the 30% reduction seen in death or MI among those undergoing percutaneous revascularization is similar to that reported from other (interventional) studies testing IIb/IIIa inhibitors. Taken together, these observations confirm a primary treatment effect of IIb/IIIa on the ruptured plaque of unstable angina and an amplified benefit during further plaque disruption (i.e., angioplasty).

"Toxic Doses" vs Dose-Dependent Responses

Because nearly all IIb/IIIa inhibitors are fully platelet specific, "toxicity" has been manifest as excess bleeding. Fortunately, the overwhelming majority of bleeding is at the site of vascular puncture. Less commonly, gastrointestinal and genitourinary bleeding occur. On the other hand, the question has arisen whether IIb/IIIa receptor antagonists can otherwise become "toxic." Several antithrombotics, such as heparin, hirudin, and fibrinolytics have been associated with a narrow therapeutic window and higher doses have been associated with both heightened bleeding and mortality. Among some IIb/IIIa trials, "high-dose" antagonism has been associated with an increase in bleeding and a neutral or negative effect on ischemic outcome. In IMPACT-II (Chapter 6)

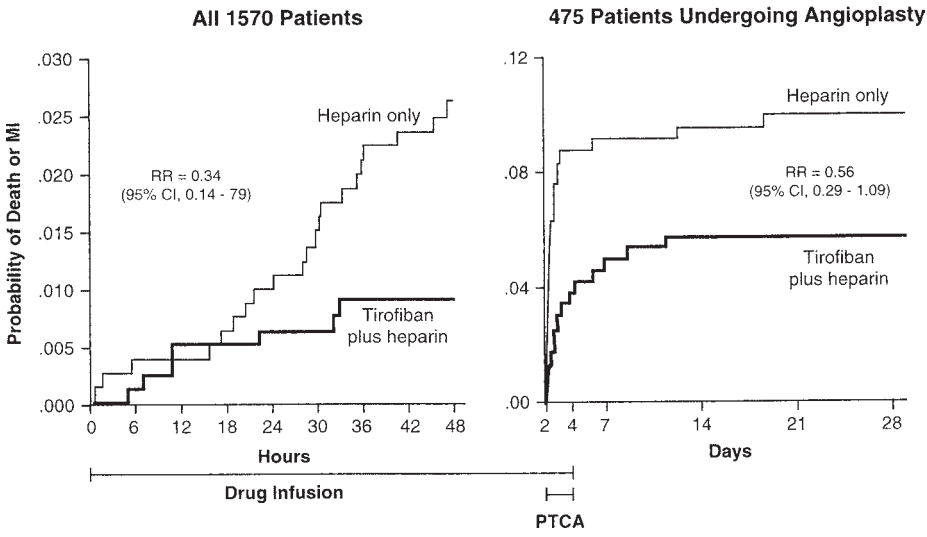

Fig. 15. Kaplan-Meier curves showing the ecumulative incidence of death or MI among patients randomly assigned to heparin or heaprin plus tirofiban. The left panel shows events during the initial 48 h of medical stabilization and prior to recommended angiography and intervention. The right panel shows the incidence of death or MI from the time of the procedure to 30 d. Reproduced from *(18)* with permission.

angioplasty patients receiving the "higher-dose" (0.75 μg/kg) eptifibatide infusion were found to have a similar or worse outcome compared with those receiving "lower-dose" (0.5 μg/kg) eptifibatide or placebo. Subsequent to this trial's completion, both eptifibatide doses were found to only modestly inhibit platelet function. Thus, rather than a toxic effect, the lack of demonstrated benefit from the higher dose regimen could be speculated to be from sampling error or yet to be understood "toxic" or counter-balancing effects.

In distinction, however, the high dose IIb/IIIa treatment arms of PARAGON and PRISM-PLUS should have provided extensive platelet inhibition (>90% inhibition of ADP-induced aggregation). These also did not show benefit over low-dose treatment or placebo. Lamifiban at 5 μg/kg in the Canadian Lamifiban Study lowered 30-d death or nonfatal MI 70% compared with placebo. In this dose-exploring study, 41 patients received the 5 μg/kg dose. In PARAGON, 769 patients received this dose, and the 30-d death or MI rate was nonsignificantly 3% higher than placebo. The death and MI composite at 6 mo was also not dissimilar from placebo, and 1-yr mortality was also not improved by high-dose lamifiban. A similar seeming discrepancy between trials with high-dose antagonists is seen with 0.6 μg/kg-min of tirofiban in PRISM and PRISM-PLUS. In PRISM, this high dose of antagonist without heparin reduced adverse events (death, MI, and refractory ischemia) 32% at 48 h and by 7% at 30 d. These findings were considered favorable. On the other hand, this same dose of tirofiban in PRISM-PLUS was prematurely discontinued because of an increase in mortality at 7 d compared with placebo (4.6 vs 1.1%, respectively). At 30-d follow-up, the effect was statistically neutral (6.1 vs 4.0%, respectively). Thus, the question remains why did high-dose tirofiban and lamifiban perform worse than their respective moderate doses and no better than placebo? These observations are in contrast to previous large studies of this class of

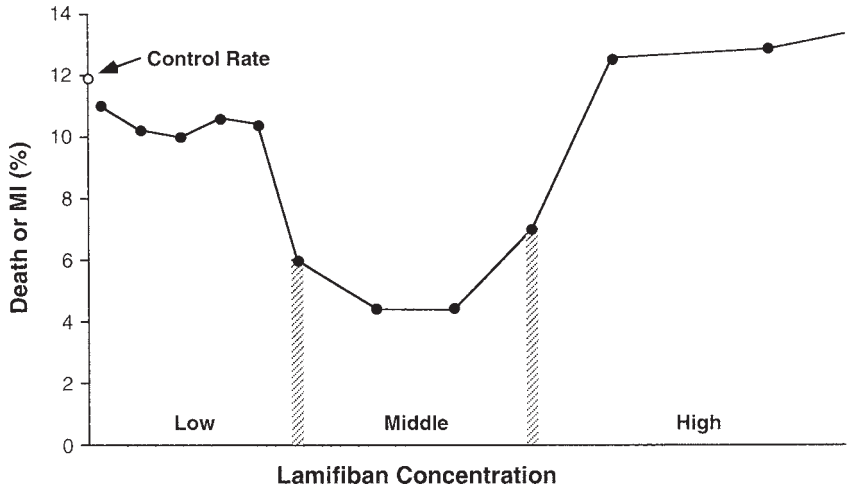

Fig. 16. Plasma levels of lamifiban collected from 810 patients. Levels were separated into three groups (low, middle, and high) and compared with clinical outcome. Patients with a middle range plasma level had the lowest incidence of the composite of death or MI at 30 days. Data from *(34)* with permission.

antithrombotic therapy and suggests an upper limit of benefit or potential "toxicity." Theoretical explanations for any apparent toxicity of high doses of IIb/IIIa antagonists and lamifiban include intraplaque hemorrhage, paradoxical platelet activation, or an interplay between excessive bleeding and clinical outcomes.

An intriguing recent analysis from the PARAGON trial was performed to better understand the dose-response relationship. The lamifiban plasma concentration was measured in 810 lamifiban-treated patients *(34)*, and this was correlated with clinical outcome. The lamifiban level at steady state was directly related to the patient's renal function, and a middle concentration range (18–42 ng/mL: corresponding to 80–90% GP IIb/IIIa receptor occupancy), was associated with a 38% reduction in death and MI at 6 mo. The benefit was also manifest at 30 d (40% reduction) in this concentration range (Fig. 16). While it remains understandable why lower concentrations were less efficacious, it remains unclear why higher plasma levels were not beneficial. This "optimal therapeutic range" is being tested in the ongoing PARAGON B trial of 5000 patients.

IIa/IIIa and Concomitant Heparin Thearpy

PARAGON was the only trial that factorially tested the benefit of antithrombin (heparin) therapy administered concomitantly with IIb/IIIa therapy. Overall, heparin provided no consistent benefit when combined with lamifiban. This may reflect that bleeding events were increased among those receiving heparin with high-dose lamifiban but not with low-dose lamifiban. Likewise, at 30 d and 6 mo, the composite ischemic event rate was numerically highest among those receiving heparin with high-dose lamifiban and lowest for those receiving heparin with low-dose lamifiban. The group assigned to high-dose lamifiban with heparin had no improved parameter and had the highest percentage of patients discontinuing drug prematurely, usually due to bleeding or an ischemic event. In the 4-P trials, as well as in previous IIb/IIIa angioplasty studies,

bleeding and adverse events were linked to the combination of potent platelet inhibition and full-dose heparin.

Although PARAGON alone was not adequately powered to assess the extent of clinical benefit provided by concomitant heparin, collectively assessing these data in combination with other observations is helpful. These include the 1) meta-analysis by Oler et al. *(27)*; 2) the largest relative benefit in the ACS trials was with moderate-dose tirofiban with heparin; 3) intermediate-term benefit present in PRISM-PLUS with heparin added to tirofiban was not seen in PRISM's tirofiban without heparin group; and 4) PARAGON's low-dose lamifiban with heparin group had the best early, intermediate, and long-term outcome. These suggest that heparin adds a modest benefit to antiplatelet therapy in ACS; however, further data are clearly needed.

SUMMARY AND FUTURE

Despite contemporary medical therapies, patients presenting with unstable angina and electrocardiographic evidence of ischemia or serologic evidence of myocardial necrosis have a substantial 30-d rate of death or important nonfatal MI. Although antiplatelet agents, such as aspirin, ticlopidine, and clopidogrel have substantially improved this outcome compared with placebo, intermediate and long-term follow-up shows plaque passivation is not complete. The IIb/IIIa receptor antagonist are much more potent than previous antiplatelet therapies, and they have been shown to be markedly beneficial in making quiescent the plaque disruption of percutaneous coronary revascularization. The PARAGON, PRISM, PRISM-PLUS, and PURSUIT trials have alone and collectively demonstrated reducing ischemic events beyond that of aspirin and heparin therapy. These agents reduce the 30-d occurrence of death or MI by ~10% and this benefit is durable at late (6-mo to 1-yr) follow-up. Approximately 16 fatal or life-threatening events per 1000 treated patients can be prevented with the addition of IIb/IIIa receptor blockade. Benefit appears highest among patients who have active atherothrombotic plaques and are aggressively treated with early percutaneous revascularization. Whether optimal plasma levels of receptor antagonists (lamifiban, PARAGON-B), prolonged IIb/IIIa receptor blockade by transitioning from intravenous to oral therapy (Klerval, TIMI-15B), or stratification to an early invasive versus a conservative approach, while maintaining IIb/IIIa antagonism (tirofiban, TIMI-18: TACTICS) will provide heightened benefit, are all being tested in ongoing clinical trials. The largest oral IIb/IIIa receptor trial for unstable angina, Sibrafiban versus Aspirin to Yield Maximal Protection from Ischemic Heart Events Post-Acute Coronary Syndromes (SYMPHONY), has recently completed enrollment of 9000 patients. The results from extended-duration sibrafiban, the oral congener of lamifiban, will provide valuable insight into the feasibility and proof-of-concept of long-term plaque stabilization and atherthrombotic passivation.

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11 The Use of Glycoprotein IIb/IIIa Inhibition in Acute Myocardial Infarction

Adam B. Greenbaum, MD,
Robert A. Harrington, MD,
and E. Magnus Ohman, MD

Contents

INTRODUCTION

Current Reperfusion Therapy

Complete and timely reconstitution of coronary blood flow is the principal mechanism by which reperfusion therapy improves outcomes of acute myocardial infarction (AMI) *(1–3)*. Fibrinolytic agents improve survival by as much as 30% when begun within 6 h of symptom onset and by as much as 50% if given within 1 to 2 h *(4,5)*. Yet current regimens fail to achieve Thrombolysis in Myocardial Infarction (TIMI) grade 3 flow at 90 min in almost 50% of cases and concede early reinfarction in 4–6% of patients despite successful reperfusion *(1,3,4)*. Percutaneous transluminal coronary angioplasty (PTCA) during AMI may achieve TIMI grade 3 flow in only 75% of cases when performed globally, conceivably less in complicated patients, and carries a

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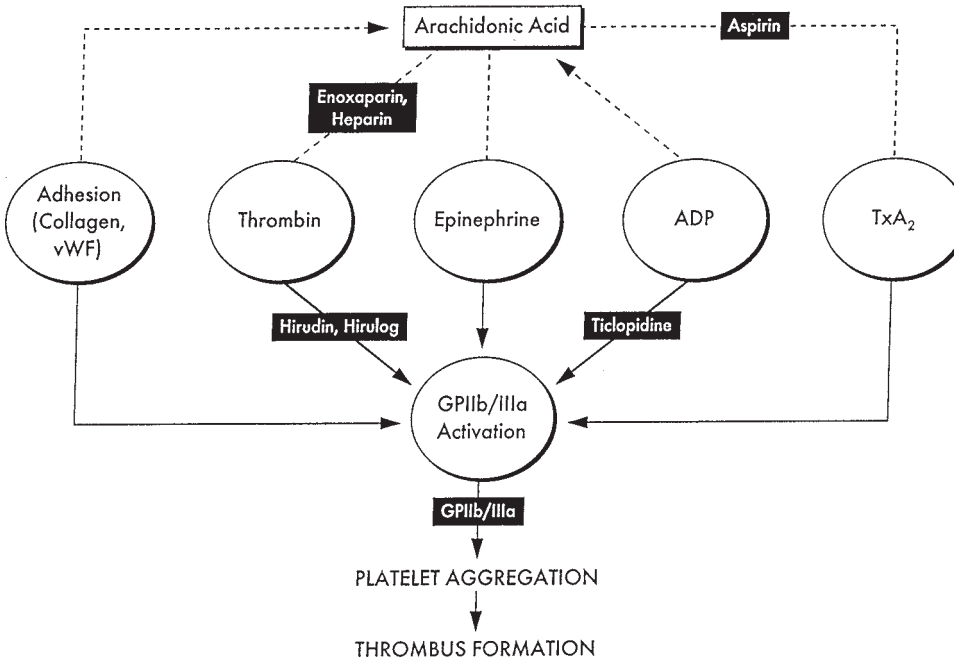

Fig. 1. Pathways of platelet activation and inhibition by various therapies.

10–13% risk of reocclusion *(6–9)*. Investigation surrounding AMI therapy continues to focus on achieving earlier, more successful, and more complete reperfusion, as well as less reinfarction *(10)*.

The Platelet in AMI

Plaque rupture, subsequent platelet aggregation, and thrombosis are the pathophysiologic mechanisms involved in acute coronary syndromes including AMI. The process begins with the exposure of highly thrombogenic plaque contents, including collagen, von Willebrand factor, and fibronectin, to circulating platelets. These substances, along with local inflammatory cell tissue factor and shear forces from the narrowed lumen, lead to platelet adhesion, activation, and aggregation. The platelet aggregate in turn provides the rich phospholipid surface on which thrombin is generated and fibrinogen converted to fibrin. The local generation of thrombin and fibrin further stimulates platelet activation and aggregation, permitting the cycle to continue (*see* Chapter 1). Ultimately, complete thrombotic occlusion of the coronary artery results in AMI *(11)*.

Aspirin (ASA) irreversibly inhibits platelet activation by inhibiting cyclooxygenase, thereby preventing the conversion of arachidonic acid to thromboxane A_2. After vascular injury, platelet activation still occurs by other pathways, regardless of inhibition by ASA (Fig. 1) *(12)*. Activation by any pathway is followed by aggregation. Unlike activation, aggregation occurs through one pathway only and requires the binding of fibrinogen to the platelet via the glycoprotein (GP) IIb/IIIa receptor *(13)*. Between 50,000 and 80,000 GP IIb/IIIa receptors lie on the surface of the platelet. Platelet activation, regardless of the mechanism, induces a conformational change in the GP IIb/IIIa

receptor, allowing binding to ligands such as fibrinogen, fibronectin, and von Willebrand factor, facilitating platelet aggregation and subsequent thrombosis.

Platelet Activation During Fibrinolysis

Current thrombolytic therapy, more appropriately named fibrinolytic therapy, dissolves fibrin, exposing underlying thrombin to circulating blood elements and actually enhancing thrombin activity *(14)*. Markers of thrombin activity, including fibrinopeptide A (FPA), plasminogen activator inhibitor type 1 (PAI-1), and β-thromboglobulin, are all elevated after treatment of AMI with fibrinolytic therapy *(15)*. As a potent activator of platelets, the newly exposed thrombin stimulates platelet aggregation. The activated platelets, in addition to catalyzing more thrombin formation, are also rich in PAI-1, one of the most potent naturally occurring inhibitors of fibrinolysis. This seemingly paradoxical effect of fibrinolytic therapy may account for worse outcomes after administration in patients with non-ST-segment elevation myocardial infarctions *(16)*. More importantly, this phenomenon has been implicated in both failure to achieve thrombolysis and early reocclusion after successful thrombolysis during ST-segment elevation AMI *(17)*.

Antiplatelet Therapy during AMI

Intuitively, platelet inhibition appears essential in the treatment of acute coronary syndromes (ACS) and as an adjunct to fibrinolytic therapy. The Second International Study of Infarct Survival (ISIS-2) documented the efficacy of ASA (a relatively weak platelet inhibitor) in reducing mortality during AMI. ASA alone resulted in a remarkable 23% reduction in mortality, a reduction similar to that produced by the thrombolytic agent streptokinase (SK) alone *(18)*. By meta-analysis, ASA therapy also reduces the risk of early reinfarction (11 vs 25%, $P < 0.001$) and recurrent ischemia (25 vs 41%, $P < 0.001$) *(19)*. In current practice, ASA is the principal antiplatelet agent used during the treatment of AMI *(20)*.

Other antiplatelet agents have also shown promise. Ridogrel, a selective thromboxane-A2 synthetase inhibitor and thromboxane A2/prostaglandin endoperoxide receptor blocker, was studied as an adjunct to thrombolysis for AMI. When 50 patients were treated with intravenous ridogrel in addition to t-PA and heparin, 90-min angiography revealed total patency rates of 86% *(21)*. Of the latest advances in AMI therapy, the GP IIb/IIIa antagonists have shown the most promise as an adjunct to both fibrinolytic therapy and primary angioplasty.

GP IIb/IIIa INHIBITORS DURING PERCUTANEOUS REVASCULARIZATION FOR AMI

Primary Angioplasty

Experimental Evidence

Primary angioplasty, a term applied to immediate cardiac catheterization and PTCA in patients with evolving AMI, although a successful strategy, is still complicated by ischemia-related events such as death, reinfarction, and urgent repeat procedures in as many as 25% of patients *(9)*. When tested in animals undergoing angioplasty, GP IIb/IIIa inhibitors resulted in less platelet aggregation, thrombosis, and acute closure than other

antiplatelet agents. Bates et al. randomized 24 dogs to placebo, ASA (325 mg), or antibody 7E3 (fragments of a monoclonal antibody against the platelet GP IIb/IIIa receptor) (0.8 mg/kg), prior to arterial injury with balloon catheters and external clamps simulating angioplasty. Arterial occlusion occurred in 5 of 8 of the control dogs, with nonocclusive thrombotic material seen in the remaining 3. One of 8 dogs in the ASA group completely occluded, with 5 of the remaining 7 dogs exhibiting nonocclusive thrombus. None of the dogs receiving antibody 7E3 occluded, and no dog treated with antibody 7E3 had thrombotic material visualized *(22)*. The superiority of antibody 7E3 to ASA in inhibiting thrombus formation in this model hinted at the possible benefits of stronger antiplatelet therapy, specifically GP IIb/IIIa receptor inhibition, in improving outcomes after percutaneous intervention during AMI.

Clinical Evidence

The first landmark study in the evaluation of GP IIb/IIIa inhibitors during coronary intervention in humans was the Evaluation of 7E3 for the Prevention of Ischemic Complications (EPIC) Study. EPIC was designed to assess the usefulness of the GP IIb/IIIa inhibitor abciximab, a monoclonal antibody to the beta 3 (IIIb) subunit, during high-risk PTCA and is discussed in detail elsewhere (*see* Chapter 5). Patients were classified as high risk based on lesion anatomy or if they presented with a coronary syndrome associated with coronary thrombus, such as unstable angina or AMI. Patients received standard therapy and were randomized to receive either an abciximab bolus, an abciximab bolus plus infusion, or placebo, prior to percutaneous intervention.

Of the 2099 patients enrolled in EPIC, 64 underwent the procedure as direct ($n = 42$) or rescue ($n = 22$) angioplasty within 12 h of AMI. Procedural success was high in both groups (91 and 96%, respectively), and there were no major differences in angiographic or procedural characteristics among those who received placebo, bolus abciximab only, or bolus and infusion of abciximab. Analysis of the clinical outcomes of these patients suggested a possible greater efficacy with abciximab use with AMI than with all other groups, with a reduction in the composite endpoint of death, reinfarction, emergency coronary artery bypass surgery (CABG), or repeat emergency angioplasty by 83% compared with 35% in the overall trial *(23,24)*. Two individual components of the composite endpoint, MI, and the need for urgent intervention, were also observed less in the abciximab bolus and infusion group than in the placebo group (Table 1). By 6 mo, the difference had increased to 91% (47.8 vs 4.5%, $P = 0.002$). There was also a clear dose response with abciximab in the reduction of subsequent MI (17.4, 5.6, and 0%, $P = 0.05$ for placebo, bolus, and bolus and infusion) and in the incidence of repeat revascularization (34.8, 11.6, and 0%, $P = 0.003$). In fact, there were no further events in the 22 patients who had received bolus and infusion of abciximab. The magnitude of benefit from abciximab bolus and infusion during AMI is even more impressive considering that patients undergoing the procedure for AMI were at greater risk for recurrent ischemic events as evidenced by a higher incidence of clinical "restenosis" in the placebo arm of the AMI patients compared with the placebo arm in the overall trial. Although the numbers of patients with AMI in EPIC were small, a role for GP IIb/IIIa inhibition in conjunction with percutaneous intervention for AMI in humans clearly seemed possible.

Unfortunately, the benefit of GP IIb/IIIa inhibition during percutaneous intervention for AMI in EPIC did not come without a cost. Of the three deaths that occurred, all had received abciximab; one of these underwent rescue angioplasty after failed t-PA, also

Table 1
Overall and AMI Subgroup Outcomes in EPIC *(23,24)*

End point	*Abciximab*	*Placebo*	*RR*	P *value*
Composite[a]				
Overall	8.3%	12.8%	35%	0.008
AMI	4.5%	26.1%	83%	0.06
MI				
Overall	5.2%	8.6%	40%	0.013
AMI	0%	8.7%	100%	0.17
Urgent revascularization				
Overall	3.2%	8.1%	60%	<0.001
AMI	0%	17.4%	100%	0.05

[a]30-d death, re-MI, or need for urgent revascularization. RR = relative reduction.

received intracoronary urokinase, and had major subsequent bleeding. Major nonfatal bleeding events were also more frequent with abciximab, occurring almost twice as often (24 vs 13%, $P = 0.7$). Furthermore, the only hemorrhagic stroke and all three spontaneous bleeds occurred in the abciximab-treated patients. Of the 13 major bleeding episodes, 9 (including the hemorrhagic stroke) occurred in patients who underwent the procedure as rescue angioplasty, having received fibrinolytic therapy within 12 h *(23)*.

Two additional trials, although not specifically targeted at intervention for AMI, also shed light on the subject of GP IIb/IIIa inhibition during angioplasty for acute infarction. The Integrilin™ to Minimize Platelet Aggregation and Coronary Thrombosis-II (IMPACT-II) and the Randomized Efficacy Study of Tirofiban for Outcomes and Restenosis (RESTORE) trials both sought to evaluate the use of GP IIb/IIIa inhibitors during percutaneous interventions *(25,26)*. Of the 4010 patients enrolled in IMPACT-II, 1663 (41%) were classified as high risk, with 126 (3%) undergoing direct or rescue angioplasty. In RESTORE, 139 (6.4%) of the 2141 patients were also enrolled during AMI and underwent direct PTCA. Prospectively specified subgroup analysis of these patients revealed trends similar to those in the entire study population toward a reduction in ischemic events and without any increased risk of bleeding.

Evaluations of GP IIb/IIIa inhibition in patients specifically during AMI and rescue interventions, although fewer in number, similarly suggest benefit with aggressive antiplatelet therapy. Of 387 such patients in the Global Use of Strategies To Open Occluded Coronary Arteries (GUSTO) III trial, 81 received abciximab prior to undergoing early angioplasty within a few hours of receiving thrombolytic therapy. Although patients were not entirely matched in baseline characteristics, there was a 62% reduction in the 30-d incidence of death, reinfarction (re-MI), or stroke in the 81 patients receiving abciximab compared with the 306 who did not (3.7 vs 9.8%, $P = 0.04$) *(27)*. There also appeared to be a lower 30-d incidence of death, re-MI, or stroke with the combination of abciximab and r-PA compared with abciximab and t-PA (8 vs 21%, $P = 0.07$), suggesting noteworthy interactions between GP IIb/IIIa inhibitors and specific fibrinolytic agents. Most importantly, there was no increase in the risk of intracranial hemorrhage with administration of abciximab during early angioplasty given soon after thrombolytic therapy (median administration: 3.5 h after thrombolysis) (0 vs 0.7%, abciximab

Table 2
Death, Reinfarction or Ischemic-Driven Target Vessel Revascularization in RAPPORT *(29)*

Time	*Abciximab*	*Placebo*	*RR*	P *value*
7 d	3.3%	9.9%	67%	0.003
30 d	5.8%	11.2%	48%	0.03
6 mo	11.6%	17.8%	35.1%	0.048

vs no abciximab group, respectively). Nor was there any significant increase in moderate or severe bleeding with the addition of abciximab to fibrinolytic and antithrombin therapy, despite the further risks posed by percutaneous access sites and intervention (16 vs 15% for severe bleeding, 3.7 vs 1%, $P = 0.08$ for moderate bleeding) *(27)*.

Retrospectively collected data from the Mayo Clinic PTCA registry not only support the observations from GUSTO-III but also suggest long-term benefit associated with GP IIb/IIIa antagonism in the setting of AMI *(28)*. From January 1995 (the first year abciximab became available) to August 1997, 52 patients receiving abciximab in addition to standard therapy prior to primary PTCA were compared with 240 who underwent direct angioplasty without adjunctive GP IIb/IIIa blockade. The 1-yr incidences of both death and the composite of death, re-MI, or the need for CABG were significantly reduced with abciximab (5.8 vs 17.1% and 5.8 vs 28.8%, $P < 0.05$).

The first prospective, randomized evaluation of GP IIb/IIIa inhibitors during primary angioplasty for AMI was in the ReoPro in Acute Myocardial Infarction and Primary PTCA Organization and Randomized Trial (RAPPORT). Hypothesizing that GP IIb/IIIa blockade would reduce both acute ischemic events (death, reinfarction, and urgent revascularization) as well as late recurrences (elective revascularization), 483 patients who presented within 12 h of AMI were randomized to abciximab or placebo prior to primary PTCA. Stent use was discouraged except for true "bail-out" purposes. Maintaining ACT ≥300 during the procedure was recommended, as was early sheath removal. The primary endpoint was the 6-mo incidence of death, recurrent MI, or any repeat target vessel revascularization. Acute phase endpoints included 7- and 30-d composites of death, reinfarction, or urgent target lesion revascularization, as well as safety data. On an intent-to-treat basis, administration of abciximab prior to direct angioplasty resulted in 67 and 48% reductions in ischemic-driven events at 7 and 30 days, respectively, mainly driven by a 78% reduction in the need for urgent target vessel revascularization (Table 2) *(29)*. Abciximab administration prior to primary angioplasty also reduced the need for bail-out stenting by 33% (17.4 vs 11.6%. $P = 0.057$) *(29)*. Although the 6-mo composite endpoint including all target lesion revascularization was not statistically different, reductions in ischemic-driven endpoints remained significant (11.6 vs 17.8%, $P = 0.048$) *(29)*.

Of the 438 patients enrolled in RAPPORT, 33 placebo-treated patients and 21 patients receiving abciximab had no angioplasty performed whereas 22 patients in the placebo group and 11 patients assigned to the abciximab group had no study drug administered. When analysis was performed on only the patients who underwent angioplasty and received study drug, results were even more striking than in the intent-to-treat analysis, with 73% and 62% reductions in the 7- and 30-d composite endpoints, respectively, and

Table 3
Clinical Outcomes with GP IIb/IIIa Inhibitors During Direct or Rescue Angioplasty

GP IIb/IIIa	*Trial*	*Enrolling procedure*[a]	*# Patients (%)*	*Result*[b]
Abciximab	EPIC *(24)*	P/R	64 (3%)	83% Reduction ($P = 0.06$)
Eptifibatide	IMPACT-II *(25)*	P/R	126 (4%)	Trend toward reduction
Tirofiban	RESTORE *(26)*	P	139 (7%)	Trend toward reduction[c]
Abciximab	RAPPORT *(29)*	P	429 (89%)	48% Reduction ($P = 0.03$)

[a]P = primary PTCA; R = rescue PTCA.
[b]30-Day composite of death, MI, or need for urgent revascularization.
[c]Included both urgent and elective repeat revascularization.

a 49% reduction in the 6-mo incidence of death, reinfarction, or urgent target vessel revascularization (10.6 vs. 19.9%, $P = 0.004$). "Bailout" stenting was now reduced by 42% (11.9 vs 20.4%, $P = 0.008$) *(29)*.

Again, the benefits with GP IIb/IIIa inhibition during percutaneous intervention came at a price, this time in isolation from concomitant fibrinolysis. Abciximab-treated patients in RAPPORT had almost twice the incidence of major bleeding (16.6 vs 9.5%, $P = 0.02$), mostly access-site related, and needed more blood products (13.7 vs 7.9%, $P = 0.04$) despite no significant differences in time to sheath removal. Encouragingly, no intracranial bleeds occurred in either group. The increased bleeding found in RAPPORT may simply relate to significantly higher ACTs at the time of angioplasty in the abciximab-treated patients (364 s vs 337 s, $P = 0.024$) and may be easily overcome by lowering heparin doses when adjunctive GP IIb/IIIa antagonism is used *(29)*.

Table 3 summarizes the information available from completed trials involving GP IIb/IIIa use prior to either direct or rescue PTCA. Although small in sample size and not randomized based on presentation, EPIC, IMPACT-II, and RESTORE all point toward reductions in death, reinfarction, or the need for repeat intervention with GP IIb/IIIa inhibitor use. RAPPORT, the first direct evaluation of GP IIb/IIIa inhibitors during intervention for AMI, confirms benefit with regard to both short-term and 6-mo ischemic complications.

Primary Stenting

Clinical Evidence

The remaining 5% of early ischemic complications seen despite the addition of GP IIb/IIIa inhibition to direct PTCA in RAPPORT and the lack of improvement on late restenosis might both be addressed with intracoronary stent placement during intervention for AMI. Despite initial concern over the addition of a metallic surface to the highly thrombogenic environment, primary stenting has been shown to be safe and may also result in improved clinical outcomes compared with primary PTCA alone *(30,31)*. The role of antiplatelet therapy with stenting for AMI has also been well established. In the Intracoronary Stenting and Antithrombotic Regimen (ISAR) trial, the combination

of ASA and ticlopidine reduced the rate of subacute stent thrombosis from 9.7 to 0% ($P = 0.03$) and was superior to anticoagulation in improving 6-mo survival free from recurrent MI (100 vs 90.3%, $P = 0.03$) *(32)*. Antithetically, ASA and ticlopidine may be insufficient antiplatelet therapy during stenting for AMI. In the recently completed Primary Angioplasty in Myocardial Infarction (PAMI-Stent) trial, there was actually less TIMI grade 3 flow reported after stenting compared with PTCA alone as measured by two core laboratories (89 vs 93%), and there was light imbalance toward increased mortality in the stented group (3.5 vs 1.8%, $P = 0.15$) *(33)*.

The role of GP IIb/IIIa antagonism during primary stenting for AMI was only recently evaluated in the Munich Randomized Trial, in which 200 patients within 48 h of AMI were randomized to either abciximab or standard therapy with heparin prior to primary stenting. Both groups received ASA and ticlopidine after successful stenting. The primary endpoints included differences in coronary blood flow velocities as measured by flow wire and wall motion index scores. Thirty-day clinical outcomes were also followed. Improvement in peak-flow velocity in the infarct-related artery was significantly greater in the abciximab-treated patients than in those receiving heparin alone (18.1 cm/s vs 10.4 cm/s, $P = 0.024$) *(34)*. Because no differences were detected in percent residual stenoses between the two groups, the improvement in peak flow is attributed to a microvascular mechanism. This improvement correlated with greater recovery of left ventricular (LV) function, both in the infarct region and globally, as well as with improvements in clinical outcomes. During the 30-d follow-up, 9 patients in the control group had clinical events, whereas only 2 in the abciximab-treated group reached an endpoint (odds ratio 5.1, 95% confidence interval 1.1–24, $P = 0.031$). The mechanism of improvements in microvascular perfusion seen with GP IIb/IIIa blockade during stenting for AMI may relate to inhibited formation of platelet aggregates in the distal vascular bed as well to possible inhibition of leukocyte function *(35)*. Although they have yet to be confirmed in larger clinical trials, the results seen in the Munich Randomized Trial imply beneficial effects of GP IIb/IIIa antagonists during primary stenting for AMI in addition to primary PTCA and/or bail-out stenting.

Ongoing Trials

The role of GP IIb/IIIa inhibitors during primary PTCA and primary stenting will be evaluated in the upcoming Controlled Abciximab and Device Investigation to Lower Late Angiographic Complications trial (CADILLAC). CADILLAC will be an open-label, randomized, multicenter, parallel design trial enrolling approximately 1720 patients within 12 h of AMI. Patients will be randomized to one of four arms: PTCA alone, PTCA and abciximab, stent (ACS Multilink™) alone, or ACS Multilink™ stent and abciximab. The primary endpoint of the study will be the 30-d composite of death, re-MI, need for target vessel revascularization, or stroke comparing stenting vs PTCA. A secondary endpoint will be the 30-d composite of the same events with regard to the use of preprocedural abciximab accompanied by early hospital discharge.

Unlike unfractionated heparin with a half-life of only 60 to 90 minutes, complete recovery of platelet function after discontinuation of abciximab requires 2 to 7 days. Successful revascularization using adjunctive GP IIb/IIIa inhibition may therefore allow for safer and earlier discharge. As the use of both primary stenting and GP IIb/IIIa inhibition carries significant monetary implications, attempts to reduce health care resource consumption are becoming increasingly important. CADILLAC will be the

first trial powered to directly evaluate the beneficial effects of GP IIb/IIIa antagonists prior to immediate intervention for AMI and will allow for comparisons to be made regarding any benefit of abciximab to primary stenting, a question left unanswered by EPIC, IMPACT-II, RESTORE, and RAPPORT. As the future direction of intervention for AMI appears headed toward primary stenting, the results of this trial will be eagerly awaited.

GP IIb/IIIa INHIBITORS AS AN ADJUNCT TO FULL-DOSE FIBRINOLYTIC THERAPY FOR AMI

Experimental Evidence

From 1988 to 1993 numerous studies combined GP IIb/IIIa inhibition with fibrinolytic therapy to evaluate its safety and its effect on reperfusion in animal models. In a canine model Gold et al. showed an increase in the speed of reperfusion and a decrease in the dose of fibrinolytic required with a combination of 7E3 and t-PA *(36)*. Studies of similar design with other GP IIb/IIIa inhibitors and fibrinolytic agents have reproduced these findings *(37)*. Elegant animal studies also confirm prevention of rethrombosis and reocclusion after successful thrombolysis with administration of GP IIb/IIIa inhibitors *(38,39)*. As safety and efficacy data accumulated, investigation in human subjects began.

Clinical Evidence

The first evaluation of GP IIb/IIIa inhibitor use during thrombolytic therapy in humans was the Thrombolysis and Angioplasty in Myocardial Infarction (TAMI) 8 pilot study. Sixty patients presenting within 6 h of onset of symptoms consistent with AMI were treated with a bolus of murine-derived monoclonal antibody 7E3 Fab (m7E3 Fab) starting at 0.1 mg/kg administered 15 h after initiation of t-PA (Activase, Genentech) and sequentially increasing to 0.25 mg/kg. As safety data accrued, the timing of m7E3 Fab administration shortened to 6 and then 3 h after t-PA initiation. The t-PA was given as 60 mg in the first hour and 40 mg over the next 2 h. ASA was given as early as possible, and full-dose heparin was given 90 min after t-PA initiation in the 15-h m7E3 Fab and control groups. Reduced-dose heparin was started after completion of t-PA in the 3- and 6-h m7E3 Fab groups. Primary endpoints were TIMI grade flow and clinical parameters including death, recurrent ischemia, re-MI, and the need for urgent intervention. Safety endpoints consisted of bleeding events and the incidence of thrombocytopenia. With the highest two doses of m7E3 Fab, recurrent ischemia occurred in only 4 of 42 patients (9.5%) vs 2 of 10 (20%) in the control patients. Angiography performed on average 5 d after enrollment revealed patency rates (TIMI grade 2 or 3 flow) of 56% (5 of 9 patients) in the control group vs 92% (34 of 37 patients) and 96% (25 of 26 patients) in patients receiving any m7E3 Fab or the highest two doses of m7E3 Fab, respectively *(40)*. The improvements in reperfusion were achieved at little hemorrhagic cost, with comparable changes in hemoglobin levels, frequency of major bleeding events, and number of transfusions required. However, CABG-related bleeding tended to be higher after m7E3 Fab administration, and of the 20 patients suffering major hemorrhagic events, 8 were related to bypass surgery; 7 of those 8 received m7E3 Fab *(40)*. Although the small number of patients does not allow conclusions to be drawn, this study opened the possibility for an expanded role of GP IIb/IIIa inhibitors during thrombolytic therapy for AMI.

Eptifibatide was subsequently evaluated as an adjunct to t-PA, ASA, and heparin during AMI in the IMPACT-AMI trial. From 1993 to 1995, 180 patients within 6 h of

AMI were randomized in a 2:1 ratio to receive open-label eptifibatide in increasing doses (as safety data accrued) or placebo within 10 min of "accelerated" t-PA, in addition to ASA and weight-adjusted heparin. In a second phase, patients were randomized to either the highest dose of eptifibatide from phase I or placebo in a 3:1 ratio to gain more experience with adjunctive eptifibatide therapy. In addition to continuous 12-lead digital ECG monitoring, 90-min angiography was performed. The primary endpoint evaluated was the 90-min TIMI grade 3 flow rate; secondary endpoints included the time to steady state ST-segment recovery, as well as the composite of death, re-MI, stroke, percutaneous or surgical coronary revascularization, new onset congestive heart failure, and bleeding. Patients allocated to the highest dose of eptifibatide achieved more complete and more sustained reperfusion than placebo-treated patients. TIMI grade 3 flow was present in 66% of eptifibatide-treated patients vs 39% of control patients ($P = 0.006$). During clinically driven follow-up angiography, preserved TIMI grade 3 flow was found in 79% of eptifibatide-treated patients vs 64% of control patients. Reperfusion also occurred more rapidly with eptifibatide. Median time to ST-segment recovery was reduced from 116 min in placebo-treated patients to 65 min for those receiving the highest dose of eptifibatide ($P = 0.05$) *(41)*. This benefit in IMPACT-AMI occurred at no significant increase in bleeding or adverse events, although moderate bleeding, defined as any bleed requiring transfusion, did occur more frequently with eptifibatide (14 vs 9%). The trial was not powered to detect differences in clinical outcomes, and no differences were observed. However, time to reperfusion has been positively correlated with survival and outcomes, and these results would suggest a possible clinical benefit to adjunctive GP IIb/IIIa inhibition in AMI *(3,42)*.

Eptifibatide was also evaluated as an adjunct to SK in a similar double-blinded placebo-controlled pilot study performed by Ronner et al. In this study, 181 patients with AMI were randomized to receive escalating doses of eptifibatide (180 μg/kg bolus followed by either 0.75, 1.33, or 2.0 μg/kg/min × 24 h) in addition to 1.5 million U of SK. Total reperfusion (TIMI grade 2 or 3 flow) was again improved with eptifibatide (75, 73, and 88% vs 65%), as was complete reperfusion (TIMI grade 3 flow) with rates of 53, 44, and 52% in the patients randomized to the three doses of eptifibatide compared with only 38% of the placebo-treated patients *(43)*. Unfortunately, bleeding events were also increased with combination therapy, including a 15% moderate bleeding rate with eptifibatide compared with 0% in the placebo-treated patients, and reached an unusually high rate of 40% in the highest doses, requiring discontinuation of those regimens. The contrasting safety data between the combination of eptifibatide and t-PA and the combination of eptifibatide and SK again suggest that the precise interactions between GP IIb/IIIa agents and specific fibrinolytic agents are not yet well understood.

The Platelet Aggregation Receptor Antagonist Dose Investigation and Reperfusion Gain in Myocardial Infarction (PARADIGM) trial was another dose exploration study using lamifiban in conjunction with fibrinolytic therapy during AMI. The three-phase trial enrolled patients presenting within 12 h of AMI. Initially, all patients received open-label lamifiban in addition to either t-PA or SK as well as ASA and heparin in order to find a dose resulting in 85–95% adenosine diphosphate (ADP)-induced inhibition of platelet aggregation. Subsequently, patients were randomized in a 2:1 fashion to either lamifiban (bolus and 24- or 48-h infusion) or placebo. The primary endpoints included safety issues, clinical markers of reperfusion, and clinical outcomes. Of the 353 patients enrolled, 236 received the GP IIb/IIIa inhibitor; in these patients, there was a significant

Table 4
Patency Rates with Combined GP IIb/IIIa Inhibitors and Full-Dose Thrombolytic Therapy

				90-Min patency (%)	
Trial	*GP IIb/IIIa*	*Fibrinolytic*	*Patients*	*Complete*[a]	*Total*[b]
TAMI-8 *(40)*	m7E3 Fab	t-PA	60	—	92
IMPACT-AMI *(41)*	Eptifibatide	t-PA	180	66	87
Ronner *(43)*	Eptifibatide	SK	181	52	88
PARADIGM *(44)*	Lamifiban	t-PA or SK	353	—	80[c]

[a]TIMI grade 3 flow.
[b]TIMI grade 2 or 3 flow.
[c]By continuous ST-segment monitoring.

