

REVIEW

Potential Future Clinical Applications for the GPIIb/IIIa Antagonist, Abciximab in Thrombosis, Vascular and Oncological Indications

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Abciximab (ReoPro[®]) is a mouse-human chimeric monoclonal antibody Fab fragment of the parent murine monoclonal antibody 7E3, and was the first of these agents approved for use as adjunct therapy for the prevention of cardiac ischemic complications in patients undergoing percutaneous coronary intervention (PCI). Abciximab binds with high avidity to both the non-activated and activated form of the GPIIb/IIIa receptor of platelets, the major adhesion receptor involved in aggregation. Additional cardiovascular indications for abciximab are unstable angina, carotid stenting, ischemic stroke and peripheral vascular diseases. Abciximab also interacts with two other integrin receptors; the $\alpha_v\beta_3$ receptor, which

is present in low numbers on platelets but in high density on activated endothelial and smooth muscle cells, and $\alpha_M\beta_2$ integrin which is present on activated leukocytes. Cell types that express integrins GPIIb/IIIa and $\alpha_v\beta_3$ such as platelets, endothelial and tumor cells have been implicated in angiogenesis, tumor growth and metastasis. Since abciximab interacts with high avidity to integrins GPIIb/IIIa and $\alpha_v\beta_3$, it is reasonable to assume that it may possess anti-angiogenic properties in angiogenesis-related diseases, as well as anti-metastatic properties in case of disseminating tumors expressing the target integrin receptors. (Pathology Oncology Research Vol 6, No 3, 163–174, 2000)

Keywords: Abciximab, ReoPro[®], cardiocascular disease, angiogenesis, tumor metastasis

Introduction

Platelet activation and subsequent platelet thrombus formation play a pivotal role in the pathophysiology of arterial thrombosis and subsequent acute coronary syndromes (ACS)^{1,2} and have been strongly implicated in the development of noncardiac vascular diseases such as ischemic stroke,^{3,4} carotid artery occlusion,⁵ and peripheral vascular disease.⁶ Platelet thrombus formation can be propagated by a number of complex, independent pathways and physiological stimuli.^{1,7} Regardless of the mechanistic or chemical stimuli utilized, the end product of each of these pathways is the induction of a conformational change in the platelet glycoprotein (GP) IIb/IIIa receptor, which then allows the receptor to bind fibrinogen and other multivalent, adhesive proteins, ultimately resulting in platelet-

platelet crosslinking and aggregate formation. GPIIb/IIIa antagonists have been developed to inhibit the interaction of fibrinogen and other ligands to the activated GPIIb/IIIa receptor, thereby inhibiting platelet aggregation and subsequent thrombus formation. GPIIb/IIIa antagonists are more versatile anti-platelet agents than those designed to inhibit one pathway of activation (e.g. heparin, ticlopidine, clopidogrel and aspirin) since they inhibit the final consequence of platelet activation – the interaction of fibrinogen with the activated GPIIb/IIIa receptor.² Validity of the concept of GPIIb/IIIa antagonism has been demonstrated in patients with symptomatic coronary artery disease undergoing percutaneous interventions.⁸⁻¹² In these studies, periprocedural administration of a parental GPIIb/IIIa antagonist markedly reduced the incidence of death or non-fatal myocardial infarction over the ensuing 30 days.

Abciximab (ReoPro[®]) is a mouse-human chimeric monoclonal antibody Fab fragment of the parent murine monoclonal antibody 7E3,¹³ and was the first of these agents approved for use as adjunct therapy for the prevention of cardiac ischemic complications in patients undergoing

Received: April 15, 2000; accepted: May 15, 2000

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percutaneous coronary intervention (PCI), or in unstable angina patients when PCI is planned within 24 hours. Abciximab binds with high avidity to both the non-activated and activated form of the GPIIb/IIIa receptor and the recommended dose induces 80% blockade of the GPIIb/IIIa receptor throughout the duration of treatment.¹⁴⁻¹⁶ A number of large prospective clinical studies have established that abciximab affords long-term reduction (up to 3 years) in ischemic complications associated with PCI.^{17,18} Additional cardiovascular indications for abciximab currently being explored are medical treatment for unstable angina, and combined therapy with reduced doses of the fibrinolytic Retavase®, in the setting of both acute myocardial infarction and facilitated PCI (see below). In addition, the utility of abciximab in non-cardiac vascular indications such as carotid stenting, ischemic stroke and peripheral vascular disease is also being explored.

