Platelets and Acute Coronary Syndromes

You may not think it is important, I know, but it is, and I'm bothering telling you so.

The Sleep Book
Dr. Seuss

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Rank: Assistant Professor

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Areas of Interest:

(1) Ischemic heart disease including unstable angina and antiplatelet therapy

(2) Percutaneous methods of coronary artery revascularization

(3) Aortic Dissection

This is to acknowledge that Joaquin E. Cigarroa has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

INTRODUCTION:

Acute coronary syndromes constitute a spectrum of clinical presentations including unstable angina, non-Q wave myocardial infarction and Q wave myocardial infarction. These disease entities affect greater than one and a half million patients per year in the United States alone, resulting in significant morbidity , mortality and utilization of resources [1,2]. Although medical therapy has improved patient outcome in acute coronary syndromes, a significant number of patients experience recurrent ischemia and/or mortality. In order to understand the role of antiplatelet therapy in acute coronary syndromes, their current applications and limitations, one must first understand the pathophysiology which precipitates these clinical syndromes.

PATHOPHYSIOLOGY:

The current belief regarding the initiation of the atherosclerotic process has been advocated by Ross as the "response to injury" hypothesis [3]. Vascular injury resulting in the formation of thrombus results in lesion progression and /or occurrence of an acute coronary syndrome. Fuster et. al. have classified vascular injury into three groups in order of increasing severity of injury: type I, type II and type III.

Type 1 injury often occurs in areas where there is a chronic disturbance in laminar blood flow such as bending points which results in minimal endothelial injury and the accumulation of macrophages and lipids [4]. Type II injury is characterized by macrophages which release toxic substances which, in turn, promote platelet aggregation. A combination of growth factors released by the endothelium, macrophage cells and platelets stimulate the migration and proliferation of smooth muscle cells resulting in a fibrointimal lesion. Type III injury occurs when a lipid lesion is disrupted resulting in the formation of thrombus. This may be a silent event causing plaque progression or a catastrophic event which precipitates unstable angina, myocardial infarction or sudden death. The dynamic interaction between ongoing thrombosis and endogenous thrombolysis are influenced by the severity of injury, endogenous lysis and our treatment which, in turn, may alter the clinical outcome.

The propensity to develop atherosclerosis , the rate of progression and the tendency for plaque rupture, are, in turn, affected by cardiac risk factors such as hypercholesterolemia, smoking history, genetic predisposition, diabetes mellitus, gender, estrogen status and other lesser factors.

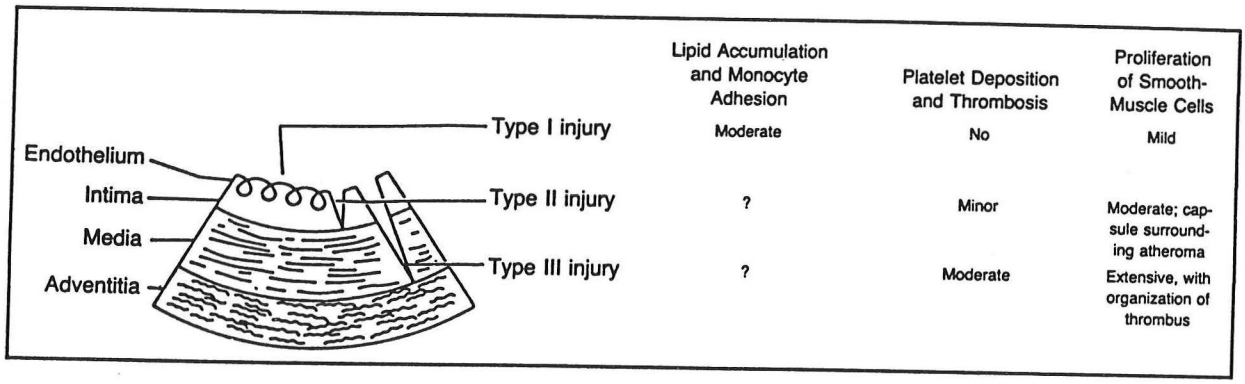

Figure 1. Classification of Vascular Injury or Damage and Vascular Response.
See the text for details. Adapted from Ip et al.[5] with the permission of the publisher.

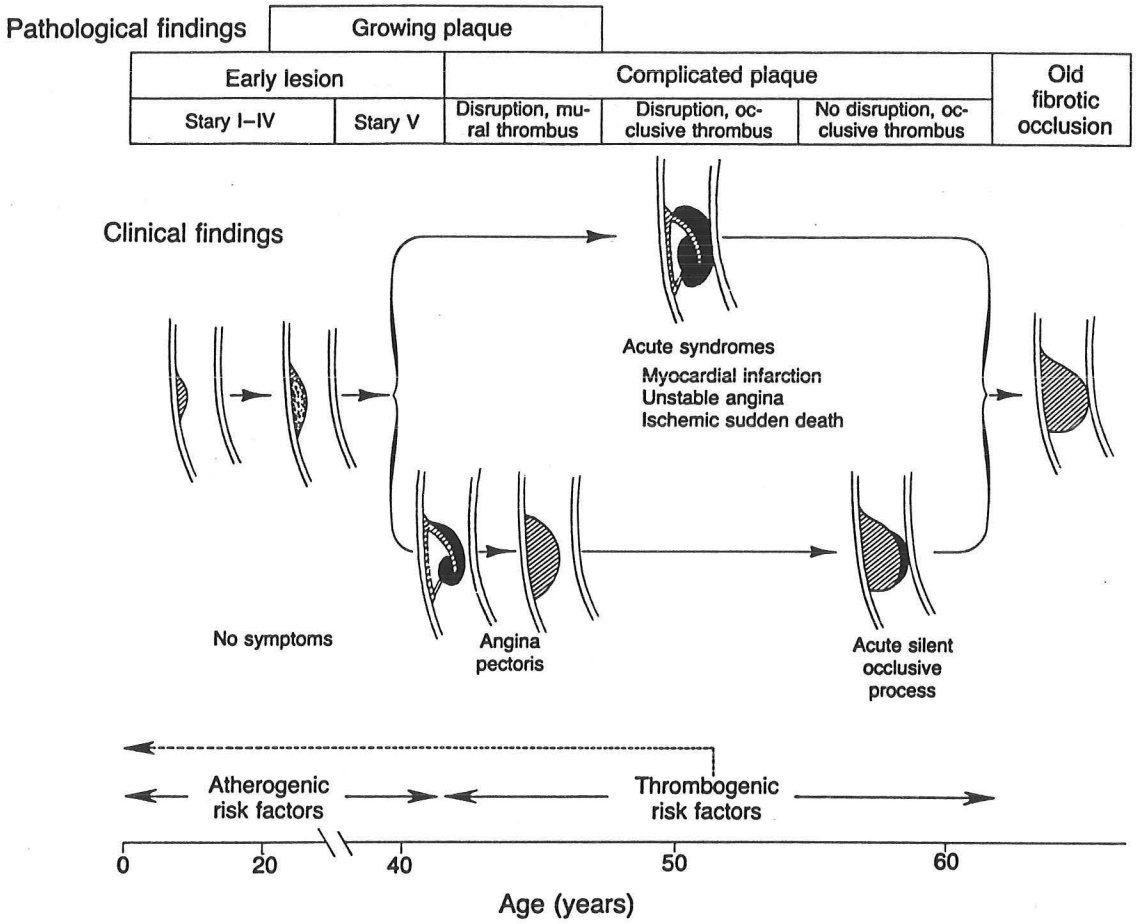

Figure 2. Typical Early Evolution and Progression of Coronary Atherosclerosis, According to the Pathological and Clinical Findings.