Table 5
Rapidity of Reperfusion with Concomitant
GP IIb/IIIa Inhibition and Full-Dose Thrombolytic Therapy

	IMPACT-AMI (41)		*PARADIGM (44)*	
	Eptifibatide[a]	*Placebo*	*Lamifiban*[a]	*Placebo*
Time to 50% recovery (min)[b]	—	—	53 (16.0,91.3)	56.6 (19.0,107.0)
Time to steady state (min)[b]	65 (40,135)	116 (64,209)[c]	88 (28.7,151.3)	122.3 (45.0,253.0)[d]

[a]Highest dose arm.
[b]By continuous ST-segment monitoring, median (25th, 75th).
[c] $P = 0.05$.
[d] $P = 0.03$.

improvement in the speed and stability of reperfusion as detected by continuous electrocardiographic monitoring during the first 24 h after reperfusion therapy *(44)*. Time to steady-state ST-segment resolution was 88 min in the lamifiban-treated patients compared with 122 min in the placebo arm ($P = 0.003$). Patency at 90 min as measured by continuous monitoring was also significantly greater with lamifiban (80.1 vs 62.5%, $P = 0.005$).

Again, no differences in clinical outcomes were detected in PARADIGM, as this phase II trial was not powered to detect those differences. Yet, bleeding complications are still noteworthy. Two intracranial hemorrhages occurred in patients receiving both full-dose thrombolytic therapy and lamifiban, whereas none occurred in the placebo group. Likewise, intermediate and major bleeding (16.2 vs 7.2%), mainly gastrointestinal (5.6 vs 0.9%) and CABG-related (3.4 vs 3.4%), was more common in patients who received lamifiban. Although most of the intermediate bleeding in IMPACT-AMI could be attributed to femoral access sites, no such correlation existed in PARADIGM, where only a subset of patients received catheterizations (2.6% femoral access-site bleeding with lamifiban vs 1.7% in those treated with placebo) *(44)*. In addition, increased bleeding with lamifiban was seen with both t-PA and SK despite a greater degree of platelet aggregation inhibition with the combination of SK and lamifiban than with t-PA (96-97 vs 83–89%, depending on the dose of lamifiban investigated) *(44)*.

Tables 4 and 5 summarize the angiographic and ST-segment monitoring results of the

trials combining GP IIb/IIIa inhibition and fibrinolytic therapy. Taken as a whole, they suggest quicker and more complete reperfusion with combination therapy but not without a few notable concerns. Despite the apparent improvements in the speed and totality of reperfusion based on either 90-min angiography or continuous ST-segment monitoring, all three trials were too small to prove a benefit in clinical outcomes. Furthermore, by using specific exclusion criteria, all three trials were performed in select populations with low risk of adverse events including stroke. Combination therapy may result in higher and possibly unacceptable bleeding rates if this safety profile is generalized to the entire population.

GP IIb/IIIa INHIBITORS AS SOLE REPERFUSION THERAPY

Thrombolytic Properties of GP IIb/IIIa Therapy

Experimental Evidence

The strategy of initiating coronary reperfusion in the setting of AMI using GP IIb/IIIa platelet inhibition without exogenous plasminogen activators has also been considered by many investigators. Gold et al., in a model of left anterior descending occlusion, treated 14 dogs with heparin to prevent fibrin generation, followed by 10 min thereafter by either placebo, ASA, or ASA followed by murine 7E3 (0.8 mg/kg). Of the dogs treated with the combination of GP IIb/IIIa antagonists, ASA, and heparin, 4/5 (80%) achieved reflow at 50 min, whereas none of the animals in either of the remaining groups achieved any degree of reflow *(45)*. The cumulative in vitro evidence of actual clot dissolution properties of GP IIb/IIIa antagonists aroused thoughts regarding potential benefits of GP IIb/IIIa inhibitor administration alone, without percutaneous intervention or fibrinolytic therapy.

Early Human Experience

In the same publication as their canine model, Gold et al. reported the retrospective analysis of angiographic outcomes in a subset of humans with AMI given abciximab. Between 1995 and 1996, 34 patients presenting with AMI referred for direct angioplasty were given abciximab according to FDA-approved guidelines. Of the 34 patients given abciximab prior to direct angioplasty and after angiographic documentation of TIMI grade 0 or 1 flow, 13 had no prior infarctions, were between 30 min and 6 h of presentation, and had also received ASA and heparin. In these 13 patients, flow was found to have increased at least one TIMI grade in 11 (85%) patients and reached TIMI grade 2 or 3 in 7 (54%). Twelve of the 13 patients went on to receive successful angioplasty with 5 patients receiving stents *(45)*.

Motivated by attempts to compensate for delays in getting patients with AMI to catheterization laboratories, the Glycoprotein Receptor Antagonist in MI Patency Evaluation (GRAPE) study evaluated the usefulness of earlier administration of GP IIb/IIIa inhibitors in inducing reperfusion. Sixty patients with AMI referred for direct angioplasty were given abciximab (0.25 mg/kg bolus followed by 10 μg/min infusion) in the emergency department in addition to ASA (160 mg) and heparin (5000 U). Total patency (TIMI grade 2 or 3 flow) at median catheterization time of 45 min after drug initiation approached 40%, with complete reperfusion occurring in 18% of patients *(46)*. This compares favorably to known overall patency rates of 27% with standard therapy and

Table 6
Reperfusion Capabilities of Various Therapies for AMI

Treatment[a]	*Trial*	*Patients*	*Minutes to angiography*	*Patency*	
				Complete[b]	*Total*[c]
None	DeWood *(49)*	126	360	—	3–13%[d]
ASA	HEAP *(50)*	108[e]	90	9%	18%
ASA/H	GUSTO-IIb *(6)*	510	90	8%	25%
ASA/↑H	HEAP *(50)*	108	90	31%	51%
ASA/H/SK	GUSTO-I[1]	283	90	32%	60%
ASA/H/t-PA	GUSTO-I[1]	292	90	54%	81%
ASA/H/A	Gold et al. *(45)*	13	10	8%	54%
ASA/H/A	ReoMI *(51)*	34	34	13%	25%
ASA/H/A	GRAPE *(46)*	60	45 min	40%	—
ASA/H/A	TIMI-14 *(47)*	32	90 min	32%	—
ASA/H/A	SPEED *(48)*	63	60 min	28%[f]	48%[f]

[a]ASA = aspirin; H = heparin; SK = streptokinase; t-PA = Activase; A = abciximab; ↑ = high-dose.
[b]TIMI grade 3 flow.
[c]TIMI grade 2 or 3 flow.
[d]87% total and 10% subtotal occlusion.
[e]Collected from a large angioplasty database.
[f]Preliminary results

TIMI grade 3 flow rates of 8% and suggests that GP IIb/IIIa antagonists exert at least modest thrombolytic properties *(6)*.

Randomized Clinical Trials

Randomized trials comparing GP IIb/IIIa inhibitors alone with fibrinolytic agents in the setting of AMI are underway. Preliminary results from two such trials shed some additional light on the reperfusion capabilities of GP IIb/IIIa antagonists. The Thrombolysis In Myocardial Infarction (TIMI-14) trial and the Strategies for Patency Enhancement in the Emergency Department (SPEED) trial contain control arms where patients receive abciximab in addition to ASA and heparin without concomitant fibrinolytic therapy prior to catheterization. Interim results from TIMI-14 reveal a 32% TIMI grade 3 flow rate at 90 min with abciximab; analysis based on available angiographic data from the first 305 patients from SPEED indicates 28% complete and 48% total reperfusion at 60 min *(47,48)*. Again, these rates are still considerably higher than historical controls with ASA and heparin for AMI and similar to that reported with the fibrinolytic SK *(1,6)*. Since TIMI-14 and SPEED both include control arms consisting of fibrinolytic therapy alone (t-PA in TIMI-14 and r-PA in SPEED), completion of the trials will allow for the first direct comparisons between GP IIb/IIIa inhibitors and fibrinolytic agents in terms of reperfusion capabilities during acute ST-segment elevation infarcts.

Table 6 compares patency rates with various therapies during AMI. Although GP IIb/IIIa inhibitors alone will most likely possess only moderate thrombolytic properties, the attribute is not unimportant *(49–51)*. This is particularly true in centers where direct angioplasty is the preferred reperfusion strategy. At these centers, early administration of a GP IIb/IIIa inhibitor in the emergency room might induce and shorten time to

reperfusion. Based on the well-established association between time to reperfusion and outcome, improvements in mortality would potentially follow *(3)*. In the event of no reperfusion, the potential complications of fibrinolytic therapy have still been avoided, and the patient expeditiously proceeds to mechanical intervention, where the benefits of less reinfarction, less recurrent ischemia, and lessened need for repeat revascularization are evident. In centers where angioplasty is not available, the possible synergistic interaction between GP IIb/IIIa inhibitors and fibrinolytic agents (in reduced doses to avoid bleeding complications) may also significantly improve reperfusion and clinical outcomes compared with current thrombolytic therapy.

GLYCOPROTEIN IIb/IIIa INHIBITORS AS AN ADJUNCT TO REDUCED-DOSE FIBRINOLYTIC THERAPY FOR AMI

Bleeding issues surrounding the combination of GP IIb/IIIa inhibition and full-dose fibrinolytic therapy, along with in vitro evidence suggesting lower fibrinolytic dose requirements with adjunctive GP IIb/IIIa antagonism, and the concept that higher patency rates may actually be achieved with lower doses of fibrinolytic therapy (less platelet activation) have prompted the next wave of GP IIb/IIIa AMI trials. Three trials are presently underway: TIMI-14 is evaluating the combination of abciximab and low-dose t-PA or SK; the GUSTO-IV pilot study, SPEED, is combining abciximab with r-PA; and INTRO-AMI will focus on eptifibatide and t-PA.

TIMI-14 is the first large-scale trial evaluating the combination of abciximab and reduced-dose fibrinolytic therapy with preliminary results. In the dose-finding phase, 584 patients were randomized to receive low-dose t-PA (20, 35, 50, or 65 mg) plus abciximab, or low-dose SK (500,000, 750,000, 1.25 million, or 1.5 million U) plus abciximab. All patients also received ASA and heparin. Updated results from the study were presented at the 47th annual scientific session of the American College of Cardiology in Atlanta, GA, in March 1998. Of patients treated with the various doses and infusions of t-PA and abciximab, 62 to 71% achieved complete restoration of flow (TIMI grade 3 flow), compared with 58% in the conventional fibrinolytic arm *(52)*. Figure 2 displays 90-min reperfusion rates in patients receiving abciximab combined with either t-PA or SK. Major hemorrhagic events were low in all groups except the group receiving 1.25 million U SK. Except for the high-dose SK group, there again appear to be greater TIMI grade 3 flow rates with the combination of abciximab and t-PA than with SK and abciximab (62–71% compared with 39–47%) *(52)*. In either case, the modest but persistent improvements in TIMI grade 3 flow with GP IIb/IIIa inhibition and reduced dose fibrinolytic therapy continue to stir interest among cardiologists.

SPEED is a 530-patient, phase II, randomized, open-label, angiographic pilot trial designed to evaluate the safety and benefit of abciximab in addition to reduced dose r-PA. Patients are being randomized within 6 h of AMI in a 1:4 ratio to receive abciximab alone or abciximab and single or double boluses of r-PA (5, 7.5, and 10 mg) prior to acute intervention. The primary endpoint will be TIMI grade 3 flow in the infarct-related artery at 60-min catheterization (rather than 90 min, as evidence of the importance of 60-min reperfusion rates continues to accrue). Secondary endpoints include the 30-day composite clinical efficacy outcomes of a) death and recurrent MI and b) death, re-MI, and recurrent ischemia requiring revascularization; and the safety of abciximab (14-d incidence of hemorrhage, thrombocytopenia, and need for red blood cell or platelet

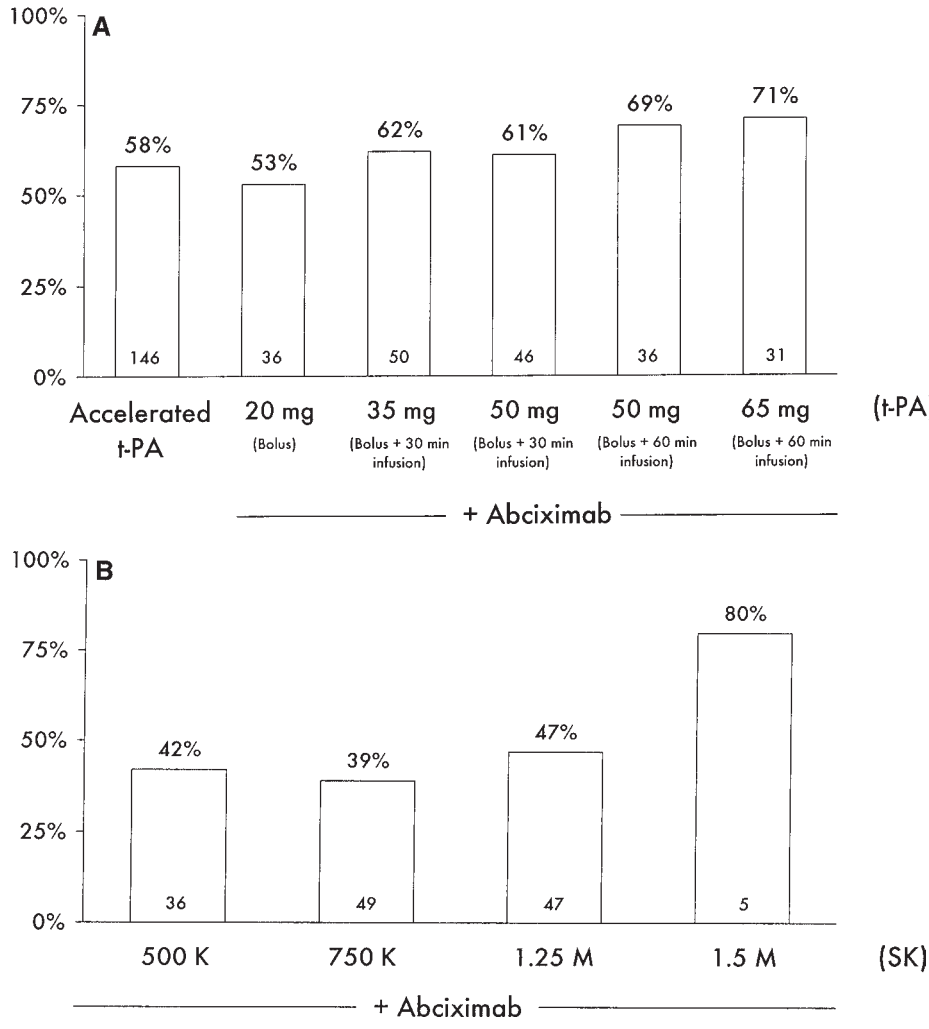

Fig. 2. 90-Minute complete patency rates among patients in the dose-finding phase of TIMI-14 *(52)*. **(A)** shows patency rates among patients who received accelerated t-PA alone and those who received t-PA plus various doses of abciximab. **(B)** shows patency in those patients who received SK plus abciximab.

transfusions, and 30-d incidence of hemorrhagic stroke or intracranial hemorrhage). Once an appropriate dose of r-PA is determined, a confirmation stage will compare the combination of abciximab and r-PA directly with conventional r-PA therapy (10 U + 10 U) in 150 patients with the same endpoint (60-min TIMI grade 3 flow). Eventually, GUSTO IV, the phase III, large-scale, international mortality trial comparing the combination of abciximab and r-PA with conventional r-PA, will be launched.

Preliminary results based on available angiographic core laboratory data from the first 305 patients enrolled in SPEED were presented at the 20th annual scientific session of the European Society of Cardiology. Results of angiography performed between 60 and 90 min showed complete patency rates approaching 63% with the combination of

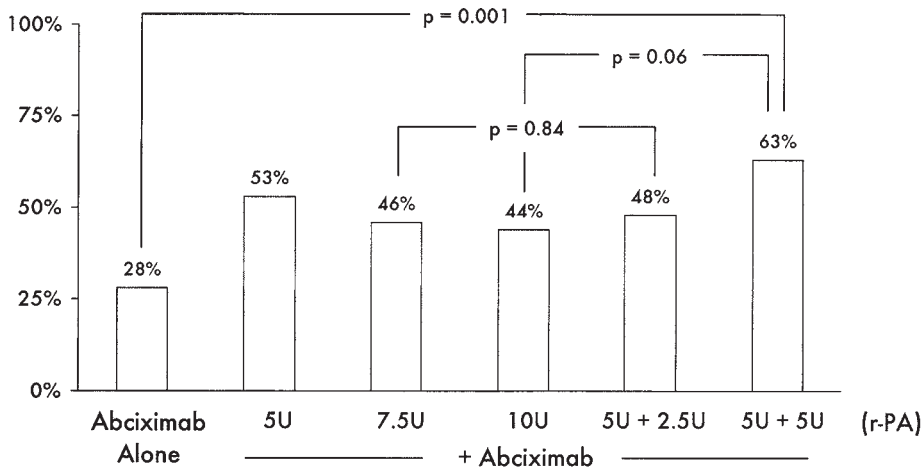

Fig. 3. 60- to 90-Minute complete patency rates among patients in the dose-finding phase of SPEED *(48)*.

abciximab and (5 U + 5 U) r-PA (Fig. 3) *(48)*. Total patency (TIMI grade 2 or 3 flow) was achieved in 83% of patients. In TIMI-14 and SPEED, respectively, the highest patency rates have been achieved with the 30-min continuous infusion of t-PA and the divided bolus of r-PA. As GP IIb/IIIa inhibition is added to reperfusion therapy, issues regarding the manner and length of fibrinolytic administration will likely resurface. Results from TIMI-14 and SPEED should clarify many of the questions surrounding the interaction between fibrinolytic therapy and GP IIb/IIIa inhibition.

Ultimately, GUSTO IV will be the definitive phase III trial needed to evaluate the efficacy of combined GP IIb/IIIa antagonism and reduced-dose fibrinolysis during ST-segment elevation AMI. GUSTO-IV AMI is designed to test the hypothesis that a further approximately 20% improvement in TIMI grade 3 flow will reduce 30-d mortality by 1%. Approximately 17,000 patients presenting within 6 h of symptoms with ECG changes consistent with AMI will be randomized to either conventional r-PA (10 U + 10 U) with standard heparin, or reduced r-PA (5 U + 5 U), abciximab (0.25 mg/kg bolus followed by a 0.125 mg/kg/min infusion for 12 h), and weight-adjusted heparin (Fig. 4). The primary endpoint will be all-cause mortality at 30 d. Enrollment is scheduled to begin in early 1999.

Need for Concomitant Antithrombin Therapy

With only modest reperfusion rates seen thus far with combined GP IIb/IIIa inhibition and reduced dose fibrinolysis, and unacceptable bleeding rates as the dose of fibrinolytic agent increases, there has been speculation that eliminating antithrombin therapy might be the most appropriate next step in improving outcomes, i.e., by replacing antithrombin therapy with GP IIb/IIIa inhibition. By inhibiting the final pathway in platelet aggregation, fibrinolytic-induced augmentation of thrombin generation is kept to a minimum with GP IIb/IIIa antagonism, and the need for heparin may be eliminated.

Platelet aggregation studies from IMPACT-AMI do not support this theory. In IMPACT-AMI, patients assigned to the eptifibatide 108 μg/kg bolus + 0.6 μg/kg/min infusion group or placebo, and the first group receiving eptifibatide 135 μg/kg bolus

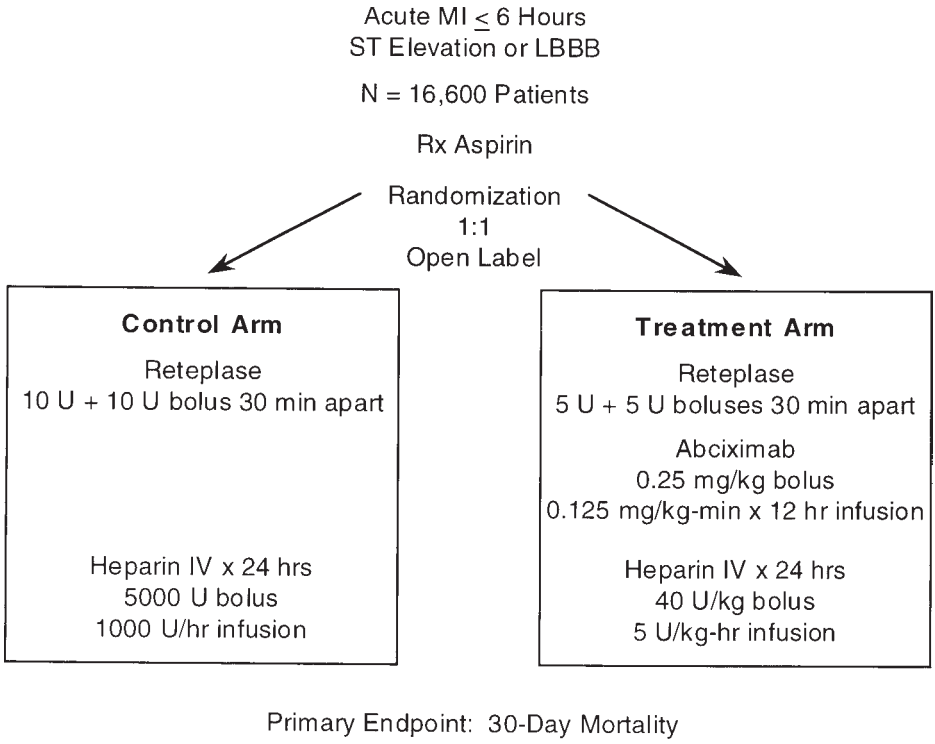

Fig. 4. Study algorithm for the GUSTO-IV AMI trial.

followed by 0.75 μg/kg/min or placebo, did not receive any heparin until 60 min after the t-PA therapy was begun, at which time a 15 U/kg heparin infusion was initiated. The second group receiving eptifibatide 135 μg/kg followed by 0.75 μg/kg/min or placebo received adjunctive heparin (40 U/kg) immediately followed by a 15 U/kg infusion. Half of the 42 patients randomized to eptifibatide in these groups had adequate baseline and 60-min coagulation studies prior to heparin initiation, and all patients underwent 90-min angiography. When evaluated, eptifibatide administration appeared to have no effect on thrombin generation. No differences were found in levels of fibrinopeptide A (FPA), thrombin-antithrombin complexes (TAT), or prothrombin fragment 1.2 (F1.2) between eptifibatide- and placebo-treated patients. No differences were found in any of these parameters in patients with or without recurrent ischemia or in patients with varying TIMI flow grades. When eptifibatide-treated patients were evaluated, the levels of FPA, TAT, and F1.2 were considerably higher in the patients not receiving any heparin bolus, whereas patients receiving heparin had relatively low levels of these parameters *(53)*. In addition, there was somewhat less TIMI-grade 3 flow in the patients who had not received heparin.

Therefore, despite GP IIb/IIIa inhibition, thrombin is still generated and catalyzes fibrin formation in patients undergoing thrombolysis with t-PA for AMI, and increased levels of FPA, TAT, and F1.2 continue to be produced following thrombolytic therapy. These findings should not diminish to any extent the overall improvements in the speed, completeness, and durability of reperfusion with eptifibatide in IMPACT-AMI, but imply not yet understood interactions among fibrinolytic therapy, GP IIb/IIIa inhibition,

and antithrombin therapy. Improvements in outcomes with GP IIb/IIIa inhibition during thrombolysis may stem from some mechanism other than prevention of thrombin formation, and antithrombin therapy may remain an essential component of fibrinolytic therapy. In fact, experimental studies in canine models suggest markedly facilitated fibrinolysis with the combination of a low dose of a GP IIb/IIIa inhibitor and the direct thrombin inhibitor hirudin *(54)*. If nothing else, the relationship between GP IIb/IIIa inhibition and antithrombin therapy and the possible role of lower-dose combination therapy warrants further investigation.

CONCLUSION

Over the last two decades a number of angiographic and phase III trials have explored different adjunctive therapies to improve both reperfusion and outcomes in AMI. The increased knowledge over this period has made clear that platelet inhibition forms one of the key components for successfully enhancing reperfusion and clinical outcomes in AMI. Although the pendulum has swung towards emphasizing platelet inhibition, the value of thrombin inhibition or passivation of the coagulation cascade remains to be completely understood. As our investigation in this field continues, specific targets such as the GP IIb/IIIa receptor will likely form a key component for the management of AMI. Future work needs to explore to what extent the coagulation cascade can be disrupted to maximize the ability to reperfuse infarct-related arteries.

Primary angioplasty with GP IIb/IIIa receptor blockers is an attractive therapy for patients with AMI. However, this therapy is severely limited by a lack of access in most hospitals worldwide. As future reperfusion strategies are being developed, the "friendliness" for allowing mechanical interventions to be performed will be important. The ultimate strategy would be to develop reperfusion therapies where mechanical interventions can be incorporated in the overall treatment strategy not only in the first few hours but also within the first 24 h to maximize the benefit.

As we move on to the next millennium, the future of the management of AMI could not be brighter. Clinical trials using either pharmacological or mechanical approaches are now ready to converge into an optimized treatment strategy for all patients with AMI. Although the journey has been long since reperfusion therapies were initially used in the 1960s, our knowledge base has expanded tremendously in the last decade. The future looks bright for further enhancing outcomes for patients with AMI.

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IV Practical Issues and Future Applications

12 Economics of Glycoprotein IIb/IIIa Inhibition

Daniel B. Mark, MD, MPH

CONTENTS

ECONOMIC CONCEPTS AND MEASUREMENTS

Basic Concepts of Economic Analysis

Medical cost analysis is a hybrid field that incorporates concepts from both accounting and economics *(1)*. Economics provides much of the theoretical and conceptual framework for cost analysis while accounting provides us with practical measurement tools and concepts. One consequence of this admixture is that there are several distinct, but overlapping sets of terminology used in cost analysis. We will start our review of this area by considering some of the basic concepts and terms that the clinician is likely to encounter in reading the medical cost literature.

When most people hear the term "cost," they think of money. An economist, on the other hand, thinks of cost in a much more theoretical way *(2,3)*. A major precept of economics is that any societal decision to employ the resources of society in the production of goods and services is necessarily accompanied by lost opportunities to do something else with those resources *(4,5)*. Health care services and programs engender a "cost" through their consumption of resources which are then lost to other uses. The technical term for this concept is "opportunity cost" *(1,6)*. Economists are far more concerned with this notion of opportunity cost than they are with measuring dollars spent.

Another important related concept from economics holds that society's collective resources are finite. Consequently, there will not be sufficient resources to fulfill all of society's goals, forcing the need for choices to be made among competing priorities. Economics therefore strives to provide a set of tools to help policy and other decision makers decide how to allocate societal resources efficiently. In the most general terms,

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resources are divided into large categories such as land, labor, and capital (e.g., stores of raw material, factories, machines). Technology allows society to convert these raw resources into desired goods and services including things such as medical care. In order to understand societal alternatives for the use of such resources, it is necessary to assess the opportunity cost of various types of health care, as well as that of investments in defense, public education, transportation, and other societal priorities. To compare the resource requirements of these needs, a common metric must be found. Some societies still use barter to match goods and services, but most industrialized societies employ markets (collections of sellers and buyers), with money used as the standard exchange intermediary. The market price in these societies represents the monetary equivalent in value of the total resource inputs used in the production of the particular good or service. Money then is a convenient shorthand for valuing all these resources, but it is the underlying resources themselves that are of primary concern for the economist.

Unfortunately, the economic concept of opportunity cost, while representing the purest definition of "true cost," is actually a theoretical construct that does not have a practical measurement analog *(1)*. Accountants, being more concerned with measurement than economists, have made some important simplifications to allow approximation of opportunity cost. In each costing exercise, because the cost information itself has a cost, the analyst must decide how much detail is necessary to satisfy the particular study requirement. In addition, cost needs to be assessed from an explicitly defined perspective. In health care, a variety of valid perspectives are possible. If a patient receives a $10,000 cardiac procedure but only pays her deductible and copayment amounting to $1000 (with the remainder paid by insurance), then for that patient the cost of the procedure is $1000. The cost to the insurance company may be only $6000 if they have negotiated discounted payments with the hospital. The cost to the hospital of delivering the care in question is different from both these figures. The societal perspective is the one most often used by cost analysts in medicine. This is the broadest possible perspective because it incorporates all costs and health benefits created by the contemplated change in medical care *(5)*.

Costs can be classified according to their behavior as production is increased or decreased (variable vs fixed costs) and in terms of their traceability to the production of health care services (direct vs indirect costs). Variable costs increase in direct proportion to production of extra health care. Disposable supplies in a catheterization laboratory are an example of variable costs. Fixed costs, as the name implies, do not change with the volume of care provided (e.g., the depreciation of the catheterization facility itself) *(1)*. Direct costs can be readily linked to the production of a particular healthcare product or service (e.g., disposable supplies, labor) whereas indirect costs (or overhead) cannot (e.g., heating, electricity, laundry, medical records, hospital administrators). Two terms are used to describe the effect on costs resulting from shifts in the use of health services. Marginal cost refers to the extra cost involved in generating one extra unit of a good or service (e.g., performing one extra PTCA) and is equivalent to variable cost. Incremental cost is the cost involved in shifting from one clinical strategy to an alternative (e.g., from thrombolysis to direct angioplasty); this is a fundamental notion that underlies cost effectiveness analysis.

Another important economic concept for the clinician is that of induced cost or induced savings. If one performs a cardiovascular procedure, it is clear that the cost of that procedure includes the cost of the equipment consumed in the procedure (such as coro-

nary stents or balloon catheters). It may be less clear that the cost of the procedure also includes things that happen later in the patient's follow-up as a consequence of the decision to do that procedure. For example, the need to have a second procedure to treat restenosis is an induced cost of the initial procedure. The reduced need for a repeat procedure when a coronary stent is substituted for a standard balloon catheter procedure is an induced savings of the coronary stent procedure. The key concept is that it's not sufficient in most cases of medical care to look only at the immediate costs of care. Downstream costs and cost savings must also be assessed in order to provide an accurate picture of the total economic effects of a particular technology or strategy.

One other term that is often used in economic analysis is indirect cost. This term actually has two meanings, an accounting meaning in which it refers to overhead (as described above) and an economic meaning in which it refers to costs associated with lost productivity. Because of this confusion of terminology, many modern investigators substitute the term "productivity costs" when they are referring to the latter type of indirect costs *(5)*. In medical care, typical productivity costs include those associated with time lost from work. Difficulties encountered in assessing productivity costs relate in part to the proper methods to assess such costs for individuals who are retired from the workforce or those who are working at home (not for pay). In practice, productivity costs are infrequently examined in medical economic studies.

In an ideal competitive economic market, prices for goods and services are established by the dynamic interplay between sellers and buyers. Such markets are characterized by competition on price among many sellers (none of whom is large enough to control prices), easy entry into the market by new sellers, and knowledgeable buyers who pay themselves for the goods and services they consume *(2)*. The "medical marketplace" has none of these features. Patients are usually insulated from most of the cost of the care they consume by insurance and they do not have the technical knowledge to discriminate among the care options they face. In most local healthcare markets, there are only a few buyers (insurance companies) and sellers (medical care systems) of health care. Whereas managed care has introduced price competition to medicine, it is not the ideal competition of economic textbooks. Managed care companies often control large blocks of business and providers may be forced to accept a heavily discounted price in order to retain this portion of their patient population. Because of these and related issues and because of the fragmentation of the medical marketplace, providers may have one price for self-pay patients (full charges, usually their highest price), another price for discounted fee-for-service business, and yet another price for managed care. Typically, none of these reflect the opportunity cost of the care provided. Thus, there is no market price for medical care in the way that automobiles and refrigerators have a market price. Consequently, several different approaches have been developed to estimate medical costs (or prices). These are reviewed briefly in the next section.

Cost Measurement

Two general approaches are available to measure the costs of medical care services: bottom-up and top-down. The bottom-up methods are all based on counting individual resources consumed in a particular episode of care and assigning appropriate cost weights. The gold standard bottom-up method is known as microcosting and involves a very detailed enumeration of all resources consumed, estimation of cost weights for each individual resource and calculation of total costs through multiplication of the individual

resources by their cost weights. Whereas microcosting analysis is possible for simple types of medical care, such as administration of an intravenous antibiotic, it can be very laborious and expensive for complex forms of care. Even things such as an admission for coronary angioplasty or acute myocardial infarction (MI) are relatively complex when considering doing a microcosting analysis. Fortunately, an increasing number of hospitals in the United States have installed computer-based detailed cost accounting systems that provide an approximate version of the gold standard bottom-up cost analysis. These systems have not been well studied from a research point of view, but they do appear to offer a better estimate of true underlying costs than is available through alternatives. Like any costing methodology, they should not be accepted without question, and component inputs should be carefully scrutinized for reasonableness whenever possible.

The simplest version of the bottom-up approach is the so called "big ticket" method. This is based on an identification of a subset of resources consumed that are considered the most important and/or costly (the "big tickets"). This approach is the most inexpensive method of costing and allows the analyst to concentrate on generating cost estimates for a small subset of the entire portfolio of resources consumed. There are several potential disadvantages to this simple method, however. Most importantly, the analysis may oversimplify the true resource effects of the strategies under study and therefore may miss important cost differences. There are rarely sufficient prior data available to assure the analyst that he or she has captured all the important resources in the appropriate amount of detail. This approach also assumes that all big ticket resources of a particular type have the same cost, which of course is clearly not true. Cost weights for this type of analysis are often chosen more for convenience than established suitability for the task.

The other major approach to cost estimation is the "top-down" approach. There are two different methods that can be used here. The most common method used in medical cost research applications involving hospital-based care is the collection of hospital billing information and the conversion of the charges on the hospital bills into costs using correction factors that are published in each hospital's annual cost report. The vast majority of hospitals in the United States generate the type of bills (UB-92) and the Medicare Cost Reports that are necessary to accomplish this conversion. (Notable exceptions are Veteran Administration Hospitals and some fully capitated HMOs.) Physician costs can also be collected as billing information, although this is generally onerous because physician billing for a particular medical center is often done from individual offices rather than from a centralized billing office. In addition, there are no standardized conversion factors to correct physician charges back to costs. For that reason, many current analysts use the Medicare Fee Schedule as a national standard for physician costs.

The Medicare Fee Schedule also provides the best current source for costs of outpatient visits and testing. A much simpler top-down approach that is used primarily when there are limited details available in the database about resources consumed is to assign each in-patient episode of patient care to a diagnosis related group (DRG) category and assign price weights to the entire episode of care based on this type of aggregating classification. This approach has many of the same limitations described earlier for the big ticket bottom-up approach. Clearly not all episodes of hospitalization for coronary angioplasty involve the same amount of resources or should be given the same cost

weight, just as different hospitalizations for MI or coronary bypass surgery should not be viewed as economically uniform.

Cost Measurement in Clinical Trials

Over the last decade, several groups have pioneered the use of empirical cost data collection in large-scale randomized trials and in large single site observational studies as the principal method for estimating medical costs *(7–11)*. By generating an empirical database of costs similar to the empirical clinical database that is typically generated in clinical trials and outcome studies, we have a much richer source of investigation and can perform analyses that do not appear as arbitrary and subject to analyst biases as earlier literature and expert-based opinion analyses often did. We have shown that it is feasible to collect hospital billing information on large numbers of patients enrolled in the United States and that these data can reliably provide an estimate of medical costs. Ideally, we would prefer to have access to a detailed hospital accounting system in every hospital in which we enroll patients in a particular study, but this is rarely feasible. Consequently, we anticipate that for at least the next five years we will continue to be measuring hospital costs through the use of medical bills and correction factors as described above.

Cost-Effectiveness Analysis

Many clinicians tend to confuse cost-effectiveness analysis with economic analysis in general, but these two concepts are not equivalent. Cost-effectiveness analysis is a specific type of economic analysis that attempts to assess the value of medical care by relating in a structured fashion the added costs of a particular strategy to its added medical benefits. Typically, cost-effectiveness analyses express their results in terms such as the extra dollars required to add an additional life year with the new strategy relative to standard care. The goal of cost-effectiveness analysis is not to assist the clinician at the bedside, but rather to give policy analysts a tool to use in allocating societal resources. Although theoretically attractive, this use of cost effectiveness is rarely attempted in actual practice and the challenge for modern analysts is to find ways of doing cost-effectiveness analysis that are more relevant to the challenges and concerns of practitioners, administrators, health plans, and patients in the modern era. Additional details on cost-effectiveness methodologies are provided elsewhere *(1,5)*.

ECONOMIC ANALYSIS OF GP IIb/IIIa RECEPTOR ANTAGONISTS

Studies of Percutaneous Coronary Revascularization

The three currently available intravenous GP IIb/IIIa platelet receptor blockers have all been studied in large scale trials involving percutaneous revascularization patients as described in earlier chapters in this book. Four trials with prospective economic analysis of GP IIb/IIIa inhibitors in percutaneous intervention populations have been completed, EPIC and EPILOG (both involving abciximab), IMPACT II (eptifibatide), and RESTORE (tirofiban).

The EPIC (Evaluation of Monoclonal Antibody to Prevent Ischemic Complications) trial randomized 2099 high-risk PTCA patients to one of three arms, placebo, bolus abciximab, and bolus plus infusion abciximab. At 30 d, bolus plus 12-h infusion abciximab reduced ischemic endpoints by 35% relative to placebo but doubled major in-hospital bleeding episodes (from 7 to 14%) *(12)*. All patients received aspirin and a

Table 1
Major Baseline Hospital Resource Consumption and Hospital Costs in the EPIC Study

	Placebo (n = *696*)	*Bolus only* (n = *695*)	*Bolus and Infusion* (n = *708*)
Resource consumption			
Urgent rePTCA	3.6%	2.6%	0.7%
Non-urgent rePTCA	3.3%	2.3%	3.7%
Coronary stent	0.6%	1.7%	0.6%
Urgent CABG	3.6%	2.0%	2.4%
Non-urgent CABG	1.1%	1.4%	2.3%
Intra-aortic balloon pump	3.3%	2.4%	2.9%
RBC transfusion	7%	13%	15%
Medical costs and length of stay (means)			
Hospital costs	$11,430	$11,141	$11,562
Physician fees	$2004	$1993	$2015
Total medical costs	$13,434	$13,135	$13,577
Total length of stay	5.9	6.1	6.4

PTCA = coronary angioplasty, CABG = coronary bypass surgery, RBC = red blood cell.
From Mark et al. *(7)*.

fixed-dose regimen of intravenous heparin. Six-month ischemic episodes were further reduced by 23% *(13)*. The economic substudy of EPIC was conducted prospectively as part of the overall EPIC research effort *(7)*. Hospital costs were estimated using hospital bills and Medicare conversion factors, as described above. Physician fees were estimated from the Medicare Fee Schedule and the record of physician services contained in the case report form. The cost of abciximab was estimated from the per vial cost of the drug and the weight of the patients in the EPIC trial, assuming that unused portions of vials would be wasted.

During the index hospitalization, the baseline costs in EPIC (excluding the cost of abciximab) were not significantly different (Table 1). Including the cost of abciximab ($1407), the baseline hospital plus physician costs were $14,984 for the bolus and infusion abciximab arm and $13,434 for placebo arm. To understand the dissociation evident between the beneficial effect of abciximab on the primary study endpoint (reflecting a composite of adverse ischemic events) and the absence of a related economic benefit, we developed an "explanatory" regression model. This model demonstrated that during the initial hospitalization, the decrease in ischemic events due to abciximab generated a potential cost saving that was estimated at $622 per patient (Table 2). However, the excess major bleeding in the bolus and infusion abciximab arm generated a $521 excess in costs for this arm.

During the 6-mo follow-up, the abciximab arm experienced a 23% decrease in rehospitalization and a 22% decrease in repeat revascularization procedures (Table 3) *(7)*. The associated cost savings from these resource reductions averaged $1270 per patient ($P = 0.02$). Combining the baseline and follow-up costs for each treatment arm yielded a net 6-mo cost for the abciximab bolus and infusion arm of $293 per patient. Based on these data, we projected that if the EPIC results could be replicated in terms of efficacy

Table 2
Multivariable Linear Regression Model from the EPIC Economic Substudy

		Placebo incidence		*Bolus and infusion incidence*		*Savings (costs) associated with bolus and infusion treatment*	
Event	*Estimated cost*	*Intent to treat*	*Rx received*	*Intent to treat*	*Rx received*	*Intent to treat*	*Rx received*
Efficacy							
Non-urgent PTCA	$4973	3.3%	3.4%	3.7%	3.4%	($25)	($1)
Urgent PTCA	$8852	3.6%	3.8%	0.7%	0.7%	$268	$273
Non-urgent CABG	$14,750	1.1%	0.9%	2.3%	1.8%	($164)	($130)
Urgent CABG	$27,349	3.6%	3.8%	2.4%	2.1%	$365	$479
Total	—	—	—	—	—	$444	$622
Bleeding[a]							
Major bleeding	$5896	3.3%	3.4%	10.6%	10.5%	($430)	($418)
Minor bleeding	$1327	9.2%	9.4%	16.8%	17.1%	($101)	($102)
Total						($531)	($521)

From Mark et al. *(7)*.

All variables coded 0 (condition absent)/1 (condition present).

[a]Bleeding not associated with CABG.

Note that the incidence of urgent PTCA, urgent CABG, major and minor bleeding reflect outcomes as of hospital discharge, whereas the clinical reports from this study provide 30-d outcomes.

PTCA = coronary angioplasty, CABG = coronary bypass surgery, Rx = treatment.

Table 3
Follow-up Medical Resource Consumption and Hospital Costs from EPIC Study

	Placebo (n = 672)	*Bolus (n = 672)*	*Bolus and infusion (n = 684)*
Medical Resource Consumption			
Follow-up hospitalizations			
1	21.3%	22.9%	17.3%
>1	10.0%	10.1%	6.9%
Follow-up procedures			
Cardiac catheterization	23.7%	24.1%	17.5%
PTCA	15.3%	15.8%	11.3%
CABG	6.5%	6.7%	5.1%
PTCA or CABG	19.9%	20.4%	15.6%
Medical Costs and Length of Stay (Means)			
Hospital costs	$3988	$3649	$2881
Physician fees	$614	$626	$451
Total costs	$4,602	$4,275	$3,332
Hospital days	2.3	2.4	1.7

From Mark et al. *(7)*

Table 4
Baseline Medical Resources and Costs in the EPILOG Trial

	Heparin (n = *939*)	*Abciximab plus low-dose heparin* (n = *935*)
Resources		
Repeat PTCA	4.0%	2.0%
Stent placed	26%	16.7%
CABG	2.9%	1.2%
ICU LOS	1.4 d	1.3 d
Total LOS	3.5 d	3.4 d
Costs		
Hospital Costs	$8291	$7485
MD fees	$1341	$1273
Total	$9632	$8758
Total plus abciximab	$9632	$10,125

From Lincoff et al. *(16)*.

of therapy, with a simultaneous reduction in the rate of major bleeding, then abciximab had the potential to pay for itself over the 6-mo observation period.