During its development, it was reported that abciximab also interacts with two other integrin receptors (*Table 1*); the $\alpha_v\beta_3$ receptor (also known as the vitronectin receptor),¹⁹ which is present in low numbers on platelets (100 copies per cell)²⁰ and in high density (500,000 copies per cell),¹⁹ on activated endothelial and smooth muscle cells and $\alpha_M\beta_2$ integrin, also known as MAC-1, which is present on activated leukocytes.^{21,22} Numerous *in vitro* and animal studies have associated $\alpha_v\beta_3$ and Mac-1 with a variety of pathophysiologic processes associated with acute coronary syndromes. Thus, it is postulated that some of the clinical benefits derived from abciximab in PCI patients could be correlated with cross-specificity with one or both of these receptors. Additionally, these receptors have been associated with non-cardiac disease processes, indicating that abciximab, or other compounds with equivalent specificity, may confer clinical benefit in other conditions where receptor activation is proposed. This article summarizes the scientific rationale and clinical evidence for the use of abciximab in additional cardiovascular and non cardiac vascular indications.

Potential Role for Abciximab as an Anti-Cancer Agent

The challenge for the oncology field today is to develop therapies that will combat advanced disseminated disease. Developing agents that will block the metastatic cascade and the growth of disseminated tumors are areas of research that are undergoing scrutiny from academic institutions and the pharmaceutical industry. One hopeful approach is anti-angiogenic therapy, where it is speculated that depleting the tumor of its blood supply may ultimately shrink the tumor and prolong patient survival. Cell types that express integrins GPIIb/IIIa and $\alpha_v\beta_3$ such as platelets, endothelial and tumor cells have been implicated in tumor growth, angiogenesis and metastasis. Since abciximab interacts with high avidity to integrins GPIIb/IIIa and $\alpha_v\beta_3$, it is reasonable to assume that it may possess anti-angiogenic properties. The following sections will provide a brief review of the importance of GPIIb/IIIa and $\alpha_v\beta_3$ integrins in tumor growth and metastasis, and the rationale for the development of abciximab as a therapeutic agent for certain cancers.

Role for $\alpha_v\beta_3$ Integrin in Tumor-Induced Angiogenesis

The outcome of solid tumor growth is closely associated with vascular density. Blockade of neo-vascularization can result in a significant decrease in solid tumor growth.^{24,25} Tumor-secreted growth factors and inflammatory cells that infiltrate the tumor can initiate tumor-induced angiogenesis. Two such angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), stimulate endothelial expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which then results in new vessel formation. A variety of studies have indicated that blockade of $\alpha_v\beta_3$ function by antagonists suppresses angiogenesis and tumor growth in animal models.²⁶⁻²⁸ One such antagonist LM609, a murine monoclonal IgG antibody that specifically blocks $\alpha_v\beta_3$, inhibits angiogenesis by pro-

Table 1. Integrin specificity characteristics of abciximab

Receptor	Cell Expression	Receptor Density (molecules/cell)	Abciximab Affinity K_D
$\alpha_{IIb}\beta_3$ (GPIIb/IIIa)	Platelets	≈80,000 (platelets) ¹	6.2 nM (platelets) ²
$\alpha_v\beta_3$ (vitronectin receptor)	Platelets, fibroblasts, osteoclasts, PMN's, lymphocytes, tumors, endothelial and smooth muscle cells	≈100 (platelets) ³ ≈500,000 (cultured human endothelial cells [HUVECS]) ⁴	9.8 nM (HUVECS) ⁴
$\alpha_M\beta_2$ (Mac-1; CD18/CD11b)	Activated lymphocyte ^{5,6}	200,000 (peripheral blood monocytes [PBM's]) ^{6,7}	160 nM (PBM's) ^{6,7}

¹Reference number (23). ²Reference number (14). ³Reference number (20). ⁴Reference number (19). ⁵Reference number (21).