Although DeWood et. al. [5] demonstrated in 1980 that total coronary occlusion is a dynamic process secondary to thrombosis present during the initial hours after myocardial infarction, it was not until later that thrombosis as a cause of atherosclerotic lesion progression was realized.

Davies et. al. [6] demonstrated atherosclerotic plaque fissuring with superimposed thrombi in a post-mortem study of 29 patients who died of noncardiac etiologies with evidence of atherosclerosis. When the coronary arteries of patients dying of ischemic heart disease were analyzed, there was evidence of fissures with superimposed thrombus. Some lesions displayed evidence of thrombus organization. These findings provided support for the concept that lesions rupture, form thrombus and may seal and result in plaque progression.

Angiographic studies have supported the post-mortem findings of thrombus present in patients with unstable angina. Gotoh et. al. [7] performed coronary angiography during spontaneous chest pain in 37 patients with prolonged rest chest pain. Angiography revealed that 57% (21/37 patients) had angiographic features consistent with thrombus. These findings were later confirmed in a separate study which utilized coronary angioscopy [8]. Ambrose et. al. performed angioscopy in 31 patients admitted with either unstable angina or acute myocardial infarction and revealed thrombus in 29 of the 31 patients. Of note, white, non-occlusive thrombus was more often present in unstable angina consistent with a platelet rich thrombus,
whereas red, occlusive thrombus consistent with fibrin, erythrocytes and platelets was more often present in acute myocardial infarction.

Patients with the diagnosis of unstable angina have been demonstrated to have a progression of stenosis severity which translates into increased adverse clinical events. A study by Chen et. al. [9] included 102 patients who had undergone coronary angiography, had evidence of a stenosis>50%, stabilized on medical therapy and were scheduled for an elective angioplasty at a later date. During the initial angiogram 198 stenoses >50% were identified of which 85 were believed to be ischemia-related. At follow-up angiography (mean 8 months) , 21/85 ischemia-related lesions progressed compared to only 8/113 non ischemia lesions. Of note, 17/21 ischemia lesions which progressed developed a total occlusion and more often had complex stenoses at initial angiography. This rapid disease progression may account for the high likelihood of recurrent ischemia and infarction in patients with unstable angina.

Thus, patients with plaque rupture and thrombosis are at high risk for disease progression, recurrent ischemia and infarction. The realization that platelet-rich thrombi are central in the progression of coronary artery lesions and are involved in acute coronary syndromes, allows us to target platelets in an effort to alter the natural history of disease progression and clinical events.

EARLY CLINICAL TRIALS OF ANTIPLATELET THERAPY

Aspirin has been demonstrated to be effective in the primary prevention of myocardial infarction, unstable angina, the treatment of acute myocardial infarction and in the secondary prevention of myocardial infarction [10-16].

Unstable Angina:
Aspirin was first noted to be effective in the reduction of myocardial infarction and probably death in the early 1980's (see Table 1). The Veterans Administration Cooperative Study [11] evaluated the efficacy of aspirin in men admitted with unstable angina. The study included 1,266 men who were assigned, by random allotment, to receive aspirin or placebo treatment for a 12 week period. Aspirin was effective in reducing non-fatal infarction from 6.9% to 3.4% while producing a trend in decreased mortality (3.4% vs 1.6%). These findings were confirmed the RISC study [12] and by a double randomized trial comparing aspirin, sulfinpyrazone or both vs placebo with an 18 month follow-up performed by the Medical Research Council of Canada in 1985[13]. Compared to placebo, aspirin resulted in a significant reduction in the incidence of cardiac death and nonfatal myocardial infarction (17%vs 8.6%). When evaluating the effect of aspirin on cardiac death alone, aspirin resulted in a 71% reduction in mortality (11.7% vs 3%); sulfinpyrazone did not add an additional benefit to aspirin. Finally, Theroux et al [14] evaluated the effects of aspirin, heparin, or both vs placebo in patients admitted with unstable angina followed for 6 to 9 days. Major endpoints were defined as refractory angina, myocardial infarction, or death which occurred in 23%, 12%, 1.7% of the placebo group. Whereas heparin decreased the occurrence of refractory angina, the

incidence of myocardial infarction was decreased by all active treatment groups: aspirin (3%), aspirin plus heparin (1.6%) and heparin (0.8%). A subsequent analysis of the data revealed that early clinical reactivation (heparin rebound) occurred in 14/107 patients treated with heparin alone whereas it occurred in only 5 patients treated with aspirin. Thus, aspirin plus heparin is associated with a decrease in the incidence of myocardial infarction. The use of heparin without aspirin may result in early reactivation of unstable angina upon discontinuation of heparin which may be attenuated by the use of aspirin.

TABLE 1: ANTIPLATLET THERAPY IN UNSTABLE ANGINA

STUDY	THERAPY	DOSE	F/U	Card Death/Nonfatal MI(%)
VA Study	ASA vs Placebo	324 mg	3 mo	5.0 vs 10.1*
RISC	ASA	75mg	90 days	7.4 vs 17.1*
	Heparin			16.6 vs 17.1
	ASA+Heparin			5.7 vs17.1*
Canadian Mult Trial	ASA	1000mg	24 months	8.6 vs 17.0*
	ASA+Sulfinpyrazone	800mg		
Theroux	ASA	650	6 days	3.3
	Heparin			0.8 vs 12.0*
	ASA+Heparin			1.6 vs 12.0*
Belsano	Ticlopidine	500mg	6 months	7.6 vs 13.6*

$p<0.05$

Thus, aspirin is effective in both reducing the progression of unstable angina to nonfatal myocardial infarction and decreasing cardiac mortality during the index hospitalization and long term follow-up. It also, appear that ticlopidine may be an effective alternative [15] in aspirin intolerant patients.

Acute Myocardial Infarction:

Since aspirin resulted in a decrease in cardiac morbidity and a reduction in myocardial infarction in patients admitted with unstable angina, people wondered about its role in acute myocardial infarction. This fundamental question was addressed in the Second International Study of Infarct Survival(ISIS-2) published in 199017, [16]. ISIS-2 included 17,187 patients admitted $\leq$ 24 hours of symptom onset suspected of experiencing an acute myocardial infarction. Patients were randomized to one of four arms: placebo, aspirin, streptokinase or aspirin plus streptokinase. The treatment with aspirin vs placebo was

continued for 5 weeks with the primary endpoint being total vascular mortality with secondary endpoints including nonfatal reinfarction and stroke.

TABLE 2	F/U	MORTALITY(%)
Placebo	5 wks	13.2
ASA(160mg)	5 wks	9.4*
Streptokinase	5 wks	9.2*
Streptokinase+ASA	5 wks	8.0*

*$p<0.05$ compared to placebo

Thus, aspirin alone decreased mortality by 21% and nonfatal reinfarction by 44%. The addition of streptokinase to aspirin had an additive effect with a further decrease in mortality. The benefit of aspirin persisted for the subsequent 24 months of follow-up.

ISIS-2 established aspirin as an effective pharmacological agent in the treatment of infarction independent of the use of thrombolytic agents.