EPIC was the first large-scale trial of a GP IIb/IIIa platelet inhibitor and demonstrated that this class of drugs provided a major therapeutic advance as adjunctive therapy for PTCA. However, the doubled risk of major bleeding provided a significant damper on the clinical and economic attractiveness of this therapy. The EPILOG trial was conducted to validate and extend the findings of EPIC to a broader percutaneous intervention population *(14)*. Importantly, it used a weight-adjusted lower-dose heparin regimen to evaluate whether bleeding could be controlled with preserved efficacy. A total of 2792 urgent or elective percutaneous coronary intervention patients were enrolled in EPILOG before the study was stopped early by the Data and Safety Monitoring Board. Clinically, the trial demonstrated a 57% reduction in major ischemic complications consisting of death, MI, or urgent revascularization in the abciximab/low-dose heparin arm by 30 d. The modified heparin regimen was successful in reducing the rate of major bleeding to that of the placebo arm (2% for abciximab vs 3.1% for placebo plus standard-dose heparin).

A prospective cost analysis was performed in EPILOG using the same methods previously described for EPIC *(15)*. The analysis showed that total baseline medical costs for the abciximab arm (including $1457 costs for the abciximab regimen itself) was $10,215 vs $9,632 for placebo, a $583 net excess cost for the abciximab strategy (Table 4). Thus, EPILOG confirmed the prediction made in the EPIC economic analysis that a reduction in ischemic complications with control of the excess bleeding risk would result in a cost offset (induced cost savings) of about $600 during the initial hospitalization.

The follow-up picture from EPILOG was significantly different from that seen in EPIC (Table 5) *(15)*. Unlike the earlier trial, EPILOG did not show any reduction in subsequent hospitalizations or cardiac procedures. In fact, there was a nonsignificant increase in hospitalizations and medical costs for the abciximab/low-dose heparin arm relative to placebo. Thus, the net cost of abciximab in the more modern EPILOG study

Table 5
Follow-up Medical Resources and Costs in the EPILOG Trial

	Heparin (n = *939*)	*Abciximab plus low-dose heparin* (n = *935*)
Resources		
Follow-up hospitalizations	47.2%	51.1%
Follow-up hospital days	1.7 d	2.1 d
Costs		
Hospital costs	$3152	$3731
MD fees	$415	$490
Total	$3568	$4221

From Lincoff et al. *(16)*.

(approximately $600) is approximately twice that initially predicted by EPIC (approximately $300) *(16)*.

The CAPTURE trial compared abciximab bolus plus infusion for 24 h vs placebo in 1265 unstable angina patients scheduled for PTCA *(17)*. Unlike EPIC and EPILOG where the abciximab bolus was given just prior to the procedure, CAPTURE patients received almost all of their abciximab prior to the procedure with drug continued for only 1 h after the procedure. At 30 d, the abciximab arm showed a 29% reduction in the primary endpoint of death, MI, or urgent revascularization ($P = 0.012$). Unfortunately, no economic data were collected in this European trial. On the basis of the resource use data published, it seems likely that the economics of abciximab use during the index hospitalization would be consistent with the results of EPIC and EPILOG, namely a partial offset of the cost of abciximab due to reduced ischemic events.

The most recent trial in the series of abciximab studies is the EPILOG-Stent trial *(18)*. The clinical data from this study have recently been published and the results from the economic analysis from EPILOG-Stent are expected to be presented at the American College of Cardiology meeting in November 1999.

The IMPACT (Integrilin to Minimize Platelet Aggregation and Prevent Coronary Thrombosis) II trial tested two doses of eptifibatide (integrilin) against placebo in a cohort of 4,010 PTCA patients *(19)*. At 30 d, the lower-dose eptifibatide bolus and infusion regimen demonstrated a reduction in 30-d ischemic events (death, MI, urgent revascularization) ($P = 0.06$) whereas a somewhat smaller effect was seen in the high-dose regimen ($P = 0.22$). When reanalyzed by treatment received (instead of intention-to-treat), the 30-d event rate for the lower dose regimen was significantly lower than for the placebo group ($P = 0.035$). At 30 d, the low dose eptifibatide group had a very modest reduction in urgent/emergent percutaneous revascularization (2.6% vs 2.8% for placebo), bailout stent use (0.5% vs 1.4% for placebo), and urgent/emergency coronary artery bypass surgery (1.6% vs 2.8% for placebo) *(19)*. The associated cost offset was small and not statistically significant. Importantly, there was no increase in major bleeding seen. In follow-up, there was no evidence of a differential effect of eptifibatide on rehospitalization or repeat procedures. As discussed elsewhere in this volume, analysis of the platelet effects achieved by the two eptifibatide doses used in IMPACT II revealed

an inhibition level of 40 to 60%, substantially lower than the target of ≥80% inhibition. These findings led to the change in dosing of eptifibatide used in PURSUIT.

The RESTORE (Randomized Efficacy Study of Tirofiban for Outcomes and Restenosis) trial randomized 2212 patients with an acute coronary syndrome (acute MI, unstable angina) who were referred for percutaneous coronary intervention to tirofiban or placebo *(20)*. The drug was given as a bolus plus 36-h infusion. There was a significant 38% reduction in the combined ischemic endpoint at 48 h, but at 30 d the results had converged somewhat leading to a net 16% reduction by tirofiban ($P = 0.16$). When the primary endpoint was redefined as death, MI, or urgent revascularization, the tirofiban arm demonstrated a 24% reduction at 30 d ($P = 0.052$). There was no excess of major bleeding seen with tirofiban *(20)*. Thus, the results of RESTORE and IMPACT II are similar. Recently published 6-mo follow-up from RESTORE has shown no evidence of a post-30-d effect of the drug on outcomes *(21)*. Economic analysis of this trial was performed using hospital billing data for a subset of patients in the United States with imputation of cost for the remaining United States patients *(22)*. Although there was a trend toward a reduced need for resource use to treat recurrent ischemia and an attendant estimated $300–$400 cost offset, these results were not statistically significant. The cost of the tirofiban regimen used in RESTORE was not reported.

Studies of Patients with ACS

Four major studies have been completed using intravenous GP IIb/IIIa receptor blockers in acute coronary syndrome (ACS) patients, CAPTURE (abciximab), PURSUIT (eptifibatide), PRISM, and PRISM Plus (both involving tirofiban). Of these, only PURSUIT has included an economic analysis. PURSUIT randomized 10,948 patients with an ACS (unstable angina, non-ST segment elevation MI) between November 1995 and January 1997 *(23)*. As described elsewhere in this text, PURSUIT had three treatment arms, two with bolus and 72-h infusion of eptifibatide regimen and one with bolus and infusion placebo. As mandated by protocol, the Data and Safety Monitoring Board determined that the higher-dose eptifibatide regimen had an acceptable safety profile and the lower-dose regimen was dropped after 3126 patients had been randomized *(23)*. Adjunctive therapy and use of invasive procedures were left to the discretion of the individual investigators, but all were encouraged to use daily aspirin (80 to 325 mg) and some form of heparin therapy.

The 30-d results from this trial have recently been published *(23)*. The bolus plus 72-h infusion eptifibatide regimen produced a 1.5% absolute reduction in the composite primary endpoint (death or MI) (15.7% in the placebo arm vs 14.2% in the eptifibatide arm, $P = 0.042$). The therapeutic benefit of eptifibatide was fully established by 96 h and was preserved out to 30 d. Using investigator-defined rather than Clinical Events Committee-defined MIs in the primary endpoint yielded a slightly larger treatment effect (placebo event rate 10% vs 8.1% for eptifibatide, $P = 0.001$). Major bleeding was increased in the eptifibatide arm (10.6% vs 9.1% for placebo, $P = 0.016$), but strokes were not increased.

As a prospective part of the PURSUIT research effort, we collected medical cost data in 2464 of the 3519 patients enrolled in the United States using the methods described earlier in this chapter *(24)*. The cost of eptifibatide was estimated from the drug's Average Wholesale Price (AWP) and the amount of drug actually administered to each United States patient. For the 1055 United States patients without hospital billing data, we used

Table 6
Index Hospitalization Resource Consumption and Costs from the PURSUIT Trial

	Eptifibatide (n=1754)	*Placebo (n=1765)*
Medical resource consumption		
Dx Cardiac catheterization	85%	85%
Percutaneous intervention	35%	36%
Coronary bypass surgery	20%	20%
ICU LOS (mean)	3.7	3.7
Total LOS (mean)	6.5	6.5
Medical costs		
Hospital costs	$12,420	$12,617
MD costs	$2309	$2340
Total costs	$14,729	$14,957

From Mark DB *(24)*.

a resource-based regression model to impute their costs. Thus, the primary cost comparisons in PURSUIT were based on the entire United States cohort (measured plus imputed).

During the index hospitalization, we found no evidence of an effect of eptifibatide on major resource consumption (Table 6). Diagnostic catheterization was performed in 85% of both groups, percutaneous revascularization in one-third, and coronary bypass surgery in one-fifth. Thus, the baseline medical costs in the two arms (excluding the costs for the eptifibatide regimen) were equivalent at approximately $14,800 ($P = 0.78$).

Resource consumption after discharge and out to the 6-mo anniversary of enrollment (when follow-up was terminated) also did not show any treatment-related differences. Diagnostic cardiac catheterization was used in 14% of both groups, percutaneous intervention in 7% and bypass surgery in 4%. The corresponding medical costs averaged about $3800 ($P = 0.60$), whereas the cumulative 6-mo costs were approximately $18,600 ($P = 0.78$).

In the second phase of the economic analysis of PURSUIT, we created a cost effectiveness model to relate the incremental clinical benefits demonstrated for eptifibatide by PURSUIT to its incremental costs. The cost effectiveness ratio used for this purpose can be simply expressed as:

$$CE = \frac{C_E - C_P}{LE_E - E_P}$$

where $C =$ the lifetime cost of each treatment arm, and $LE =$ their life expectancy, $E =$ eptifibatide arm, and $P =$ placebo arm.

The cost side of this analysis was simplified by the fact that our empirical cost data showed no significant cost difference between the two arms out to 6 mo, so the numerator of the cost effectiveness ratio consisted only of the cost of the eptifibatide regimen. Using the drug's AWP, we estimated this at $1217.

Projecting life expectancy for each treatment arm from the empirical 6-mo PURSUIT

clinical data was more complex and was based on a methodology previously developed by us for the GUSTO I study *(25)*. Our method projected an overall life expectancy for the PURSUIT patients of almost 16 years and an incremental effect of eptifibatide on life expectancy of 0.111 per patient (equivalent to 1 extra survivor per 100 patients shifted to eptifibatide who lives an extra 11.1 yr).

With these two components, the resulting cost effectiveness ratio was $16,292 per yr of life saved (discounted at 3%). Conventional thinking in this field is that any cost effectiveness ratio smaller than $50,000 per added life year is "economically attractive," whereas a ratio greater than $100,000 is "economically unattractive" *(1)*. By these criteria, use of eptifibatide therapy in ACS patients is quite economically attractive. Furthermore, these results were not sensitive to reasonable variations in the starting parameters of the model.

The PRISM (Platelet Receptor Inhibition for Ischemic Syndrome Management) Plus trial compared tirofiban plus heparin in 1915 ACS patients and demonstrated a 27% reduction in death or MI at 30 d from 11.9% with placebo to 8.7% with tirofiban ($P = 0.031$) *(26)*. No economic analysis of this trial has been conducted. However, given the roughly comparable pricing of tirofiban and eptifibatide and the comparable clinical effectiveness, it seems likely that formal cost effectiveness analysis of this trial would yield a cost effectiveness ratio smaller than $50,000 per added life year.

Two additional trials are underway that test intravenous GP IIb/IIIa inhibitors in ACS patients, GUSTO IV (abciximab), and PARAGON B (lamifiban). Economic analysis of the latter trial is planned.

Studies of Patients with ST-Elevation MI

To date only phase II trials have been completed in this area and none have incorporated economic analysis. Several phase III trials of abciximab and modified dose thrombolytic therapy are ongoing.

Secondary Prevention

As detailed elsewhere in this text, phase III trials have been initiated to evaluate the use of oral GP IIb/IIIa inhibitors in high-risk atherosclerosis patients. The SYMPHONY trial is evaluating two regimens of sibrafiban against placebo and has a prospective economic component.

SUMMARY

GP IIb/IIIa platelet receptor inhibitors represent a major advance in the pharmacotherapy of acute coronary disease, whether occurring spontaneously or following percutaneous intervention. In the percutaneous intervention population, the greatest amount of economic data is available for abciximab. This agent costs on average $1400–$1450 per case, but has a net cost of only $600 due to the cost savings associated with reduced ischemic events. Eptifibatide (IMPACT II) and tirofiban (RESTORE) demonstrated more modest clinical benefits in this population and smaller, nonsignificant cost offsets.

In ACS patients, only eptifibatide (PURSUIT) has demonstrated both clinical effectiveness and cost effectiveness. Over the next few years, important additional data will become available on the clinical effects and economics of GP IIb/IIIa antagonists as adjuncts to thrombolysis in acute MI and as secondary prevention agents in high-risk atherosclerosis patients.

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13 Monitoring Platelet Aggregation

Neal S. Kleiman, MD, Wojciech Mazur, MD, and Nikki Graziadei, BS

CONTENTS

INTRODUCTION

The value of inhibition of platelet function to prevent morbid events in individuals with atherosclerosis has been unequivocally established over the last two decades. The series of observations establishing efficacy began with trials of aspirin for secondary prevention *(1–3)*, was extended to patients with cerebrovascular disease *(4,5)* and acute myocardial infarction *(6)*, and those undergoing percutaneous *(7)* and surgical revascularization procedures *(8)*. Subsequently, a novel set of antiplatelet agents—the thienopyridines ticlopidine and clopidogrel—were shown to be useful in a set of similar circumstances *(9,10)*. Because the latter agents are more potent inhibitors of platelet aggregation, their utility, beyond that of aspirin alone, suggested that even more profound inhibition of platelet aggregation might prove useful in preventing arterial occlusion and its consequences. Consequently, the last several years have witnessed the unequivocal demonstration that intravenous antagonists of the platelet glycoprotein (GP) IIb-IIIa offer further protection against the ischemic consequences of percutaneous coronary interventions and recurrent events in patients with acute coronary syndromes *(11)*. Further areas that are likely to be explored include patients with cerebrovascular disease and peripheral vascular disease, and those undergoing cardiopulmonary bypass. In addition, a number of orally active antagonists of platelet glycoprotein IIb-IIIa are undergoing exploration for chronic therapy.

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Potential Utility of Monitoring Platelet Aggregation

The demonstration that treatment with these agents results in clinically meaningful benefits coupled with the knowledge that overly vigorous platelet antagonism is associated with elevation of the bleeding risk *(12,13)* implies that monitoring platelet aggregation may be important for several reasons. First, development of new agents and new treatment regimens for clinical study requires that appropriate doses be selected based on their demonstrable effects on platelet aggregation. Occupancy of 80% or more platelet GP IIb-IIIa receptors has been a target about which most dose regimens of GP IIb-IIIa receptor antagonists have been designed. Occupancy of 80% of platelet surface GP IIb-IIIa by the murine antibody 7E3 F(ab′)2 (a precursor of abciximab) abolishes adenosine diphosphate (ADP)-induced platelet aggregation in platelet-rich plasma *(14)*. The obvious implication is that this degree of blockade is required to prevent arterial thrombosis. This has been true in experimental models that include a potent stimulus for platelet aggregation, such as a thrombolysis-rethrombosis model *(15)*. However, in models that involve a less potent stimulus, such as a Folts model of cyclic flow variations, doses of the same antibody that do not abolish turbidimetric aggregation are able to limit cyclic flow variations. Unfortunately, accurate quantification of GP IIb-IIIa occupancy by nonantibody antagonists is difficult for reasons described in the following section. Therefore, inhibition of ADP-induced aggregation has been used to define target doses with these agents.

The development plans for all the currently available agents have targeted inhibition of 80% of ADP-induced aggregation in 80% or more of patients for dose selection. Although there is little clinical evidence that this target is optimal, all three currently available agents have proven effective at doses that achieved this target level of inhibition. Although the correlation between the degree of platelet inhibition and clinical efficacy probably holds true in a general population, the value of platelet monitoring to tailor dosing regimens in individual patients has not been demonstrated and is only now under investigation. As such, the usefulness of monitoring platelet aggregation in the clinic must still be regarded as theoretical.

It is also very possible that refinement of current dosing may increase the efficacy of current regimens. Studies of volunteers and of patients undergoing treatment with intravenous GP IIb-IIIa antagonists has indicated that the response to infusion of a GP IIb-IIIa antagonist is heterogeneous. A simple example can be seen in patients treated with abciximab. This drug is, in general, administered as a bolus of 0.25 mg/kg followed by an infusion of either 10 μg/min or 0.125 mg/kg/min. In general, the initial bolus of abciximab appears to occupy 80% or more receptors in the vast majority of patients. However, as time progresses, the dispersion around the median degree of inhibition increases. During the course of the infusion, as many as 15% of patients may fall below the 80% level *(16)*. Abciximab has a prolonged effect lasting several weeks *(17)*. In volunteers given a 0.25-mg/kg bolus, the degree of receptor occupancy has been studied up to 2 wk following the bolus. As time progresses following the bolus, the degree of dispersion around the mean degree of receptor blockade increases. Thus, as the time from a bolus dose increases, the variability of the remaining receptor blockade increases. This property may influence the selection of subsequent doses in the setting of staged angioplasty performed one or more days after an initial percutaneous coronary intervention, or may also guide dosing of subsequent oral agents.

Another use for monitoring platelet aggregation in patients treated with GP IIb-IIIa antagonists concerns the use of concomitant anticoagulants. In patients receiving abciximab during percutaneous coronary intervention, reduced doses of heparin (70 U/kg) are associated with less frequent bleeding complications and earlier removal of the arterial access sheath *(18)*. If the level of platelet inhibition in a given patient can be documented to be adequate, it may be possible to reduce the dose of heparin even further.

In patients who have been treated with GP IIb-IIIa antagonists, monitoring the degree of platelet inhibition may also be useful if a surgical procedure is indicated and it is desirable to know when platelet function has returned to a normal state. If oral antagonists of GP IIb-IIIa prove to be useful clinically, the case for monitoring platelet aggregation will become considerably stronger. In addition to variations in excretion of these agents, inter- and intraindividual absorption is also likely to vary. Concerns about the tolerability of chronic therapy have caused the goals of inhibition with oral agents to be more variable. However, a range of levels of inhibition has been selected and is currently undergoing evaluation. As other platelet antagonists undergo development, it is likely that they will undergo similar scrutiny. Because many of these agents have high K_Ds (dissociation constants), monitoring plasma levels may serve as a surrogate for receptor occupancy. However, the delay involved in obtaining these levels in the clinic makes a strong case for assessing platelet aggregation instead if a reliable and easily usable "point of care" instrument becomes available.

The remainder of this chapter reviews the various methods of assessing platelet aggregation that have come into use in modern cardiology.

Sources of Interindividual Variation

Differing physiologic parameters may lead to variations among individuals in the response to a given dose of an antiplatelet agent. These sources include variations in metabolism and excretion of the free form of the drug, variations in the way the drug has been administered, i.e., whether the infusion has been started promptly following the bolus, variations in platelet activation state resulting in differing numbers of free receptors available for ligand binding, and differences in the platelet count. The latter two etiologies are likely to be of prime importance in patients receiving abciximab, as the drug has a short plasma half-life and low steady-state plasma concentration, and is largely bound to the platelet GP IIb-IIIa receptor. Following activation with a strong agonist, such as thrombin, GP IIb-IIIa receptors stored internally in the alpha granules are exteriorized *(19,20)*. These newly available receptors may adsorb free abciximab, thus decreasing the degree of receptor occupancy per platelet, as an equal number of abciximab molecules must now be bound to a larger total number of receptors. This concern is largely theoretical and has not been demonstrated clinically. However, a qualitatively similar finding has been demonstrated in patients with elevations in the platelet count. There are at least two reports in the literature of patients with thrombocytosis who have had diminished responses to abciximab *(21,22)*. Such studies have not yet been performed using eptifibatide or tirofiban, however, it seems likely that given the shorter biologic half-lives of these agents, their higher K_D, and their dependence on renal function for excretion *(23,24)*, that patient-to-patient variation will be at least equally as great, whereas the effect of the activation state of circulating platelets may be less prominent.

METHODS OF ASSESSING PLATELET AGGREGATION

For the reasons described in the preceding section, measurement of platelet aggregation has come into increasing prominence in the last several years. This section describes the various methods that are commonly used. It must be remembered that arterial thrombosis is an extremely complex process that involves disruption of laminar flow within a blood vessel, hemodynamic alterations within the arterial system, alterations in the content of arterial blood, activation of the soluble clotting cascade, elaboration of native anticoagulation and plasmin, activation of platelets through several different pathways, and often inflammation of the vessel wall. In this context, each method of assessing platelet function must be regarded as a model. Each has its own advantage and imperfections; it is likely that none of the methods described truly represents the process that occurs when an atherosclerotic plaque is disrupted.

Platelet Agonists

Platelets are activated and stimulated to aggregate through a variety of pathways. Recognition of these pathways and the receptors and ligands required to initiate them has greatly facilitated the assessment of platelet aggregation. Several platelet agonists have been identified and have become accepted agents in assessing aggregation responses. Agonists are generally classified as being either weak or strong. Strong agonists lead to platelet degranulation and often exteriorization of GP IIb-IIIa receptors and fibrinogen stored within the alpha granules.

ADP was the first platelet agonist described and has been the agonist most commonly used for turbidimetric aggregation studies. The physiologic importance of ADP is believed to derive from the lysis of red blood cells as they pass through areas of high shear in a narrowed coronary artery. In addition, ADP secretion by activated platelets recruits more platelets into a developing thrombus. There are at least two *(25,26)* and possibly three *(27,28)* platelet surface receptors specific for ADP, only one of which has been well characterized *(29)*.

The aggregation response to ADP is concentration-dependent. Aggregation in response to low concentrations of ADP is spontaneously reversible, whereas aggregation that follows the addition of higher concentrations is not *(30)*. In studies performed in our laboratory, agonist titration indicates that approximately 50% of patients have a maximal aggregation response between 5 and 10 μ*M*. Many early studies of antiplatelet drugs including the thienopyridines ticlopidine and clopidogrel and most physiologic studies of congenital platelet defects are performed using concentrations of 1–5 μM ADP, or less *(31–33)*. Most studies of GP IIb-IIIa antagonists have required more intense stimulation of the platelet in order to discriminate among doses and have been conducted using concentrations of 10–20 μ*M (34–39)*, although 5 μ*M* have also been used *(40)*. The implications of selecting a dose of a platelet inhibitor based on a lower versus a higher concentration are obvious.

Collagen is an agonist that has been classified as both weak and strong, depending on the concentration used. In normal platelet donors, maximal release of dense granule content occurs at collagen concentrations of 5 mg/mL *(41)*. The physiologic importance of collagen derives from the abundance of types IV and VI collagen in the subendothelium exposed at the time of arterial injury. Unlike ADP-induced aggregation, aggregation in response to collagen is sensitive to the effects of aspirin. Generally, concentrations of

1–5 μg/L are used in platelet aggregometry. Unfortunately, because collagens are biologic products, their use is limited by variability between production lots that occurs at the time of harvest.

Arachidonic acid is a fatty acid that is metabolized to thromboxane A2 and prostacyclin as well as a number of other eicosanoids. The physiological importance of these prostaglandins is beyond the scope of this chapter. The importance of arachidonic acid in monitoring platelet aggregation, however, is due almost exclusively to its exquisite sensitivity to the effects of aspirin. In volunteers given doses of aspirin as low as 81 mg, arachidonic acid-induced aggregation and thromboxane A2 production are inhibited as rapidly as 15 min after a dose is given, whereas this rapid a response cannot be observed when other agonists are used *(42)*. For this reason, arachidonic acid-induced platelet aggregation is often used to assess compliance with aspirin regimens.

Thrombin is the prototype of strong agonists. Among its many biologic functions, thrombin is the most potent biologic activator of platelets. In addition to stimulating platelet aggregation, thrombin stimulates platelet degranulation, and leads to a "flipflop" or "scrambling" of phosphatidyl serine residues in the platelet membrane. These residues reside on the inner layer of the plasma membrane of the resting platelet. Following platelet activation, phosphatidyl serine residues are expressed externally and provide a phospholipid surface that permits assembly of the tenase and prothrombinase complexes, which in turn catalyze the activation of Factor X to Xa and Factor II (prothrombin) to IIa (thrombin), respectively *(43–47)*. The use of thrombin (α thrombin rather than γ thrombin is generally used) as an agonist to assess aggregation has been well accepted for years. However, assessment of thrombin-induced platelet aggregation suffers from several limitations including lot-to-lot variability, storage requirements, and thrombin's ability to interfere with the assessment by activating the soluble clotting cascade. These difficulties have been circumvented partially by experiments elucidating the mechanism through which thrombin stimulates the receptor.

A basic understanding of the thrombin receptor's physiology is useful in understanding the use of thrombin and its surrogates in monitoring platelet aggregation. The platelet thrombin receptor was poorly characterized for many years. It was originally suspected that thrombin interacted with the Ia receptor, which is also stimulated by collagen. In the early 1990s, however, Coughlin and co-workers isolated and cloned a receptor whose activation shared many characteristics with thrombin stimulation of the platelet *(48,49)*. The receptor is the first identified member of a receptor family that is known as the "protease activated receptors" (PARs) *(50–53)*. Unlike mechanisms functioning in conventional receptors, ligation of thrombin by the receptor does not lead directly to activation. Instead, the extracellular domain of the receptor contains an amino acid sequence homologous to that of hirudin. This region subsequently recognizes the hirudin-binding domain of thrombin. Once thrombin attaches to the receptor, its active site cleaves the amino terminus of the thrombin receptor's extracellular domain. The new amino terminus is then inserted into the pocket of the receptor and thus stimulates the receptor *(48,54)*. Since the initial discovery of this receptor, at least two more protease activated receptors have been identified. PAR-1 is probably not the sole receptor for thrombin on the human platelet. Human platelets in which the PAR-1 receptor is eliminated are able to respond to thrombin stimulation. Another receptor, PAR-3, appears to play a role that is at least as important *(53)*. Nonetheless, the amino acid sequence of the newly created amino terminus of the PAR-1 receptor has been identified. Because it is this sequence

that is inserted into the receptor pocket, fragments of this sequence can be used as platelet agonists. These fragments, known as the thrombin receptor-activating peptides (TRAPs), mimic the action of thrombin on the platelet. Additionally, they do not suffer from the logistic limitations affecting the use of thrombin, and can be produced inexpensively. The TRAPs consist of between 6 and 14 amino acids beginning at the amino terminus of the sequence NH_2-SFLLRNPNDKYEPF. The per weight potency of these sequences varies *(55)*. The six amino acid sequence is used most commonly. Recently, the substitution of isoserine for serine has led to the development of iso-TRAP *(56)*, which is more resistant than TRAP to degradation by the plasma enzyme aminopeptidase and is thus likely to yield more reproducible aggregation responses.

Platelet stimulation with either thrombin or a TRAP causes more vigorous aggregation than other commonly used agonists and leads to the exteriorization of GP IIb-IIIa receptors from within the alpha granules. Flow cytometric studies in our laboratory have indicated that after stimulation with TRAP, fluorescence of GP IIb increases between 20% and 40% compared with ADP. Thus, when aggregation in response to ADP or collagen is blocked, stimulation with TRAP appears able to recruit more activated receptors to the platelet surface and may still produce aggregation *(57,58)*. Increasing the plasma concentration of a GP IIb-IIIa antagonist can further block this aggregation *(57)*. The TRAPs are thus useful for discriminating effects of higher doses of GP IIb-IIIa antagonists when ADP-induced aggregation appears to be inhibited maximally.

TECHNIQUES PERFORMED USING PLATELET-RICH PLASMA

Turbidimetric Aggregation

The turbidimetric light transmittance method is the most widely used measure of platelet aggregation. This technique is based on the observation that light transmittance through a specimen of plasma is linearly related to the platelet count in that specimen. First described by Born and Cross, the turbidimetric technique measures *ex vivo* aggregation as an increase in light transmittance through a turbid sample of platelet-rich plasma *(59)*. As platelets are induced to aggregate in this system, they settle to the bottom of a reference cuvet. As a result, the turbid sample becomes clarified and light transmittance increases. The measurement is performed in a device known as an aggregometer. In this device, light is passed through the sample, and a curve of light transmittance measured with a photocell is used to represent aggregation.

Platelet aggregation in plasma depends on the platelet count, the concentration of circulating ligands such as fibrinogen and von Willebrand factor, and the availability of activated GP IIb-IIIa receptors. It is therefore necessary to standardize or "correct" the concentrations of platelets and plasma in the final sample that is prepared for study. Thus, comparison of samples before and after a given treatment is possible. Platelet-rich plasma is prepared by centrifugation of blood at (in our laboratory at 1700 rpm for 6 min). The centrifuged platelet-rich mixture is then diluted with autologous platelet-poor plasma (prepared in our laboratory at 3500 rpm for 15 min) to achieve a final platelet count of 225,000–275,000/mm^3. Most aggregometers have several wells that allow several platelet samples to be assayed at once. The use of multiple channels allows comparison of several agonists, or concentrations of the same agonist on aliquots of a single blood sample. This technique eliminates the variation that may be induced by variations in blood collection techniques and differential aging of samples.

The aggregation response is determined by using the nadir of the light transmittance curve at a predetermined point in time after the addition of the agonist. Times between 4 and 5 min are generally selected. Platelet aggregation is recorded as the maximum percent aggregation achieved. This is reported using a reference of value of 100% for light transmittance through platelet-poor plasma that is used initially to calibrate the aggregometer. In some laboratories, the slope of the aggregation curve over the first 4–5 min is recorded. When platelet-inhibiting drugs are studied, changes in aggregation are frequently reported as a percentage of the pretreatment value. Thus, the value that is already calibrated against 100% light transmission in platelet-poor plasma is again normalized for a baseline aggregation value of 100%. This method of reporting has been quite useful in studying the short-term effects of intravenous agents. However, when chronically administered agents are studied, a normalized value is less useful, as it requires that all subsequent values be linked to a remote state of aggregation, and thus ignores the many intercurrent events that may have occurred.

The advantage of turbidimetric aggregation is that it is currently well accepted and clearly understood. There is a cumulative experience of approximately 35 yr in measuring and reporting platelet aggregation with this technique. On the other hand, there are several important disadvantages as well. The equipment required consists of several components–a centrifuge, a cell counter, and an aggregometer–all of which are relatively expensive and require meticulous maintenance and frequent quality control checks. The time and training required of operators to perform the determinations are extensive and vary from operator to operator. In addition, preparation of platelet-rich plasma by centrifugation can alter the physiologic status of the sample by activating the platelets. The delay involved in the centrifugation process can also result in a loss of platelet reactivity.

The layer of platelet-rich plasma formed during centrifugation is not uniform and sampling platelets from various levels of this layer may introduce artifacts. The size and density of platelets is heterogeneous and the densest platelets may sediment below the platelet pellet after centrifugation. When the platelet-rich plasma is sampled, withdrawing plasma from the bottom of the centrifugation tube can also yield inordinately high platelet counts. In addition, the centrifugation process itself can induce platelet shape change and can activate platelets as well. Additional physiologic factors as hemolysis, lipemia, and thrombocytopenia can also hinder analysis. These factors become especially problematic in clinical studies when samples must be collected at precise time points and may not be able to be re-collected.

Preservation of samples for this technique has also been recognized recently to alter the degree of aggregation observed. Samples have been traditionally collected in tubes anticoagulated with sodium citrate (3.2–3.8%). Sodium citrate interferes with coagulation by chelating calcium, which is a necessary cofactor for activation of the soluble coagulation cascade. Unfortunately, hypocalcemia can also alter the binding of fibrinogen to the GP IIb-IIIa receptor and can alter the affinities of GP IIb-IIIa antagonists for the receptor as well. This effect is probably not equivalent for all antagonists *(60)* and appears to be most prevalent with eptifibatide *(61)*.

Radiometric Receptor Assays

This technique is useful for estimating the number of GP IIb-IIIa receptors present on the platelet surface. The technique is essentially a radioimmunoassay, which measures

displacement of an iodine-labeled antibody from the receptor. The theory underlying its use is that platelets' ability to participate in thrombosis is most directly dependent on the number of GP IIb-IIIa receptors available *(62)*. Unfortunately, it is not clear whether the absolute number of available receptors or the proportion of receptors remaining unbound after a therapeutic intervention is more important.

The principal advantage of the technique is that it provides a window through which the cellular mechanism of action of GP IIb-IIIa antagonists can be observed. When performed concomitantly with platelet aggregation studies, it allows the effects of GP IIb-IIIa receptor occupancy to be separated from those of other factors (such as aspirin or thienopyridine therapy) that affect platelet aggregation. The principal disadvantage is that the technique requires dilution of the platelet mass. Thus, it can only be used to assess agents that bind to the receptor with high affinity, such as abciximab. Agents that bind with less affinity cannot be assessed using this technique. Because binding of these agents to the GP IIb-IIIa receptor is highly dependent on plasma concentration, dilution of the platelets leads to alterations in the degree of binding. Additionally, low-affinity agents may be displaced by the anti-GP IIb-IIIa probe. The other disadvantage of this technique is that it requires relatively sophisticated equipment to perform radioactive counting on the platelet mass and requires a well-trained technician to perform it adequately. In addition, it also suffers from the same limitations of platelet-rich plasma sample preparation as turbidimetric aggregometry.

TECHNIQUES PERFORMED USING WHOLE BLOOD

Electrical Impedance Aggregometry

Introduced in 1980 by Cardinal and Flower *(30)*, the electrical impedance technique can be used in either whole blood or platelet-rich plasma. This technique detects platelet aggregation by passing a very small electric current between two platinum electrodes immersed in a sample diluted with an equal volume of isotonic saline and measures the impedance between the electrodes. Electron microscopy indicates that during the initial contact with the blood the electrodes become coated with a monolayer of platelets. If no aggregating agents are added, then no further interactions occur between the platelets and the electrode, and the conductance between the two electrodes is constant. In the presence of aggregating agents, however, platelets aggregate to the platelet monolayer coated on the electrodes and there is a gradual accretion of platelets on those sites. This layering coats the electrodes and impairs conduction between them. The increase in impedance is directly proportional to the platelet mass deposited on the electrode probe assembly. Using a standard protocol, aggregation is monitored for 6 min and the final increase in resistance over this period is displayed as a numeric readout. For each specimen sampled, the percent of baseline aggregation is determined by calculating the maximum change (in ohms) of the test sample indexed for the maximum change (in ohms) of the baseline sample (Fig. 1). A more recent iteration of the device is able to produce numeric readouts of the aggregation slope.

The impedance technique is advantageous in that it can measure platelet aggregation in the presence of other cellular and plasma blood components that may be physiologically important in vivo. The device can be used easily by clinicians in order to monitor the effect of antiplatelet agents and hence is potentially useful in the point-of-care setting. Unlike turbidimetric aggregometry, electrical impedance aggregometry requires

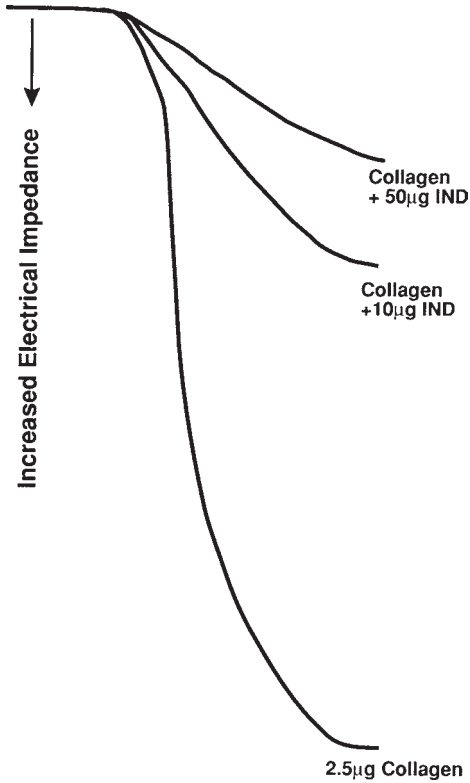

Fig. 1. Electrical impedance aggregometry. Partial reversal by indomethacin of collagen-induced aggregation in platelet-rich plasma. Adapted from ref. *30*.

minimal preparation time and can be implemented relatively quickly, as it requires no cell separation and minimal operator attention and time. Impedance aggregometry can be completed in as little as 6 min after an initial 5-min incubation of the whole blood/isotonic saline sample volume. An additional advantage of this technique is the lesser amount of blood than is needed for a routine platelet test. Approximately 5 mL are needed to complete as many as 10 assays, whereas 20 mL are needed when platelet-rich plasma (PRP) is used.

Several important points concerning impedance aggregometry should be mentioned. The hematocrit of the sample studied using whole blood aggregation influences the outcome of the measurement. Thus, hematocrit is generally standardized to a value of 0.30, which does not cause excessive platelet dilution. Dilution may limit the applicability of this technique to agents with rapid off-rates. It is of interest that a small dilution of whole blood leads to an increase in measured platelet aggregation by impedance, whereas in PRP a decrease in optical aggregation results. This effect is probably due to a combination of the removal of impeding red cells and the difference in conductivity between saline and platelet-poor plasma. Whole blood aggregation is particularly more suitable than use of platelet-rich plasma for measuring platelet aggregation in patients with low platelet counts. Good responses can be obtained at platelet counts as low as 50,000/mm^3.

Electrical impedance aggregometry was originally compared with turbidimetric

assays in platelet-rich plasma *(30,63–65)*. Collagen-induced aggregation measured by electrical impedance has been reported by several investigators to be more sensitive to the effects of aspirin than is turbidimetric aggregometry *(30,63,64)*. Mascelli et al. *(66)* studied patients treated with abciximab and aspirin and reported that the electrical impedance aggregation response to 5 μg/mL of collagen corresponded with radiometric assays of receptor occupancy more closely than did turbidimetric techniques. Electrical impedance aggregometry also appeared to be more sensitive to the effects of abciximab. Thirty-six hours after an infusion of abciximab was completed, turbidimetric aggregation appeared to be returning to normal more rapidly than impedance aggregation. Electrical impedance aggregometry has also been reported to more useful than turbidimetric aggregometry in detecting platelet hyperaggregability, as it is more sensitive to the formation of large platelet aggregates *(67)*.

The principal disadvantage of electrical impedance aggregometry is that the aggregometer must be maintained meticulously, as small changes in the temperature of the medium or in the resistance of the electrodes lead to large changes in measured aggregation.

Fluorescent Bead Aggregometry

This technique is currently becoming an extremely popular method of assessing platelet aggregation at the bedside. When platelets are stimulated with an agonist, activated GP IIb-IIIa receptors ligate fibrinogen. Based on techniques developed by Coller and colleagues *(68)*, this technique utilizes fluorescent polystyrene beads coated with immobilized fibrinogen. When the beads are mixed with a blood sample, the immobilized fibrinogen on the beads competes with circulating fibrinogen for binding to platelet GP IIb-IIIa receptors. Because much of the available fibrinogen is fixed on the beads, platelets subsequently agglutinate directly with beads rather than other platelets. As platelet aggregation increases, more beads agglutinate. Consequently, the fluorescence of the agglutinated mass increases. This fluorescence can be measured and expressed as platelet aggregation. GP IIb-IIIa antagonists prevent platelets from attaching to the fluorescent beads and thus decrease the total fluorescence of the specimen.

As the technique is now practiced, whole blood samples (approximately 5 mL) are collected into an anticoagulant tube. The tube is then attached directly to the fluorescent bead aggregometer. The blood sample is then drawn directly into a cuvet that contains a series of wells. One well is used to measure fluorescence of an unstimulated sample while another well contains the agonist. A curve of fluorescence versus time is generated and the slope of this curve is measured. A baseline sample is studied and the slope of its fluorescence curve is stored for comparison with a subsequent sample taken after the antiplatelet therapy is administered. The difference between the two curves is then reported as percentage inhibition of platelet aggregation.

Generally, iso-TRAP is used to assess the effects of GP IIb-IIIa antagonists, as it is insensitive to the effects of aspirin and ticlopidine. However, other agonists such as ADP can be used as well; these agonists may prove useful in detecting effects of ticlopidine or clopidogrel. In the device's current iteration, the concentration of iso-TRAP has been selected to mimic a 20 μM concentration of ADP. However, it is likely that a variety of further cuvets will be produced that contain other agonists. Correlation data obtained in samples spiked with abciximab indicate near-linear correlation with ADP-induced aggregation over a broad range of platelet inhibition.

This device offers the advantage of becoming a point-of-care instrument for dosing of GP IIb-IIIa antagonists. A sample can be obtained and processed in 5 min or less. In contrast to other techniques, minimal training is required to operate the device, and the result is available as a numeric printout. On the other hand, fluorescent bead aggregometry is only now entering the clinical arena, and the breadth of experience obtained with it does not yet match that of turbidimetric aggregometry. However, bedside aggregometry is being measured in a number of clinical trials, and experience is accumulating rapidly. At least one study has been completed and indicates that if the aggregation measurement obtained with this device is taken as the standard, then a bolus of abciximab given during a percutaneous intervention achieves nearly complete inhibition of platelet aggregation in virtually all patients, however, during the course of an infusion, recovery of aggregation occurs in some patients and nearly 15% become "subtherapeutic" during a 12-h period *(16)*.