⁶Reference number (22). ⁷Estimates obtained with the bivalent form of abciximab (7E3 IgG)

moting programmed cell death in activated, but not resting, endothelial cells via a P-53 dependent pathway.²⁹ In addition to integrins, proteinases may also be involved in angiogenesis by remodeling the sub-endothelial matrix, thus facilitating endothelial attachment and movement. Integrin $\alpha_v\beta_3$ can bind to matrix metalloproteinase-2 and inhibition of this interaction also inhibits angiogenesis in tumor model systems.³⁰ Immunohistological analysis indicated that expression of $\alpha_v\beta_3$ is preferentially enhanced in blood vessels of patients with human breast carcinoma.³¹ These investigators also reported that integrin expression was significantly higher in tumors of patients with metastasis than patients without metastasis.

A variety of $\alpha_v\beta_3$ antagonists such as small molecular weight inhibitors, peptidomimetics, or monoclonal antibodies are in various stages of development as anti-cancer therapeutics. A selective peptidomimetic antagonist of $\alpha_v\beta_3$ SC-68448, inhibited endothelial cell proliferation and tumor growth in mice. In a mouse Matrigel model of angiogenesis, an $\alpha_v\beta_3$ small molecule antagonist SM 256, inhibited bFGF stimulated blood vessel formation.²⁷ A phase I clinical trial of the humanized version of LM609 (Vitaxin[®]) in patients with stage IV disease with breast, colon, lung, kidney and ovarian carcinoma has recently been completed with no reported major toxicity.³³ These findings collectively suggest that $\alpha_v\beta_3$ antagonists may be effective and well-tolerated anti-angiogenic agents.

Based on the available data supporting the role of $\alpha_v\beta_3$ in angiogenesis, abciximab was evaluated for anti-angiogenic activity. The parent antibody of abciximab, m7E3 IgG was compared to LM609 in a severe combined immuno-deficient (SCID) mouse human chimeric skin angiogenesis model.³⁴ In this system, $\alpha_v\beta_3$ negative human melanoma cells were injected into full-thickness human skin grafted onto SCID mice. The resulting tumors induced an angiogenic response that enhanced growth of tumor cells in an orthotopic microenvironment. Regular administration of 7E3 prevented or significantly inhibited growth of tumors, and this effect directly correlated with a reduction in the number of blood vessels supplying the tumors. Since 7E3, like LM609, does not crossreact with mouse integrins, its anti-angiogenic effect was attributed to blockade of human $\alpha_v\beta_3$ receptors in the vasculature of the human skin.

Role for Platelet GPIIb/IIIa Integrin in Tumor Growth and Metastasis

The rationale and evidence supporting the development of anti-platelet agents in metastasis has been reviewed in detail elsewhere.³⁵ Despite uncertainties as to how anti-platelet agents may function as anti-cancer therapeutics, some have postulated that these agents inhibit the adherence or trapping of cancer cells to capillary walls, expos-

ing circulating tumor cells for a prolonged period of time to host anti-tumor entities. The involvement of platelets in experimental models of metastasis was recognized almost 30 years ago,³⁵ as an integral part of the microthrombus that is thought to be involved in the arrest of circulating tumor cells.

Certain tumor cells induce platelet aggregation (TCIPA) *in vitro*, which directly correlates with their metastatic potential.³⁶ It is hypothesized that TCIPA may be required during the hematogenous spread of tumor cells and the resulting aggregates induce endothelial cell retraction and facilitate tumor extravasation. This idea was supported by a finding that reconstituting thrombocytopenic mice with human platelets dramatically increased the lung colonization ability of tumor cells *in vivo*.³⁷ TCIPA can be blocked by mAbs directed to platelet GPIIb/IIIa integrin, 10E5 and AP-2^{36,38-40} and abciximab (unpublished results, Trikha, M). The initial tumor cell-platelet bridging event requires β_3 integrins and this results in a rapid induction of platelet aggregation. Furthermore, blockade of human platelet GPIIb/IIIa by 10E5 blocked the increase in lung colonization of tumor cells.³⁷ These results suggested that platelets use GPIIb/IIIa integrin to interact with circulating tumor cells and blockade of this receptor could prevent tumor cell arrest and/or extravasation.