Primary Prevention:

The Physician's Health study included 22,071 male physicians who did not have evidence of coronary artery disease by clinical measures who were randomized to aspirin (325mg po qod) versus placebo. These patients were followed for five years. Compared to placebo, the use of aspirin decreased the incidence of myocardial infarction by 44% (n=139 vs 239) while not affecting mortality [10]. An observational study involving female nurses revealed that women older than 50 years had a significant reduction in myocardial infarction [17].

Aspirin is effective in decreasing the risk of a first myocardial infarction in both men and women; individuals with risk factors appear to benefit the most.

LIMITATIONS OF ASPIRIN:

Although aspirin is clearly effective in reducing cardiac morbidity and mortality in various clinical syndromes, patients with acute coronary syndromes treated with aspirin continue to experience significant morbidity and mortality.

A review of the GUSTO trial [18] assessing four thrombolytic strategies to treat acute myocardial infarction revealed that front-loaded tPA in conjunction with aspirin and heparin yielded the lowest mortality at 30 days (6.3%). Despite this optimal medical treatment of acute myocardial infarction, significant limitations occurred. The GUSTO angiographic investigators [19] revealed that at 90 minutes, 81% of the front-loaded tPA group had a patent vessel while only 54% had normal TIMI 3 flow. Furthermore, 6% of these patients developed a reocclusion of the infarct-related vessel. Recurrent ischemia and or reinfarction occurred in 23% of the patients. Thus, early restoration of normal flow which is associated with improved clinical

outcomes fails in a significant percentage of patients while recurrent ischemia and or infarction occurs in approximately 25% of patients.

It appears that the administration of thrombolytic therapy, despite the utilization of aspirin, results in platelet activation [20,21] which may limit the efficacy of the therapy. When tPA or streptokinase is administered to a patient with an acute myocardial infarction, a paradoxical activation of platelets occurs. This effect may be due to direct platelet activation and/or due to the generation of thrombin, a potent platelet activator. In addition, thrombin also stimulates the production of thromboxane.

Since acute myocardial infarction is secondary to an occluding thrombus composed of platelets and fibrin, it may be that the failure to achieve recanalization in the 20% who fail lytic therapy initially is due to a platelet rich clot whereas those who fail to achieve normal flow and or develop recurrent ischemia is secondary to thrombolytic activation of platelets with the accumulation of platelets on the clot surface resulting in the formation of a new, platelet rich thrombus [22-23].

I suspect that this paradoxical activation of platelets by thrombolytic therapy explains the higher incidence of MI in unstable angina patients treated with lytic therapy (8.3% vs 4.6%) in TIMI 3B [24] and the increased risk of death or reinfarction in routine, immediate PTCA vs delayed PTCA (12.8% vs 8.8%) following thromboytic therapy in acute MI demonstrate by TIMI 2A [25] . These findings were later confirmed in the TAUSA [26] trial (adjunctive thrombolytic therapy during angioplasty for ischemic rest trial). Patients with unstable angina were randomized to urokinase or placebo during angioplasty. Patients treated with adjunctive lytic therapy in addition to PTCA had worse outcomes with an increased rate of abrupt closure (10.2% vs 4.3%) and increased rates of ischemia, infarction and emergent CABG (12.9% vs 6.3%).

A more effective antiplatelet agent might be able to offset the effects of lytic agents on platelet activation and improve clinical outcomes in the setting of acute myocardial infarction by improving initial perfusion, decreasing recurrent ischemia/occlusion and potentially improving mortality . A more effective antiplatelet agent might also improve the efficacy of angioplasty in both unstable angina and rescue angioplasty.

In order to understand the limitation of aspirin in acute coronary syndromes and in angioplasty, one must first understand the mechanism of platelet adhesion and aggregation.

PLATELET ACTIVATION, ADHESION AND AGGREGATION:

Activation:

Damage to the vessel wall with exposure of the subendothelial matrix stimulates platelet adhesion which, in turn, triggers platelet aggregation. The process of platelet adhesion and aggregation enhances the coagulation cascade resulting in platelet-fibrin clots. Various stimuli including collagen, thrombin, serotonin, epinephrine and adenosine diphosphate can stimulate platelet aggregation. These agonists may release arachidonic acid which is metabolized via cyclooxygenase and thomboxane synthase into thromboxane A2 (TxA2). TxA2 is a vasoconstrictor and directly stimulates the release of ADP and serotonin from platelet granules thereby stimulating further platelet aggregation. In addition, agonists such as thrombin, collagen and epinephrine can directly promote platelet aggregation [28].

Platelet aggregation

Agonists	Transducing mechanisms	Effectors
1. Adhesion	1. Arachidonic acid	1. Activated GPIIb/IIIa
2. Thrombin	2. Protein kinase C	
3. Thromboxane A_2	3. ?	
4. ADP		
5. Epinephrine		
6. Serotonin	cAMP or cGMP	
7. Vasopressin		
8. Plasmin	PGI_2 NO*	
9. t-PA/SK*		
10. Shear		

* t-PA, tissue plasminogen activator; SK, streptokinase; NO, nitric oxide.

FIG 2. Table of platelet aggregation. A variety of agonists, including adhesion itself, and agents either synthesized or released at the site of injury, can initiate platelet aggregation. Thrombolytic agents and shear forces can also cause platelet activation. All of the agonists operate through several transducing mechanisms, including arachidonic acid metabolism and protein kinase C, but it is almost certain that other unknown transducing mechanisms exist. These transducing mechanisms ultimately result in activation of the GPIIb/IIIa receptor, which is the final common event. The transducing mechanisms can be inhibited by prostacyclin (PGI_2), which increases cAMP, or nitric oxide (endothelium-derived relaxation factor [EDRF]), which increase cGMP. From Coller BS. Antiplatelet agents in the prevention and therapy of thrombosis. *Ann Rev Med.* 1992;43:171-180. Reproduced with permission.

Mechanism of Aspirin:

The antiplatelet effects of aspirin are due to the irreversible acetylation [29] of a serine residue which thereby inhibits cycooxygenase and hydroperoxidase reaction preventing the formation of thromboxane A2. Although this produces an antiplatelet effect, it is a relatively weak effect because thrombin, collagen and epinephrine are able to directly stimulate platelet aggregation. In the setting of acute coronary syndromes, the presence of collagen via plaque rupture, thrombin and increased catecholamines may overcome aspirin's antiplatelet effect.