Thromboelastography

Thromboelastography (TEG) is a technique first developed by Harter in 1948. This technique measures the tensile strength of a developing clot. Consequently, it can be used to display the temporal formation and breakdown of a whole blood clot and, as such, provides a visual pattern of functional clotting status. Thromboelastography had little clinical use for many years until it was reevaluated in the surgical setting in 1974. Subsequently, it was developed further and has been more recently applied in both liver transplant and cardiac surgery *(69)*.

The thromboelastograph involves two mechanical parts, a cuvet and a piston. Freshly drawn blood is placed in the cuvet, which oscillates through 4°45′ at 37°C (Fig. 2). The piston is suspended in the blood sample by a torsion wire whose motion is transduced to a chart recorder. When no clot exists, the motion of the cuvet does not affect the piston and the chart records a straight line. As strands of fibrin form and attach to the piston, it becomes coupled to the motion of cuvet. Hence, the shearing elasticity of the evolving clot is transmitted to the recording paper. The TEG pattern has component variables, which are shown in Fig. 3. Reaction time (r) is the interval between the start of recording and the time at which the amplitude of recording is 2 mm. It reflects the function of intrinsic clotting pathway. Coagulation time ($r + K$) is the time required for the amplitude to reach 20 mm and gives information about not only intrinsic factors but also platelets and fibrinogen, which are also represented by the clot formation rate α. Maximum amplitude (MA) on the thromboelastogram is a measure of clot strength and elasticity, reflecting the properties of platelets and fibrinogen as well as factor XIII. Whole blood clot lysis index (WBCLI) is the amplitude 60 min after MA is achieved (A60) as a percentage of MA and it denotes the amount of clot retraction or lysis *(70)*.

TEG, as mentioned earlier, is particularly useful in liver transplant surgery. In combination with clinical bleeding assessment, it facilitates selective use of component therapy (fresh frozen plasma, platelets, and cryoprecipitate) and specific drug treatment only if appropriate (Fig. 4). It was noted that there is an imperfect correlation between TEG variables and laboratory coagulation tests, which can be explained by the fact that TEG variables are interdependent, measuring the interaction of the coagulation cascade and platelets in whole blood rather than specific endpoints in centrifuged plasma samples. In addition to its contribution to management of coagulation during liver transplantation, TEG is very useful as a liver function test. Normal TEG within 60 min posttransplant

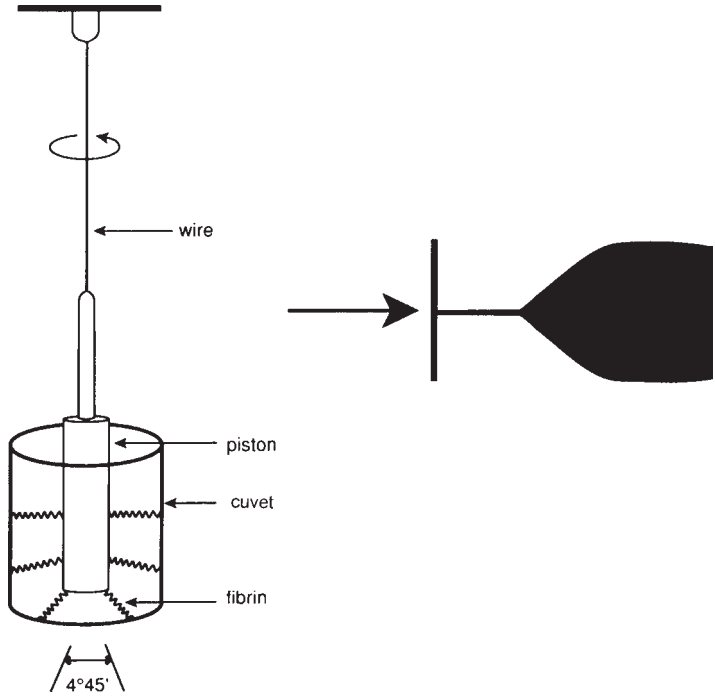

Fig. 2. Principle of thrombelastography. Adapted from ref. *69*.

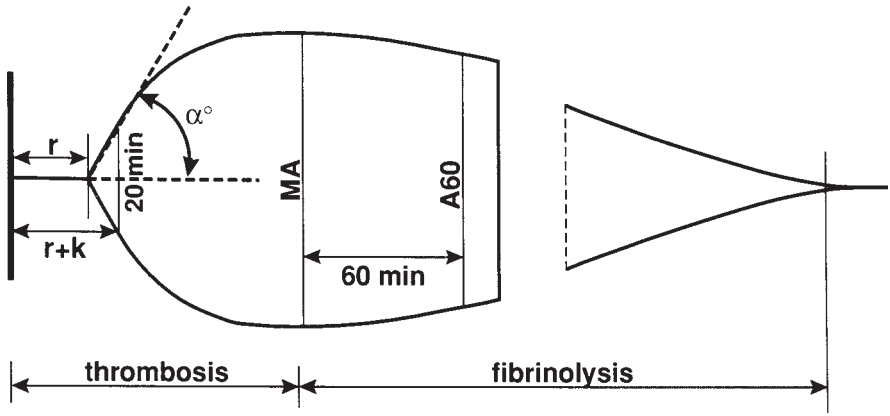

Fig. 3. Component variables of the thrombelastography: r = reaction time; (r +K) = coagulation time; α = clot formation time; WBCLI = whole blood clot lysis index; MA = maximum amplitude. Adapted from ref. *64*.

indicates reperfusion of the organ; conversely, a poor thromboelastogram indicates need of surgical revision of vascular supply.

Thromboelastography has recently been used to study the effects of treatment with abciximab in patients undergoing percutaneous coronary intervention. Priming the thromboelastograph with tissue factor or a TRAP activates platelets and allows measurement of the ability to inhibit platelet aggregation maximally. At least one recent study

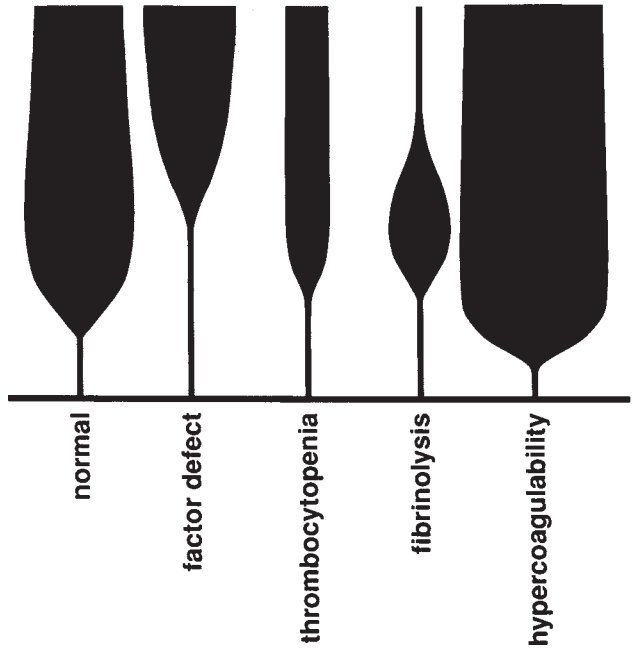

Fig. 4. Thrombelastographic patterns. Adapted from ref. *69*.

has emphasized the heterogeneity of platelet response to abciximab when the platelet contribution to thrombosis is measured using thromboelastography *(71)*.

Shear Chamber Devices

Most methods of assessing platelet function rely on the response of the platelet to biochemical agonists. However, platelets are also induced to form thrombi in the arterial system by mechanical forces. As blood flow velocity increases, the growth rate of hemostatic plugs has been observed to increase *(72)*. The disruption of laminar flow at the site of arterial narrowing is referred to as shear. A variety of cells including platelets, neutrophils, and monocytes undergo membrane changes when exposed to shear *(73)*. In the case of the platelet, shear leads to activation and adhesion within seconds of exposure to the shearing force *(74)*. This process is believed to be mediated by the interaction of vWF and the GP Ib/IX receptor *(75–77)*. The filamentous structure of the latter receptor may make it more sensitive than other receptors to alterations in the pattern of flow surrounding the platelet.

Fluid shear stress is a measure of the force required to produce a certain rate of flow of viscous liquid. Shear refers to the parallel motion between fluid planes during flow. Average levels of shear stress in the arterial circulation are approximately 20 dynes/cm^2. Fluid shear stress may reach levels of 200–400 dynes/cm^2 in small arteries and arterioles that are partially occluded. Levels of shear in the venous circulation are considerably lower and probably do not activate platelets.

The standard instrument to investigate shear-induced platelet activation is a rotational viscometer with cone-and-plate geometry *(78)* (Fig. 5). This instrument has important experimental advantages. It can apply uniform shear fields to test samples by generating

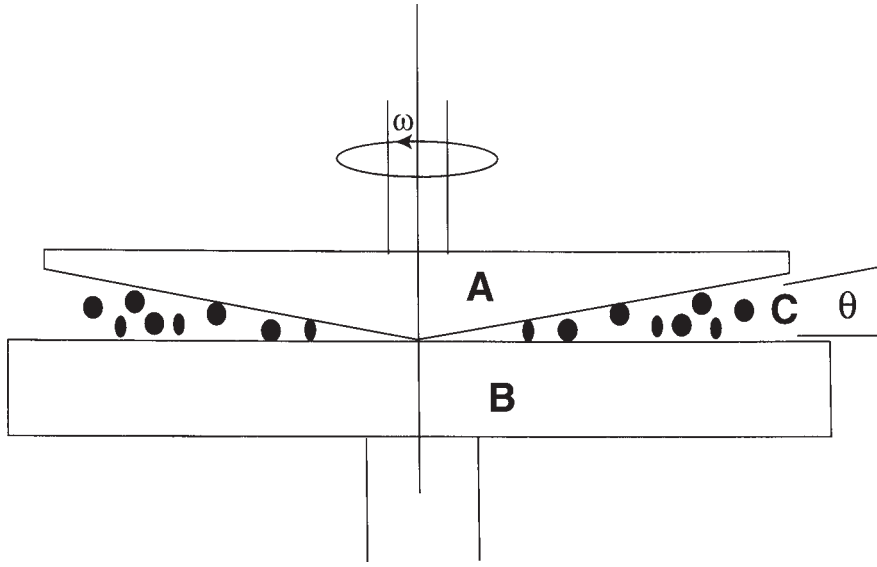

Fig. 5. Cutaway schematic view of a cone-plate viscometer showing **(A)** rotating truncating cone, **(B)** stationary plate, **(C)** platelet suspension. A platelet suspension between the cone and the plate is subjected to a known, uniform shearing stress for a specified period of time. Subsequently, a specimen is fixed and its particle size distribution determined electronically. Adapted from ref. *82*.

specified shear rates rapidly under conditions in which blood cell interactions with the viscometer surface have little influence on the experimental results. Collection of the effluent after blood has passed through the viscometer allows the detection of platelet aggregates, and thus the determination of inhibition of platelet aggregation *(79)*. Aspirin and heparin have little effect on shear-induced aggregation, and ticlopidine has a moderate effect on shear-induced aggregation *(80)*, whereas abciximab and eptifibatide have been shown to inhibit it dramatically *(79,81)*.

Huang et al. *(82)* developed a mathematical model (population balance equation) incorporating both particle coalescence and breakage process applied to platelet aggregation. Only a small fraction of platelet collisions result in the binding together of involved platelets. The modified collision efficiency is approximately zero for shear rates below 3000 s^{-1} and increases to 0.001 for shear rate of 8000 s^{-1} (in the latter case, about 1 collision in 100 results in binding). Added chemical platelet agonists can increase the collision efficiency by one or more orders of magnitude. Under the test conditions, the breakage rate constant is observed to be independent of the shear rate, and the breakage processes are of only secondary importance. The lack of dependence of the breakage rate constant on the shear rate suggest a competition between two opposing influences: increasing platelet activation vs increasing shear forces acting to destroy platelet aggregates. In addition, the collision efficiency for shear-induced platelet aggregation is about an order of magnitude less than 37°C than at 23°C *(82)*.

Initially, this technique could only be performed at the bench, which required removal of blood samples from the bedside. However, a bedside shear chamber is now undergoing evaluation. This device can be connected to an arterial cannula and can be used

to determine in real time the effect of various interventions on shear induced aggregation *(83)*.

CONCLUSION

As antagonists of platelet aggregation become used more frequently and in a broader population, the likelihood increases that monitoring the effect of these agents will become more important. Although assessment of receptor occupancy or of platelet aggregation is now primarily a laboratory procedure, developments currently underway are likely to bring this form of monitoring to the bedside in the near future.

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14 Oral Platelet Glycoprotein IIb/IIIa Blockade

Dean J. Kereiakes, MD
and Christopher P. Cannon, MD

CONTENTS

INTRODUCTION

Oral antiplatelet therapy has demonstrated efficacy in reducing recurrent vascular ischemic events in patients with vascular disease *(1,2)*. Clinical evidence suggests that the benefit of secondary prevention is more widespread and extends beyond the regional circulation responsible for the signal vascular event to more generalized atherothrombotic manifestations. In support of the concept of widespread vascular benefit are data from the antiplatelet trialists collaboration, which show a 27% risk reduction in the composite outcome of myocardial infarction, ischemic stroke, and vascular death from a meta analysis of 145 clinical trials involving more than 73,000 high-risk patients in multiple disease categories *(2,3)*. Relative risk reductions for this composite outcome were 25% with aspirin and 33% with ticlopidine *(2,4,5)*. Both ticlopidine and a chemically related compound, clopidogrel, block platelet activation by selectively and irreversibly inhibiting the binding of adenosine diphosphate (ADP) to its receptor on platelets, thus affecting ADP-mediated activation of the glycoprotein (GP) IIb/IIIa complex–the final common pathway for platelet activation *(6–8)*. When compared with aspirin, these thienopyridine derivatives have achieved a further 8–10% risk reduction for the composite outcomes of stroke, myocardial infarction, and vascular death *(9)*. Synergism for inhibition of collagen-induced platelet aggregation has been demon-

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strated for ticlopidine and aspirin *(10)*. In addition, this combination has proven superior in reducing indices of platelet activation *(11–13)* and thrombotic clinical events *(14,15)* in patients following coronary stent deployment when compared to therapy with aspirin alone or the combination of aspirin and coumadin anticoagulation.

Nevertheless, significant limitations in current oral antiplatelet therapy exist. From the antiplatelet trialists collaborative data base, the rate of recurrent vascular ischemic events in follow-up ranged from 4.5% in "low-risk" patients to 18.4% in patients with a prior cerebrovascular ischemic event despite treatment with currently available antiplatelet agents *(1)*.

In addition, aspirin has been relatively ineffective in reducing the recurrence of ischemic events following discontinuation of antithrombin therapy from patients after an acute myocardial infarction *(16,17)* or percutaneous coronary intervention *(18–20)*. As previously noted, the relative clinical benefit achieved with ticlopidine and clopidogrel when compared with aspirin suggests the potential for greater benefit to accompany therapy with even more potent platelet inhibitors. Recent clinical trials of parenteral competitive small molecule inhibitors of the platelet GP IIb/IIIa receptor administered during percutaneous coronary intervention and for the treatment of acute coronary syndromes (ACS) have demonstrated efficacy for these agents in reducing ischemic outcomes including death, myocardial infarction, and the need for urgent repeat coronary intervention *(20–23)*. However, small molecule GP IIb/IIIa inhibitors have a relatively short duration of action at the platelet receptor, thus limiting the duration of clinical benefit in proportion to the duration of parenteral infusion. For example, although a 24-h infusion of eptifibatide (IMPACT II) *(22)* or a 36-h infusion of tirofiban (RESTORE) *(23)* reduced ischemic outcomes following coronary intervention to 48 h and 7 d, respectively, neither drug achieved a significant reduction in primary study endpoint (composite of death, myocardial infarction, urgent revascularization) by intention to treat at 30 d compared with placebo. Evidence from both clinical trials and platelet studies suggest the need for more prolonged platelet inhibition to both preserve and possibly amplify the early benefits achieved by parenteral therapy with shorter acting agents, making the prospect for "benefit extension" by oral GP IIb/IIIa inhibitors evident.

Additional support for longer term IIb/IIIa inhibition in unstable coronary syndromes comes from large Phase III trials of intravenous IIb/IIIa inhibitors. In the PURSUIT trial *(25)*, a 72-h infusion of eptifibatide led to significant reduction in the composite occurrence of death or myocardial infarction at 30 d (primary study endpoint) 14.2% vs 15.7% ($P = 0.042$). The absolute reduction of 1.5% in death or infarction was achieved at 96 h (7.6% vs 9.1%) with no loss of benefit from day 4 to 30 (as might be expected since patients were not receiving study drug during this time frame). Similar observations were made in the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM PLUS) trial *(26)* in which 1915 patients were randomly assigned to treatment with intravenous tirofiban or heparin and all patients received aspirin. The tirofiban-plus-heparin-treated patients had a significantly lower composite event rate at 7 d (death, myocardial infarction, recurrent ischemia) compared with heparin-treated patients, 12.9% vs 17.9% (34% risk reduction; $P = 0.004$). The 30-d rate of death or infarction was also reduced by 31%, from 11.9% to 8.7% ($P = 0.031$). Again, the absolute 3.5% benefit in death or infarction achieved early was preserved at 30 d. The average duration of intravenous tirofiban infusion was

Table 1
P Selectin Results from TIMI 12

Agonist	*Baseline (%)*	*Day 7 (%)*	*Day 28 (%)*
Spont.	28 ± 19	[a]17 ± 13	[b]20 ± 14
0 μM ADP	36 ± 17	[a]24 ± 17	[a]25 ± 15
1 μM ADP	48 ± 19	[b]44 ± 20	[c]40 ± 17
5 μM ADP	65 ± 19	68 ± 18	64 ± 18

[a] $P < 0.001$.
[b] $P < 0.05$.
[c] $P = 0.005$.

approximately 70 h. Thus, prolonged administration of intravenous IIb/IIIa inhibitors has been shown in several large trials to achieve significant benefit during the infusion period that is preserved during follow-up. This observation raises the question of whether continuation of IIb/IIIa inhibition by oral agents might accrue further ongoing clinical benefit.

The need for longer term IIb/IIIa inhibition is also supported by data from the TIMI 12 and TIMI 3B trials in which a marker of platelet activation, p-selectin, was measured *(24,27)*. In TIMI 12, platelet activation was assessed in patients with acute coronary syndromes (ACS; average 3 days post the onset of unstable angina or acute myocardial infarction). As shown in Table 1, "spontaneous" activation was observed in 28% of platelets from subjects with an ACS. This percentage is much higher than observed in normal subjects studied in the TIMI 3B trial in whom only 2% of platelets showed spontaneous activation *(27)*. Following 28 d of GP IIb/IIIa blockade by sibrafiban in the TIMI 12 study, the percentage of activated platelets remained significantly reduced compared with baseline *(24)*. Of significance is the fact that even after 28 d of oral IIb/IIIa treatment, platelets from patients with ACS remain more spontaneously activated and have an exaggerated response to exogenous agonist than observed in normal subjects. This observation lends pathophysiologic support to the strategy of even longer term oral IIb/IIIa inhibition.

Potential Indications for Oral IIb/IIIa Inhibition

Currently established indications for IIb/IIIa blockade therapy involve acute treatment with intravenous IIb/IIIa inhibitors during percutaneous coronary revascularization procedures or unstable angina pectoris *(24,25,28,29)*. Expanded application of this therapy to the treatment of acute myocardial infarction, stroke, transient cerebrovascular ischemia, and during peripheral or cerebrovascular intervention is currently being evaluated (Table 2). The potential exists for oral agents to duplicate the benefit observed with parenteral therapy (just as aspirin is used in acute treatment) and oral therapy may prove easier to administer than a prolonged intravenous infusion. Oral agents offer the unique opportunity to provide secondary prevention for all vascular indications. In addition, potent chronic platelet inhibition with an oral IIb/IIIa inhibitor could reduce thrombus formation in response to plaque rupture *(30)*. Thus, plaque growth and the process of athero(thrombo) sclerosis could be favorably influenced.

Table 2
Indications for IIb/IIIa Inhibition: Current and Future

• Acute treatment	Intravenous IIb/IIIa Inhibitors
Percutaneous coronary intervention	? Oral IIb/IIIa Inhibitors
Acute coronary syndromes (ACS)	? Intravenous + Oral Inhibitors
(unstable angina; acute myocardial infarction)	
Stroke	
Peripheral vascular/cerebrovascular intervention	
• Secondary prevention	
Percutaneous coronary intervention	
ACS	
Stroke	? Oral IIb/IIIa Inhibitors
• *Both* early treatment and secondary prevention	
• Inhibition of atherosclerosis (atherothrombosis)	

RANDOMIZED TRIALS

Prior to the Oral Glycoprotein IIb/IIIa Receptor Blockade to Inhibit Thrombosis (ORBIT) trial, experience with xemilofiban was gained in two smaller pilot trials *(31,34)*. First, in a single center, single-blind, placebo-controlled study, 30 patients with unstable angina undergoing percutaneous coronary interventions were randomized to placebo or 35 mg xemilofiban orally before, and 20 to 25 mg P.O. TID for 30 d after angioplasty *(31)*. Bleeding events, platelet aggregation, and pharmacokinetic and hematologic parameters were assessed in-hospital and at 2 and 4 wk of chronic study drug administration. In this pilot trial, xemilofiban produced rapid, sustained, extensive inhibition of platelet aggregation for a period of 30 d. At the dose tested, however, acute major bleeding and mucocutaneous bleeding during chronic administration were encountered. Secondly, in a multicenter, randomized, placebo-controlled dose-ranging trial, xemilofiban was administered to patients following intracoronary stent deployment *(34)*. Study medicine was initiated on the morning following the interventional procedure on a randomized basis at doses of 5, 10, 15, or 20 mg on a twice-daily schedule. Patients randomized to placebo also received ticlopidine 250 mg orally twice daily begun immediately after the procedure. All patients received 325 mg of aspirin orally daily. In patients who required intraprocedural abciximab at the discretion of the investigator, xemilofiban was begun 8–18 h following the discontinuation of abciximab. Study medication was administered twice daily for 2 wk and ex vivo platelet aggregation studies were performed following the inital dose and at 7 and 14 d of chronic oral therapy. In this trial, oral xemilofiban resulted in a dose-dependent inhibition of platelet aggregation that was sustained through 2 wk of chronic therapy. Doses of xemilofiban required to achieve ≥ 50% inhibition of platelet aggregation were ≥ 10 mg and the duration of inhibition lasted 8–10 h. No significant hemorrhagic episodes or transfusions were observed.

The ORBIT trial was a multicenter, placebo-controlled randomized trial of xemilofiban in doses of 15 and 20 mg administered to 549 patients following successful percutaneous coronary intervention *(35)*. Study drug was administered TID for 2 wk, then BID for 2 wk (total 28 d) and all patients were followed clinically for 90 d. A stratification procedure based on the type of intervention performed was employed.

Patients undergoing balloon angioplasty received double-blind study medication. Patients undergoing stent deployment had study medication administered in a single-blind fashion to accommodate therapy with ticlopidine 250 mg orally twice daily for 4 wk in individuals randomized to receive placebo. Concomitant administration of xemilofiban and ticlopidine was not permitted. All patients were treated with oral aspirin (325 mg/d). The primary objective of ORBIT was to asess the pharmacodynamic effect of two dose levels of xemilofiban compared with placebo on inhibition of platelet aggregation. Secondary objectives were to evaluate the pharmacokinetic profile of xemilofiban after 2 and 4 wk of therapy; the safety profile of xemilofiban administered for 1 mo following coronary intervention; and the incidence of clinical cardiovascular events (death, myocardial infarction, urgent bypass surgery, urgent repeat angioplasty, or nonhemorrhagic stroke) in follow-up at 3 mo following randomization.

Results from phase II pilot studies previously cited indicated that single oral doses of 10 mg or more of xemilofiban were required to achieve peak levels exceeding 50% (50–80%) inhibition of platelet aggregation in response to 20 μ*M* ADP. The duration of platelet inhibition following a single dose of study drug was 8–10 h. Thus, dosing xemilofiban three times daily was anticipated to maintain 50–80% inhibition of ADP induced platelet aggregation throughout 24 h. The hypothesis was set forth that following 2 wk of chronic therapy, twice daily dosing would suffice in providing an acceptable degree of platelet inhibition to prevent recurrent cardiovascular events. The ORBIT trial was also designed and treatment arms were further stratified to accommodate abciximab administration at the discretion of the investigator during the interventional procedure. In both stented and nonstented patients, a blinded dosing strategy of xemilofiban was available to accommodate procedural abciximab administration with either xemilofiban or placebo therapy initiated at least 8 h following discontinuation of abciximab. Abciximab treated patients received a reduced dose of xemilofiban (10 mg orally, 3 times daily for 2 wk) because of the previously described potentiated dose response to xemilofiban when administered in close temporal sequence with abciximab therapy as observed in the phase II xemilofiban-stent pilot trial *(33,34)*.

The Thrombolysis in Myocardial Infarction (TIMI) 12 trial was a phase II, double-blind dose-ranging trial designed to evaluate the pharmacokinetics (PK), pharmacodynamics (PD), safety, and tolerability of sibrafiban in 329 patients post ACS *(32)*. In the PK/PD cohort of TIMI 12, 106 patients were randomized to receive 1 of 7 dosing regimens of sibrafiban, ranging from 5 mg daily to 10 mg twice daily or aspirin for 28 d. In the safety cohort, 223 patients were randomized to 1 of 4 dose regimens of sibrafiban (ranging from 5 mg twice daily to 15 mg once daily) or aspirin for 28 d.

GENERAL CONSIDERATIONS

Pharmacokinetics and Pharmacodynamics

A number of orally active platelet GP IIb/IIIa inhibitors have been studied or are under evaluation in clinical trials (Table 3) *(31–38)*. These agents are administered in the prodrug form and require hepatic conversion to an active moiety. Absolute bioavailability is generally low as shown in Table 3. Currently available data suggest these agents produce inhibition of ex vivo platelet aggregation in response to various agonists (ADP; collagen; TRAP) that correlates closely with plasma level of active metabolite. In addition, the dose/concentration response is maintained without evidence for tolerance or

Table 3
Trials of Oral GP IIb/IIIa Agents

Oral agent	*Company*	*Trial*	*Study population*	*Drug half-life*	*Absolute bioavailability*	*Metabolism*	*Renal elimination*
Xemilofiban	G.D. Searle	ORBIT EXCITE	ACS/PTCA Coronary Intervention	4.1 h	13%	Hepatic	90%
Sibrafiban	Roche Genentech	TIMI 12 Symphony	Post-ACS ACS	11 h	na	na	na
Orbofiban	G.D. Searle	SOAR OPUS TIMI 16	ACS	10 h	19%	na	na
Lotrafiban	Smith-Kline Beecham	APLAUD	ACS TIA, Post-Ischemic Stroke	4–8 h	na	na	na
Klerval	Rhône-Poulenc Rorer	TIMI 15	ACS	4–5 h	na	na	na
Roxifiban	DuPont Merck	GAP	Coronary Intervention	11–12 h	na	na	na

na = not applicable.

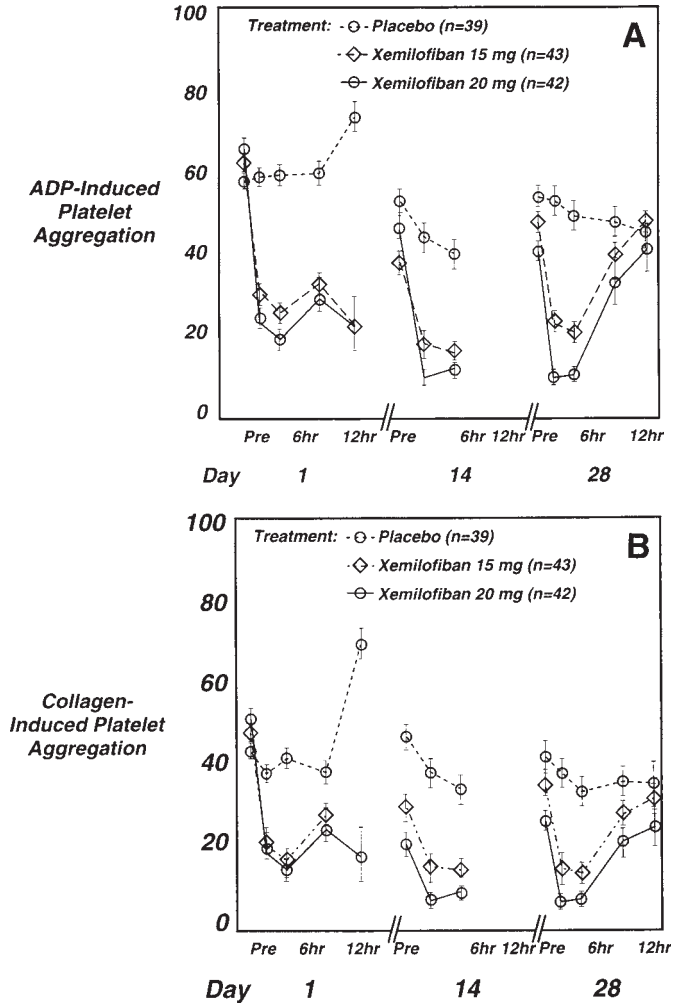

Fig. 1. Ex vivo platelet aggregation in response to 20 μM ADP **(A)** and 4 μg/mL collagen **(B)** assessed over time on days 1, 14, and 28 of study drug administration in the ORBIT trial.

tachyphylaxis over time. Differences in drug half-life may result in drug accumulation and more pronounced platelet inhibition during chronic therapy depending on the dose interval employed. The pharmacokinetic and pharmacodynamic response to most oral GP IIb/IIIa inhibitors can be illustrated by comparing and contrasting the responses of short (xemilofiban, half-life 4.1 h) and longer (sibrafiban; half-life 11 h) acting agents.

The dose response curves over time for xemilofiban at doses of 15 and 20 mg vs placebo from the ORBIT trial are shown in Fig. 1. Peak inhibition of platelet aggregation was similar following the same dose of xemilofiban administered on days 14 and 28 of the trial. The time to peak blood level following the same dose of xemilofiban was reduced from 4 h following the first dose of drug to 2 h with steady-state dosing during chronic therapy (Fig. 2A,B). In comparison, twice daily administration of sibrafiban in the TIMI 12 trial was associated with drug accumulation during 28 d of chronic oral administration (Fig. 3). Consistent with the previously noted correlation between blood

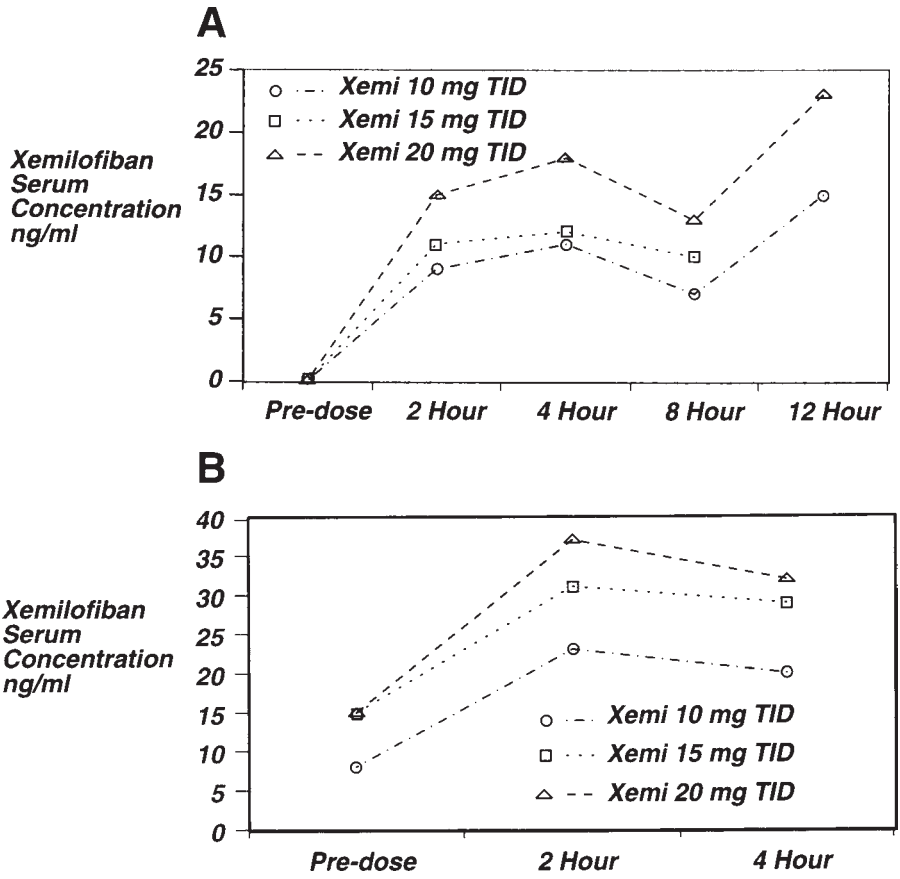

Fig. 2. Pharmacodynamic profile of xemilofiban on day 1 **(A)** following the first dose of study drug and during chronic "steady-state" study drug administration on a 3-times-daily dosing regimen **(B)**. The time to peak blood level was reduced from 4 to 2 h during steady-state administration.

level and degree of platelet inhibition, more marked inhibition of ex vivo platelet aggregation in response to agonists (both ADP and TRAP) occurred on day 28 than on day 1 of twice daily sibrafiban administration (Fig. 4). A similar correlation between blood level of active drug and the degree of platelet inhibition would be expected for all orally active competitive antagonists. Drug accumulation and exaggerated platelet inhibition would likewise accompany multiple daily dosing of a longer acting agent.

As the major route for elimination of oral platelet GP IIb/IIIa antagonists is renal excretion (Table 3), drug accumulation may also accompany changes in renal function or perfusion. Clinical trials of these agents have in large part excluded patients with serum creatinine values exceeding 1.6 mg% and the safety of their use is in this context is not known. Similarly, multiple active metabolites with variable serum half-lives, as observed following oral administration of Klerval, may demonstrate variable renal excretion.

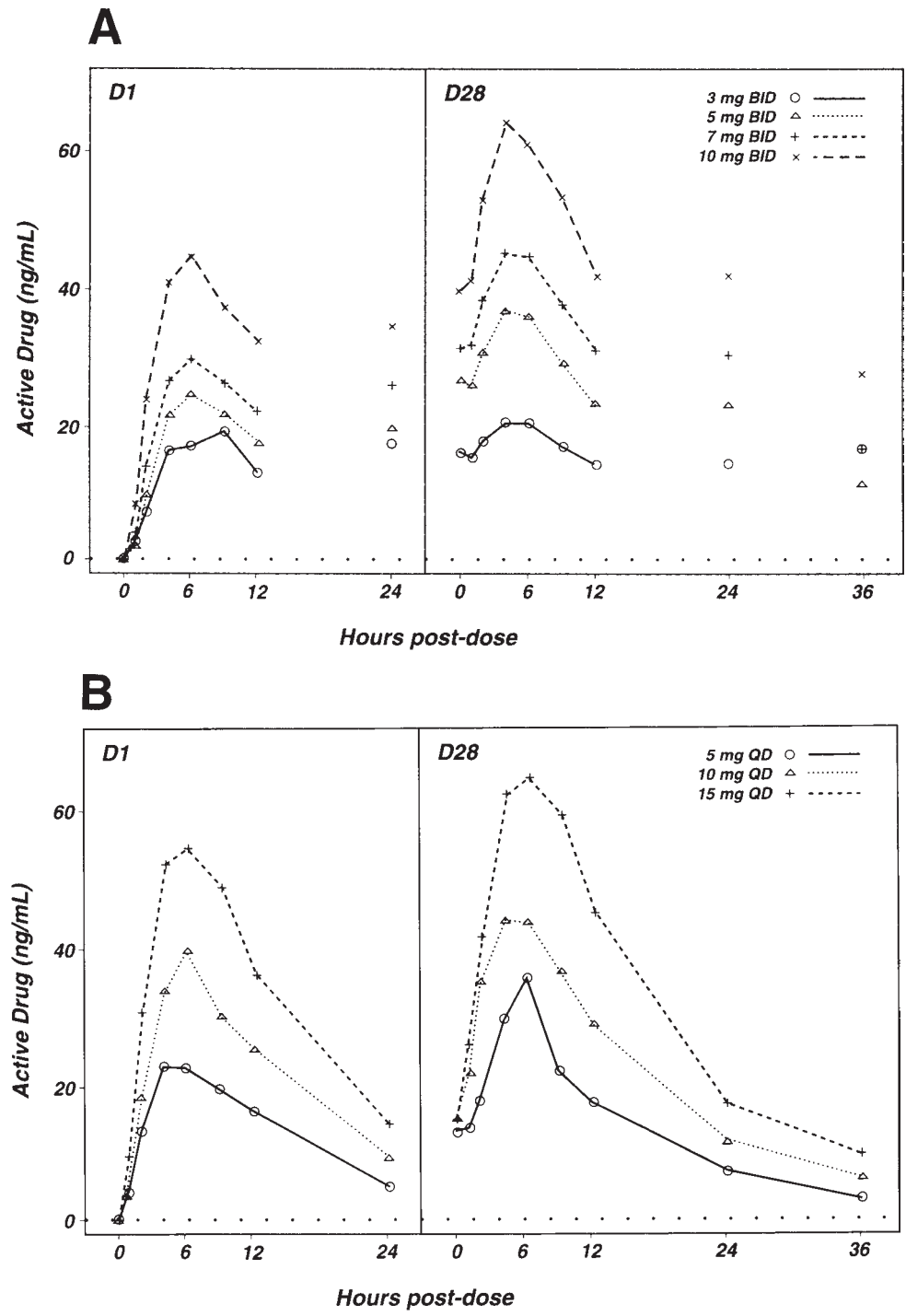

Fig. 3. Concentration of sibrafiban active metabolite (RO 44-3888) measured over time on day 1 (D1) and day 28 (D28) following twice daily dosing regimens **(A)** and daily dosing regimens **(B)** administered in the TIMI 12 trial.

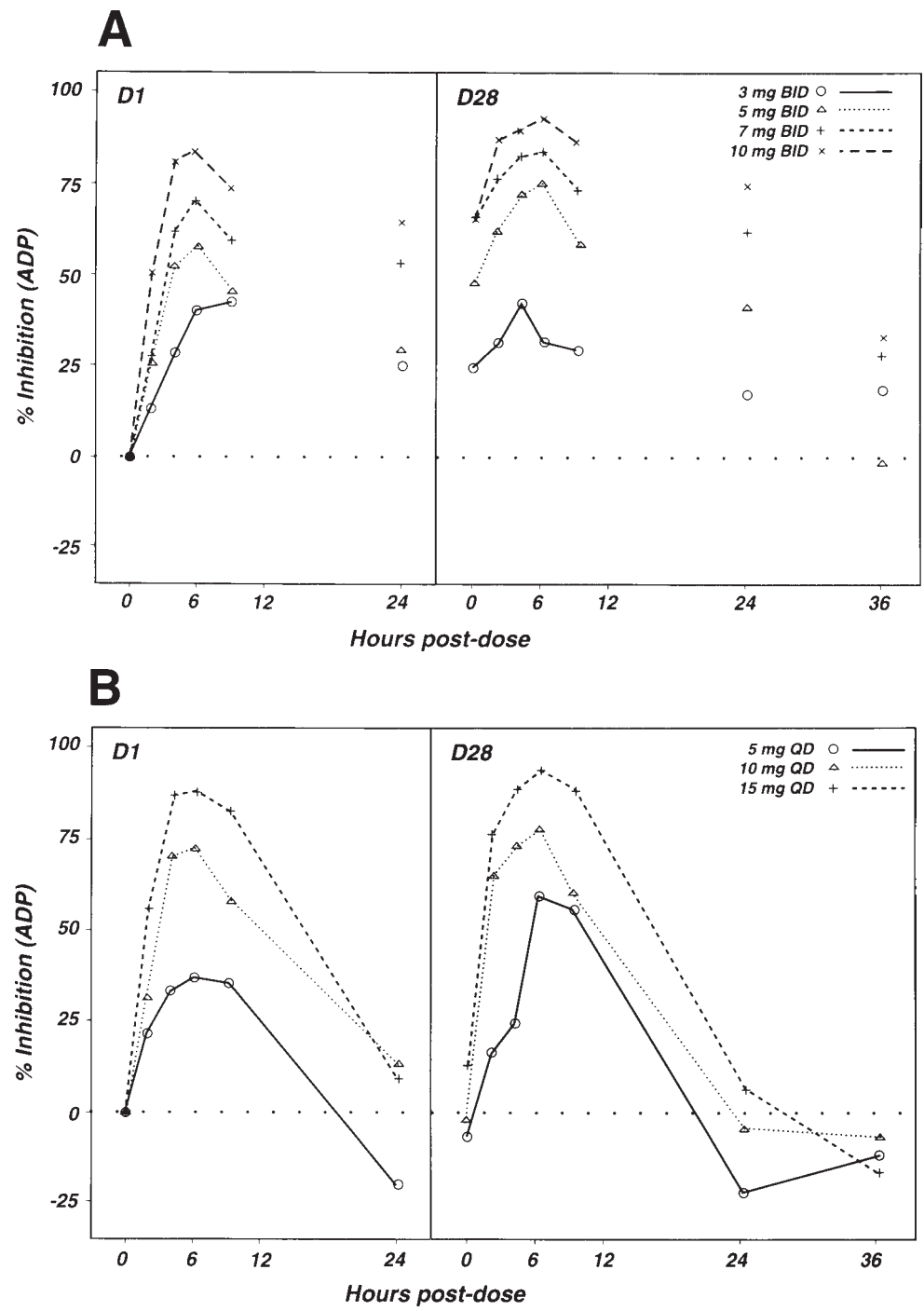

Fig. 4. Inhibition of ex vivo platelet aggregation in response to 20 μM ADP **(A,B)** on day 1 (D1) and day 28 (D28) for twice-daily (BID) dosing regimens (A) and during-daily (QD) dosing regimens (B) of study drug administered in the TIMI 12 trial. Similar observations for ex vivo inhibition of TRAP-induced platelet aggregation during twice-daily **(C)** and daily **(D)** dosing regimens on days 1 and 28 of the TIMI 12 trial are shown.