Recently, Amirkhosravi et al. demonstrated that murine tumor cells injected intravenously into nude rats rapidly induced thrombocytopenia.⁴¹ The murine F(ab')₂ version of abciximab, 7E3 F(ab')₂ that crossreacts with rat, but not murine, GPIIb/IIIa and $\alpha_v\beta_3$ prevented tumor cell-induced thrombocytopenia. The functional consequence of blocking tumor cell-induced thrombocytopenia was a near complete eradication of experimental metastasis. The authors speculated that when tumor cells are shed into the circulation, they rapidly recruit platelets to form tumor cell-platelet aggregates which results in a transient decrease in circulating platelet count. These aggregates help tumor cells survive the hostile environment and facilitate in their arrest at distant sites. These studies in conjunction with results obtained from 10E5 experiments suggest that abciximab could block hematogenous metastasis.

In addition to facilitating hematogenous metastasis, platelets may also participate in angiogenesis and growth of primary and disseminated tumors. Pinedo and Folkman have postulated that a true anti-angiogenic therapy must target platelets.⁴² Platelets contain one of the largest stores of angiogenic and mitogenic factors, and with a circulating half life of ~5-7 days,⁴³ they could provide tumors with a continuous supply of growth factors. Tumor vasculature is leaky and extravasated fibrin(ogen) that is deposited on the tumor surface can provide an ideal substrate for platelet binding. Platelet granules contain a variety of factors such as VEGF, PDGF, TGF- β , and fibrinogen, and these modulators are immediately secreted after platelet

activation. Abciximab can block platelet aggregation and adhesion to fibrin(ogen), and it also inhibits platelet degranulation. By blocking granule release, abciximab inhibits secretion of serotonin, TGF- β , PDGF AB⁴⁴ and VEGF.^{41,45,46} Most of these factors have been implicated in various steps of tumor progression and metastasis. VEGF is one such angiogenic factor that is stored in large amounts in circulating platelets. Abciximab inhibits ADP-stimulated platelet secretion of VEGF.⁴⁶ In addition, tumor cells induce platelets to secrete VEGF and this secretion is also blocked by abciximab.⁴¹ Blockade of VEGF secretion by abciximab is due to its ability to inhibit both platelet aggregation and tumor cell-platelet binding that is mediated by $\alpha_v\beta_3$ and platelet GPIIb/IIIa. It is tempting to speculate that when administered to patients with cancer, abciximab could directly block $\alpha_v\beta_3$ and GPIIb/IIIa function and indirectly block VEGF function. This multi-receptor binding of abciximab may distinguish it from other anti-angiogenic antagonists that are unable to inhibit platelet GPIIb/IIIa. However, the safety of this dual effect of abciximab remains to be defined.

Role of Tumor β_3 Integrins in Tumor Growth and Metastasis

In addition to participating in host cell mediated tumor growth, angiogenesis and metastasis, β_3 integrins are also upregulated in certain tumors. A large body of literature indicates that β_3 integrins play a critical role in mediating human melanoma cell adhesion, spreading, invasion, and tumor cell survival *in vitro* and in animal tumor models.³⁶ The clinical significance of β_3 integrin expression was suggested in a prospective study that examined the expression of this integrin in patients who were followed for a mean of 98 months post-diagnosis with intermediate thickness malignant melanoma.^{47,48} This study concluded that tumors in 64% of the patients expressed β_3 integrin, with greater mortality in patients with β_3 positive melanomas when compared to those with β_3 negative tumors (45% vs. 8%). Presence of β_3 integrin was also associated with subsequent lung metastasis. In an earlier study, Hsu et al., demonstrated that adenoviral gene transfer of the β_3 integrin subunit into non-tumorigenic radial growth phase primary human melanoma cells converted the cells into tumorigenic and invasive vertical growth phase primary melanoma.⁴⁹ Collectively, these observations suggest that blockade of β_3 integrin function in human melanoma may suppress tumor growth and metastasis.