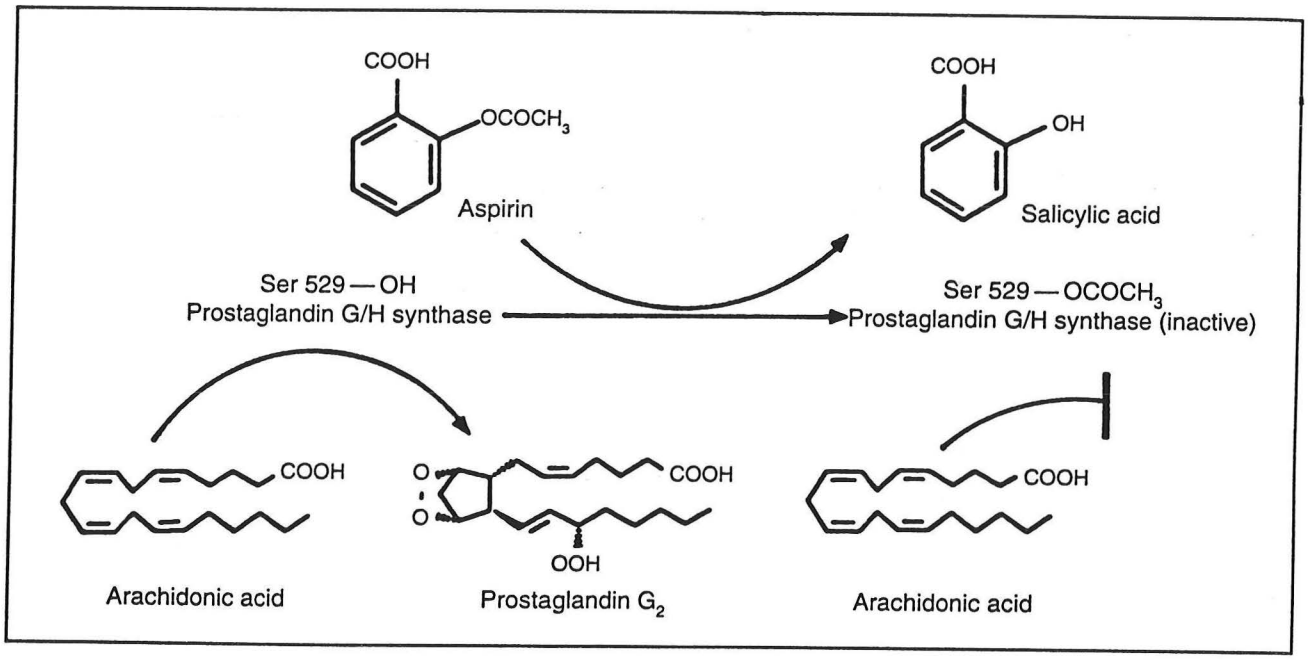

Figure 1. Mechanism of the Antiplatelet Action of Aspirin.
Aspirin acetylates the hydroxyl group of a serine residue at position 529 (Ser529) in the polypeptide chain of human platelet prostaglandin G/H synthase, resulting in the inactivation of cyclooxygenase catalytic activity. Aspirin-induced blockade of prostaglandin G_2 synthesis will result in decreased biosynthesis of prostaglandin H_2 and thromboxane A_2.

An understanding of the mechanisms involved in platelet activation, adhesion and aggregation may lead to more effective antiplatelet agents.

Platelet Receptors:

Platelet adhesion and aggregation involve platelet receptors, some of which belong to the integrin family. Each receptor class is composed of entities, an alpha and beta subunit. Members of each class contain a common beta subunit coupled to a distinct alpha subunit.

Platelet Receptors

Receptor	Ligand
Adhesion	
GPIa/IIa	Collagen
GPIc/IIa	Laminin
Av/IIa	Vitronectin, fibrinogen, vWF, thrombospondin
GPIIb/IIIa	Fibrinogen, fibronectin, VWF, vitronectin, ?thrombospondin
GPIb	von Willebrand factor
Aggregation	
GPIIb/IIIa	Fibrinogen, fibronectin, vWF, vitronectin, ?thrombospondin

Adhesion:

Platelet adhesion to the vascular wall is regulated by the intact endothelium, not the platelets. When the endothelium is disrupted secondary to plaque rupture with the exposure of underlying von Willebrand factor and collagen, platelets adhere rapidly, form a platelet monolayer and achieve hemostasis. This rapid response is ensured by the fact that platelet receptors responsible for adhesion are always active, thereby leaving the regulation of adhesion to the endothelium. The glycoprotein Ib receptor which binds to the ligand, von Willebrand, factor is the most important platelet surface receptor involved in adhesion.

Aggregation:

Platelet aggregation is dependent upon the glycoprotein IIb/IIIa receptor (GPIIb/IIIa) which is found only on platelets and megakaryocytes. This receptor is inactive and present in large numbers (>50,000/platelet). The inactive, resting state of the receptor precludes aggregation and occlusion in the absence of stimuli. The large number of receptors, ensures, upon activation that rapid aggregation will occur at the injury site. When activation occurs, the receptor changes conformation and exposes sites for ligand binding. The GPIIB/IIIa receptor binds with high affinity to fibrinogen resulting in platelet cross linking and the formation of a platelet rich plug. Thus, the activation of the GPIIb/IIIa receptor is the final mechanism by which platelets aggregate, independent of the initial agonist [30-32].

Two peptide sequences [33-34] govern ligand binding by the GPIIb/IIIa receptor and ensure specificity. The Arg-Gly-Asp (RGD) sequence is present on fibrinogen, von Willebrand factor, fibronectin and vitronectin. The Lys-Gln-Ala-Gly-Asp-Val sequence is located in the fibrinogen gamma chain and is primarily responsible for binding of fibrinogen to the GPIIb/IIIa receptor. These two binding sites provide

high affinity binding by GPIIb/IIIa of fibrinogen, which in turn, results in platelet cross-linking and the formation of a platelet rich plug.

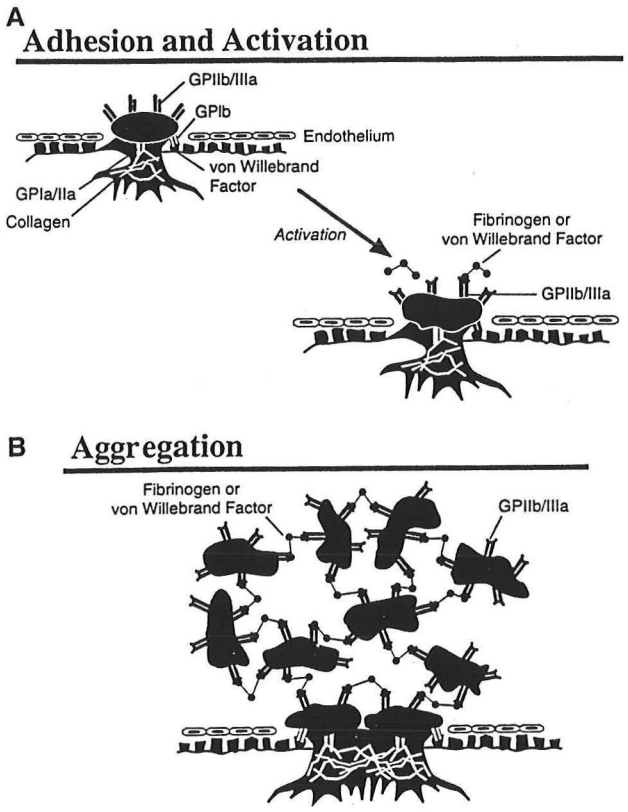

FIG 1. Schematic of platelet adhesion, activation, and aggregation. A, Damage to an atherosclerotic plaque or normal blood vessel results in exposure of subendothelial proteins such as von Willebrand factor and collagen, among others. Platelets have on their surface receptors that bind these proteins (Table), and this results in platelet adhesion. After adhesion, a variety of agonists (Fig 2) can induce platelet activation, resulting in a conformational change in the GPIIb/IIIa receptor that allows it to bind fibrinogen, von Willebrand factor, and perhaps other glycoproteins with high affinity. B, Fibrinogen and von Willebrand factor are multivalent, so they can bind to more than one platelet at a time, resulting in crosslinking. This results in platelet thrombus formation. From Braunwald E, ed. *Inhibitors of Platelet Aggregation: GPIIb/IIIa Antagonists: Heart Disease, Update 4.* Philadelphia, Pa: WB Saunders; in press. Reproduced with permission.