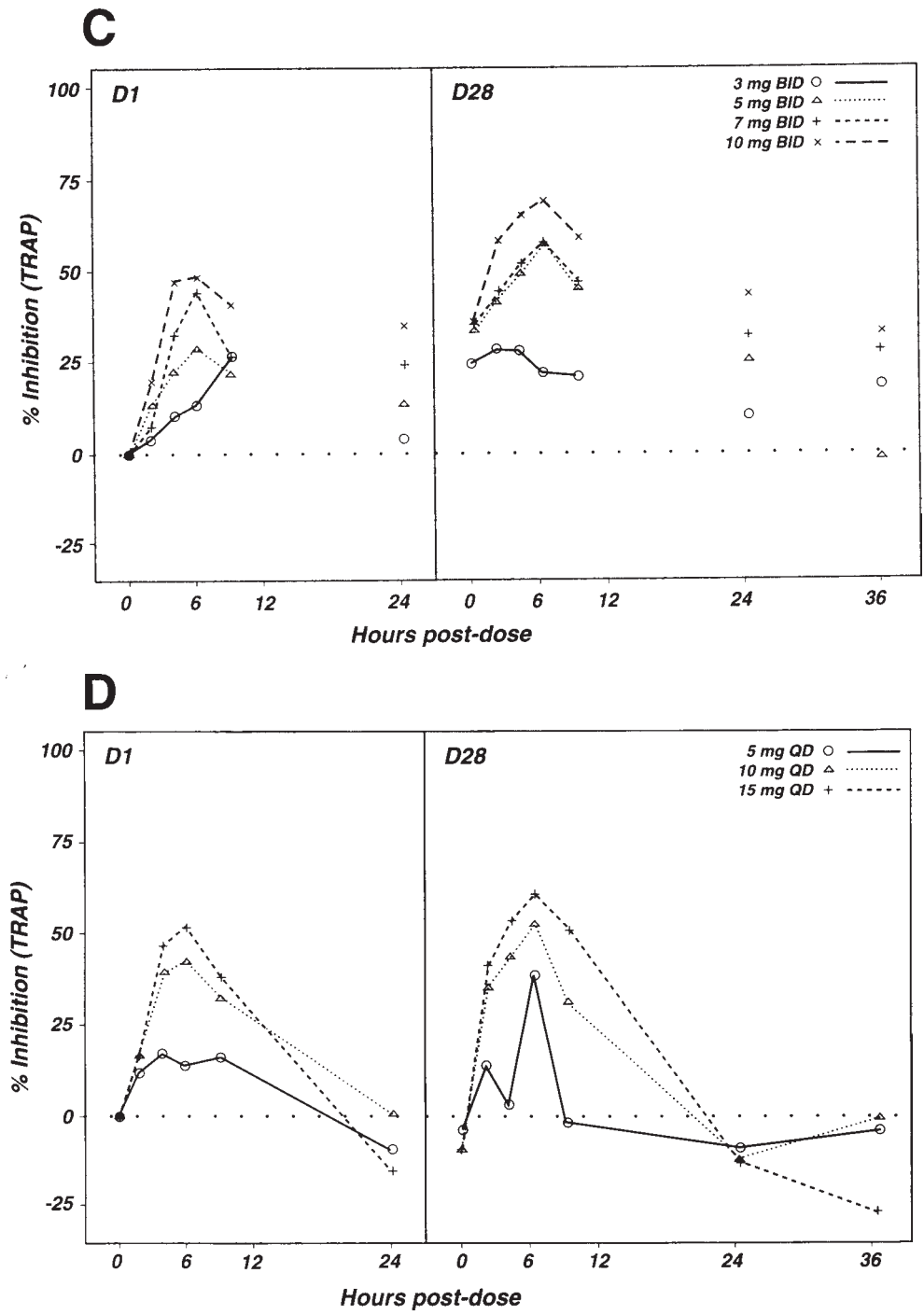

Fig. 4. *(continued)*

Table 4
Bleeding Events and Blood Transfusion by Drug Treatment Allocation In the ORBIT Trial

	Placebo		*Xemilofiban 10/15 mg*[a]		*Xemilofiban 10/20 mg*[a]	
	Abcix	*No Abcix*	*Abcix*	*No Abcix*	*Abcix*	*No Abcix*
n	*52*	*124*	*56*	*132*	*53*	*132*
Bleeding Event						
Vascular access N (%)	2 (4%)	7 (6%)	1 (2%)	8 (6%)	1 (2%)	9 (7%)
Gastrointestinal	0	1 (1%)	3 (5%)	6 (5%)	0	10 (8%)
Genitourinary	2 (4%)	3 (2%)	1 (2%)	10 (8%)	2 (4%)	4 (3%)
Oral/ gingival	0	1 (1%)	3 (5%)	13 (10%)	2 (4%)	7 (5%)
Epistaxis	3 (6%)	6 (5%)	9 (16%)	20 (15%)	12 (23%)	30 (23%)
Blood (RBC) Transfusion	3 (6%)	0	1 (2%)	2 (2%)	1 (2%)	3 (2%)
No. units per patient	4/1/2	0	2	3/7	2	3/4/5

[a] Xemilofiban dosing regimen as outlined in Figs. 1 and 2; and in text; RBC = red blood cells (Abcix = abciximab).

Bleeding Events

A major limitation of oral GP IIb/IIIa blockade therapy has been the occurrence of bleeding complications *(31,32,35,37)*. Although most of the bleeding events observed have been mild in severity, termination of study drug because of bleeding has at times been required and is more frequent at higher doses. The origin of bleeding is most often mucocutaneous with epistaxis being most common (Table 4). The incidence and severity of bleeding events to 2 and 4 wk of therapy by pharmacologic treatment regimen in the ORBIT trial is shown in Table 5.

Most bleeding events were observed during the first 2 wk of therapy on a three-times daily dosing regimen *(35,37)*. Further bleeding events were uncommon during the final 2 wk of treatment on a twice-daily dosing regimen and the requirement for blood transfusion was infrequent.

Patient tolerance of bleeding may be influenced by the pharmacodynamic profile of GP IIb/IIIa inhibition. For example, although epistaxis was common (23%) at the highest dose of xemilofiban (20 mg) tested, discontinuation of study drug for bleeding was infrequent (5%) at this dose. This apparent tolerance of mucocutaneous bleeding may be secondary to cyclic recovery in platelet function to baseline between doses of xemilofiban. Although marked inhibition of platelet aggregation (70–80% to 20 μM ADP) was achieved during steady state with both 15 and 20 mg doses of xemilofiban, this level of inhibition was sustained for only 2–6 h after each dose and was followed by rapid and complete recovery of platelet aggregability prior to the next dose of medication. This sequence is illustrated by the pharmacodynamic response to a 20 mg dose of xemilofiban as reflected by the inhibition of ex vivo platelet aggregation in response to 20 μM ADP (Fig. 5). Complete recovery in platelet function to predose levels was observed within 8–10 h of a dose on both a twice and three times daily dosing regimen.

Table 5
Incidence and Severity of Bleeding Events at 2 and 4 Wk of Study Drug Therapy by Treatment Allocation In the ORBIT Trial

	Placebo		*Xemilofiban 10/15 mg*[a]		*Xemilofiban 10/20 mg*[a]	
	Abcix	*No Abcix*	*Abcix*	*No Abcix*	*Abcix*	*No Abcix*
Event at 2 wk (%)						
Insignificant	12	19	18	26	23	32
Mild	0	5	11	10	4	11
Moderate	6	0	2	1	0	2
Severe	0	0	0	2	2	0
Event at 4 wk (%)						
Insignificant	17	24	23	32	34	38
Mild	4	6	13	11	6	13
Moderate	6	0	2	1	0	2
Severe	0	0	0	2	2	0

[a] Xemilofiban dosing regimen as outlined in Figs. 1 and 2 and in text (Abcix = abciximab).

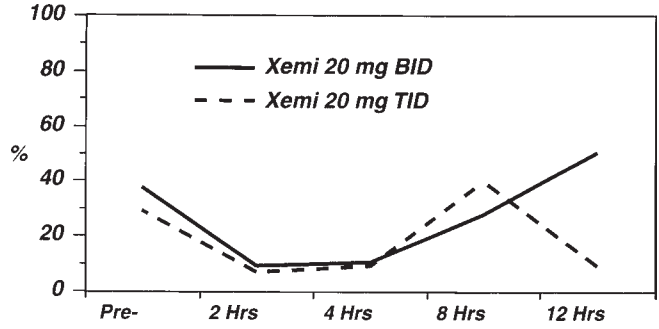

Fig. 5. Ex vivo platelet aggregation in response to 20 μM ADP following chronic, steady-state administration of xemilofiban (XEMI) 20 mg on a twice daily (BID) or 3 times daily (TID) dosing regimen. Rapid and profound inhibition of platelet aggregation is followed in short order by rapid recovery in platelet aggregability.

In contrast, discontinuation of study drug for bleeding during sibrafiban therapy in the TIMI 12 trial appeared to occur more frequently despite an incidence of mucocutaneous hemorrhage similar to that observed in the ORBIT trial. The longer half-life of this medication and drug accumulation particularly during twice daily dosing makes it less likely for significant recovery in platelet function to occur between doses. In addition, the longer half-life of sibrafiban provides a much more sustained duration of platelet inhibition. Thus, epistaxis once initiated, may be less likely to spontaneously resolve in the context of protracted and profound inhibition of platelet aggregation particularly following higher doses of sibrafiban or any other longer acting GP IIb/IIIa blocking agent.

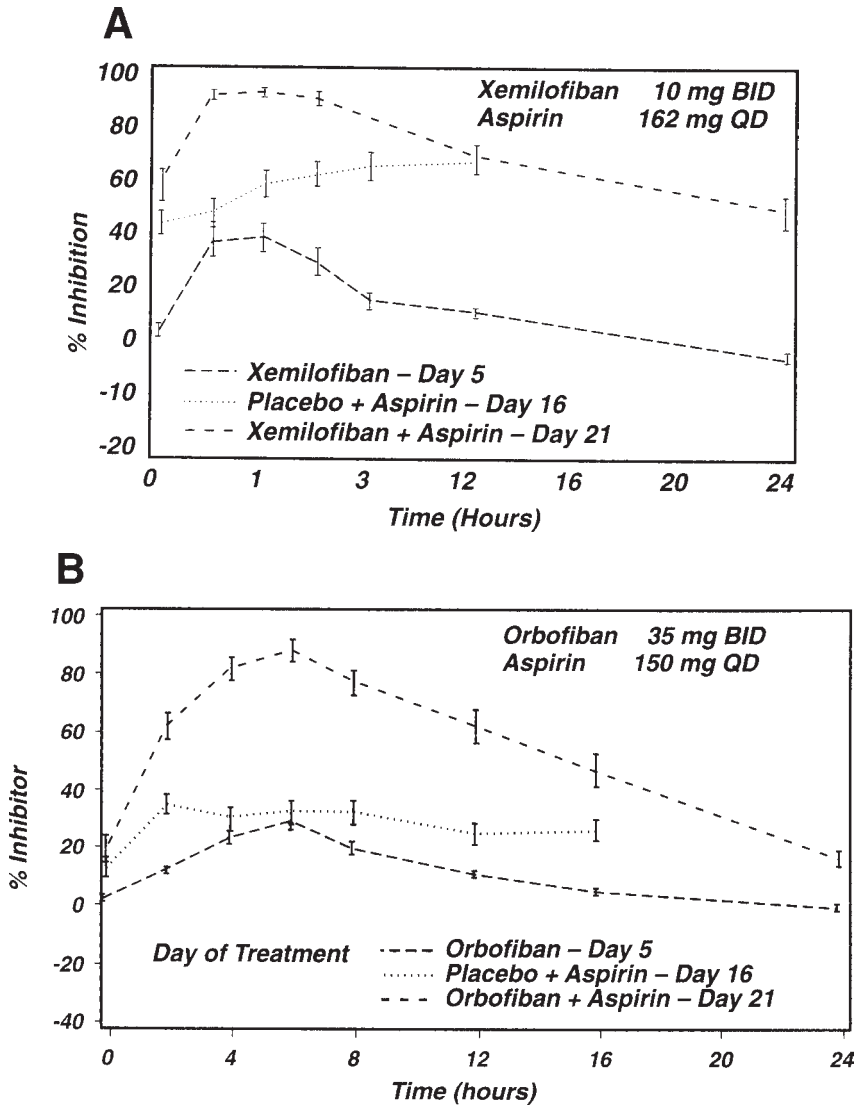

Fig. 6. Inhibition of ex vivo platelet aggregation in response to 4 μg/ml collagen following aspirin alone, xemilofiban **(A)** or orbofiban **(B)** and the combinations of xemilofiban or orbofiban and aspirin is shown. Marked enhancement of platelet inhibition follows the combination of aspirin and oral GP IIb/IIIa inhibitor therapy.

Concomitant Antiplatelet and Antithrombotic Therapy

Aspirin

An important aspect of oral GP IIb/IIIa inhibition is concomitant therapy with aspirin. Synergism between aspirin and oral GP IIb/IIIa blockers has been demonstrated for inhibition of platelet aggregation in response to collagen *(39,40)*. The combination of GP IIb/IIIa inhibitor and aspirin produces more marked platelet inhibition than either agent alone as illustrated for both xemilofiban and orbofiban (Fig. 6A,B). Enhanced

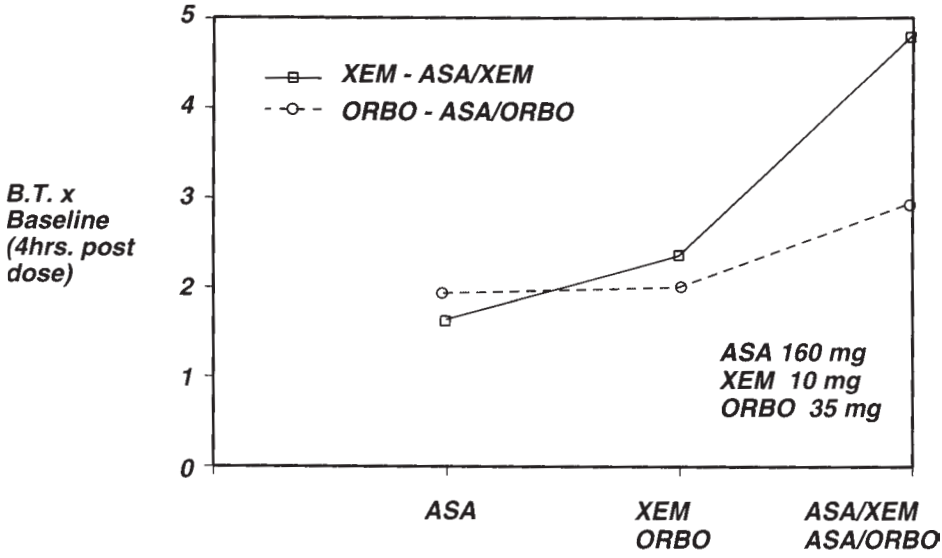

Fig. 7. Ivy template bleeding time measured following aspirin alone, xemilofiban (XEM) or orbofiban (ORBO) alone and the combination of aspirin and oral GP IIb/IIIa blockade therapy. More marked prolongation of the bleeding time follows combination aspirin and oral GP IIb/IIIa blockade therapy.

inhibition of ex vivo platelet aggregation in response to 20 μ*M* ADP has also been observed for Klerval in combination with aspirin (F. Catella-Lawson, MD ISTH Meeting, Florence, Italy, June, 1997). The potentiation of platelet inhibition by aspirin is particularly evident at trough blood levels of Klerval (Rhône-Poulenc Rorer, Collegeville, PA). Template bleeding time may also be prolonged by the combination of GP IIb/IIIa blockade and aspirin (Fig. 7). Thus, both efficacy and potential safety of oral GP IIb/IIIa blockade therapy may be influenced by concomitant aspirin administration. In view of the fact that aspirin has proven efficacy for primary and secondary prevention of ischemic events, is well tolerated, inexpensive, and displays synergism with GP IIb/IIIa blockade, the most likely future scenario would entail combination therapy with aspirin and GP IIb/IIIa blockade. Adjunctive treatment with aspirin could facilitate the therapeutic efficacy of lower doses of GP IIb/IIIa blockers and might provide additional platelet passivation during the troughs in GP IIb/IIIa blockade that occur between doses of a short acting agent. Data are lacking on the interaction and potential synergism between other nonsteroidal antiinflammatory drugs and GP IIb/IIIa blocking agents. The potential adverse effect of concomitant aspirin (or other nonsteroidal antiinflammatory agents) is increased bleeding, particularly gastrointestinal in origin. We believe the beneficial aspects of concomitant aspirin therapy will outweigh any potential harm. Data will be available in the future on oral GP IIb/IIIa inhibitors both with (EXCITE and OPUS/TIMI 16 Trials) and without aspirin (SYMPHONY Trial), which may help to clarify these issues.

Abciximab

Although parenteral IIb/IIIa blockade during percutaneous coronary intervention followed subsequently by oral therapy appears to represent a logical therapeutic

sequence to "extend" clinical benefit particularly for small molecule antagonists, few data are available in this regard. The sequential administration of the monoclonal antibody abciximab (c7E3; ReoPro™ Centocor, Inc., Malvern, PA) followed by an oral competitive GP IIb/IIIa inhibitor has been studied *(33)*. Unlike available parenteral competitive short acting GP IIb/IIIa blocking agents, abciximab has a prolonged duration of action at the platelet receptor. Redistribution and equilibration of abciximab across platelets has been observed. At 8 and 15 d following abciximab therapy approximately 29,000 and 13,000 Fab fragment molecules, respectively, persist on each circulating platelet, which reflects 30 and 10% GP IIb/IIIa receptor occupancy, respectively *(41)*. Thus, the potential for an extended drug–drug interaction with a sequentially administered oral IIb/IIIa antagonist is evident. When xemilofiban was administered 8–18 h following cessation of abciximab therapy, a differential dose response was observed. As compared with patients who had not received antecedent abciximab for percutaneous coronary intervention, abciximab treated patients demonstrated additive inhibition of ADP induced platelet aggregation. Synergism for collagen induced platelet aggregation was observed following the first dose of oral drug *(33)*. The exaggerated dose response to xemilofiban following antecedent abciximab was no longer evident after 1 wk of continued oral drug therapy (Fig. 8A,B). This observation formed the basis for a reduced dosage of xemilofiban to be administered following abciximab therapy in the ORBIT trial protocol *(37)*. The sequential administration of a shorter acting parenteral agent with an orally active competitive antagonist, both of which provide platelet inhibition in parallel to blood concentration, should allow for a more predictable and less protracted interaction. Data on the parenteral-oral administration sequence for a competitive GP IIb/IIIa antagonist will be available from the TIMI 15 trial of Klerval.

Heparin

Although few data are available on the interaction of heparin with oral GP IIb/IIIa inhibitors, extrapolation from the experience with parenteral GP IIb/IIIa blockade may be instructive. Via a direct effect on the platelet GP IIb/IIIa receptor *(42)*, GP IIb/IIIa blocking drugs prolong the activated clotting time (ACT) response to heparin *(43)*. This exaggerated response to heparin appears to be most marked following abciximab therapy but has been described to occur with parenteral small molecule competitive antagonists of the GP IIb/IIIa receptor as well *(44)*. A similar interaction with heparin would be expected following orally administered GP IIb/IIIa inhibitors. In the EPIC trial of abciximab therapy for high-risk coronary intervention, the high ACT levels observed following the administration of nonweight-adjusted heparin were associated with frequent bleeding events and transfusion requirement *(20)*. Subsequent evaluation of a lower dose weight-adjusted strategy for administering heparin in conjunction with abciximab demonstrated a marked reduction in bleeding complications *(45)*. The safety and efficacy of low-dose weight- adjusted heparin (70 U/kg bolus dose) in combination with abciximab was confirmed in a large, multicenter randomized trial (EPILOG) *(21)*. These studies have led to the current recommendation for administering heparin as a weight-adjusted bolus (70 U/kg) in conjunction with abciximab *(46)*. Thus, in conjunction with either parenteral or oral GP IIb/IIIa inhibitors it would appear prudent to use lower, weight-adjusted doses of heparin to avoid bleeding complications.

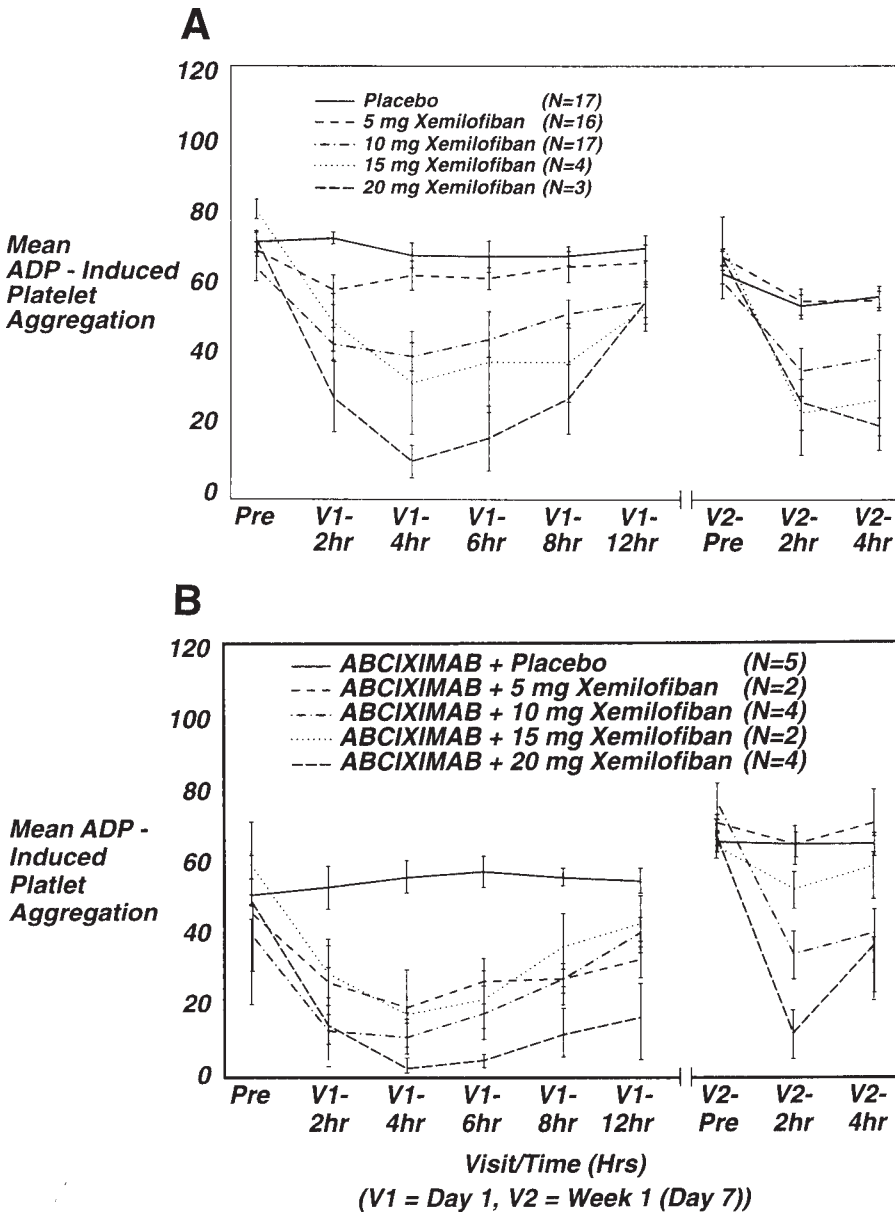

Fig. 8. Dose response inhibition of ex vivo platelet aggregation in response to 20 μM ADP for incremental doses of xemilofiban (5, 10, 15, and 20 mg) or placebo on days 1 and 7 of study drug therapy in patients without **(A)** or with **(B)** antecedent abciximab therapy at the discretion of the investigator at the time of coronary intervention. (Reproduced from *(33)* with permission).

Thienopyridine Derivatives

The thienopyridine derivatives ticlopidine and clopidogrel irreversibly bind adenosine diphosphate (ADP) receptors on the platelet, thus inhibiting ADP-mediated GP IIb/IIIa receptor activation and platelet aggregation. They may also have an effect on intracellular signaling of the ADP-pathway in expressing IIb/IIIa receptors. A relatively weak interaction to enhance platelet inhibition has been described between abciximab and ticlopidine *(47)*. As yet unpublished in vitro "interaction" studies with ticlopidine and either xemilofiban or orbofiban suggest marked synergism for inhibition of ADP-induced platelet aggregation (R. Anders, GD Searle; personal communication). Thus, combined therapy with a thienopyridine derivative and an oral GP IIb/IIIa inhibitor cannot be recommended at this time.

Warfarin

The interaction of oral GP IIb/IIIa blockade and concomitant warfarin therapy has not been evaluated to date. Patients receiving warfarin have been excluded from clinical trials of oral GP IIb/IIIa agents because of concern for bleeding risk. The experience with parenteral GP IIb/IIIa blockade with abciximab in the context of warfarin anticoagulation may be instructive *(48)*.

Approximately one-third of all intracranial hemorrhages observed following abciximab therapy have occurred in patients treated with warfarin who have an international normalized ratio (INR) of ≥1.5 *(49)*. Specific recommendations to avoid abciximab therapy in this context have been made *(46)*. If concomitant GP IIb/IIIa blockade must be given to this group of patients, rapid reversal of warfarin anticoagulation with intravenous vitamin K and fresh frozen plasma should be considered. It appears prudent to adopt similar recommendations for the combination of oral GP IIb/IIIa blockade and warfarin until more data are available.

LESSONS FROM THE PRE-ORBIT, ORBIT, AND TIMI 12 TRIALS

The obvious limitation to therapy for the oral platelet GP IIb/IIIa inhibitors is patient tolerance of side effects, particularly bleeding. In the ORBIT trial, doses of a short-acting oral GP IIb/IIIa inhibitor were targeted to achieve levels of ex vivo platelet inhibition of ADP (20 μM) induced aggregation approaching 80%. The short half-life and rapid onset of action of xemilofiban exaggerates the peak and trough relationship of blood level and platelet inhibition. The pharmacodynamic profile of xemilofiban provides cyclic, rather than smooth, continuous platelet inhibition and, thus the ORBIT trial provides a valuable tolerance (safety) and efficacy profile for the strategy of cyclic platelet inhibition.

In the TIMI 12 trial, high levels of platelet inhibition were also achieved: mean peak values ranged from 47 to 97% inhibition of 20 μM ADP-induced platelet aggregation on day 28 across the seven doses (Fig. 4A,B). Twice daily dosing provided more sustained platelet inhibition (mean inhibition 36–86% on day 28), whereas platelet inhibition returned to baseline levels by 24 h with once daily dosing. Major hemorrhage was rare (1.5%) in patients treated with sibrafiban or aspirin (1.9%). However, protocol-defined "minor" bleeding, usually mucocutaneous, occurred in 0 to 32% of patients in the various sibrafiban groups, compared with none of the aspirin-treated patients. In a multivariate model, minor bleeding was related to total daily dose ($P = 0.002$), once- vs

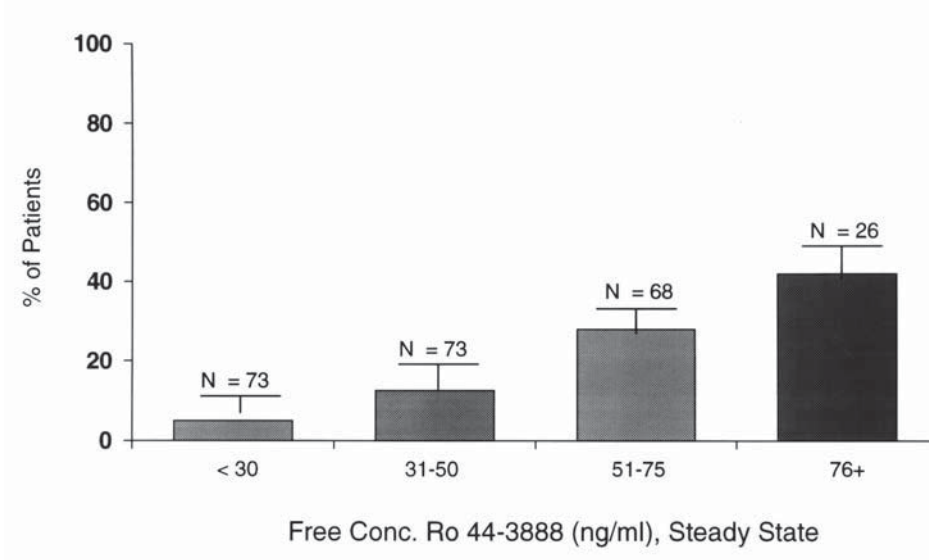

Fig. 9. Relationship of major or minor hemorrhage to peak plasma concentration level of sibrafiban active metabolite (RO 44-3888) in the TIMI 12 trial.

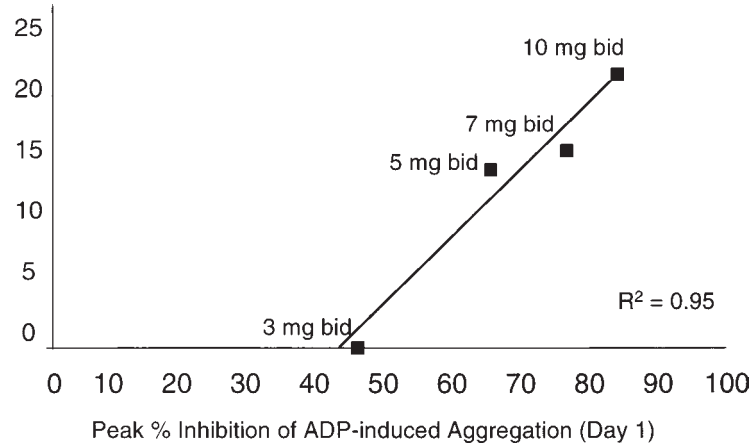

Fig. 10. Relationship between major or minor hemorrhage (% of patients) and peak % inhibition of ADP (20 μM) induced platelet aggregation on day 1. Mean levels of platelet inhibition achieved by twice-daily (BID) study drug administration are shown.

twice-daily dosing ($P < 0.0001$), renal function ($P < 0.0001$), and presentation with unstable angina ($P < 0.01$). Thus, the oral IIb/IIIa antagonist sibrafiban achieved effective, chronic platelet inhibition with a clear dose-response, but at the expense of a relatively high incidence of minor bleeding. The mucocutaneous bleeds appeared to be related to plasma drug concentration, the degree of platelet inhibition (*see* Figs. 9 and 10), and other patient factors (weight, renal function).

Another interesting observation from the TIMI 12 trial was that the rate of minor bleeding with once-daily dosing was approximately twice that seen with a similar total

daily dose administered on a twice-daily dosing interval (e.g., 15 mg once daily vs 7 mg twice daily). This suggests that higher peak drug concentrations and degree of platelet inhibition (sometimes 100%) may be related to the bleeding episodes observed. The timing of the bleeding events appeared to occur approximately 6 h after study drug ingestion, thus correlating with peak blood drug level. These data suggest that dosing regimens that avoid sustained high peak levels of platelet inhibition may decrease the risk of bleeding.

Interestingly, although patients in the ORBIT trial had cyclic and profound (≥ 80%) platelet inhibition and frequently recorded bleeding events, the majority of these events were not clinically significant and did not prompt medical evaluation or discontinuation of study medication. Indeed, the simple relationship between total bleeding event incidence (53%) and discontinuation of study drug for bleeding (5%) at the highest dose (20 mg) of xemilofiban tested reflects a "tolerance" ratio of 10-fold. Although similar levels of peak platelet inhibition (≥ 80%) were achieved following the 10 mg dose of sibrafiban administered twice daily (bid) in the TIMI 12 trial, the longer half-life of this agent resulted in a more continuous pattern of platelet inhibition that remained ≥50% throughout the entire dosing interval. The total incidence of bleeding events (21%) during chronic therapy with 10 mg bid Sibrafiban bears a closer relationship (<2:1) to the incidence of study drug discontinuation because of bleeding (13%) reflecting a lower tolerance "threshold" for this pattern of more continuous and marked platelet inhibition. Cyclic complete recovery of platelet function between doses of the shorter-acting xemilofiban appears to confer tolerance to greater peak levels of inhibition than might otherwise be tolerated for more extended periods of time during chronic oral therapy. A low tolerance threshold was also observed following a single, larger dose (15 mg) of sibrafiban administered daily. Marked inhibition of platelet aggregation was sustained (≥70% to 20 μ*M* ADP) for 14 h or more following this dose. A close relationship between the incidence of bleeding events (38%) and the discontinuation of treatment because of bleeding (32%) with this dose again suggests that a sustained high level of platelet inhibition may be poorly tolerated. Minor and otherwise insignificant bleeding events are likely exacerbated by a pattern of continuous and profound platelet inhibition. If a prolonged or continuous profile of platelet inhibition is desired, it would appear prudent to target lower degrees of peak inhibition. Studies are ongoing to determine the tolerability of 30–50% inhibition of ex vivo platelet aggregation to 20 μ*M* ADP when sustained over protracted periods of time.

A consideration important to long-term therapy with oral GP IIb/IIIa inhibitors is the "therapeutic window" provided by these agents. Agents that provide a continuous or sustained profile of platelet inhibition may be particularly likely to be associated with patient intolerance when high peak levels of platelet inhibition are targeted. Variations in renal blood flow, hydration status, and drug–drug interactions may exaggerate the degree of platelet inhibition and precipitate a bleeding event. For example, simply ingesting a meal can reduce xemilofiban and orbofiban absorption. Concomitant therapy with cimetidine can alter the pharmacodynamic profile of xemilofiban to resemble that of orbofiban, a much longer acting GP IIb/IIIa inhibitor.

Given the interpatient variability observed in drug level and degree of platelet inhibition, another potential strategy for dosing any IIb/IIIa antagonist is to monitor the degree of platelet inhibition or drug level achieved in individual patients and adjust

the dose to a target level, as is currently done with anticoagulant therapy. By avoiding very high levels of platelet inhibition, bleeding complications might be reduced. Such monitoring may be accomplished with a rapid bedside assay for platelet inhibition. An alternate strategy for adjusting the dose of an oral IIb/IIIa inhibitor is to initiate therapy with a fixed dose and to lower the dose if the patient experiences minor bleeding. These dosing strategies may improve the overall safety profile of these potent platelet antagonists.

Although synergy for inhibition of collagen induced platelet aggregation exists between aspirin and oral GP IIb/IIIa inhibitors as previously described, the nature and extent of interaction with other nonsteroidal antiinflammatory agents are not known. Thus, "pushing the therapeutic envelope" by targeting protracted and profound levels of platelet inhibition with oral GP IIb/IIIa blocking agents will likely "push the tolerance envelope" as many of the abovementioned concomitant influences cannot be controlled in a large and variable patient population.

Another concept pertinent to oral GP IIb/IIIa inhibitor therapy for patients with ACS or percutaneous coronary intervention is to balance potential benefit in reducing ischemic events with the risk of hemorrhage, i.e., treat patients with a higher degree of platelet inhibition early in the course of an ACS (as well as during or immediately following coronary intervention) followed by a lower level of inhibition for prolonged secondary prevention. This strategy would match the degree of inhibition with the overall risk for recurrent ischemic events, thus counterbalancing any increased bleeding risk associated with higher levels of platelet inhibition.

The optimal pharmacodynamic profile(s) for platelet inhibition to achieve long term clinical benefit has not been determined. Despite concerns that complete recovery of platelet function between doses of GP IIb/IIIa inhibitor might precipitate thrombosis, no excess of ischemic events was observed in patients receiving variable doses of xemilofiban in the ORBIT trial on a twice or three times daily regimen. The concomitant administration of aspirin to these patients and the synergism provided may have been protective in the "troughs" of GP IIb/IIIa inhibition. Furthermore, concerns that cyclic inhibition of platelet function might not provide "optimal" suppression of cardiovascular ischemic events is assuaged by the ORBIT trial clinical follow-up. A striking trend for reduction in the composite cardiovascular event rate at 90 d in patients receiving the highest dose (20 mg) of xemilofiban tested (11% placebo, 5% xemilofiban; $P = 0.04$) was observed *(35,37)*.

Two important dosing issues that must be definitively evaluated in future trials include: which pharmacodynamic profile (cyclic vs continuous) of platelet inhibition provides optimal clinical benefit, especially when high levels of platelet inhibition are targeted, and the potential for extended clinical benefit to follow a more limited duration of pharmacotherapy. The concept that a brief duration of platelet and arterial passivation may beget a longer duration of clinical benefit is supported by a recent analysis of the PARAGON trial 6-mo follow-up *(50)*. In this trial, 72 h of therapy with low dose lamifiban, a parenteral GP IIb/IIIa inhibitor in combination with heparin was associated with late clinical benefit at 6-mo follow-up *(51,52)*. Ongoing phase III trials, including OPUS-TIMI 16, EXCITE, and SYMPHONY are testing several of these dosing strategies and will no doubt add considerably to our knowledge of this important new class of drugs.

SPECIFIC ISSUES

Oral Therapy Prior to Coronary Intervention

Clinical investigation is ongoing to test the strategy of oral GP IIb/IIIa blockade prior to percutaneous coronary intervention. Both DMP 754 (DuPont Merck: GAP trial) and xemilofiban (G.D. Searle; EXCITE trial) are being administered in this fashion with percutaneous intervention performed 30–90 min following initial dose of drug. As peak blood level and platelet inhibition following the initial dose of xemilofiban occurs 3–4 h following drug administration, coronary intervention is being performed at submaximal levels of inhibition as illustrated in Fig. 11. Whether levels of platelet inhibition less than those achieved (≥ 80%) during trials of parenteral GP IIb/IIIa blockade can be expected to confer similar degrees of benefit remains to be determined *(53–55)*. Studies in animals suggested that maximum benefit of GP IIb/IIIa inhibition occurs when the degree of platelet inhibition achieved exceeds 80% *(56,57)*. In clinical studies, abciximab was shown to reduce major cardiovascular ischemic events 1 mo following coronary angioplasty by 35% in the EPIC trial *(20)* and 56% in the EPILOG trial *(21)*. The degree of platelet inhibition achieved by abciximab was evaluated in a separate trial. Abciximab, in currently recommended doses, was shown to inhibit 20 μM ADP-induced platelet aggregation by 76% on average (25th, 75th percentiles 65%–90%). For unstable angina, the doses of tirofiban and eptifibatide used in the PRISM-PLUS and PURSUIT trials, respectively, targeted similar levels (approximately 80%) of platelet inhibition *(25,26)*. However, uncertainty exists as to whether lesser degrees of platelet inhibition may also be beneficial. For example, the IMPACT II study *(22)* showed a strong trend toward the reduction of recurrent ischemic events after coronary angioplasty following a dose of eptifibatide that achieved only 50–60% platelet inhibition. Thus, it remains to be determined whether 80% inhibition is needed as a "threshold level" to achieve clinical benefit with GP IIb/IIIa inhibitors or whether there is graded benefit across a wider range of platelet inhibition.

In addition, significant logistical challenges are posed by oral therapy in a busy catherization laboratory where *ad hoc* intervention at the time of diagnostic angiography is common. Clearly, if a predictable, high degree (≥ 80%) of platelet inhibition of desired in a time-efficient manner, the most likely scenario would involve the initial administration of an intravenous GP IIb/IIIa blocking agent. Another pressing and potentially life-threatening clinical issue involves emergency coronary bypass surgery following failed percutaneous intervention after oral GP IIb/IIIa blockade has been administered *(31)*. As removal of these agents involves renal clearance, changes in renal perfusion induced by ischemic left ventricular dysfunction, hypotension, or cardiopulmonary bypass may increase blood levels and prolong drug half-life. Unlike parenteral therapy with small molecule inhibitors, oral GP IIb/IIIa therapy creates a potential gastrointestinal depot and tissue reservoir, which can result in continued absorption and recirculation of active drug. For these reasons, platelet transfusion is ineffective in reversing the platelet dysfunction that follows oral GP IIb/IIIa therapy *(58)*. In addition, hemodialysis may not remove adequate quantities of drug to facilitate hemostasis. Charcoal hemoperfusion appears to be the most efficient method for removal of xemilofiban with a clearance of approximately 200 mL/min (R. Anders, Aziz Kareem, GD Searle: personal communication). The prospect of safely performing emergency coronary bypass

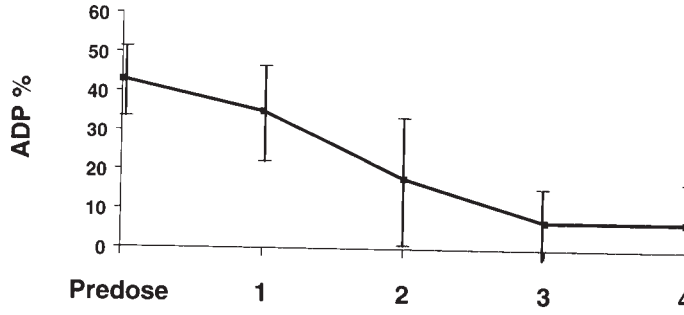

Fig. 11. Ex vivo inhibition of platelet aggregation in response to 20 μM ADP measured over time following administration of the first dose of study drug in the EXCITE trial. Peak inhibition of platelet aggregation following the first dose of study drug occurs at 3–4 h.

surgery or even vascular repair may thus require emergency charcoal hemoperfusion to facilitate to hemostasis and minimize transfusion requirement. A monoclonal antibody to xemilofiban that will rapidly reverse platelet inhibition has been developed and will hopefully enhance the safety of emergency surgical procedures (G.D. Searle: data on file).

Receptor Affinity

Most oral agents evaluated to date have been focused on blockade of the platelet GP IIb/IIIa receptor. Recent data have suggested that αvβ3 (vitronectin) receptor blockade may prevent smooth muscle cell migration and proliferation (restenosis) *(59)* and dual receptor blockade (αvβ3 and GP IIb/IIIa) may be more effective in inhibiting platelet prothrombinase and platelet supported thrombin generation *(60)*, an important pathophysiologic mechanism of ACS. The potential exists for αvβ3 blockade alone or in combination with GP IIb/IIIa blockade to provide specific clinical benefit. New orally active agents with dual receptor affinity are entering clinical testing (XV 454, DuPont Merck) *(61)*.