An important role of tumor expressed $\alpha_v\beta_3$ was demonstrated when function blocking mAb LM609 induced apoptosis of human melanoma cell growth in a collagen gel.⁵⁰ Native type I collagen does not bind $\alpha_v\beta_3$ integrin, but tumor cell secreted matrix metalloproteinases can degrade type I collagen to expose cryptic sites that rec-

ognize $\alpha_v\beta_3$. LM609 inhibits melanoma cell binding to these exposed sites in denatured collagen thereby promoting apoptosis. Chersesh and colleagues demonstrated that M21-L cells that do not express $\alpha_v\beta_3$ are significantly less tumorigenic in nude mice when compared to the parental M21 cells that are positive for $\alpha_v\beta_3$ integrin.⁵¹ Subsequently, they demonstrated that repeat administration of LM609 inhibits growth of M21 ($\alpha_v\beta_3$ positive) cells in mice. Since LM609 does not recognize mouse $\alpha_v\beta_3$, these data suggest that $\alpha_v\beta_3$ -expressed in human melanoma may contribute to tumor growth independent of its role in angiogenesis.⁵²

In addition to the important role of $\alpha_v\beta_3$ integrin in tumor growth and metastasis, a subpopulation of tumors abnormally express the platelet GPIIb/IIIa integrin. Puerschel et al., evaluated human melanoma specimens for expression of GPIIb and GPIIIa subunits on cells from patients with metastatic and non-metastatic malignant melanoma over a 6 year period.⁵³ They observed that the GPIIb subunit was present exclusively on metastatic melanoma cells, but not on non-metastatic melanomas or benign melanocytes. As expected, GPIIIa (also known as β_3) was heterogeneously expressed in both primary and metastatic melanoma. The absence of contaminating platelets in the tumor specimens was ruled out by staining with the platelet specific GPIb antibody. Others studies corroborating these findings reported that in an experimental model of metastatic melanoma, expression of GPIIb/IIIa directly correlated with metastatic potential.^{36,38,39} Further, GPIIb/IIIa expression can be detected in some solid tumor cell lines and function blocking mAbs directed to GPIIb/IIIa block tumor cell adhesion, metastasis and invasion.⁵⁴⁻⁵⁸ Taken together, these findings suggest that targeting GPIIb/IIIa and $\alpha_v\beta_3$ may be a more effective therapy for certain cancers than targeting either integrin alone.

Considerations

An important message from the findings reviewed above is that combined blockade of integrins GPIIb/IIIa and $\alpha_v\beta_3$ may be more effective than a therapy that targets only single receptors. Abciximab inhibits GPIIb/IIIa and $\alpha_v\beta_3$ with equivalent affinity and has been administered to over a million patients with atherosclerotic disease with minimal complications. However, the safety of chronic administration of abciximab in patients with advanced cancer (ie bleeding, formation of restenosis, an immune response to abciximab) as well as the ability to administer an effective concentration of the drug at the tumor site needs to be evaluated. The pharmacodynamics of abciximab within the tumor microenvironment must be closely examined to ensure adequate drug delivery. Currently, there are few effective therapies available for patients with advanced cancer. As described above, *in*

in vivo tumors interact with a variety of host cells such as platelets and endothelial cells, and these interactions help them to grow and metastasize. The ability of abciximab to block many such interactions suggests a novel approach with the potential to inhibit tumor progression.

Sickle Cell Crisis

Sickle cell disease is an autosomal dominant genetic disorder characterized by red cells that transform into a sickle cell shape upon deoxygenation.⁵⁹ The genetic defect is a point mutation which substitutes a valine for glutamic acid in the sixth position of the β -globin chain and results in the abnormal polymerization of sickle hemoglobin under hypoxic conditions.⁶⁰ The pathophysiology of the disease is related to the intracellular polymerization of sickle cell hemoglobin and the abnormal interaction of sickle erythrocytes with the microvascular endothelium.⁶¹ The clinical manifestations of sickle cell disease are highly diverse, but are all ultimately linked to hemolytic anemia and recurring episodes of painful vascular occlusion. Such occlusions are associated with multiple organ damage and increased susceptibility to infection, primarily with polysaccharide-encapsulated organisms due to splenic infarction. The vascular occlusion can involve every organ of the body.