DEVELOPMENT OF A NEW ANTIPLATELET AGENT:

A clinical entity known as Glanzmann thrombasthenia was described in 1918[35] and later determined to be a genetic disorder. This clinical syndrome is characterized by mucocutaneous hemorrhage and abnormal laboratory studies. Specifically, these patients have prolonged bleeding times and their platelets fail to aggregate in response to platelet agonists including adenosine diphosphate, epinephrine, serotonin, collagen and thrombin. Despite these abnormalities, these patients rarely have internal bleeding .
In the mid 1970's two groups [36-37] identified deficiencies of two glycoproteins designated glycoprotein IIb and glycoprotein IIIa which, subsequently, were determined to exist as a complex on the platelet surface.

Fibrinogen binding to the platelet surface was determined, in the 1970's, to be necessary for in vitro platelet aggregation [38-40] . Therefore, Coller et al performed studies using murine hybridomas to develop antibodies against platelet glycoproteins which would prevent the interaction between platelets and immobilized fibrinogen [41]. Multiple antibodies were developed but it was the antibody 7E3 which was selected for further study because it cross reacted with canine platelets and could be assessed in canine models of arterial thrombosis.

In order to decrease the immunogenecity of 7E3 and to decrease the likelihod that platelets coated with 7E3 would be cleared by the spleen, the Fc region was cleaved and a mouse/human chimeric 7E3 Fab was formed.

Subsequent agents which are not antibodies have been developed to interfere with the glycoprotein IIb/IIIa receptor. Tirofiban is a pseudopeptide which mimics the geometric, stereotactic and charge characteristics of the arginine-glycine-aspartate (RGD) sequence and interfere with platelet aggregation. The synthetic peptides bind competitively to the RGD sequence and interfere with platelet aggregation. Both the pseudopeptides and synthetic peptides have a rapid reversal after drug discontinuation, unlike c7E3.

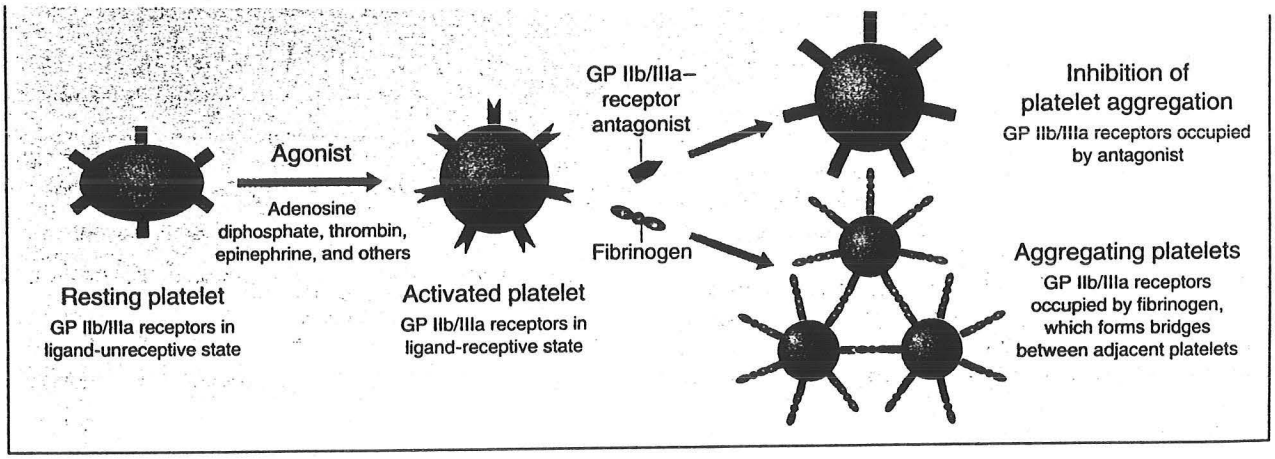

Figure 1. Overview of the Processes of Platelet Activation and Aggregation and the Inhibition of Platelet Aggregation by Inhibitors of Glycoprotein (GP) IIb/IIIa Receptors.

Platelet activation causes changes in the shape of platelets and conformational changes in glycoprotein IIb/IIIa receptors, transforming the receptors from a ligand-unreceptive to a ligand-receptive state. Ligand-receptive glycoprotein IIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of glycoprotein IIb/IIIa receptors also bind to glycoprotein IIb/IIIa receptors, blocking the binding of fibrinogen and thus preventing platelet aggregation.

Initial Animal Studies:

Dr Folts created a cyclical flow model of coronary artery flow in dogs which involved creating an external stenosis with a cylindrical device and damaging the artery with a hemostat. This results in platelet activation, formation of platelet thrombi and the occurrence of cyclical reduction in flow . Aspirin can partially normalize arterial flow but further reductions in flow occur with the addition of a platelet agonist such as epinephrine. The addition of the chimeric 7E3 antibody inhibited cyclic flow reduction from occurring even after the addition of platelet agonists such as epinephrine and further vascular damage [42-44].

Dr Gold developed a canine model which evaluated the mechanism of reocclusion following successful thrombolysis with tissue-type plasminogen activator(tPA). This model involved creating a severe stenosis with the addition of fresh thrombus in the left anterior descending artery. The artery was successful reperfused with tPA but reocclusion often developed within minutes [45]. The use of aspirin resulted in some benefit in decreasing reocclusion. The use of the chimeric antibody 7E3, however, prevented reocclusion from occurring.

Subsequent studies revealed that the chimeric antibody 7E3 decreased the time to complete reperfusion, decreased the dose of tPA required to achieve reperfusion and lysed platelet rich thrombi which were not lysed by rtPA.

Thus, it appears that the chimeric antibody 7E3 has significant advantages over aspirin in in vitro and in

vivo animal studies since it is able to effectively block all platelet aggregation when >80% of the GPIIb/IIIa receptors are blocked. It does not, however, interfere with platelet adhesion, the deposition of a platelet monolayer and the attainment of hemostasis.

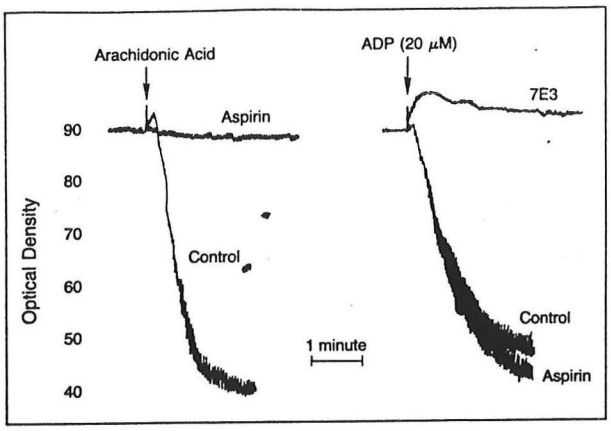

Figure 2. Effects of Aspirin and Monoclonal Antibody 7E3, Directed at Platelet GPIIb/IIIa, on Platelet Aggregation Induced by Arachidonic Acid and ADP.

Citrated platelet-rich plasma was treated with buffer (control), aspirin (final concentration, 0.8 mM), or antibody 7E3-F(ab')$_2$ (final concentration, 10 μg per milliliter) for 30 minutes at 22°C. Aggregation was initiated with either arachidonic acid (0.8 mM) or ADP (20 μM). The aspirin-treated platelets completely failed to aggregate in response to arachidonic acid, indicating widespread inhibition of platelet cyclooxygenase. Despite this, the platelets responded as well as control platelets to a relatively high dose of ADP. In contrast, the monoclonal antibody (7E3-F(ab')$_2$) reacting with the platelet GPIIb/IIIa receptor completely blocked platelet aggregation despite activation with a high dose of ADP, indicating the central role of this receptor in platelet aggregation. The increase in optical density observed when ADP was added to antibody-treated platelets reflects the change of shape produced by ADP; this response was not seen in the absence of antibody because it was overwhelmed by the decrease in optical density produced by platelet aggregation.