Monitoring Therapy

Many factors contribute to variability in response to oral GP IIb/IIIa blocking agents. These agents exhibit differences in absorption, bioavailability, half-life, metabolism, active metabolite production, and excretion. In addition, patients vary with respect to platelet count, GP IIb/IIIa receptor density and affinity, platelet mass, and basal platelet function. These factors are further complicated by potential drug–drug interactions that may cause further variability in the degree of platelet inhibition. The advantage of a point-of-care assessment of platelet function is apparent *(62,63)*. A rapid, "bedside" assay that closely correlates with standard aggregometry and receptor blockade could facilitate dose adjustment within minutes to enhance both safety and efficacy *(64)*. Considering the multiple concurrent variables influencing blood level, degree of platelet inhibition and thus, risk for bleeding events, these devices will likely play an integral role in oral GP IIb/IIIa blockade therapy. The correlation between a point-of-care rapid platelet function assay (RFPA) device and standard aggregometry is shown in Fig. 12.

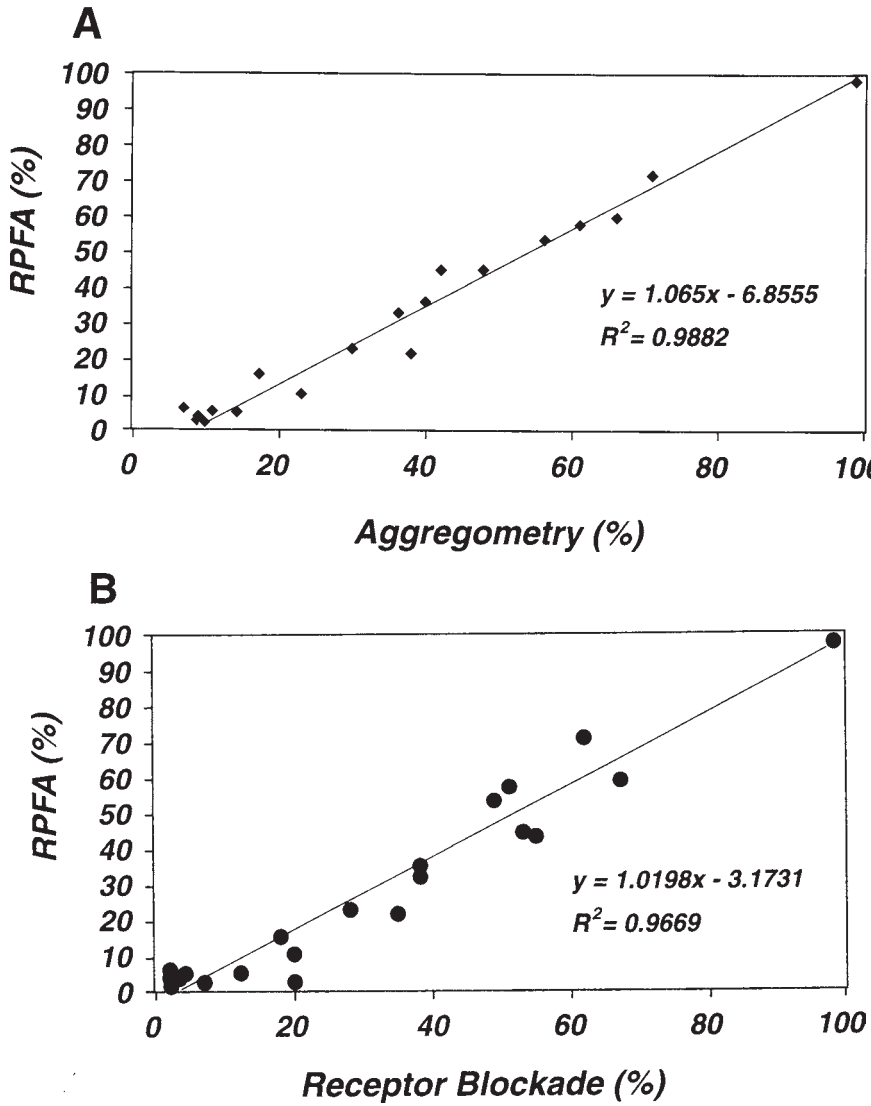

Fig. 12. Correlation between the Rapid Platelet Function Assay (RPFA; Accumetrics, Inc., San Diego, CA) and **(A)** standard aggregometry and **(B)** standard measurement of platelet GP IIb/IIIa receptor blockade. A close correlation was observed.

FUTURE QUESTIONS

Many questions remain for oral GP IIb/IIIa blocking therapy. What degree of platelet inhibition and over what duration of time will be clinically tolerated, or more importantly, will be required to achieve clinical benefit remains to be determined. The optimal pharmacodynamic profile for platelet inhibition (continuous vs cyclic) to enhance clinical tolerance and still suppress vascular ischemic events is not known. What duration of treatment (days, months, years, or lifetime) will be required? Can extended long-term benefit follow a more limited duration of therapy? How should therapy be monitored and

at what intervals should monitoring be performed. Hopefully, many of these questions will be answered by the results of ongoing clinical trials.

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15 Cerebrovascular Aspects of Glycoprotein IIb/IIIa Inhibitors

Cathy A. Sila, MD

Contents

INTRODUCTION

The cerebrovascular complications of cardiac interventional trials are comprised of heterogeneous groups of ischemic and hemorrhagic brain injuries. These occur with varying frequencies determined by the characteristics of the patient population under study, the procedures involved and the combination and intensity of antitplatelet, antithrombotic, and fibrinolytic agents employed. Unraveling this complex interplay of factors contributing to all stroke subtypes is important not only to improve the overall outcome for patients with coronary disease, but also to develop successful treatments for patients with occlusive cerebrovascular disease.

CEREBROVASCULAR COMPLICATIONS OF GP IIb/IIIa RECEPTOR INHIBITORS

Cerebral Infarction Related to Underlying Cardiac Presentation

In the prethrombolytic era, cerebral infarction (nonhemorrhagic stroke, ischemic stroke) followed 0.8–5.5% of acute myocardial infarctions (MI) *(1)*. Embolism of cardiac thrombus as a mechanism of cerebral infarction is supported by features including a predominance of middle cerebral artery territory involvement, relationship to cardiac rhythm and ventricular function, and declining risk over time. With the introduction of thrombolysis for acute MI, the risk of cerebral infarction dropped to 0.1–1.3% *(1)*. The

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timing of cerebral infarction is similar whether thrombolysis is used or not with virtually all events occurring within the first month, 1/3 in the first day, 1/3 in the first week, and the remaining 1/3 clustered in the second week *(2)*. Risk factors for cerebral infarction include advanced age, higher heart rate, history of prior cerebrovascular events, diabetes mellitus, previous angina, history of hypertension, worse Killip class, coronary angiography, bypass surgery, and atrial fibrillation/flutter *(3)*. Of the 247 patients with nonhemorrhagic stroke from the GUSTO-I trial, 17% died and another 40% remained disabled at 1 mo *(4)*.

Cerebral Infarction Related to Cardiac Interventional Procedures

Cerebral infarction complicates 0.1–1.0% of cardiac catheterization or coronary percutaneous transluminal angioplasty (PTA) procedures although even without specific therapy, half resolve within 48 h *(5)*. The vertebral-basilar circulation accounts for 60–70% of focal deficits presenting as confusion, visual field defects, and brainstem signs whereas the carotid circulation accounts for 30–40% with combinations of hemiparesis, hemisensory deficits, dysphasia, and retinal ischemia *(6)*. The mechanism is attributed to cerebral embolism during manipulation of the guidewire or catheter flushing, although the propensity to involve the vertebral-basilar circulation has never been adequately explained *(7)*. As the confusional states can mimic medication effects and metabolic encephalopathy, the true incidence may be underestimated *(8)*.

The occurrence of cerebral infarction or nonhemorrhagic stroke has been variably recorded in trials of glycoprotein (GP) IIb/IIIa receptor inhibitors with little insight into their specific causes. Potential mechanisms of cerebral infarction include cardioembolism related to the presenting cardiac condition, the catheterization procedure, preexisting cerebrovascular lesions that were aggravated by hemodynamic instability, or other unrelated causes of stroke that occurred within the 30-d time frame. Aggregate data from four large phase III trials (Table 1) using abciximab or eptifibatide with aspirin and various heparin regimens during coronary artery angioplasty or atherectomy reported nonhemorrhagic stroke in 11/3598 (0.3%) of placebo-treated patients and 20/6568 (0.3%) of patients who received active drug *(9–12)*. These low rates of cerebral ischemic complications may be attributable to the technical expertise of study interventionalists and the protective effect of combined antiplatelet and antithrombotic therapy, but the numbers are too small to address a specific protective role of abciximab or eptifibatide.

Intracranial Hemorrhage Following Therapies for Coronary Artery Disease

Intracranial hemorrhage after acute MI treated with aspirin or heparin is typically because of hemorrhagic transformation (or conversion) of an initially "bland" cerebral infarct. Although the clinical use of the terms bland or ischemic and hemorrhagic infarct refer to the absence or presence of blood within the region of infarction seen on computed tomographic (CT) scans of the brain, histological examination frequently detects microscopic extravasation of erythrocytes that results from ischemic endothelial damage to capillary blood vessels. The risk of hemorrhagic transformation of a cerebral infarct is increased in the presence of any antiplatelet or antithrombotic therapy and increases with the intensity of such therapy. Risk factors for hemorrhagic transformation include advanced age, hypertension, embolic mechanism, volume of cerebral infarct by

Table 1
GP IIb/IIIa Receptor Inhibitors: Cerebrovascular Events in Phase III Trials

	Nonhemorrhagic stroke		*Intracranial hemorrhage*[a]	
Agent	*Placebo*	*Active drug*	*Placebo*	*Active drug*
Abciximab				
EPIC	2/696 (0.3%)	5/1403 (0.4%)	2/696 (0.3%)	4/1403 (0.3%)
EPILOG	0/939 (0%)	2/1853 (0.1%)	0/939 (0%)	4/1853 (0.2%)
CAPTURE	2/635 (0.3%)	0/630 (0%)	1/635 (0.2%)	1/630 (0.2%)[b]
EPISTENT	1/809 (0.1%)	5/1590 (0.3%)	0/809 (0%)	0/1590 (0%)
Eptifibatide				
IMPACT II	7/1328 (0.5%)	13/2682 (0.5%)	1/1328 (0.1%)	3/2682 (0.1%)
PURSUIT	33/4696 (0.7%)	27/4679 (0.6%)	3/4696 (0.1%)	5/4679 (0.1%)
Tirofiban				
RESTORE	N/A	N/A	3/1070 (0.3%)	1/1071 (0.1%)
PRISM	N/A	N/A	2/1616 (0.1%)	1/1616 (0.1%)
PRISM-PLUS	N/A	N/A(1 fatal)	0/350 (0%)	0/681 (0%)
Aggregate	45/9103 (0.49%)	47/12,837 (0.37%)	12/12,139 (0.10%)	19/16,205 (0.12%)

[a]Includes intracerebral hemorrhage, other intracranial hemorrhage, and hemorrhagic transformation of nonhemorrhagic infarcts.
[b]Includes a stroke of unknown type.
N/A = not available.

neuroimaging or severity of clinical deficit, early anticoagulant therapy of large cerebral infarcts, use of bolus dosing of heparin and excessive prolongation of the activated partial thromboplastin time *(13–15)*. Most of these data are derived from studies using heparin in the setting of acute cerebral infarction and no consensus on the relative importance of these factors or their predictive value is available.

Thrombolytic therapy for acute MI reduces the incidence of all stroke, but significantly increases the proportion of hemorrhagic stroke subtypes *(1)*. With thrombolysis, the risk of hemorrhagic transformation of cerebral infarcts is increased and spontaneous intracranial hemorrhage including intracerebral, subdural, subarachnoid, and intraventricular hemorrhage can occur *(16)*. Hemorrhagic transformation of varying severity occurs in 30% of cerebral infarcts complicating coronary thrombolysis and in two-thirds is severe enough to produce neurological deterioration *(2)*.

The risk of intracranial hemorrhage in recent thrombolysis trials varies from 0.3 to 1.0% and is increased by higher dosages and combination therapy with aspirin and heparin *(17)*. Although two-thirds of thrombolysis-related intracranial hemorrhages are solitary lesions involving the deep nuclei or lobar white matter, there is great diversity with multiple hemorrhages in 15% and another 15–20% affecting the subdural space as well. These coagulopathy-related hemorrhages differ from spontaneous hemorrhages by neuroimaging features of blood-fluid levels in 80% and an overall mottled appearance that implies a fundamental difference in their genesis and evolution *(16)*. Although the risk of intracranial hemorrhage persists for days, most occur within 24 h and many are recognized during the infusion or shortly afterward. Risk factors for intracranial hem-

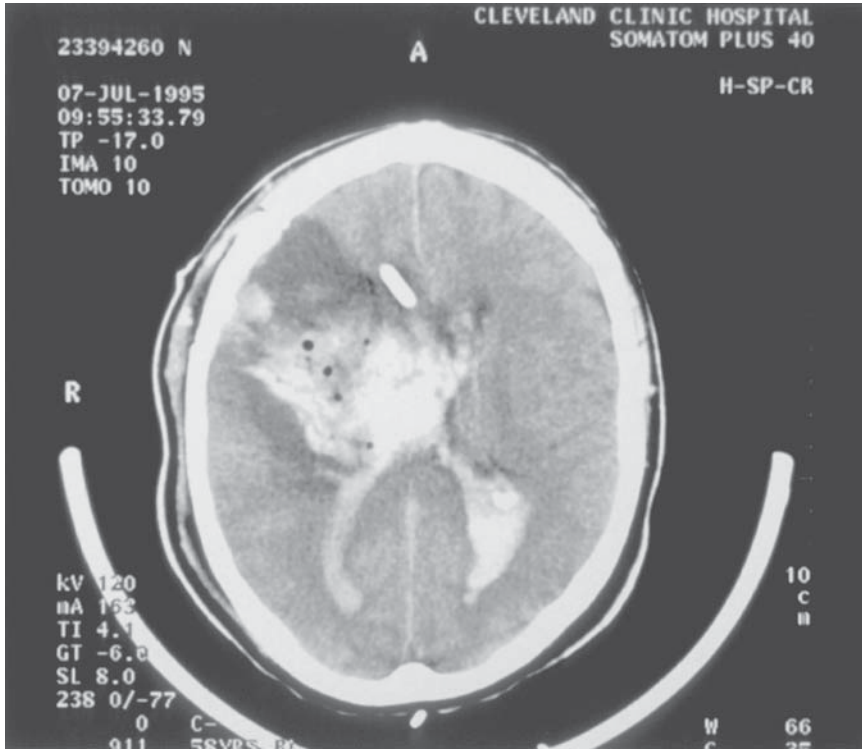

Fig. 1. Fatal intracerebral hemorrhage from the EPILOG trial.

orrhage include advanced age, hypertension, prior neurological disease, as well as the intensity of the coagulopathy induced by type, dosage, and concomitant therapies administered *(18)*. Amyloid angiopathy has been detected in neuropathologic specimens from several case reports *(19)*. As amyloid angiopathy is a degenerative, age-related phenomenon, rare before age 50, but common in octogenarians, its presence and associated cerebral vascular fragility may explain the increased cerebral hemorrhage risk with advanced age.

The fear of stroke complicating coronary thrombolysis emanates from the dismal outcome of the subset of patients with intracranial hemorrhage. Although 60% die and an additional 25% remain disabled, there still remains an overall benefit for thrombolysis produced by improved myocardial salvage and reduction in ischemic, thromboembolic stroke *(4,20)*.

Given this background, hemorrhagic stroke subtypes were specifically addressed in the safety analyses of trials of GP IIb/IIIa receptor inhibitors in coronary interventions *(21)*. Borrowing from past experience with thrombolysis trials, patients were excluded from entry if they had a history of stroke within the preceding 2 yr, intracranial or intraspinal surgery within 2 mo, or known intracranial neoplasm, arteriovenous malformation, or aneurysm. Aggregate data (Table 1) from nine large phase III trials of abciximab, eptifibatide, and tirofiban reported intracranial hemorrhage in 12/12,139 (0.10%) of control patients and 19/16,205 (0.12%) in patients who received the GP IIb/IIIa receptor inhibitor *(9–12,22–24)*. The intracranial hemorrhage category included

intracerebral hemorrhage, other intracranial hemorrhages, as well as hemorrhagic transformation of cerebral infarcts. All patients also received aspirin and various heparin regimens. Although overall bleeding rates were significantly lower for the weight adjusted heparin regimens in EPILOG and CAPTURE compared to the fixed-dose heparin regimen in EPIC, there are too few patients with intracranial hemorrhage in each group to permit analysis.

Although intracranial hemorrhagic events were rare with this combination therapy, the clinical profile is consistent with coagulopathy-related hemorrhage as illustrated by (Fig. 1) this case of a 58-year-old man with unstable angina and a recent non-Q-wave MI who received abciximab in the EPILOG trial. Dysarthria and weakness of his right limbs developed insidiously during drug infusion and within 2 h a herniation syndrome required emergent surgical evacuation. Analysis of limited neuropathological material did not demonstrate amyloid angiopathy or other underlying vascular abnormality.

Two small trials have investigated escalating doses of eptifibatide or abciximab in combination with rt-PA, aspirin, and heparin *(25,26)*. One fatal intracerebral hemorrhage (2%) occurred among 51 patients who received the highest eptifibatide dose tier. Although the numbers are too small to predict overall risk, it might be expected that escalating to profound platelet inhibition after thrombolysis will produce higher rates of intracranial bleeding than with current thrombolysis regimens and future studies should proceed with careful patient selection.

THERAPEUTIC POTENTIAL OF GP IIb/IIIa RECEPTOR INHIBITORS IN CEREBROVASCULAR DISEASE

Rationale for Antiplatelet Therapy in Cerebrovascular Diseases

Although ischemic stroke is largely because of atherothrombosis or thromboembolism, there are several stroke subtypes that can be distinguished by clinical profile, response to treatment, and rate of recurrence stroke. The proportion of stroke subtypes in a specific patient population under study varies, modified by age and race, as well as by imposed factors such as inclusion and exclusion criteria for clinical trials. In population studies, causes of ischemic stroke include extracranial atherosclerosis (<10%), intracranial atherosclerosis (<10%), small vessel or lacunar disease (25–30%), cardioembolism (20%), cryptogenic (30–40%), and other determined cause (<5%). The 30-d risk of recurrent stroke varies from 7 to 8% for the extracranial and intracranial atherosclerosis groups, 2–4% for cardioembolic, and <2% for the other subtypes *(27,28)*. Risk of recurrent events is highest in the period after the index event and decreases over time, and among patients with extracranial carotid atherosclerosis, the stroke risk also increases with the severity of carotid stenosis *(29)*. The rate of recurrent events observed in any population will be modified by the varying natural history of the underlying diseases in the patients at study, as well as the best medical therapies offered in addition to the agent under study, as illustrated by the broad range of 4–12% in recurrent stroke or death observed in the placebo groups. Although some clinical trials have been devoted to one subtype of ischemic stroke, such as severe carotid atherosclerosis and atrial fibrillation, most trials have selected an index event (TIA, transient ischemic attack; minor, or moderate stroke) as a starting point with little attention to documenting the underlying mechanism of the event. Given these limitations of stroke subtype mix,

shifting risk over time, and variable performance of placebo patients, it is difficult if not impossible to make meaningful indirect comparisons of efficacy among the various antiplatelet trials. These factors are largely responsible for the ongoing controversy regarding the optimum antiplatelet agent and dose for patients with cerebrovascular disease.

Despite all this, aspirin remains the standard preventive therapy for patients at risk for stroke. Its indications have grown from the initial FDA approval in 1980, which specified its use in men with TIAs "due to fibrin platelet emboli" based on a trial using 1300 mg daily *(30)*. In a metaanalysis of 31 randomized trials involving more than 29,000 patients (of which only 13 trials and 8689 patients were specifically entered for various cerebrovascular index events), aspirin at dosages from 300 to 1500 mg daily afforded a 23–24% relative risk reduction in the combined endpoint of stroke, MI, or vascular death *(31)*. The UK-TIA trial compared aspirin at 300 mg daily or 600 mg twice daily and could detect no difference in outcomes between the two dosage levels, but as the rate of gastrointestinal side effects was significantly lower in the low-dose group, the trend of lower aspirin dosages began *(32)*. Very-low-dose aspirin was investigated in the Scandinavian (SALT) trial where the 17% relative risk reduction of the combined endpoint of stroke and vascular death with aspirin 75 mg daily reached significance *(33)*. The Dutch TIA trial could not demonstrate a difference in the frequency of stroke, MI, or vascular death between aspirin dosages of 30 mg/d and 283 mg/d although major bleeding complications were slightly fewer *(34)*.

Aggregate data from several trials comparing the combination of aspirin plus dipyridamole to placebo have demonstrated a 31% relative risk reduction in the combined endpoint of stroke, MI, or vascular death, which was not statistically different from trials using aspirin alone and did not support the use of combination therapy *(31,35,36)*. The European Stroke Prevention Study II evaluated aspirin 50 mg, dipyridamole 400 mg, their combination, and placebo, selecting endpoints of stroke, or stroke and death. The relative risk reduction in the combined endpoint of stroke and death was 13% for low-dose aspirin alone, 15% for dipyridamole alone, and 24% in combination. For MI or vascular death, the combination was not superior to aspirin alone *(37)*.

The thienopyridine derivatives ticlopidine and clopidogrel inhibit platelet aggregation by selectively and irreversibly inhibiting the ADP-dependent activation of fibrinogen binding via the GP IIb/IIIa complex. The Canadian-American Ticlopidine Study (CATS) demonstrated a 34% relative risk reduction in recurrent stroke and 30% in the combined risk of stroke, MI, or vascular death in patients with moderate brain infarction given ticlopidine 250 mg twice daily vs placebo *(38)*. The Ticlopidine Aspirin Stroke Study (TASS) compared ticlopidine 250 mg twice daily to high-dose aspirin 650 mg twice daily in patients with TIAs or minor strokes and reported a relative risk reduction of 19% for recurrent stroke and 9% for the combined endpoint of stroke, MI, or vascular death *(39)*. Significant neutropenia (absolute neutrophil count <450) occurred in <2% of ticlopidine treated patients requiring hematologic monitoring within the first three months of therapy. Recently, 60 cases of thrombotic thrombocytopenic purpura related to ticlopidine were reported, abrupt in onset and 80% within the first month of therapy *(40)*. This has further raised concern about the safety and efficacy profile of this agent. The Clopidigrel Aspirin Prevention of Recurrent Ischemia (CAPRIE) study compared clopidogrel 75 mg daily to aspirin 325 mg daily in patients with cerebrovascular, cardio-

Table 2
Antiplatelet Agents for Cerebrovascular Disease

Trial	*No. pts*	*f/u*	*Index event*	*Endpoints*	*Rx regimens*	*RRR*
SALT	1360	2.7 yr	TIA, stroke	S/M/VD	asa 75 mg, vs P	17%
Dutch	3131	2.6 yr	TIA, stroke	S/M/VD	asa 30 mg, 283 mg	NS
UKTIA	2435	4 yr	TIA, stroke	S/M/D	asa 300 mg, 1200 mg, vs P	18%
ESPS II	6602	2 yr	TIA, stroke	S/D	asa 50 mg	13%
					Dipyridamole 400 mg	15%
					Combination vs P	24%
CATS	1072	2 yr	Stroke	S/M/D	Ticlopidine 500 mg vs P	23%
TASS	3069	3.3 yr	TIA, stroke	S/D	Ticlopidine 500 mg, asa 1300 mg	12%
CAPRIE	19,185	1.9 yr	MI, stroke, PAD	S/M/VD	Clopidogrel 75 mg, asa 325 mg	9%

S= stroke, M= myocardial infarction, VD= vascular death, D= all cause death, PAD= peripheral arterial disease, P= placebo, asa= aspirin, RRR= relative risk reduction, TIA= transient ischemic attack.

vascular, or peripheral vascular index events and demonstrated a 9% relative risk reduction in the combined endpoint of stroke, MI, or vascular death *(41)*. As hematologic problems have not been encountered with clopidogrel, it has largely replaced ticlopidine as an aspirin alternative.

As summarized in Table 2, therapeutic options abound, but with limited efficacy, more effective strategies are warranted. Chronic oral therapy with the GP IIb/IIIa receptor SB 214857 was evaluated a dose escalation safety and feasibility study and included 169 patients with TIA or stroke of less than 6 mo duration. Although patients noted minor bleeding, particularly easy bruising in a dose-related fashion, none experienced thrombocytopenia or any intracranial bleeding and a large, phase III trial is planned (AM Lincoff, Cleveland Clinic, personal communication). True progress in understanding the optimal preventive strategies for patients with cerebrovascular disease begs guidance from clinical trials specifically addressing stroke subtypes and the specific mechanisms of recurrent events, and correlating clinical outcomes with accurate and reliable in vitro measurements of platelet function.

Angioplasty and Stenting for Patients with Cerebrovascular Disease

Angioplasty and stenting techniques are promising treatment alternatives for cerebral occlusive lesions but experience thus far is limited. Most of the reported case series involve the treatment of extracranial carotid artery stenosis *(42)*. Although clinical trials have been in development for several years, there remains no standard approach to patient selection, procedure, or use of concomitant antiplatelet, or antithrombotic therapy. Acute procedure-related thrombosis of extracranial vessels appears to be low, in the range of 1%, likely influenced by the large vessel size. However, thrombosis may be more of an issue in the intracranial carotid and basilar artery where vessel diameters range 2–5 mm.

Governed by concerns of excessive intracranial hemorrhage risk in patients with established cerebrovascular disease, experimentation with GP IIb/IIIa inhibitors has moved slowly. The first reported case was a success born out of desperation *(43)*. A patient with acute basilar artery thrombosis and stroke-in-evolution was initially treated

Table 3
Current Experience with Abciximab for Cerebrovascular Angioplasty[a]

Indication for treatment	*No. patients*	*Procedural complications*
Intracranial vertebral-basilar stenosis with recent TIA or minor stroke	3	One minor ischemic stroke
Intracranial vertebral-basilar thrombosis with acute stroke	2	Two persistent moderate ischemic strokes
Intracranial carotid stenosis, asymptomatic	2	One dissection with minor ischemic stroke
Extracranial carotid stenosis, asymptomatic, bilateral	2	One hemodynamically unstable during procedure

[a](J Perl, Cleveland Clinic, personal communication.) All patients received abciximab (0.25 mg/kg IV bolus followed by 10 mcg/kg infusion for 12 h) with aspirin 325 mg qd and/or ticlopidine 250 mg bid.

with intraarterial thrombolysis and basilar PTA. Basilar artery rethrombosis recurred after each of several angioplasty attempts and patency was maintained only after the addition of abciximab.

Percutaneous transluminal angioplasty of symptomatic intracranial vertebral-basilar stenoses was described in four additional patients *(44)*. All patients received abciximab (0.25 mg/kg bolus followed by a 10 mcg/min infusion for 12 h) prior to PTA. Three patients had recurrent transient cerebral ischemic events (TIA) failing anticoagulant therapy and one patient was neurologically unstable with acute midbrain and cerebellar infarcts and received intraarterial thrombolysis prior to PTA and abciximab. In this small series, the patient with the stroke-in-evolution had no improvement and died, but there were no procedural strokes or intracranial hemorrhage. At 3 mo, two of the survivors had persistant TIAs with restenosis.

Anecdotal experience from the Cleveland Clinic (Table 3) includes eight additional patients with various high-risk cerebral occlusive lesions who underwent percutaneous transluminal angioplasty with abciximab, aspirin, and ticlopidine, but without heparin infusions. There were no systemic or intracranial hemorrhages in this small group, but two patients suffered a minor ischemic stroke related to the procedure and one patient was hemodynamically unstable during the procedure (J Perl, Cleveland Clinic, personal communication).

This small experience illustrates how safety concerns have limited the initial cerebrovascular use of the GP IIb/IIIa receptor inhibitors to extremely high-risk cerebrovascular patients with promising initial results. As angioplasty and stenting technology for treatment of cerebrovascular disease advances, it is likely that modifications of therapeutic approaches piloted in coronary angioplasty will emerge and the focus will shift somewhat from the overwhelming concerns about intracranial hemorrhage to address procedural success and long-term issues of vessel patency.

Treatment of Acute Cerebral Infarction

Proven treatments for acute cerebral infarction includes aspirin and IV rt-PA. Aspirin has been shown in two large trials to be effective in reducing death and recurrent stroke when given within 24 h. The International Stroke Trial and Chinese Acute Stroke Trial

combined experience with more than 40,000 patients demonstrated that 160–300 mg aspirin begun within 48 h of acute ischemic stroke significantly reduced absolute early mortality by 0.5–0.6% and recurrent ischemic stroke by 0.5–1.1%, while increasing the risk of symptomatic hemorrhagic stroke by a nonsignificant 0.1–0.2% *(45,46)*.

The International Stroke Trial also evaluated combination therapy with subcutaneous heparin (25,000 U/d or 10,000 U/d) with or without aspirin 325 mg/d, and placebo. Heparin was not effective in reducing the rates of death and nonfatal recurrent stroke in that population because the rate of hemorrhagic stroke (1.2–0.4%) was increased and it offset the significant early reduction in recurrent ischemic stroke (2.9–3.8%) *(46)*. Although these trials have been subjected to many subset analyses and criticized for their somewhat superficial data collection, they have fostered a shift away from heparin to aspirin for patients with acute ischemic stroke. More focused studies are warranted to address what subset of patients experienced benefit from heparin and ways to reduce hemorrhagic transformation risks.

Progress in developing thrombolytic strategies for acute cerebral infarction has been methodical, but slow and often thwarted by an increased risk of intracranial hemorrhage related to petechial or confluent hemorrhagic transformation of the infarct. The NINDS trial describes the only FDA-approved effective thrombolytic regimen for acute stroke. Weight-adjusted rt-PA, 0.9 mg/kg intravenously, 10% given as a bolus, maximum 90 mg, was given within 3 h of symptom onset under a protocol that prevented the concomitant use of any antiplatelet or antithrombotic agents for the first 24 h *(47)*. Symptomatic and fatal intracranial hemorrhage was significantly increased from 0.6% in placebo-treated patients to 6.4% for those receiving rt-PA. Elderly patients over age 77 and those with a severe neurological deficit (NIH Stroke Scale Score >22) were at increased risk for intracranial hemorrhage and for those patients the benefit-to-risk ratio for therapy was only marginally positive.

Three trials of intravenous streptokinase in acute ischemic stroke demonstrated an excess mortality due to fatal intracranial hemorrhage complications *(48–50)*. One of these trials addressed the combination of aspirin and streptokinase and was stopped because of an excessive risk of early fatality at 34% in patients receiving combination therapy, 10% because of symptomatic cerebral hemorrhage *(50)*.

In a dog model of carotid thrombosis, the addition of a short-acting platelet GP IIb/IIIa receptor inhibitor (TP-9201) to thrombolysis was successful in preventing rethrombosis without hemorrhagic complications, but this has not been tested in acute cerebral infarction *(51)*.

Although time constraints prevent extensive testing to document the mechanism of cerebral artery occlusion prior to treatment, embolic occlusion from a proximal artery or the heart was the most common mechanism reported in a study of angiographically documented acute cerebral occlusions *(52,53)*. The size of vessels involved in cerebral infarction ranges from 100–400 μm perforators that produce lacunar infarctions to 6–7 mm cervico-cephalic vessels capable of producing massive hemispheric infarction. Although local atherothrombosis of an intracranial stenosis accounts for perhaps less than 10% of acute occlusions, these are the lesions that would be expected to behave similarly to coronary lesions where the lessons learned in coronary interventional trials could be extrapolated. Given these anatomical and mechanistic considerations, it might be expected that reocclusion after thrombolysis in the treatment of acute ischemic stroke will be less of a problem than for acute MI, but this important topic has not been adequately addressed.

Despite the heterogeneity of acute cerebral occlusions, platelet aggregation is likely to be a common theme for many lesions. To address the potential utility of profound platelet inhibition in acute cerebral infarction, a multicenter dose-ranging double-blind randomized trial began in February 1998 to study abciximab in acute ischemic stroke of less than 24 h duration. If intracranial hemorrhage is not prohibitive, efficacy trials and expansion into combinations with thrombolytic therapy should soon follow.

CONCLUSIONS

A heterogeneous group of ischemic and hemorrhagic cerebrovascular events complicate coronary interventional trials governed largely by risk factors for cardioembolism and the combination and intensity of antiplatelet, antithrombotic, and fibrinolytic agents employed. Cerebral infarction is typically because of embolism from cardiac chambers related to poor ventricular function or rhythm disturbances or from embolism of catheter clot or atherosclerotic material catheterization. Hemorrhagic complications include the hemorrhagic transformation of cerebral infarcts, as well as intracerebral and other intracranial hemorrhages. The complex interplay of factors is nicely illustrated by the experience of coronary thrombolysis for acute MI where a reduction in total stroke, mostly thromboembolic cerebral infarction, is accompanied by an increase in symptomatic intracranial hemorrhages. Glycoprotein IIb/IIIa receptor inhibitors in combination with aspirin and heparin in various trials of coronary interventions do not appear to increase cerebrovascular events of either type, but the rare cases of intracranial hemorrhage are consistent with coagulopathy-related bleeding. Their consistent benefits in the setting of coronary PTA have spawned a preliminary experience with high-risk cerebral PTA with anecdotal but promising results.

In the treatment of acute cerebral infarction, hemorrhagic transformation can occur spontaneously, but is exaggerated in the setting of any agent that alters coagulation. Aspirin has minimal benefit with minimal risk, but the modest benefit conferred by heparin was offset by an increase in intracranial hemorrhage. The 30% increase in good outcome seen with intravenous rt-PA in acute stroke of less than 3 h was accomplished despite a 6.4% risk of symptomatic brain hemorrhage. The safety and feasibility of profound platelet inhibition in the treatment of acute ischemic stroke is currently under investigation.

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16 The Future of Glycoprotein IIb/IIIa Inhibitors

Robert M. Califf, MD and David F. Kong, MD

CONTENTS

INTRODUCTION

Medicine has entered a new era characterized by dramatic advances in the understanding of disease biology and a new focus on the organization of medical practice. The identification of receptors, agonists, and antagonists permits development of therapy targeted against specific biological processes. Over the next several decades, the world will reap the dividends from government and industry's recent huge investments in biomedical research. As a result, medical treatments for chronic diseases will increase exponentially. Simultaneously, the administration of medical care will increasingly organize into delivery systems that focus upon managing health care costs rather than advancing innovative therapy.

When Dr. Barry Coller and his predecessors sought an understanding of the platelet glycoprotein (GP) IIb/IIIa receptor *(1)*, clinical applications were not a consideration. However, once these pioneers had identified the receptor and developed an antibody against it, attention rapidly turned to its physiologic function and therapeutic potential. In the future, the number of molecular targets without a known medical application will increase. Many therapeutic agents will emanate from the human genome project and computer-based applications that simulate receptors, genes, and gene products. It will be increasingly possible to model receptors and synthesize agonists and antagonists

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before their biological function is known. The accumulated GP IIb/IIIa inhibitor experience should serve as an educational template for this more complex future.

In the 1980s, health care providers would have embraced any major therapeutic advance after studies of a few hundred patients, and would have eagerly applied it to a variety of indications. The continuing rigorous evaluation of the GP IIb/IIIa inhibitors demonstrates the world's changing approach to new cardiovascular therapies. Today, regulatory authorities apply immensely higher standards for proof of safety and efficacy, requiring definitive evidence that a new agent does not increase mortality in several thousand patients. Health care systems are concerned not only about the potential benefits of new therapy, but also about incremental costs. When compared to aspirin, one of the most effective and inexpensive therapies ever devised, the new antiplatelet agents face a challenging standard. As a result, clinical trials of GP IIb/IIIa antagonists have randomized more than 50,000 patients, yet only a few agents are currently marketed.

PATHOPHYSIOLOGY OF DISRUPTED ARTERIAL SURFACES AND THERAPEUTIC TARGETS

Our understanding of the pathophysiology and clinical outcomes of thrombotic cardiovascular disorders is evolving rapidly *(2)*. In order to envision the future of GP IIb/IIIa antagonists, it is necessary to comprehend the pathophysiologic rationale for their application and the quantitative measures of their effect on clinical outcomes.

Percutaneous Intervention

For the past three decades, the animal model for atherosclerosis has combined abrasion of the arterial endothelial surface with a high cholesterol diet. Developers of these models have increasingly recognized the importance of the thrombotic response to vascular injury. In many ways, balloon dilatation and mechanical debulking initiate a similar biological reaction, providing human model for cellular proliferation and thrombosis-mediated vascular occlusion. In many situations, platelets protect areas of vascular injury, but when this process becomes exuberant it causes vessel occlusion or embolic episodes. Thus, the acute thrombotic reaction to percutaneous coronary intervention is an ideal target for platelet inhibitors. Preventing the aggregation of platelets is an obvious approach to reducing complications.

With current therapy, vessels close abruptly in 2–14% of patients, depending upon multiple factors. Lesions with long length, tight stenosis, and intraluminal thrombus are more likely to occlude acutely. These factors become more important in small arteries, which are also less amenable to stenting. Indeed, several studies of abrupt closure have associated the AHA/ACC classification scheme of adverse characteristics (modified by Ellis et al.) with higher risk *(3,4)*. Each adverse characteristic typically adds a relative risk less than 2.0, limiting the usefulness of individual characteristics for stratifying risk and evaluating new therapy. Myler suggested that advances in equipment and technique may make the AHA/ACC classification scheme less applicable to current clinical practice *(5)*. However, combinations of adverse characteristics can be used to identify high-risk patients.

Although coronary stenting clearly improves vessel patency, 2–6% of patients still experience abrupt closure in trials of stenting. Most of these trials have focused on experienced, relatively high-volume operators treating patients with large vessels and

single, discrete lesions. The rate of abrupt closure is certainly higher in clinical practice, particularly when operators with less experience and skill approach complex lesions in smaller vessels. The EPISTENT trial confirmed that, in the absence of GP IIb/IIIa inhibition, 10.8% of stented patients experience death, myocardial infarction (MI), or urgent revascularization by 30 d *(6)*.

Between 8 and 30% of patients undergoing percutaneous intervention (angioplasty, atherectomy, stenting, etc.) develop myocardial necrosis with elevation of creatine kinase MB. The majority of these events can be identified by angiography, such as side-branch occlusion or abrupt vessel closure. However, a substantial number of subclinical events occur. Although no one denies that elevated enzymes represent myocardial necrosis and higher mortality, it remains hotly debated whether the additional mortality results from periprocedural myocardial injury. Alternatively, evidence of myocardial necrosis during a procedure may simply identify high-risk patients with more severe, diffuse coronary disease.

When a coronary stent is used to improve an acute result, intractable in-stent restenosis occurs in 10–25% of patients. Although this complex process is multifactorial, it is increasingly clear that growth factors elaborated by platelets stimulate the neointimal proliferation that ultimately causes restenosis. In series that have been reported to date *(7)*, 20–30% of patients had in-stent restenosis and the likelihood of recurrence was 43%.

A proportion of patients develop new or recurrent lesions in the longer term after percutaneous intervention (with or without stenting). This problem of restenosis continues to plague percutaneous revascularization. Table 1 presents commonly observed restenosis rates, both asymptomatic and symptomatic. Commonly accepted risk factors for restenosis include diabetes, unstable ischemic syndromes, and a tight or long lesion before the procedure. Failure to achieve a wide lumen at the time of revascularization is associated with the greatest risk of restenosis. Many other factors have been examined, including traditional risk factors for atherosclerosis, but the results have been either inconclusive or frankly negative.

The role of platelet activation in the process of restenosis remains controversial. Mechanical factors contribute significantly to restenosis. The buttressing effect of intracoronary stents addresses these factors by preventing vascular recoil and producing a wider lumen initially. However, cellular proliferation represents another substantial component of restenosis. Growth factors synthesized by platelets, including platelet derived growth factor, tissue growth factor beta, epidermal growth factor (EGF), and interleukin-1 (INT-1), are thought to be important components of this complex and multifactorial response to injury.

For these reasons, percutaneous intervention has been the major initial target of GP IIb/IIIa inhibitor treatment. The intense activation of platelets at a recognizable moment in time and the high event rates provide a rich environment for ancillary therapeutic intervention.