Initial Human Studies:

Dr JT Willerson demonstrated that cyclical flow occurs following angioplasty and that serotonin, thromboxane A2 and thrombin contribute key roles in the formation of platelet thrombi [46-47]. The realization that cyclic flow occurs post angioplasty secondary to platelet activation and aggregation coupled with the canine animal models which demonstrated that 7E3 prevented cyclic flow reduction in various models, led to the study of 7E3 administered to angioplasty patients which demonstrated the elimination of cyclic flow reduction. [48]. One could hypothesize that prevention of cyclic flow reduction might translate into clinical benefits to the patient.

CLINICAL TRIALS UTILIZING c7E3 (ReoPro)

Angioplasty produces endothelial denudation with activation and aggregation of platelets. Occasionally this may result in the formation of a platelet rich thrombus which becomes occlusive and results in abrupt closure in 6-15% of all angioplasty cases. Aspirin has been demonstrated to decrease, but not abolish, the rate of abrupt closure [49]. Because angioplasty simulates plaque rupture and has a high clinical event rate (abrupt closure and recurrent ischemia), the first, large, prospective randomized trial of ReoPro studied patients with unstable angina treated with angioplasty.

Evaluation of 7E3 for the Prevention of Ischemic Complications Trial (EPIC):

The EPIC trial [50] was designed to assess the efficacy of c7E3 in *high risk* patients undergoing

angioplasty with a primary composite endpoint of death, myocardial infarction, the need for bypass surgery or urgent percutaneous revascularization. Patients were followed for 6 months.

The entry criteria included patients who were considered to be at high risk of abrupt vessel closure during percutaneous coronary angioplasty. The clinical criteria included patients with unstable angina or postinfarct angina, acute evolving myocardial infarction within 12 hours of symptom onset if the patients were undergoing primary or rescue angioplasty or high risk angiographic characteristics. Unstable angina was defined as having a minimal of 2 episodes of angina with electrocardiographic changes within 12 hours of randomization.

Patients were excluded if the were 80 years or older, had a known bleeding diathesis, had major surgery within 6 weeks or a stroke within 2 years.

The study protocol randomized patients to one of three groups:

1. Group 1 included a c7E3 bolus (0.25mg/kg) plus 12 hour infusion (10 ug/min)
2. Group 2 included a c7E3 bolus (0.25mg/kg)
3. Group 3 included a placebo bolus plus placebo infusion.

All patients were treated with aspirin prior to the angioplasty and received heparin with a goal of achieving an activated clotting time (ACT) between 300 and 350 seconds. Heparin was continued for 12 hours following the completion of the procedure. Aspirin (325mg po) was continued daily.

Compared to the placebo group, c7E3 bolus plus 12 hour infusion resulted in a 35% reduction in the composite primary endpoint of death, myocardial infarction, need for bypass surgery or urgent percutaneous revascularization. This translated in the prevention of 45 events per 1000 patients treated during the first 30 days. This event reduction was maintained at 6 months (27% event rate vs 35% event rate) .

Primary Outcome Events at 30 Days in the Treatment Groups

EVENT	Placebo (n=696)	c7E3 bolus (n=695)	c7E3 bolus+infusion (n=708)
Primary endpoint	89 (12.8)	79 (11.4)	59(8.3)*
Death	12 (1.7)	9 (1.3)	12(1.7)
Nonfatal MI	60 (8.6)	43 (6.2)	37(5.2)*
Emergent PTCA	31 (4.5)	25 (3.6)	6 (0.8)*
Emergent CABG	25 (3.6)	16 (2.3)	17(2.4)
Stent placement	4 (0.6)	12 (1.7)	4 (0.6)

*p<0.05

The major reduction in the composite primary endpoint was due to mainly to a major reduction in myocardial infarction which was sustained at 6 months [51]. In addition, the need for repeat percutaneous revascularization was reduced in the bolus plus infusion group compared to placebo. There was no difference in mortality, as expected.

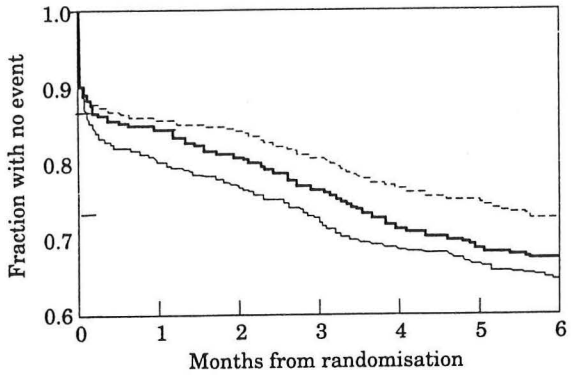

Figure 1 Kaplan-Meier curve of all events (death, myocardial infarction, coronary revascularization) in all patients enrolled. There was a significant reduction of events in the c7E3 bolus + c7E3 infusion group (- - -) compared with the active bolus only (—), or placebo treatments (—) ($P = 0{\cdot}001$). A substantial proportion of events occurred after one month. (Reproduced with permission[5]).

The three treatment groups had a difference in the timing of nonfatal ischemic events requiring urgent repeat angioplasty. The placebo group had more ischemic events during the first six hours following initial angioplasty while the c7E3 bolus group's ischemic events were delayed by several additional hours. The c7E3 bolus plus infusion groups had a profound delay in the onset of ischemic events in addition to an absolute reduction in events.

Although the bolus group without infusion had an early reduction in the primary composite endpoint, the effect disappeared early suggesting the need for a more prolonged inhibition of platelet aggregation.

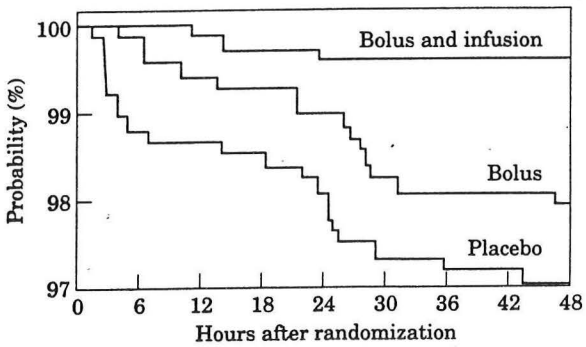

Figure 2 Probability of no urgent repeated percutaneous revascularization procedures in the three treatment groups (Kaplan-Meier plots). Events began to occur shortly after the index procedure in the placebo group, between 6 and 12 h after the procedure in the group given the bolus of c7E3 Fab, and even later in the group given both the bolus and the infusion. The y axis is truncated at 97% to demonstrate the differences in this endpoint, which occurred with low frequency. (Reproduced with permission[4].)

Of the patients enrolled into the EPIC trial, 64 patients entered the trial having an acute myocardial infarction [52]. Twenty two patients were undergoing rescue angioplasty while 42 were being treated with primary angioplasty. Compared to placebo, the bolus plus infusion c7E3 group had a significant reduction in the primary composite endpoint at 30 days (26% vs 4.5%). This reduction was maintained through 6 months (48% vs 4.5%).