ST Elevation ACS

Acute epicardial vessel occlusion with myocardial necrosis remains a major public health issue. Primary relief of total occlusions with fibrinolytic therapy or percutaneous intervention can lower mortality and morbidity substantially, but the residual death and disability rates remain unacceptable. The 30-d mortality rate remains 7–9% for patients treated with reperfusion therapy and at least 15% among patients without attempted

Table 1
Clinical Restenosis Trials with Angiographic Follow-up

Study	n	Follow-up n (%)	Therapy	Restenosis definition	Restenosis rate Active (%)	Restenosis rate Control (%)	Odds ratio (95% CI)
Aspirin vs placebo							
Schwartz	376	249 (66)	990 mg/dipyridamole 225 mg	>50% stenosis†	38	39	0.96 (0.58–1.61)
White	157	111 (71)	650 mg/dipyridamole 225 mg vs ticlopidine	>70% stenosis*†	18	20	0.83 (0.32–2.16)
Finci	40	29 (73)	100 mg	>50% stenosis*	33	14	3.00 (0.52–24.28)
Savage	752	384 (76)	325 mg	≥50% stenosis†	44	57	0.58 (0.37–0.90)
Taylor	216	212 (98)	100 mg	≥50%, loss of gain†	35	43	0.71 (0.41–1.24)
Aspirin/dipyridamole vs placebo							
Chesebro	207	171 (83)	975 mg/dipyridamole 225 mg	Mean change in MLD (mm)†	1.33	1.34	
High- vs low-dose aspirin							
Dyckmans	203	86 (42)	1500 mg vs 320 mg	>50% stenosis*	21	31	0.65 (0.24–1.72)
Mufson	453	166 (37)	1500 mg vs 80 mg	>50% stenosis*	51	47	1.15 (0.63–2.13)
Schanzenbacher	79	79 (100)	1000 mg vs 100 mg	Revasc at 6 months	21	18	1.22 (0.39–3.85)
Kadel	188	173 (92)	1400 mg vs 350 mg	N/A	31	21	1.75 (0.89–0.35)
Ticlopidine vs placebo							
White	157	119 (76)	750 mg vs ASA/ dipyridamole	>70% stenosis*†	29	20	1.61(0.70–3.88)
Bertrand	266	244 (92)	500 mg/d	Loss >50% gain*	50	41	1.44 (0.87–2.39)
Kitazume	189	189 (100)	200 mg	>50% stenosis*	27	38	0.61 (0.33–1.13)
Thromboxane A2 inhibition vs placebo							
Yabe	33	33 (100)	DP-1904 600 mg	Loss >50% gain	22	53	0.25 (0.05–1.08)
Serruys	649	575 (89)	GR32191 80 mg	Loss ≥0.72 mm†	21	19	1.13 (0.73–1.73)
Feldman	1192	705 (59)	GR32191 40–80 mg	≥70% stenosis†	28	31	0.87 (0.63–1.20)
Savage	752	385 (76)	Sulotroban 3200 mg	≥50% stenosis	57	51	1.30 (0.84–2.02)
Hattori	105	85 (81)	CV4150 200mg/d	≥50% stenosis	45	37	1.38 (0.55–3.61)
Prostacyclin/analogs vs placebo							
Knudtson	270	250 (93)	Prostacyclin 5 ng/kg/min	≥50%, loss of gain†	27	32	0.79 (0.46–1.37)
Gershlick	135	125 (93)	Epoprostenol 4 ng/kg/min	Loss >50% gain†	29	38	0.66 (0.31–1.40)
Raizner	291	248 (85)	Ciprostene 120 ng/kg/min	≥50% stenosis†	41	53	0.62 (0.37–1.02)
Anticoagulants							
Thorton	248	178 (72)	Warfarin vs ASA	Loss of 50% gain*	36	27	1.53 (0.89–2.63)

Ellis	416	255 (61)	Heparin vs placebo	≥50% stenosis†	41	37	1.20 (0.73–2.00)
Urban	110	85 (77)	Warfarin	≥50% stenosis*	29	37	0.675 (0.27–1.67
Lehmann	30	23 (77)	SQ heparin 10,000 U/d vs placebo	NR	82	33	9.33 (1.26–97.1)
Faxon	458	357 (78)	Enoxaparin 40mg/d × 30 d	Loss of 50% gain, clinical†	50	49	1.04 (0.68–1.57)
Brack	339	299 (88)	SQ heparin 12,500 U bid × 4 mo vs placebo,	Loss >50% gain, change in MLD†	39	46	0.76 (0.48–1.21)
Karsch	625	514 (83)	Riviparin SQ × 4 wk vs heparin 3,500 bid	Loss of 50% gain, clinical†	33	32	1.06 (0.76–1.49)
Cairns	653	625 (96)	Enoxaparin vs placebo	Loss ≥50% gain†	46	45	1.02 (0.74–1.39)
Serruys	1141	986 (86)	Hirudin 40 mg × 24 h and SQ × 3 d vs heparin	Change in MLD, clinical†	34	33	1.07 (0.83–1.39)
Grassman	117	99 (85)	Certoparin vs placebo	>50% stenosis*	30	51	0.41 (0.18–0.93)
			Calcium antagonist vs placebo				
Corcos	92	92 (100)	Diltiazem 90 mg tid	≥70% stenosis	15	22	0.65 (0.21–1.86)
O'Keefe	201	120 (60)	Diltiazem 360 mg	>70% stenosis†	36	36	1.02 (0.48–2.16)
Whitworth	241	198 (82)	Nifedipine 10 mg qid	>50% loss of gain†	28	30	0.93 (0.50–1.71)
Hoberg	196	172 (88)	Verapamil 240 mg bid	Loss >50% gain†	47	62	0.56 (0.30–1.02)
Unverdorben	189	170 (90)	Diltiazem 180 mg qd	≥50% stenosis*	21	38	0.44 (0.22–0.86)
			Steroid vs placebo				
Stone	102	54 (53)	125 mg IM, 60 mg qd × 7 d	>50% stenosis	59	56	1.16 (0.39–3.46)
Pepine	694	510 (73)	1 g methylprednisolone	≥50% stenosis†	43	43	1.02 (0.72–1.45)
			ACE inhibition vs placebo				
Serruys	693	595 (86)	Cilazapril 5 mg bid	>50% stenosis†	28	28	0.99 (0.69–1.42)
Faxon	1436	1077 (75)	Cilazapril 2–20 mg	>50% stenosis†, change in MLD	33	37	1.17 (0.91–1.51)
Desmet	336	304 (90)	Fosinopril 40 mg qd × 6 mo	Loss >50% gain	46	41	1.24 (0.79–1.96)
			Trapidil				
Maresta	384	254 (66)	100 mg tid ×6 mo vs ASA	≥50% loss of gain*	24	40	0.49 (0.28–0.83)
Okamoto	97	72 (74)	600 mg vs ASA/ dipyridamole	≥50% loss of gain†	19	42	0.34 (0.11–0.95)
Nishikawa	160	137 (86)	600 mg + ASA vs ASA/dipyridamole	≥50% loss of gain†	20	38	0.42 (0.19–0.89)
			Fish Oil vs Placebo				
Reis	186	68 (37)	6.0 g	≥70% stenosis, ETT†	34	23	1.76 (0.89–3.64)
Dehmer	82	82 (100)	5.4 g	≥50% stenosis†	19	46	0.27 (0.10–0.70)
Grigg	108	101 (93)	3.0 g	Loss >50% gain†	34	33	1.03 (0.45–2.37)
Nye	73	69 (95)	4.0 g	Loss >50% gain†	11	30	0.30 (0.08–0.98)

(continued)

Table 1 *(continued)*

Study	n	Follow-up n (%)	Therapy	Restenosis definition	Restenosis rate Active (%)	Restenosis rate Control (%)	Odds ratio (95% CI)
Franzen	204	175 (86)	3.2 g	>50%stenosis†	33	35	0.90 (0.48–1.69)
Bairati	119	119 (100)	4.5 g	≥50% stenosis†	31	48	0.47 (0.22–0.99)
Leaf	551	447 (81)	8.0 g	≥50% stenosis†	52	46	1.27 (0.88–1.85)
Cairns	653	625 (96)	5.4 g	Loss ≥50% gain†	47	45	1.07 (0.78–1.47)
Kaul	107	42 (38)	3.0 g	Loss 50% gain, ETT*	33	27	1.35 (0.59–3.17)
Bellamy	120	113 (94)	3.0 g	Loss >50% gain†	35	36	0.96 (0.44–2.10)
Cheng	50	43 (86)	3.0 g	Loss >50% gain†	20	34	0.63 (0.16–2.32)
Lipid-lowering agents versus placebo							
O'Keefe	200	117 (59)	Lovastatin 40 mg, Probucol 1000 mg/d	>50% stenosis, change in MLD†	51	46	1.19 (0.54–2.65)
Weintraub	354	321 (91)	Lovastatin 40 mg bid	≥50% stenosis, change in MLD†	39	42	0.89 (0.57–1.39)
Sahni	157	79 (50)	Lovastatin 20–40 mg/d	>50% stenosis	12	45	0.17 (0.05–0.50)
Nakamura	133	124 (93)	Pravastatin 10 mg bid	Loss ≥50% gain†	29	39	0.65 (0.30–1.37)
Antioxidants							
Tardiff	255	230 (90)	Probucol 1000 mg/d, multivitamin, placebo	≥50% stenosis, change in MLD†	30	43	0.56 (0.32–0.96)
Wantanabe	118	118 (100)	Probucol 500 mg/d	Loss >50% gain†	19	37	0.39 (0.16–0.88)
Demaio	100	85 (85)	Tocopherol 1200 IU/d	Loss ≥50% gain†	36	48	0.61 (0.25–1.45)
Lee	111	111 (100)	Probucol vs pravastatin pre	>50% stenosis	26	34	0.66 (0.28–1.49)
Yokoi	101	78 (78)	Probucol 1000 mg/d	>50% stenosis	23	57	0.21 (0.08–0.55)
Antiproliferative agents							
O'Keefe	197	145 (74)	Colchicine 0.6 mg bid	Loss >50% gain†	41	45	0.85 (0.43–1.69)
Serotonin antagonists							
Serruys	658	592 (90)	Ketanserin 40 mg bid	>50% stenosis†	32	32	1.00 (0.71–1.41)
Klein	43	43 (100)	Ketanserin 0.1 mg/min	>50% stenosis	33	29	1.33 (0.36–5.07)
Heik	97	86 (89)	Ketanserin 40 mg bid	>50% stenosis	22	38	0.46 (0.17–1.18)
Angiopeptin							
Kent	1054	917 (87)	190–3000 mg/d	≥50% stenosis	37	39	0.94 (0.69–1.27)
Eriksen	90	75 (83)	750 mg/d	≥50% stenosis†	12	40	0.21 (0.06–0.64)
Emanuelsson	455	423 (92)	6 mg	>50% stenosis†	36	37	0.96 (0.65–1.43)

MLD = minimal luminal diameter, CI = confidence interval, N/A = not available, NS = not significant, ETT = exercise treadmill test.
*Visual assessment.
†Quantitative coronary angiography. Each trial's definition of angiographic restenosis was used.
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Table 2
Targets for IIb/IIIa Inhibition in Acute Coronary Syndromes

Initial TIMI-3 flow after thrombolytic therapy
TIMI-3 flow after thrombolytic therapy
Flow after thrombolytic therapy
Reocclusion after thrombolytic therapy
Acute closure and restenosis after direct PTCA
Community-based early treatment strategies
Reduction of intracranial hemorrhage

reperfusion. Other important therapeutic targets include the 10–20% rate of new congestive heart failure and the 5–15% rate of recurrent infarction. Stroke is another major clinical outcome in acute MI patients, because of both embolic events resulting from the damaged left ventricle (1–2%) and the adverse effects of fibrinolytic and antithrombotic therapy (0.4–0.9%).

Spontaneous plaque rupture, with thrombin and fibrin formation, initiates complete vessel occlusion. Precipitating factors include a lipid-rich plaque, macrophage infiltration into the fibrous cap of the plaque, and an "ischemic trigger" *(8)*, which provides the immediate stimulus for plaque disruption. Platelets seem to have a particularly important role in the cyclical risk of acute coronary events, with substantial evidence for platelet hyperreactivity *(9)* in the early morning hours when these events are most common.

Five clinically important targets can be considered for the IIb/IIIa inhibitors in ST-segment elevation acute coronary syndromes (ACS) (Table 2).

1. The current ceiling of thrombolysis in myocardial infarction (TIMI) Grade 3 flow with thrombolytic therapy appears to be fixed at 50–60%. Early and sustained TIMI Grade 3 flow is critical to improved survival. A systematic overview of 3374 patients enrolled in randomized trials of thrombolytic therapy estimated a 3.0% absolute mortality reduction for patients with TIMI 3 flow at 90 min compared with those with TIMI 2 flow *(10)*. Efforts to enhance this rate through development of more fibrin-specific agents *(11)* or addition of direct antithrombin agents *(12–14)* have been limited by increased rates of intracranial hemorrhage. Based upon the initial GUSTO-I calculations, an improvement of TIMI grade 3 flow at 90 min from 31 to 54% would result in a 26% relative reduction in mortality (or a 1.6% absolute mortality reduction) *(15)*. This, of course, assumes that the other important outcomes (such as stroke or bleeding) would not also be altered, an unlikely scenario. One of the advantages of GP IIb/IIIa inhibitors is that all of the important modifiable outcomes could move in the same direction.
2. Reocclusion occurs in 4–20% of patients in the first week *(16)* after initial thrombolytic therapy and in an additional 10–20% of cases over the next 3–6 mo *(17)*. When reocclusion occurs during the initial hospitalization, it doubles mortality and substantially increases the risk of other major complications *(18)*. Little progress has been made in the prevention of reocclusion with medical therapy despite years of effort. In several trials *(19–21)*, even mechanical intervention failed to improve long-term patency after thrombolytic therapy. A recent report by White et al. *(22)* with 12-yr follow-up demonstrates a long-term survival advantage for patients with a patent infarct-related artery one month after admission.

Among patients treated with thrombolytic therapy, those receiving t-PA, with poor TIMI grade flow, right coronary artery occlusions, and bradycardia possess the highest risk of reocclusion *(23)*. Tighter residual stenoses, but not other angiographic characteristics, are associated with a higher risk of reocclusion. The combination of clinical and angiographic characteristics has poor power to discriminate patients who reocclude from those who do not. One promising avenue is continuous ST-segment monitoring; patients without stable ST segments are likely to have higher mortality and lower patency rates *(24–26)*.

It is likely that platelets are important in reocclusion, given the role of platelet activation and thrombus generation in initial events. Indeed, Yatsuda and Gold's canine preparation *(27)*, which has been used as the experimental model for reocclusion, is highly responsive to potent antiplatelet agents *(28)*.

Calculations based on the GUSTO-I experience indicate that prevention of reocclusion alone with accelerated alteplase regimens could make a modest difference in patient outcome. A reduction of 30-d mortality from 5.3 to 4.9% could be accomplished with a 33% reduction in the reocclusion rate *(18)*. Combined with a higher rate of TIMI Grade 3 flow, a concomitant reduction in reocclusion could yield a substantial improvement in outcome.

3. Acute events and restenosis after direct coronary angioplasty with or without stenting. A series of randomized trials has demonstrated that primary fibrinolytic therapy leads to inferior results compared to direct coronary angioplasty performed in a timely fashion, with experienced operators and staff, in a facility with adequate volume *(29)*. Other data show that in low volume, less-experienced, or less-efficient situations, direct coronary angioplasty may have no benefit over thrombolytic therapy *(30,31)*. More recently, clinical trials have evaluated use of coronary stents to improve the outcomes of the angioplasty strategy *(32)*.

   Despite these clinical trials data, percutaneous revascularization does not address the risk of ongoing thrombosis except by establishing a higher flow state. From the trials of GP IIb/IIIa inhibitors and ST elevation MI, including the RAPPORT trial *(33)*, it is evident that antagonizing the function of platelets reduces the number of myocardial necrotic events and the need for repeat procedures.

4. Integration of a community-based early treatment strategy with maximum use of percutaneous revascularization when needed. Fibrinolytic therapy fails to achieve timely reperfusion in 30–40% of patients. Additionally, the confusing choice between direct coronary angioplasty and primary fibrinolytic therapy is a major source of treatment delay. Cardiologists are divided on this issue, and front-line physician responders must often determine the preference of the on-call physician before administering therapy.

   For patients who fail to reperfuse, mortality is at least doubled *(18)*. One conundrum has been detection of fibrinolytic treatment failure without performing an acute angiogram. Although initial haphazard observations were disappointing, careful evaluation found continuous 12-lead ECG *(24)* or vectorcardiographic *(34)* monitoring possessed a reasonable predictive value. Multiple studies have indicated that less than 50% reduction of the peak ST elevation on a 12-lead ECG, carefully performed 90 to 180 min after initiation of fibrinolytic therapy, identifies a population with a two- to threefold increase in mortality *(35–38)*.

   Although the 12-lead ECG can identify patients who fail to reperfuse, the management of these patients remains undetermined. High failure, reocclusion, and mortality rates frustrated initial evaluations of the "rescue angioplasty" approach. In the only published prospective randomized clinical trial, Ellis et al. *(39)* demonstrated that "res-

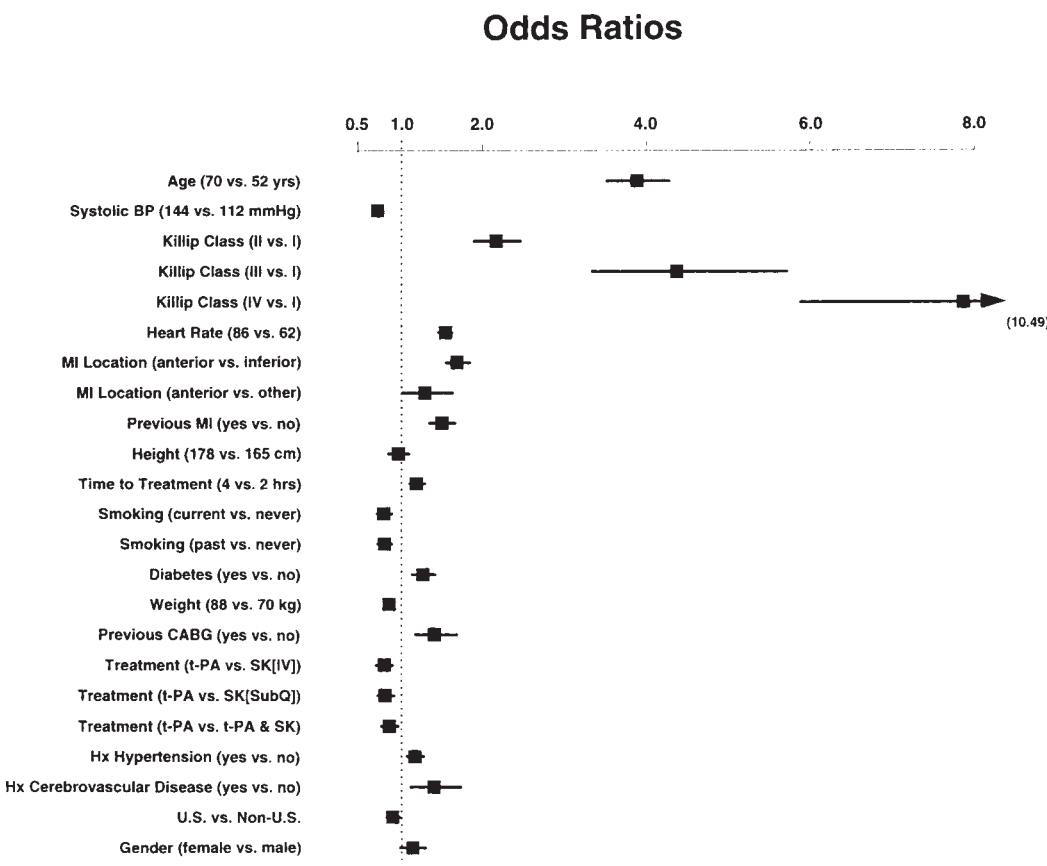

Fig. 1. Graph showing odds ratios and 95% confidence intervals for variables in the final multivariable mortality risk model in GUSTO-I. BP = blood pressure; MI = myocardial infarction; CABG = coronary artery bypass surgery; t-PA = accelerated tissue-plasminogen activator; SK = streptokinase; SubQ = subcutaneous; Hx = history. Reprinted with permission from ref. *97*.

cue angioplasty" reduced the composite endpoint of death and heart failure for patients with left anterior descending coronary occlusions.

Both the TIMI *(40)* and TAMI *(41)* trialists reported good outcomes for patients with successful "rescue angioplasty" but very high mortality for those who failed "rescue angioplasty." These results created considerable uncertainty about the benefit of percutaneous intervention after failed thrombolytic therapy. With the advent of stents, the failure rate of "rescue" procedures should decline, and the benefits identified by Ellis should become more evident.

If opening occluded arteries is beneficial, particularly when the ST segments have not resolved, a community-based strategy of early fibrinolytic and GP IIb/IIIa inhibitor administration could be followed by 12-lead electrocardiography with referral to an expert center for high-risk patients. Risk would be predictable from clinical factors, as demonstrated by the GUSTO prognostic odds ratios (Fig. 1). This approach emphasizes maximal benefit from early treatment, achieving an open artery in nearly all patients.

5. Reduction of intracranial hemorrhage. In clinical trials, the rates of intracranial hemorrhage have been 0.3–0.5% for streptokinase and 0.7–0.9% for current doses of alteplase and reteplase. Because alteplase reduces mortality better than streptokinase, it would be

Table 3
Combined Therapy for Acute MI:
Potential for Benefit Using Fibrinolytic Agent,
GP IIb/IIIa Inhibitor, and Angiographic Strategies

Perfusion
Higher rates with medical therapy
Faster achievement of reperfusion
Rescue PTCA for reperfusion failures
Prevention of reocclusion
More potent antiplatelet therapy
Stenting
Reduction in stroke

desirable to lower the risk of intracranial hemorrhage associated with alteplase therapy while preserving the reperfusion rate. Indeed, reduction of the intracranial hemorrhage rate by 50% would decrease disability and shrink the mortality rate by 0.25%. Recent experience from the TIMI 14 and SPEED trials indicates that, for half-dose fibrinolytic therapy combined with abciximab, the rate of intracranial hemorrhage may be dramatically reduced while maintaining improved TIMI 3 rates.

Combining GP IIb/IIIa inhibitors with thrombolytic therapy and a judicious strategy of planned percutaneous intervention could lead to a substantial benefit (Table 3). This combination could sum to a total of 1.5–2 lives saved per 100 patients treated, and the benefits would be even greater in high-risk patients.

Non-ST Elevation ACS

Many more patients with ACS present without ST-segment elevation than with ST-segment elevation. The pathophysiology of the underlying lesion is thought to be the same as in ST elevation ACS, except that the thrombus may be more platelet rich. Angiographic identification of thrombus is unreliable, and there may be many cases of non-ST elevation ACS without thrombus as a major component.

Therapeutic targets for ACS without ST-segment elevation are straightforward. Patients want to avoid death and MI or reinfarction. As in ST-segment elevation ACS, stroke and heart failure are important clinical issues. Although hemorrhagic stroke is infrequent, nonhemorrhagic stroke occurs equally in non-ST-segment elevation ACS and ST-segment elevation ACS. In addition, however, reductions in the need for revascularization procedures and repeat hospitalizations constitute very important endpoints for patients with ACS.

Event rates in patients with non-ST-segment elevation ACS are substantial. In clinical trials, the overall mortality rates have been 3.5% with nonfatal MI rates of 7.8% and stroke rates of 0.7% (Table 4). The variation in death and stroke rates stems from the diverse populations enrolled and differences in the measurement of nonfatal MI. The criteria for the use of revascularization procedures and for repeat hospitalizations are extremely culturally dependent.

Critical prognostic factors can identify even higher-risk populations. Patients with ST-segment abnormalities on the 12-lead ECG have the highest risk, followed by patients without ST-segment changes and then by patients with normal ECGs. A positive CK-MB, Troponin T *(42)*, or Troponin I *(43)* value is also associated with a higher risk.

Table 4
Mortality, reMI, and Stroke Rates
in the ESSENCE, GUSTO-II, PURSUIT, and PARAGON Trials

	ESSENCE		*GUSTO-II*		*PURSUIT*		*PARAGON*	
	Placebo	*Treated*	*Placebo*	*Treated*	*Placebo*	*Treated*	*Placebo*	*Treated*
Mortality	3.6%	2.9%	4.7%	4.5%	3.4%	3.4%	2.9%	3.3%
ReMI	5.2%	3.9%	6.3%	5.4%	12.0%	11.9%	10.6%	10.1%
Stroke	0.5%	0.4%	0.8%	0.9%	0.9%	0.6%	0.4%	0.8%

Table 5
Characteristics Associated with Mortality
and MI in the PURSUIT, GUSTO-IIb, and ESSENCE Trials

Characteristics	*PURSUIT*	*GUSTO-IIb*	*ESSENCE*
Age	x	x	x
ST-depression	x	x	x
MI at enrollment	x	x	
Angina class	x		
Region (SA vs US/CA/WE)			x
Hemodynamic compromise		x	

SA = South America; US = United States; CA = Canada; WE = Western Europe.

As in most cardiovascular illnesses, older age, diabetes, peripheral vascular disease, and hemodynamic distress (pulmonary edema, hypotension) are also associated with a much higher risk. Table 5 compares the characteristics associated with the composite endpoint of death and MI in ESSENCE, PURSUIT, and GUSTO-II.

A large reduction in events is not needed to be economically attractive in ACS without ST-segment elevation. In a model developed from several databases from clinical trials, a 10–15% reduction in the composite of death, MI, and revascularization was associated with a saving of over $1,000 per patient on average *(44)*. Most importantly, these cost savings are because of fewer revascularization procedures and the prevention of MI complications.

Chronic Coronary Atherosclerosis

As our knowledge about the natural history of atherosclerosis grows, the role of platelet inhibition in preventive strategies becomes more evident. Atherosclerosis progression is now thought to include gradual luminal narrowing from cellular proliferation and more sudden luminal compromise from plaque rupture, luminal thrombus formation, and incorporation of the thrombus into the plaque. The two are related by the presence of an abnormal vessel wall for initial plaque rupture and by the elaboration of growth factors from activated platelets stimulating cellular proliferation.

The clear benefit of aspirin, ticlopidine, and clopidogrel in secondary prevention trials points to platelet inhibition as a significant part of a therapeutic strategy. Increasing interest in inflammation markers as risk factors for acute ischemic events has prompted new questions. Is aspirin's benefit because of its antiplatelet attributes or its antiinflam-

Table 6
Event Rates in the CARS and CAPRIE Trials

	CARS		*CAPRIE*	
	Aspirin	*Treated*	*Aspirin*	*Treated*
Mortality	3.0%	3.3%	5.9%	5.8%
Nonfatal MI	6.8%	7.2%	3.1%	2.7%
Nonfatal Stroke	0.6%	1.1%	5.3%	4.9%

matory effects? The modest superiority of clopidogrel *(45)* over aspirin is strong evidence of a dominant antiplatelet effect *(46)*.

The therapeutic targets for chronic atherosclerosis are no different than for ACS. Patients would like to avoid death, nonfatal infarction, stroke, hospitalization, and the need for surgical procedures. The risk of these events is highly dependent on the characteristics of the population chosen.

A population with recent ACS can be expected to have a high rate of recurrent events while on aspirin therapy. Table 6 displays the event rates from the CARS study and the CAPRIE trial. Higher-risk populations can easily be identified using algorithms from available clinical databases.

Another topic of great interest is aspirin resistance. Using some measures of inhibition of platelet aggregation, up to 30% of patients may not achieve any significant evidence of inhibition of aggregation with aspirin *(47–50)*, despite evidence of adequate absorption of the drug. Although no data exist to demonstrate that these patients have a high risk of ischemic events during follow-up, they may derive particular benefit from an alternative platelet inhibitor drug.

Atrial Fibrillation

Atrial fibrillation is becoming one of the most common serious medical problems. More than 5% of the population over age 75 yr has atrial fibrillation. It accounts for substantial morbidity because of impaired cardiac capacity and major disability due to stroke. The risk of embolic stroke in patients with atrial fibrillation ranges from < 1% per year for patients with no structural heart disease to 5–7% for patients with multiple risk factors. Identified risk factors include structural heart disease, hypertension, age (especially > 65 yr), diabetes, and prior stroke or transient ischemic attack.

Traditionally, embolic stroke in the setting of atrial fibrillation has been treated with anticoagulants rather than antiplatelet therapy. Yet, many patients cannot tolerate the substantial bleeding risk associated with warfarin. Additionally, the failure rate of warfarin anticoagulation is significant. At least one study has found lower mortality for patients with valvular heart disease treated with aspirin and warfarin compared to warfarin alone, suggesting that the combination of antiplatelet and antithrombin therapy might be most beneficial *(51)*.

Cerebrovascular Disease

As with atrial fibrillation, cerebrovascular disease afflicts older adults. Whereas the age-specific incidence of stroke declines by 5% per year, the absolute incidence is

Table 7
Event Rates for Patients Meeting Entry Criteria into CAPRIE Because of Cerebrovascular Disease

		Stroke		*Myocardial infarction*				
Drug	*Patient years at risk*	*Nonfatal*	*Fatal*	*Nonfatal*	*Fatal*	*Other vascular death*	*Total*	*Event rate per year*
Clopidogrel	6054	298	17	33	11	74	433	7.15%
Aspirin	5979	322	16	37	14	72	461	7.71%

increasing because of the aging population. Conventional wisdom holds that 60% of ischemic strokes in the United States are atheroembolic, 20% are because of small vessel cerebrovascular disease, 15% are embolic from the heart, and the remaining 5% are because of a variety of unusual causes. In most of these situations, it is plausible that more effective antiplatelet therapy would reduce event rates.

Patients with cerebrovascular disease are at very high risk for coronary vascular events. The risk of MI, sudden death, and unstable angina is substantially higher in patients with both cerebral and coronary artery disease than with either alone The composite rate of stroke and death in trials of endarterectomy has ranged from 10 to 32%. Table 7 provides the event rates for patients meeting entry criteria into CAPRIE because of cerebrovascular disease.

Peripheral Vascular Disease

Increasing recognition of the amount of disability caused by peripheral vascular disease has made it a major focus of cardiovascular medicine. Little is known about the event rates for patients with peripheral vascular disease, although cardiac events are common. Early reports suggested a 6.5–28.7% incidence of nonfatal infarction, a 5–12.4% incidence of nonfatal stroke, and a 1.5–5% amputation rate *(52)*. Peripheral vascular disease reduces overall life expectancy by 10 yr, with about 75% of deaths caused by atherosclerosis (50% coronary, 15% cerebrovascular, 10% abdominal). The mortality rate is 20–30% 5 yr after diagnosis, 40–72% after 10 yr, and 74% after 15 yr *(53)*. Tunis et al. *(54)* noted that amputation rates remained constant despite increasing numbers of peripheral angioplasty procedures over a 10-yr period.

Given the beneficial effects of GP IIb/IIIa inhibitors in coronary intervention, the same benefits would likely accrue in peripheral vascular intervention. An intriguing observation from the CAPRIE trial was the significant heterogeneity in treatment responses, with a greater benefit of clopidogrel over aspirin in patients entering with peripheral vascular disease versus coronary disease or cerebrovascular disease *(45)*.

Coronary Artery Bypass Grafting

Coronary artery bypass grafting is an extremely common vascular procedure with more than 500,000 operations per year performed in the United States alone. The natural history of saphenous vein graft patency has been well described. More than 10% of grafts occlude in the first month, with an additional 10% closing over the next year. Over the

next 5 yr, 2–4% occlude per year. Beyond this point, the rate of graft closure accelerates. After 10 yr of follow-up, more than half of saphenous vein grafts are occluded and half of the patent arteries have significant stenoses.

The most recently completed angiographic study after bypass surgery was completed by the BARI Investigators *(55)*. At four centers with a high rate of angiographic follow-up, a combination of 1- and 5-yr angiograms was done in 200 CABG patients. In the 5-yr follow-up period, there were no additional CABGs and 17 additional PTCAs. Of 334 vein grafts restudied, 87% were patent and 84% were free of lesions >50%. Of 198 mammary grafts restudied, 95% were patent and 89% were free of lesions >50%.

Alternative Therapies

Platelet Inhibitors

Aspirin. The year 1997 marked the 100th anniversary of aspirin as a therapeutic agent. It has been remarkably effective in the treatment of thrombotic diseases, particularly of the arterial wall. Across the spectrum of acute and chronic arterial diseases, the Antiplatelet Trialists' Collaboration (56) demonstrated a consistent 15–25% reduction in the relative occurrence of death or recurrent MI. Despite the fact that almost none of the trials had adequate sample size to define the magnitude of the benefit, multiple trials in each disease have found a consistent reduction in death, MI, and stroke in a variety of populations. In a recent review, the Cardiorenal Advisory Panel of the FDA concluded that aspirin has been shown to reduce these critical endpoints in acute MI, in the post-MI period, in unstable angina, in acute stroke, and in the post-stroke period. Although it was agreed that antiplatelet agents are beneficial in the prevention of these events for patients with peripheral vascular disease, there was not enough specific evidence about aspirin to single it out as beneficial.

Despite irrefutable evidence of aspirin's benefit, we continue to be uncertain about the best dose. Doses between 81 and 650 mg have reduced events in a variety of conditions, but no adequate studies have meaningfully compared the benefits of one dose over another. In contrast, the risk of bleeding clearly increases at least linearly with dose. Up to 5% of patients possess true aspirin allergy, and gastrointestinal irritation prevents another 5–10% of patients from tolerating chronic aspirin therapy. Little is known about what distinguishes patients who have an event when on aspirin from those who seem to be protected. Some patients may receive very little platelet inhibitory effect as measured by platelet aggregation studies despite documented aspirin levels *(57,58)*. A series of studies have shown minimal to no inhibition of platelet aggregation with standard doses of aspirin in up to 30% of patients.

Ticlopidine. This drug interferes with fibrinogen binding and inhibits ADP-induced platelet aggregation without affecting the arachidonic acid pathway. In trials of patients with cerebrovascular disease *(59)* and unstable angina *(60)*, it has been modestly superior to aspirin. Its onset of action is believed to take several days, and it is therefore not recommended for acute use.

Perhaps the greatest use of ticolopidine in recent years has been in the prevention of stent thrombosis in conjunction with aspirin. Initially, several uncontrolled studies showed dramatic reductions in stent thrombosis for ticlopidine and aspirin compared with aspirin and warfarin. Two pivotal randomized trials were significantly positive for ticlopidine. The ISAR trial *(61)* with 517 randomized patients found a 6.2% cardiac

endpoint rate in patients treated with aspirin and warfarin compared with 1.6% in the aspirin and ticlopidine-treated patients. Hall et al. *(62)* found a 2% event rate with aspirin alone compared with 0.8% with aspirin and ticlopidine, whereas the STARS trial *(63)* found a reduction from 2.9 to 0.6% in the primary endpoint composite of death, Q-wave MI, coronary artery bypass grafting, and repeat percutaneous intervention in patients randomized to aspirin and ticlopidine compared with aspirin alone.

The small risk of granulocytopenia merits careful consideration of whether ticlopidine should be used at all beyond several weeks. This is much less of an issue for poststent patients treated for less than 3 wk. Recently concern has been raised about thrombotic thrombocytopenic purpura with ticlopidine, but the incidence is unknown.

Clopidogrel. Clopidogrel has been developed as a successor to ticlopidine (CAPRIE) *(45)*. It also inhibits ADP-induced platelet aggregation without affecting the arachidonic acid pathway, but it does not seem to cause granulocytopenia. In a large study of patients with vascular disease it was found to be modestly superior to aspirin, with an 8% reduction in the composite of vascular death, nonfatal MI, and stroke. When all-cause mortality was added to the composite endpoint, the difference was no longer statistically significant. Furthermore, significant heterogeneity was observed in the treatment effect with a greater effect in post-stroke and peripheral vascular disease than in post-MI patients.

Despite the complexity of the data, clopidogrel is at least as effective as aspirin for preventing major vascular events. Practitioners will administer clopidogrel to aspirin-allergic patients and perhaps to those who fail aspirin therapy. Several unanswered questions persist. The degree to which aspirin resistance should guide clinical therapy remains in doubt. In addition, a large successor trial to CAPRIE plans to address whether clopidogrel combined with aspirin is superior to aspirin alone. Whether clopidogrel can substitute for ticlopidine to prevent stent thrombosis also remains unknown. Clinicians will likely make varying therapeutic selections until definitive trials are completed.

Thrombin Inhibitors

Unfractionated Heparin. Unfractionated heparin therapy is supported by less clinical outcomes evidence than one might expect for a commonly used therapy. In unstable angina, heparin clearly prevents death and MI in the acute phase, but event rates increase when it is discontinued and little is known about the durability of the benefit. Heparin has the troublesome capacity to produce thrombocytopenia and occasionally malignant thrombosis. Finally, it is difficult to titrate heparin within the therapeutic range; both insufficient and excessive doses are associated with worse clinical outcomes.

Considerable angiographic data have shown improved coronary patency when fibrinolytic agents are administered with concomitant heparin therapy. However, no definitive clinical outcome data have been generated *(64)*, and a systematic overview of available randomized trials demonstrated a small trend favoring lower mortality and a larger trend towards excess stroke, MI, and bleeding *(65)*. It is unlikely that a definitive study will ever be done on this topic.

Low Molecular-Weight Heparin. Low molecular-weight heparin offers several advantages over unfractionated heparin. One potential advantage accrues from its inhibition of coagulation higher up the coagulation cascade. Low molecular-weight heparin primarily inhibits factor Xa while inactivating thrombin less intensively. It is less likely

to cause heparin-induced thrombocytopenia (HIT) or heparin-induced thrombotic thrombocytopenia syndrome (HITTS), and current standards do not require therapeutic monitoring. In several direct comparative trials of patients with non-ST-elevation ACS, low molecular-weight heparin has been found to be at least as effective as *(66)*, and perhaps superior to *(67)*, unfractionated heparin. Several additional trials will provide needed definitive information about the acute use of intravenous low molecular-weight heparin, the value of prolonged outpatient administration, and efficacy in ST-segment elevation MI and percutaneous intervention settings.

Warfarin. Warfarin is very effective for preventing recurrent cardiac ischemic events and cerebral embolic events, but it has a number of limitations. It requires considerable therapeutic monitoring and can cause life-threatening bleeding. Although its efficacy is similar to aspirin in coronary thrombotic indications, aspirin antiplatelet therapy is the preferred preventive strategy due to lower cost and less risk of bleeding. For patients with cerebrovascular disease, warfarin is frequently added when events recur during aspirin or ticlopidine therapy despite firm evidence of incremental benefit. In atrial fibrillation, the best estimates of overall benefit highly favor warfarin over aspirin.

The Thrombosis Prevention Trial *(68)* of high-risk British men without manifest coronary disease showed that aspirin prevented nonfatal events, warfarin averted fatal events, and combination therapy prevented both. In this trial, warfarin was adjusted to maintain an INR of 1.5, and dosing over time was carefully adjusted. This trial demonstrates an additive effect of antiplatelet and antithrombin therapy.

Xa Inhibitors

Several molecules that directly inhibit Factor Xa are currently in development. However none of them has reached phase III clinical trials. Inhibition of the coagulation system closer to its origin may have significant advantages.

Tissue Factor Pathway Inhibitor. Several methods that directly inhibit tissue factor have now been developed. Initial clinical trials in microvascular surgery have yielded interesting results in an effort to prevent microvascular clotting. Interesting animal and human pathology studies imply that Tissue Factor Pathway Inhibitor (TFPI) may be particularly important in vein graft occlusions.

CURRENT STATE OF THE DATA AND FUTURE USE

Percutaneous Intervention

Many trials have examined GP IIb/IIIa inhibitor therapy for percutaneous intervention indications. In general, the trial designs have been similar, randomizing broad patient populations undergoing percutaneous interventions. In most trials, patients were randomized before the procedure, and the agent was started after the operator defined the overall coronary anatomy. Primary endpoints have generally been measured 30 d after the procedure, seeking a reduction of the composite of death, nonfatal infarction, and repeat (especially unplanned) revascularization procedures. Many consider 30-d benefit to be an excellent measure of acute therapy efficacy.

Overall, the evidence for benefit in the first 48–96 h is substantial (Fig. 2). There is a highly significant reduction in the composite with a trend towards reduction in death (odds ratio 0.66), a dramatic effect on the composite of death and nonfatal MI (odds ratio 0.57), and a substantial reduction in the composite of death, nonfatal MI, and acute repeat

procedures (odds ratio 0.56). This amounts to a reduction of 1 death, 27 deaths or MIs, and 38 deaths, MIs, or repeat revascularization procedures for every 1000 patients treated.

At 30 d the benefit observed at 48–96 h is maintained but not magnified (Fig. 3). This time point provides evidence that the benefit is not lost in the early phase after discontinuation of the intravenous agents.

The first large trial, EPIC, demonstrated an increased advantage for abciximab at 6 mo, with a reduction in death, nonfatal MI, and repeat procedures *(69)*. Although the differences after the first 48 h were not quite statistically significant, the trend toward a reduction in long-term events was quite evident. Unfortunately, this effect was not replicated in the EPILOG trial, and an angiographic substudy failed to show a reduction in anatomic restenosis *(70)*.

However, the aggregate results at 6 mo continue to show a trend towards lower mortality (Fig. 4), with the maintenance of the reduction of approximately 1 death per 1000 patients treated. A large reduction in the composite of death and MI is also maintained. Furthermore, additional revascularization procedures are clearly prevented, although the relative effect is modest.

The benefit of these agents in percutaneous intervention is beyond doubt, but many secondary questions remain. One important issue is their interaction with intracoronary stents. The EPILOG, CAPTURE, and IMPACT-II results demonstrate continued benefit for GP IIb/IIIa inhibitors after stent implantation, but the majority of these stents were unplanned. The EPISTENT trial randomized almost 2400 patients to stenting alone, abciximab alone, or both. This trial found that the 30-d mortality rate was comparable in all three arms but the risks of death and MI or death, MI, and repeat revascularization were lower in the abciximab groups (Table 8).

The role of antithrombin agents in combination with GP IIb/IIIa inhibitors is complex. In the EPIC trial, full-dose heparin was used with abciximab, but the bleeding complication rate was higher than hoped. This result led to the design of the EPILOG study *(71)* in which a lower, weight-adjusted dose of heparin was found to be much safer yet at least as effective. Most GP IIb/IIIa inhibitors consistently prolong the activated clotting time (ACT) when added to heparin *(72)*. The interactions between antiplatelet agents and thrombin inhibitors prompt the question of how much thrombin inhibitor doses can be lowered without losing efficacy. In the setting of coronary intervention, it may be possible to shrink the dose of thrombin inhibitor considerably. In ST-elevation ACS, heparin doses have gradually been reduced. Clinical outcomes have been preserved as the dose of antithrombin therapy has been lowered, particularly with concomitant fibrinolytic therapy *(37,73)*.

Comparisons of these many available agents, all of which have been shown to reduce ischemic events, are desperately needed. Indirect comparisons appear to indicate a more dramatic reduction in ischemic events for abciximab. This dramatic reduction led to the early termination of the EPILOG trial. Indirect comparisons can be treacherous, however, as demonstrated by the early enthusiasm for anistreplase over streptokinase. The AIMS trial *(74)* found over a 50% mortality reduction comparing anistreplase with placebo, whereas ISIS-2 found only a 25% reduction comparing streptokinase with placebo *(75)*. Many inappropriately concluded that anistreplase was superior to streptokinase based on a systematic overview of these data *(76)*, although the authors did not intend this interpretation. Despite no apparent difference in the underlying populations, a direct comparison study of the two agents found no difference in outcome.

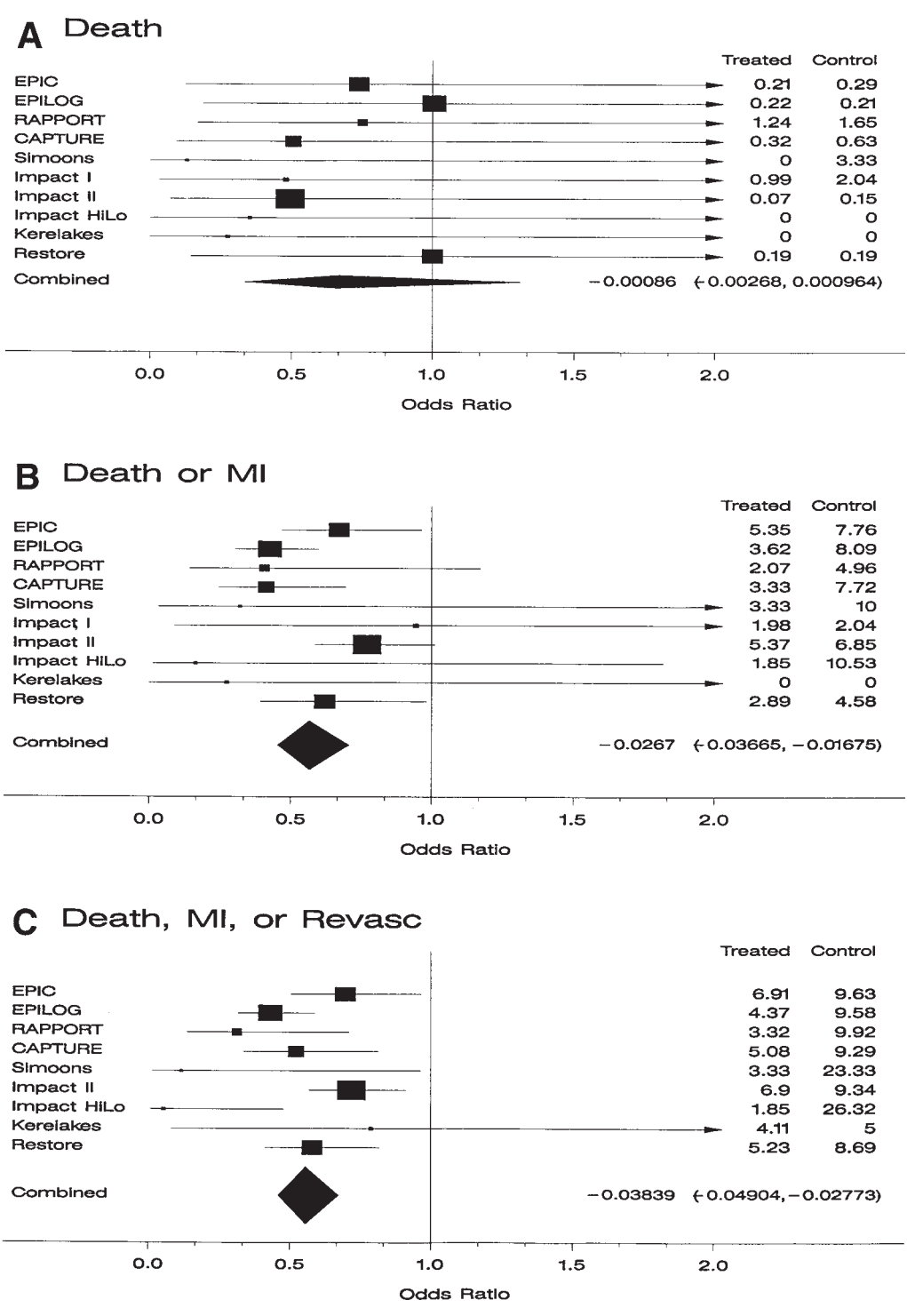

Fig. 2. Benefit of GP IIb/IIIa inhibitors in the first 48–96 h after percutaneous intervention.

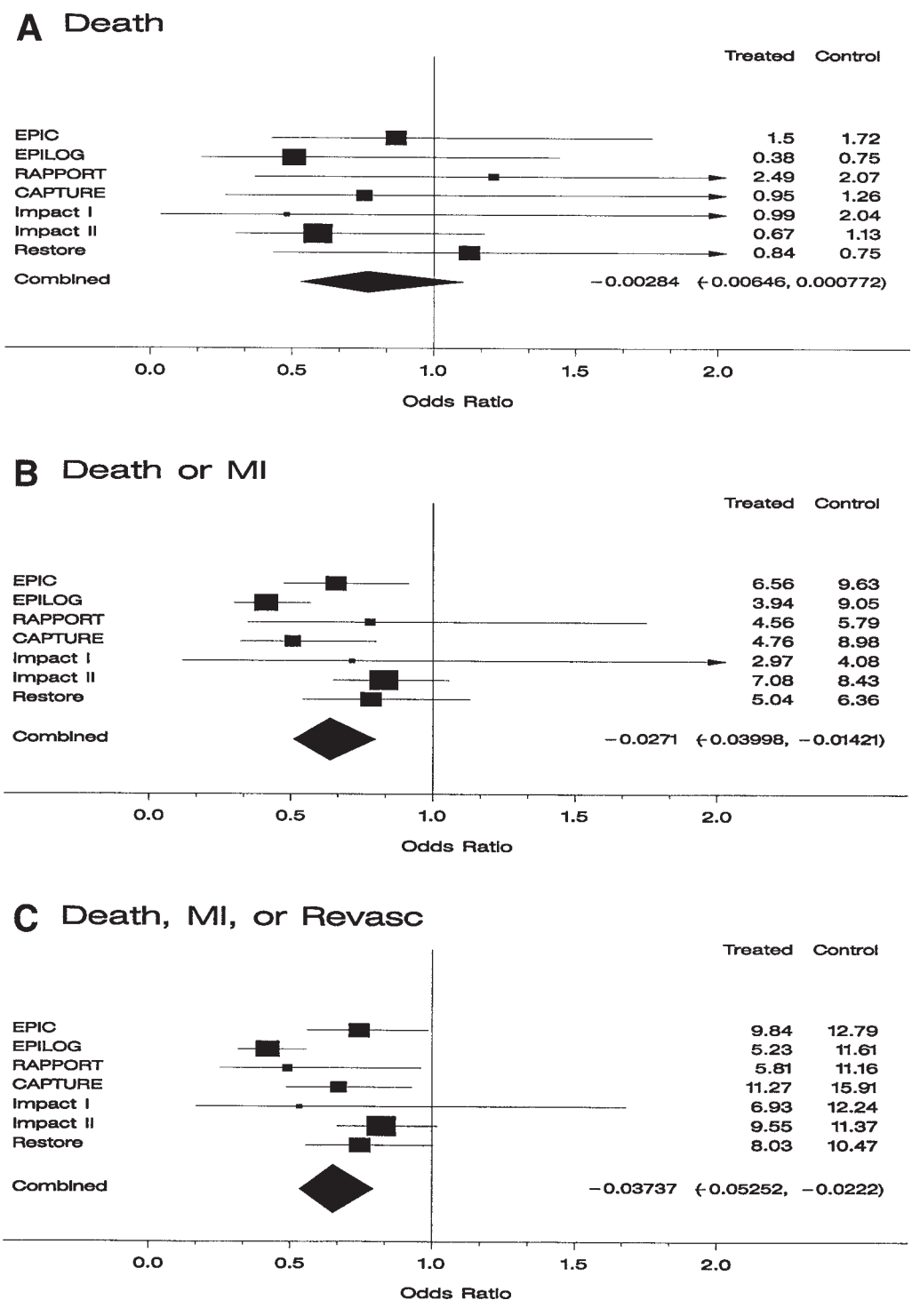

Fig. 3. Benefit of GP IIb/IIIa inhibitors up to 30 d after percutaneous intervention.

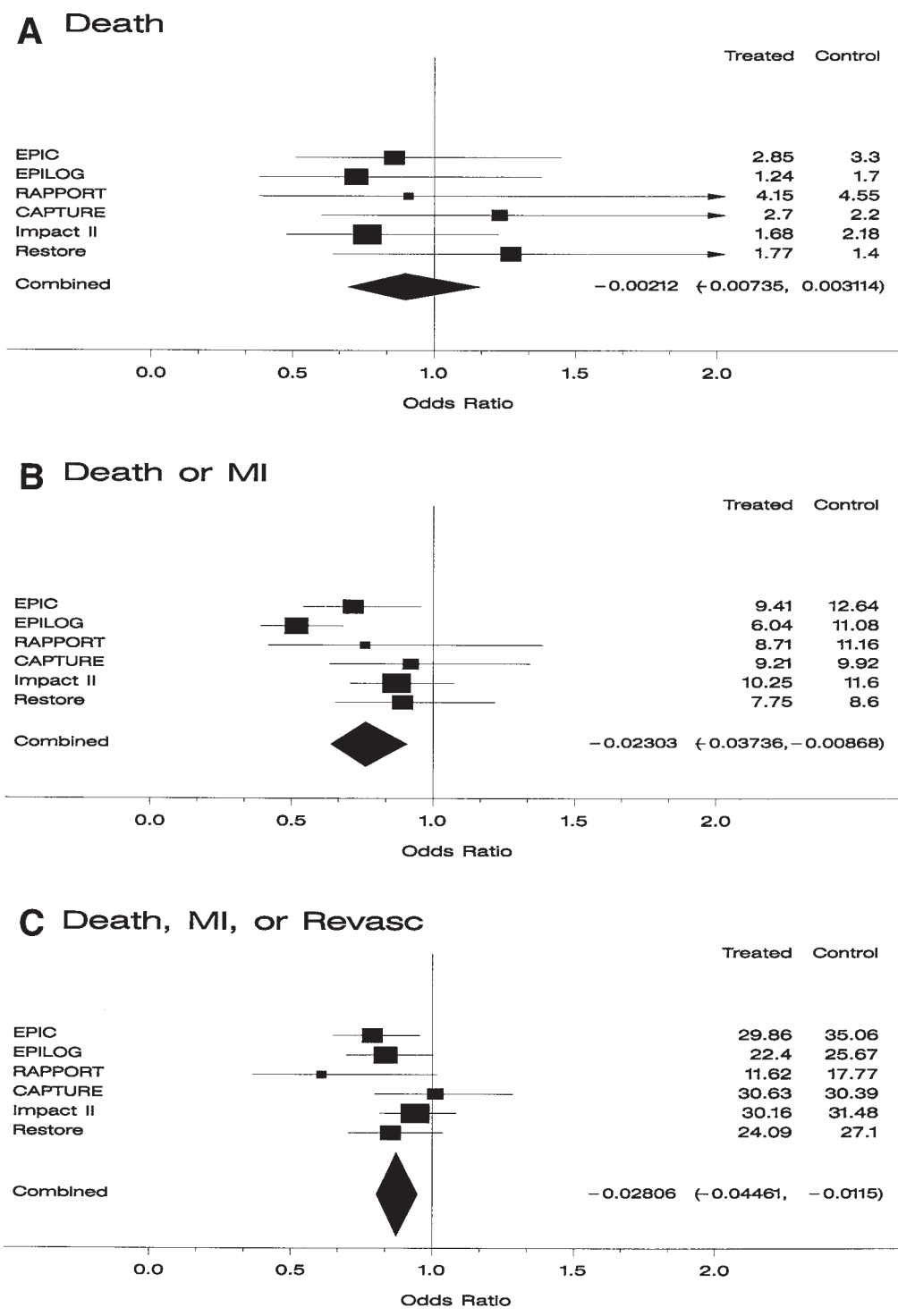

Fig. 4. Benefit of GP IIb/IIIa inhibitors 6 mo after percutaneous intervention.

Table 8
EPISTENT 30-Day Rates of Death, Death/MI, Death/MI/Repeat PTCA

	Stent + Placebo	*Stent + Abciximab*	*PTCA + Abciximab*
Death	0.6%	0.3%	0.8%
Death or MI	10.2%	4.7%	5.8%
Death/MI/repeat PTCA	10.8%	5.3%	6.9%

Whereas intravenous agents are attractive for acute situations, whether clinicians can simply use an oral agent is not known. A large trial is currently evaluating acute and long-term outcomes of xemilofiban, an oral GP IIb/IIIa inhibitor.

ST-Elevation MI

Only small studies of GP IIb/IIIa inibitors exist for patients with ST-segment elevation. Nevertheless, a few conclusions can be drawn from the accumulated experience.

Abciximab in the setting of direct angioplasty for acute MI reduces the rate of nonfatal infarction and revascularization *(33)*. The effect size is similar to the experience with percutaneous intervention in other settings.

Full-dose fibrinolytic and GP IIb/IIIa inhibitors result in improved reperfusion as assessed by angiography *(77)* and noninvasive testing *(78)*. These surrogate markers provide support for adequately sized clinical outcome trials. However, unacceptable bleeding occurs when the dose of GP IIb/IIIa inhibitor is advanced with full-dose thrombolytic therapy. A pilot study combining eptifibatide and streptokinase was terminated early because of bleeding complications *(79)*.

In an intriguing substudy of GUSTO III *(80)*, patients treated with abciximab prior to attempted rescue angioplasty had a better outcome than similar patients without abciximab treatment. These results, combined with the impressive benefit for acute MI patients in EPIC and EPILOG, make a strong case for GP IIb/IIIa inhibitors in the setting of rescue angioplasty.

The ongoing TIMI-14 and SPEED studies are evaluating different dosing combinations of abciximab and alteplase or reteplase, respectively. Preliminary results indicate that half-dose thrombolytic and full-dose abciximab can achieve increased TIMI 3 flow rates, but insufficient data exist to meaningfully measure clinical outcomes. Dose-ranging studies are planned to evaluate whether higher doses of abciximab or fibrinolytic yield even more impressive results.

The absence of any major outcome trials leaves the role of GP IIb/IIIa inhibition in ST-elevation MI uncertain. The design of such trials will be challenging, because a different angiographic strategy may be required to achieve maximum therapeutic benefit. Furthermore, regimens that produce optimal clinical outcomes will vary as a function of both fibrinolytic agent and GP IIb/IIIa inhibitor.

Clinical practice should ultimately integrate this drug class with pharmacologic and catheter-based strategies in the community. Patients reporting to a hospital with an available, experienced interventional cardiologist, support staff, and catheterization laboratory have better outcomes with catheter-based intervention. However, even in the United States, most patients with ST-segment elevation are seen in facilities without

these capabilities. The combination of immediate drug therapy with minimal risk of intracranial hemorrhage, a noninvasive method of detecting coronary reperfusion, and efficient use of percutaneous revascularization provides the best opportunity to improve acute phase outcomes.

Non-ST-Elevation ACS

More than 30,000 patients have been randomized into clinical trials evaluating IIb/IIIa inhibitors in non-ST-elevation ACS. The message from the trials is consistent. In the first 48–96 h there is a reduction in death, nonfatal MI, and revascularization procedures. Over the first 30 d, this benefit is sustained (Table 9). By 6 mo, the mortality reduction is less clear (odds ratio, 1.0; 95% CI, 0.87–1.15), but the reduction in nonfatal MI and use of revascularization procedures is obvious (Table 10). These data come from definitive studies of several agents and interesting additional studies with four different GP IIb/IIIa inhibitors.

In non-ST-elevation ACS, the PRISM trial found tirofiban alone to be superior to heparin alone at a 48-h primary endpoint *(81)*. This trial involved relatively "low-risk" patients. The complementary PRISM PLUS trial found that tirofiban and heparin in combination was better than heparin alone, but a tirofiban-only arm was terminated early because of excess death and MI *(82)*. These results leave uncertainty about the role of the combination versus GP IIb/IIIa inhibitor alone and support the recommendation that tirofiban should be used in combination with heparin. These studies recently led to the approval of tirofiban for clinical use.

The PARAGON A study investigated the role of heparin combined with different doses of lamifiban *(83)*. The factorial design evaluated heparin and no heparin with higher and lower dose lamifiban; in addition there was an "anchor" control arm of aspirin and heparin. A slight trend toward benefit of low-dose lamifiban and heparin was observed, but the result was far from definitive at the primary endpoint (30 d). By 6 mo, a larger difference in favor of lower dose lamifiban was evident, but no difference was found with regard to heparin. This trial has been followed by the 4000 patient PARAGON B trial, which is testing lamifiban doses adjusted for creatinine clearance.

The largest trial, PURSUIT, evaluated nearly 11,000 patients randomized to either eptifibatide or placebo *(84)*. All patients received concomitant aspirin. The trial was designed to be a large, simple trial with a broad spectrum of patients from a variety of health care systems and countries. In the end, a modest reduction in the composite of death and nonfatal MI was observed, with a more substantial difference in North America (Fig. 5).

The systematic overview demonstrates a substantial reduction in death, MI, and revascularization procedure use in patients with non-ST-elevation acute coronary syndromes in the first 48–96 h of treatment (Fig. 6). These benefits are sustained through 30 d for all three endpoints. By 6 mo, however, the effect on mortality is no longer evident, although there is no erosion of the benefit with regard to prevention of nonfatal MI and revascularization.

Despite the overwhelming evidence for the clinical benefit of these agents, many important questions remain:

Comparative studies among the agents need to be conducted. As with percutaneous intervention, many have assumed that abciximab will have a greater benefit. The only available study of non-ST-elevation patients is the CAPTURE trial, where all patients

Table 9
Clinical Events at 30 Days After Platelet Glycoprotein IIb/IIIa Inhibition for Non-ST Elevation ACS

Study	*Year*	n	*Agent*	*Event Rate*		*Odds ratio (95% CI)*
				Treated	*Control*	
						Death or Infarction
PRISM	1994	3232	Tirofiban	5.8%	7.1%	0.80 (0.60, 1.06)
PRISM-PLUS	1997	1915	Tirofiban	8.7%	11.9%	0.70 (0.50, 0.98)
PURSUIT	1996	10948	Eptifibatide	14.2%	15.7%	0.88 (0.79, 0.99)
Total		**16,095**		**11.7%**	**13.4%**	**0.86 (0.77, 0.95)**
						Death, Infarction, or Revascularization
PRISM	1994	3232	Tirofiban	15.9%	18.3%	0.84 (0.70, 1.01)
PRISM-PLUS	1997	1915	Tirofiban	18.5%	22.3%	0.79 (0.62, 1.01)
PURSUIT	1996	10948	Eptifibatide	18.1%	20.4%	0.87 (0.78, 0.96)
Total		**16,095**		**17.7%**	**20.1%**	**0.85 (0.78, 0.93)**

Table 10
Clinical Events at 6 Months After Percutaneous Intervention with Platelet Glycoprotein IIb/IIIa Inhibition

Study	*Year*	n	*Agent*	*Event Rate*		*Odds ratio (95% CI)*
				Treated	*Control*	
						Death or Infarction
EPIC	1994	2099	Abciximab	9.4%	12.6%	0.72 (0.54, 0.96)
EPILOG	1995	2792	Abciximab	6.0%	11.1%	0.52 (0.39, 0.68)
IMPACT-II	1997	4010	Integrilin	10.3%	11,6%	0.87 (0.71, 1.07)
CAPTURE	1997	1265	Abciximab	9.2%	9.9%	0.92 (0.63, 1.34)
RESTORE	1997	2139	Tirofiban	7.8%	8.6%	0.89 (0.66, 1.34)
Total		**12,455**		**8.5%**	**10.8%**	**0.76 (0.63, 0.92)**
						Death, Infarction, or Revascularization
EPIC	1994	2099	Abciximab	29.8%	35.1%	0.79 (0.65, 0.96)
EPILOG	1995	2792	Abciximab	22.4%	25.7%	0.84 (0.70, 1.00)
IMPACT-II	1997	4010	Integrilin	30.2%	31.5%	0.94 (0.82, 1.08)
CAPTURE	1997	1265	Abciximab	30.6%	30.4%	1.01 (0.80, 1.29)
RESTORE	1997	2139	Tirofiban	26.9%	29.8%	0.85 (0.70, 1.04)
Total		**12,455**		**28.0%**	**30.5%**	**0.88 (0.81, 0.96)**

had already failed standard therapy and percutaneous intervention was anticipated. As with the percutaneous intervention trials, indirect comparisons are treacherous and direct comparisons are needed.

The duration of therapy remains concerning. Pressure to limit the length of hospital stay in the United States has caused many studies to focus on a short duration of therapy. Conceptually, it may be more effective to prolong therapy and "pacify" the surface of the plaque. Oral agents may best achieve this effect. Observational analyses have not

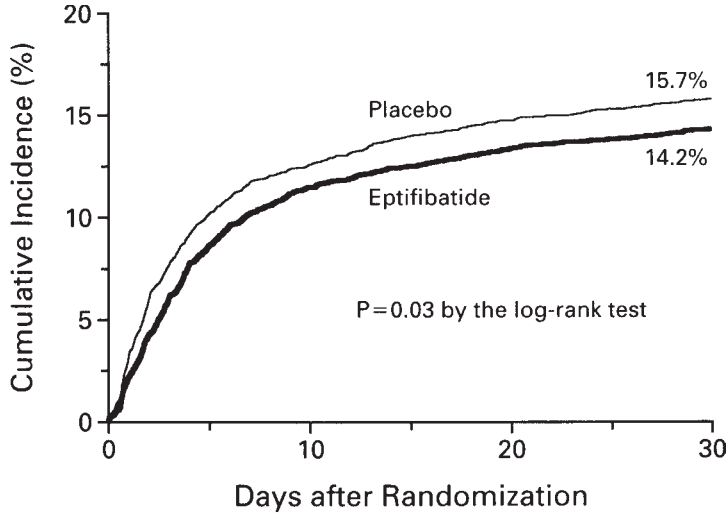

Fig. 5. Kaplan-Meier curve of the PURSUIT primary endpoint, demonstrating a moderate reduction in death and nonfatal MI. Reprinted with permission of ref. *84*.

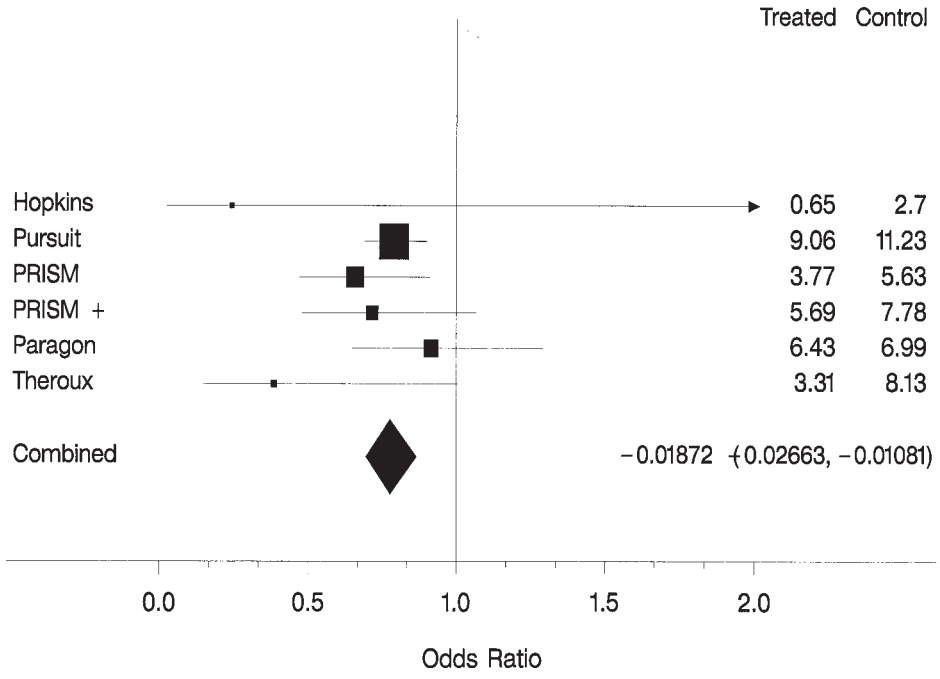

Fig. 6. Results of a systematic overview of trials of GP IIb/IIIa inhibition in non-ST-segment elevation ACS, demonstrating considerable reduction in death, MI, and need for revascularization.

detected a direct relationship between duration of therapy and event reduction. These trials have had fairly uniform duration of therapy, limiting their power.

Another major question is whether this class of drugs prevents the need for revascularization procedures or increases the efficacy and efficiency of revascularization procedures when they are done. The systematic overview shows that clinical outcomes

can be improved with fewer revascularization procedures. As previously discussed, only a small decrease in the need for revascularization procedures with preservation of clinical outcomes would be needed to create an economically attractive therapy. Both the CAPTURE and the PURSUIT trials (Fig. 7) show benefit prior to revascularization procedures. More importantly, the event rates increase at the time of revascularization, suggesting that patients may do better without procedures. On the other hand, both percutaneous revascularization and bypass surgery are associated with an increased risk of early events; the survival curves in trials comparing surgery and medical therapy do not cross until at least the first year after randomization. From this perspective, revascularization could be regarded as an "investment" that does not provide return for at least a year. GP IIb/IIIa inhibitors provide an opportunity to reduce this early hazard and thereby decrease the time until the investment pays off. This issue can only be addressed by randomized trials that assign patients to a strategy of early intervention or intervention only for specific indications. Unfortunately, the opportunity for such a factorial randomized trial has been missed. The benefit of GP IIb/IIIa inhibitors is now clear, and it would be unethical to assign high-risk patients to placebo in the future.

Chronic Coronary Artery Disease

Several small phase II studies have examined the use of GP IIb/IIIa inhibitors beyond the acute phase of percutaneous intervention or ACS, but no clinical outcome data are available. So far, all that is known is that when inhibition of platelet aggregation to 20 μM ADP consistently exceeds 90%, excess mucocutaneous bleeding occurs. Below 80% inhibition, the compounds appear to be well tolerated with regard to bleeding.

A variety of trials will compare oral IIb/IIIa agents with aspirin in the chronic setting. In some cases the IIb/IIIa inhibitor will be given with aspirin as background therapy, whereas in others the IIb/IIIa inhibitor will be used without aspirin. Currently designed trials also vary in patient populations from solely high-risk patients to the entire secondary prevention population.

Atrial Fibrillation

No studies have evaluated the use of GP IIb/IIIa inhibitors in the setting of atrial fibrillation. Trials of oral GP IIb/IIIa inhibitors will likely enroll a significant number of such patients.

Stroke and Cerebrovascular Disease

No studies have evaluated GP IIb/IIIa inhibitors for either the treatment or the prevention of stroke. Indeed, only recently has the effectiveness of aspirin been validated in acute stroke. A pilot study is now underway with abciximab in the early hours of acute ischemic stroke, and other GP IIb/IIIa agents will be evaluated in the near future in attempts to ameliorate the acute outcomes. This drug class may be valuable as an acute therapy to avert early recurrent events and as a chronic therapy to prevent the cerebrovascular and coronary events that eventually kill many acute stroke survivors.

Peripheral Vascular Disease

No studies have been conducted in patients with peripheral vascular disease. GP IIb/IIIa receptor inhibitors may be useful during peripheral vascular intervention, for prevention of new acute occlusions, and for limiting chronic peripheral disease progression.

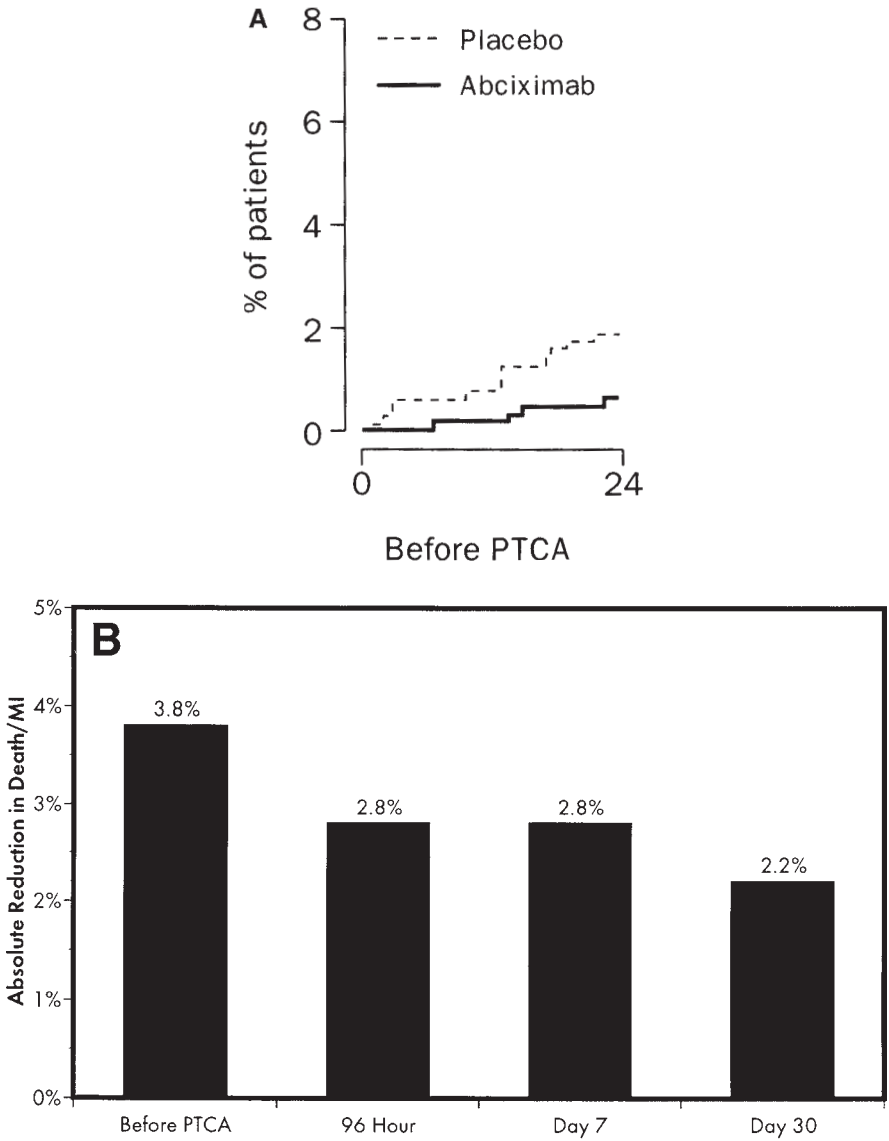

Fig. 7. Benefit of GP IIb/IIIa inhibitors in the CAPTURE trial before revascularization **(A)** and in PURSUIT before and 96 h, 7 d, and 30 d after such procedures **(B)**. CAPTURE data reprinted with permission from ref. *98*.

The prevention of large numbers of cardiac and cerebrovascular events typical of this population would be an important secondary benefit.

Coronary Artery Bypass Grafting

No trials have been done in this setting, although a growing number of patients have had bypass surgery after treatment with GP IIb/IIIa inhibitors. Data to date indicate no major increase in the risk of bleeding, although many patients have been treated with platelet transfusions. Trials will need to assess whether pretreatment with GP IIb/IIIa

inhibitors prevents platelet consumption during cardiopulmonary bypass and whether these drugs ameliorate the impact of platelet activation on systemic outcomes.

COMMON THEMES

Intensity of Platelet Inhibition

The most effective intensity of platelet inhibition remains unknown. The initial positive clinical trials with abciximab used animal model data to simulate ACS. These models indicated that 80% receptor occupancy by the antibody was required to maintain optimal continuous arterial perfusion. It may be presumed that aspirin inhibits platelet aggregation to 20 μ*M* ADP by 20%, and ticlopidine and clopidogrel inhibit aggregation by 30% under these conditions. The doses of eptifibatide used in the IMPACT II study achieved 50–60% inhibition by standard measures. A higher level of inhibition may be desirable, based on the modest benefit over aspirin achieved by clopidogrel in CAPRIE *(45)* and by eptifibatide in IMPACT II *(85)*. Indeed, this line of reasoning led to a doubling of the eptifibatide dose for PURSUIT *(84)*.

Experience with a variety of antithrombotics indicates that excessively high doses can increase the frequency of bleeding and (paradoxically) the rate of ischemic cardiovascular events. When the aPTT is prolonged by heparin *(86)*, the occurrence of death, nonfatal MI, and ischemic stroke is elevated in addition to the expected increase in bleeding. The same pattern has been observed with the direct thrombin inhibitor, hirudin. It is unknown whether the increase in ischemic events is due to hemorrhage within the plaque, cessation of drug because of bleeding, hypotension from hemorrhage, or some unexplained physiological effect of the thrombin inhibitor. The PARAGON A study, in which the higher lamifiban dose was associated with worse outcomes than lower lamifiban dose, may indicate a form of GP IIb/IIIa toxicity.

The most effective level of platelet inhibition may vary with the phase of the clinical condition. In the acute phase of ACS, high grade inhibition of platelet function seems appropriate, and the bleeding risk appears tolerable. Similarly, during the acute phase of percutaneous intervention, the degree of plaque disruption is so extensive that high degree inhibition is likely preferable. During the chronic phase, bleeding is much less tolerated, encouraging more inhibition than aspirin alone but to lesser degree than acute therapy.

Dosing

Most of the GP IIb/IIIa inhibitors are renally excreted. Accordingly, the serum levels and consequent platelet inhibition are highly dependent upon creatinine clearance. For oral agents, this problem complicates chronic administration to patients with renal insufficiency. Dose adjustment may be helpful even in cases of modest renal insufficiency. It remains to be seen whether the complexity of adjusted dosing is clinically worthwhile. The PARAGON B study will evaluate this issue with intravenous administration, and SYMPHONY will evaluate adjusted dosing for oral regimens.

Constancy of Inhibition

Most researchers have assumed that constant platelet inhibition would be preferable to cyclical inhibition. This approach makes sense because it assumes that the stimulus to thrombosis, when present, remains intense for a prolonged period of time. Alterna-

tively, we know little about modulation of the receptor and the biological response underlying clinical benefit; it is possible that variation in receptor occupancy would be beneficial. Similar reasoning has led to more effective nitrate therapy.

In fact, mucocutaneous bleeding may be proportional to the duration of high level (>80% inhibition of ADP-stimulated aggregation) inhibition. Future studies will test different dosing strategies of the orally administered IIb/IIIa inhibitors, which possess different pharmacological profiles. Those with a longer half-life will achieve a sustained level and duration of inhibition, whereas those with a shorter half-life will be administered with aspirin, cycling the degree of platelet inhibition.

Monitoring

General Issues

The most effective level of inhibition of platelet function remains uncertain. When considering the methodology for demonstrating a relationship between clinical outcomes and pharmacological parameters, the sample sizes required are potentially astronomical. To establish a clear difference in the outcomes produced by different doses requires a sample size at least as large as that required to distinguish active drug from placebo. On the other hand, a lower dose limit can be derived from a threshold using physiological measures with a proven relationship to clinical benefit. The upper dose limit can be defined by clinical bleeding. These clinical outcome boundaries can establish a therapeutic range for antiplatelet compounds.

Serum drug levels may be used as a direct assay. The ability to monitor blood samples has certain advantages. In most studies a direct relationship exists among drug levels, receptor occupancy, and physiological measures of platelet inhibition. Unfortunately, the relationship between receptor occupancy and physiological measures is variable. It might be possible for the number of receptors per platelet, the level of physiological activation, and the number of circulating platelets to disrupt the relationship between drug level and biologic effect to a clinically important degree.

Receptor occupancy has also been considered to be an important measure of effect. However, measures of receptor occupancy are cumbersome and expensive. Platelet aggregation appears not to be inhibited until approximately 50% of the receptors are occupied, and then the effect of receptor occupancy on platelet aggregation increases markedly.

Assays of platelet aggregation to ADP and TRAP agonists form the mainstay for phase II trials, which seek optimal doses for testing in clinical outcome studies. These difficult assays must be performed at the bedside; prolonged transport time may produce spurious results. Standard platelet aggregation assays do not provide a practical monitoring solution.

New functional assays for platelet aggregation using occlusion of membranes by platelet thrombi, thromboelastography, and sheer-induced platelet aggregation have been advocated *(87–96)*. Several are available at the bedside and might be used to identify patients with doses inappropriate for the clinical situation.

Bleeding and Thrombocytopenia

The only likely limitations of these agents will be mucocutaneous bleeding and thrombocytopenia. As previously discussed, the likelihood of mucocutaneous bleeding seems to increase directly with the level of platelet inhibition, with little bleeding occurring below the level of 80% inhibition after stimulation with 20 μM ADP.

Thrombocytopenia occurs in 0.4–4% of patients in different populations with different agents. Apparently all GP IIb/IIIa inhibitors are associated with some risk of thrombocytopenia. Abciximab initially brought attention to this problem with precipitous thrombocytopenia in <1% of patients. It is unclear whether the incidence of thrombocytopenia is actually increased relative to aspirin and heparin when the small molecule GP IIb/IIIa inhibitors are used. However, the oral agents are associated with a rate of profound thrombocytopenia in <1% of patients. These events occur most often within the first several doses, but episodes have been observed up to 2 wk after the first dose. Guidelines for the treatment of acute thrombocytopenia have been published and most patients have not had clinical sequelae, but the precise mechanism and optimal treatment remain unknown. Although the problem is significant in inpatients, the potential for harm in outpatients is obvious. Since all of the oral agents face this risk, a common effort to understand the thrombocytopenia they induce appears to be in order.

Bleeding with GP IIb/IIIa agents is a concern, although the intravenous agents have reasonable safety. In the EPILOG study, a statistically insignificant lower bleeding rate was observed with abciximab and low-dose heparin versus standard heparin, and bleeding may even be reduced further by decreasing the dose of thrombin inhibitor. A more concerning issue is whether chronic oral administration will be possible without unacceptable bleeding. With a variety of oral agents, unacceptable mucocutaneous bleeding seems to occur with inhibition of platelet aggregation exceeding 80% with stimulation with 20 μM ADP. Given the fact that all of the oral agents have variable absorption and many have significant renal elimination, it is likely that even with a target of over 80%, a proportion of the population will remain in excess of this degree of inhibition for significant periods of time. The impact of chronic aspirin use with its properties of gastric mucosal dysfunction in combination with more potent platelet inhibition also remains uncertain.

Readministration of these compounds remains a source of uncertainty. The only experience so far is with abciximab, which evokes concern because it is an antibody. Although the rate of thrombocytopenia appears to be modestly higher, preliminary observational data suggest no clinically important loss of efficacy. The results with readministration of the smaller molecules are unknown.

ATTRIBUTES OF MORE EFFECTIVE AGENTS

It is humbling to recognize after many years of study and more than 50,000 randomized patients that we still do not know the optimal attributes of a GP IIb/IIIa inhibitor. Physical properties such as receptor affinity, receptor specificity, half-life, and routes of metabolism and elimination have been compared hypothetically, but we lack comparative clinical outcome data to know which attributes are associated with better outcomes. In addition, the various GP IIb/IIIa inhibitors may interact with different regions of the receptor; the clinical relevance of these differences is unknown.

COST EFFECTIVENESS

As more clinical outcome data become available, the application of GP IIb/IIIa inhibitors will be shaped by measures of economic cost relative to clinical benefit. A minor reduction in the need for revascularization or duration of hospital stays could significantly reduce downstream costs. In a detailed analysis done by Eisenstein et al., a 15%

reduction in the composite endpoint of death, MI, and revascularization would save $1456 for each patients with non-ST elevation ACS *(44)*. Similar results would be expected in high-risk chronic coronary disease patients. In low-risk patients with chronic coronary disease or primary prevention, much less is known about the type of event reduction that would be economically attractive; the issue is further confounded because of the lower tolerance for bleeding complications.

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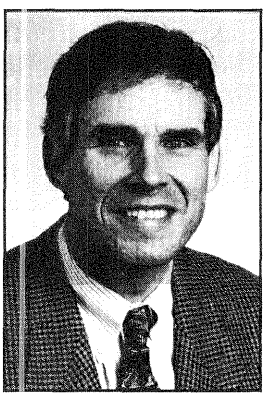
About the Editors

Dr. A. Michael Lincoff is an Associate Professor of Medicine in the Department of Cardiology and the Joseph J. Jacobs Center for Thrombosis and Vascular Biology at the Cleveland Clinic Foundation, and a practicing interventional cardiologist at the Cleveland Clinic. As such, he is involved in research activities focusing on the development of therapies to reduce the acute and long-term complications of percutaneous coronary revascularization procedures and to optimize therapy for acute coronary ischemic syndromes. As Director of the Experimental Interventional Laboratory, he supervises preclinical research projects in which animal models are used to study the mechanisms of restenosis following coronary intervention.

Dr. Lincoff has also led multicenter clinical trials in ischemic heart disease coordinated by the Cleveland Clinic Cardiovascular Coordinating Center and in collaboration with the Duke Clinical Research Institute. He is principal investigator and steering committee member for a variety of studies testing new antiplatelet and/or antithrombin therapies during coronary intervention and in the settings of unstable angina and acute myocardial infarction, including EPILOG, EPISTENT, PROLOG, IMPACT II, PURSUIT, SPEED, GUSTO IV AMI, and CACHET.

Dr. Lincoff has authored or coauthored many articles in the *New England Journal of Medicine, Lancet, Annals of Internal Medicine, Circulation, Journal of the American College of Cardiology, American Heart Journal,* and *European Heart Journal,* among others. He has also authored book chapters and reviews, including chapters in *Textbook of Interventional Cardiology and Textbook of Cardiovascular Medicine,* both edited by Eric J. Topol, and *Heart Disease,* edited by Eugene Braunwald.

Dr. Lincoff is a Fellow of the American College of Cardiology and a member of the American College of Physicians.



Dr. Eric J. Topol is Chairman of the Department of Cardiology, which has been ranked number one by *U.S. News and World Report* for the past four years, and Professor of Medicine at the Cleveland Clinic Foundation. He is also Director of the Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Vice-Chairman of the Department of Molecular Cardiology, and Codirector of the Heart Center at the same facility.

Dr. Topol is a member of the American Association of Physicians and the American Society of Clinical Investigation. He is a Fellow, American Heart Association; Fellow, European Society of Cardiology; Fellow and Trustee, Society of Cardiac Angiography and Interventions; Fellow, American College of Chest Physicians; and is listed in both *Good Housekeeping* and *American Health* magazines as being among "the country's best (heart) doctors."

He has been a principal investigator for a number of international multicenter research trials, including, GUSTO I-III, TAMI 1-9, the IMPACT trials, EPIC, EPILOG, EPISTENT, PURSUIT, PARAGON, CAVEAT, and many others. He was the first physician to administer recombinant t-PA and two different platelet glycoprotein IIb/IIIa inhibitors.

Dr. Topol serves on the editorial boards of *Circulation, Circulation Research, American Journal of Cardiology, British Heart Journal,* and the *European Heart Journal,* among others. He has edited many original publications and books, including *Textbook of Interventional Cardiology* (editions 1 to 3), *Textbook of Cardiovascular Medicine,* and *Comprehensive Cardiovascular Medicine.*