Although c7E3 resulted in a significant decrease in myocardial infarction and need for urgent revascularization, it did result in a significant increase in the rates of both major and minor bleeding.

Bleeding Complications

Variable	*Placebo*	*c7E3 plus infusion*
Major bleeding	46 (7)	99 (11)*
Site of major bleeding		
Groin	20	58
Retroperitoneal	3	12
Intracranial	2	3
Transfusion Red Cells	49 (7)	109 (15)*
Platelets<100,000	24 (3.4)	37 (5.2)

*p<0.001

This study demonstrated that a more potent anti-platelet therapy is able to significantly reduce the occurrence of myocardial infarction and the need for urgent repeat revascularization in patients admitted with unstable angina or acute myocardial infarction treated with percutaneous revascularization. This study confirms the importance of platelet aggregation in recurrent ischemia in patients treated with angioplasty. The two groups treated with c7E3 allowed us to determine that prolonged blockade of platelet receptors achieved by bolus plus infusion is essential in reducing the occurrence of recurrent ischemia and infarction. The surprising finding is that these benefits were sustained through six months of follow-up resulting in a decrease in clinical events (27% vs 35%). Whether this benefit was secondary to the effects of c7E3 on platelets during the periprocedural period or due to a cross-reactivity with vitronectin translating into decreased restenosis post procedure remains unknown. Unfortunately, the administration of c7E3 was accompanied by a marked increase in bleeding complications and the need for transfusion. An analysis of the c7E3 bolus plus infusion group revealed that the risk of major bleeding was inversely related to weight.

Whether these findings could be applied to a broader group of patients undergoing percutaneous revascularization remained unknown. Whether decreasing the dose and duration of heparin utilized might result in a decreased risk of bleeding complications also remained unknown. The EPILOG trial was performed to address these questions.

The Evaluation in PTCA to Improve Long-Term Outcome with Abciximaab GP IIb/IIIa Blockade (EPILOG):

Epilog [53] was designed to answer several questions raised by the EPIC trial. The objectives of EPILOG

were to determine whether the reduction in clinical events in high risk patients undergoing angioplasty who received c7E3 bolus plus infusion could be extended to all patients undergoing percutaneous revascularizaton. In addition, the use of low dose heparin was tested to determine whether the increased risk of hemorrhagic complications could be reduced while maintaining the efficacy of c7E3.

The entry criteria included patients undergoing elective or urgent percutaneous coronary revascularization with a severe stenosis. Patients were excluded if they had an acute myocardial infarction, unstable angina with electrocardiographic changes within 24 hours, planned stent or rotational atherectomy, PTCA within 3 months, major surgery within 6 weeks, CVA within 2 years or a known bleeding diathesis.

All patients were treated with 325 mg of aspirin daily and randomly assigned to one of three treatment groups:

1. placebo with standard-dose, weight-adjusted heparin (100 units/kg) to achieve ACT $\geq$300 seconds.
2. c7E3 with standard-dose, weight-adjusted heparin (100 units/kg) to achieve ACT $\geq$300 seconds.
3. c7E3 with low-dose, weight-adjusted heparin (70 units/kg) to achieve ACT of $\geq$seconds.

The protocol recommended that the heparin be stopped immediately following the procedure with removal of the vascular sheaths once the ACT fell below 175 seconds.

The placement of stents was discouraged and only allowed for threatened and or abrupt closure.

The primary endpoint was a composite one which included death, myocardial infarction or reinfarction , ischemia requiring urgent coronary artery bypass surgery or percutaneous revascularization within 30 days. Secondary efficacy endpoint included a composite of death, myocardial infarction, coronary bypass surgery or repeat percutaneous revascularization within 6 months.

The study was terminated at the interim analysis upon review by the Data and Safety Monitoring Committee due to a significant reduction in death or myocardial infarction in the c7E3 groups.
The incidence of the composite primary endpoint at 30 days was 11.7% for the placebo group, 5.2% for the c7E3 group plus low-dose heparin and 5.4% for the c7E3 group with standard dose heparin.

Components of the 30-Day Primary Composite End Point

Efficacy Endpoint	Placebo+Standard Dose Heparin	c7E3+Low-Dose Heparin	c7E3+Standard Dose Heparin
Composite	109 (11.7)	48 (5.2)*	49 (5.4)*
Death	7 (0.8)	3 (0.3)	4 (0.4)
Myocardial Infarction	81 (8.7)	34 (3.7)*	35 (3.8)*
Urgent Revascularization	48 (5.2)	15 (1.6)*	21 (2.3)*
PTCA	35 (3.8)	11 (1.2)*	14 (1.5)**
CABG	16 (1.7)	4 (0.4)	8 (0.9)

*p<0.001
**P<0.003

At 6 months, the cumulative incidence of death, myocardial infarction or repeat revascularization was 25.8% in the placebo group, 22.8% in the c7E3 plus low-dose heparin group (p=0.07) and 22.3% in the c7E3 plus standard-dose heparin group (p=0.04). Although both C7E3 groups maintained a significant reduction in the need for urgent revascularization or occurrence of myocardial infarction, the incidence of target-vessel revascularization was similar amongst the three groups.

Despite the use of c7E3, the risk of hemorrhagic complications was reduced by utilizing a low dose heparin protocol

Bleeding Endpoints

Bleeding End Point	Placebo+Standard Dose Heparin	c7E3+Low-Dose Heparin	c7E3+Standard Dose Heparin
Major Bleeding	29 (3.1)	19 (2.0)	32 (3.5)
Minor Bleeding	35 (3.7)	37 (4.0)	68 (7.4)*
Transfusion-Red Cells	37 (3.9)	18 (1.9)**	30 (3.3)

*p<0.001
**p<0.013

Thus, the administration of c7E3 as a bolus plus infusion with reduced , weight adjusted heparin decreased the composite endpoint at 30 days by 56% without an increase in the risk of major bleeding complications. This effect was observed independent of the patients' clinical risk profile. This translated into 65 fewer adverse effects for every 1000 patients treated with a marked reduction in rates of myocardial infarction and need for urgent revascularization. Unlike the EPIC trial which demonstrated a 26% reduction in the need for revascularization at 6 months, this trial did not demonstrate a difference in the need for revascularization at 6 months. This may be explained, in part, by the 12% of patients who received unplanned stents in the EPILOG trial.

The findings of the EPIC trial which were confirmed and extended to all patients undergoing angioplasty by the EPILOG trial has resulted in an increased utilization of c7E3. Two additional studies have provided some insights as to the future role that c7E3 may play in the treatment of unstable angina without angioplasty.

Randomized placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE study:

The Capture trial [54] was designed to determine whether the administration of c7E3 for 18-24 hours prior to percutaneous revascularization and continued for 1 hour post-procedure would decrease the composite endpoint of death, myocardial infarction or urgent revascularization at 30 days and 6 months.

The entry criteria included patients with refractory unstable angina despite therapy with intravenous heparin and nitroglycerin within 48 hours of enrollment. All patients had undergone coronary angiography which revealed a culprit lesion amenable to angioplasty. Angioplasty was scheduled 18-24 hours following the administration of the study medication.

Patients were excluded for bleeding risk factors, left main disease, recent myocardial infarction, or prior CVA within 2 years.

All patients were treated with aspirin, heparin and nitroglycerin. Patients were randomly assigned to:

Group 1: c7E3 (0.25mg/kg plus infusion of 10ug/min)
Group 2: placebo

The use of stents was discouraged.

The Capture trial was halted after the third interim analysis due to a significant reduction in the primary endpoint of death, myocardial infarction or need for urgent revascularization. A primary endpoint occurred in 15.9% of the placebo group vs 11.3% of the c7E3 group. These findings were due mainly to a reduction in the occurrence of myocardial infarction (8.2% vs 4.1%). There was a reduction in myocardial infarction prior to the procedure (2.1% vs 0.6%) and during the 24 hours following the angioplasty (5.5% vs 2.6%).

Thus, the Capture trial demonstrated a 29% lower rate of death, myocardial infarction or urgent repeat revascularization when treated with c7E3. In addition, patients treated with c7E3 demonstrated a reduction in the occurrence of myocardial infarction during the study infusion prior to the angioplasty. This reduction in progression to myocardial infarction suggests that glycoprotein IIb/IIIa receptor blockade may become an effective therapy in the medical management of unstable angina.

Platelet Receptor Inhibition for Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms PRISM-PLUS STUDY:

The PRISM-PLUS study [55] evaluated tirofiban (48-104 hour infusion) in patients with high-risk unstable angina and non-Q-wave myocardial infarction. Tirofiban is a pseudopepetide (non-peptide mimetic) which mimics the geometric, sterotactic and charge characteristics of the arginine-glycine-aspartate (RGD) sequence and prevents platelet aggregation. At 7 days, there was a significant decrease in the composite endpoint of death, refractory ischemia or myocardial infarction (17.9% vs 12.9%) which remained significant at 30 days.

Both Capture and PRISM-PLUS trials have demonstrated the ability of both c7E3 and tirofiban to decrease the event rate of refractory ischemia and myocardial infarction in patients admitted with refractory unstable angina. In these two trials, however, most patients underwent coronary revascularization. Measurements of platelet activation in patients with unstable angina reveal persistent platelet activation even one month after the initial event. It appears that more prolonged blockade of the glycoprotein IIb/IIIa receptor should be tested in patients admitted with unstable angina. The TIMI 16 trial will test the efficacy of long term administration of an oral pseudopeptide in patients admitted with unstable angina.

TAMI-8

This eighth thrombolysis and angioplasty in myocardial infarction study [56] was a phase II study which assessed the effects of a murine-derived antibody to the glycoprotein IIb/IIIa receptor. The study enrolled 70 patients with acute myocardial infarctions within 6 hours of symptom onset. Ten patients served

controlls while the remaining 60 received a 3 hour t-PA infusion plus one of 3 doses of m7E3 (3,6, 15 hours after t-PA). Angiography performed electively in some patients revealed TIMI 2/3 flow in 92% of treated patients vs 56% of t-PA patients. The study confirmed that it is safe to administer a glycoprotein IIb/IIIa receptor antagonist in the setting of lytic drugs with potential clinical benefits. The GIIb/IIIa receptor antagonist may prevent lytic induced platelet activation thereby providing a clinical advantage.

PARADIGM/IMPACT-AMI

PARADIGM was a phase II trial which assessed the efficacy of a non-peptide inhibitor of the glycoprotein IIb/IIIa receptor named lamifiban in 353 patients presenting within 12 hours of symptom onset of acute myocardial infarction treated with streptokinase or t-PA. As assessed by continuous electrocardiography, the 90 minute infarct artery patency was 80% in the lamifiban group compared to 63% in the lytic group [57]. These findings are consistent with the 90 minute angiographic findings from the IMPACT-AMI trial [58] in patients admitted with acute myocardial infarction treated with front-loaded t-PA and then randomized to placebo vs integrelin (cyclic heptapeptide). Coronary patency was observed in 87% of patients treated with the highest dose of integrelin vs 69% of placebo. In addition, the 66 percent of patients had TIMI 3 flow when treated with the highest dose of integrelin compared to 39% in the placebo group.

The findings of accelerated, more complete reperfusion of coronary arteries in the setting of acute myocardial infarction treated with lytic therapy and glycoprotein IIb/IIIa therapy is consistent with early canine studies. These studies led us to perform an observational study in patients presenting with an acute myocardial infarction referred for primary angioplasty.

Restoration of Coronary Flow in Myocardial Infarction by Intravenous Chimeric 7E3 Antibody without Exogenous Plasminogen Activators: Observations in Animals and Humans:

Animal Studies: A stable fibrin -rich coronary thrombosis was formed by local endothelial damage in the left anterior descending artery [59]. Heparin was administered to achieve an ACT of $\geq$250 seconds. The animals were then divided into three groups:

1. aspirin and c7E3(10minutes after aspirin)
2. aspirin followed by placebo
3. placebo

Only group 1(4/5 dogs) achieved stable reflow at 50$\pm$9 minutes.

Patient Observations: Patients with an acute myocardial infarction presenting within 6 hours onset of symptoms who were referred for primary angioplasty and demonstrated to have an occluded culprit artery were included. These patients received c7E3 in anticipation of angioplasty. During the 10 minutes following the administration of c7E3, The TIMI flow rate improved by at least one TIMI rade in 11 of 13 patients(85%). There was evidence of improve reflow in patients without pretreatment collateral flow. Only one patient achieved an increase in flow from TIMI 0 to TIMI 2 flow without angioplasty.

These findings suggest that c7E3 inhibits platelet accumulation and reaccumulation resulting in a clot

which is more susceptible to unopposed endogenous lysis. The c7E3 may, in fact, accelerate endothelium-based fibrinolysis, as suggested by in vitro work which reveals increased plasminogen activator, decreased plasminogen activator inhibitor-1 and decreased thrombin generation by tissue factor.

CONCLUSIONS:

1. Plaque rupture with superimposed platelet aggregation and thrombosis results in coronary artery disease progression and acute coronary syndromes.

2. Antiplatelet therapy is effective in reducing mortality in acute myocardial infarction independent of lytic therapy.

3. Antiplatelet therapy is effective in primary and secondary prevention of myocardial infarction.

4. Antiplatelet therapy is effective in decreasing the progression of unstable angina to myocardial infarction.

5. Newer antiplatelet therapies targeting the glycoprotein IIb/IIIa receptor have significant advantages over aspirin in patients undergoing angioplasty because they prevent platelet aggregation independent of the agonist.

6. Glycoprotein IIb/IIIa receptors antagonists may decrease the progression of coronary artery disease by decreasing the formation of platelet rich thrombi which contribute to plaque progression.

FUTURE DIRECTIONS:

As our understanding of risk factors and mechanisms of acute coronary syndromes increase, we may be better able to tailor our therapy to reduce a patients' likelihood of developing a clinical event such as unstable angina or acute myocardial infarction.

We now know that patients with unstable angina and acute myocardial infarction have increased platelet activation for greater than one month. In the future, we may target out degree of platelet inhibition by determining the degree of platelet activation and clinical risk factors for an event. Recent evidence has demonstrated that markers such as C-reactive protein, fibrinogen, homocysteine, fibrin degradation products and plasminogen activator inhibitor are markers for impaired fibrinolysis and potential for thrombosis. A combination of traditional risk factors coupled with markers of impaired fibrinolysis, inflammatory markers and thrombotic markers may allow us to select the appropriate antiplatelet agent and degree of platelet inhibition in an effort to decrease the silent progression of plaque formation, rupture, disease progression and clinical events.

Glycoprotein IIb/IIIa receptor antaganists will play an increasing role in the management of patients with unstable angina, acute myocardial infarction and primary and secondary prevention of acute coronary syndromes.

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