The distribution of sickle cell disease parallels that of falciparum malaria since people who carry the sickle cell trait and are infected with *Plasmodium falciparum* have a selective advantage over those not carrying the gene.⁶² Due to the selective advantage of sickle cell over the normal gene, the frequency of the trait is increased in areas where malaria is endemic. There are approximately 2.5 million people in the US and 30 million people in the world who have sickle cell trait.⁶³ The incidence of sickle cell trait in the US is 1 in approximately 600 newborns, with a significant incidence among the African American population (8%).⁶⁴

The pattern of illness and disease severity varies considerably among individuals homozygous for hemoglobin S. However, the syndrome is associated with significant morbidity and mortality that is primarily mediated by the vaso-occlusion of the microvasculature. The underlying pathophysiology of these vaso-occlusive episodes is complex and may involve adhesion receptor mediated interactions of sickle cell red blood cells (SS RBC's), other cellular constituents (e.g. platelets, leukocytes) and the endothelial cells lining the vascular bed.⁶⁵ These adhesion receptors are up-regulated by inflammatory mediators that are produced during infection or inflammation.⁶⁶

A number of *in vitro* studies have implicated several adhesion receptors (e.g. $\alpha 4\beta 1$ and CD36 on SS RBC's and VCAM-1 and $\alpha_v\beta_3$ on activated endothelial cells)⁶⁶⁻⁶⁹ and adhesive proteins (von Willebrand factor [vWf], throm-

bospondin, fibrinogen)⁷⁰⁻⁷² in mediating the association of SS RBC's with endothelial cells, yet the significance of one particular receptor or ligand in the development of microvascular occlusion has until recently, not been clearly elucidated. Kaul and colleagues,⁷³ using an *ex vivo* platelet activating factor (PAF) activated rat mesoceleal microvascularization model, demonstrated that a bivalent form of abciximab (7E3 F(ab')₂) and an $\alpha_v\beta_3$ -specific antibody LM609 appreciably reduced adhesion of human sickle cells to postcapillary venules. In contrast, a GPIIb/IIIa-specific antibody 10E5 had no effect on the hemodynamics of SS RBC's in PAF-treated-vessels. PAF, a potent inflammatory agent that is elevated in the plasma of sickle cell patients,⁷⁴ and increases endothelial vWf expression,⁷⁵ was used to stimulate the interaction of SS RBC's with the post-capillary venules. This and/or other pro-inflammatory agents with similar physiological effects that are produced during infection may play a pivotal role in the development of sickle cell-mediated vascular occlusion. These data, as well as numerous *in vitro* studies, support the role of $\alpha_v\beta_3$ in mediating SS-RBC interactions with endothelial cells lining postcapillary venules. It should be noted that certain limitations relating to the experimental design need to be addressed. This experimental model consisted of isolated cells in a plasma-free medium and does not reflect the complex hemodynamics that occur in the microcirculation during an inflammatory episode. For instance, subjects with sickle cell disease have increased numbers of circulating platelet-erythrocyte aggregates and elevated levels of platelet activation, *in vivo*.^{76,77} Adhesive glycoproteins (e.g. thrombospondin) released from activated platelets may exacerbate microvascular occlusion by promoting sickle cell adhesion to the endothelium. Thus, the current *in vitro* and *ex vivo* data provides the rationale for dual GPIIb/IIIa and $\alpha_v\beta_3$ receptor blockade as a therapeutic approach to prevent sickle cell disease-related vascular occlusion.

Stroke

Stroke is an important clinical disorder associated with significant morbidity, mortality and economic impact. It is the second leading cause of adult mortality and the leading cause of serious disability in older individuals.^{78,79} In the United States, there are an estimated 500,000 to 700,000 new cases of stroke (85% of which are ischemic), and 150,000 deaths attributed to the disease each year.⁸⁰ Twenty (20%) of stroke victims die within the first month after the episode.⁸¹ For those who survive for 6 months, approximately 15% require institutional care and 30-40% are dependent in their daily living.⁸² The combined incidence of acute ischemic stroke in the United States and Europe is in excess of 1.2 million cases per year.⁸³ The aggregate lifetime cost of ischemic stroke occurring within a single

year in the United States has been estimated at \$29 billion, and the average lifetime cost for a stroke victim from diagnosis to death is approximately \$90,000.⁸⁴ These numbers are expected to rise as the median survival age of humans increases.⁸⁵

The only approved reperfusion treatment for ischemic stroke is alteplase (Activase®), a recombinant tissue plasminogen activator (rt-PA).^{86,87} However, <5% of stroke patients are candidates for this therapy, primarily due to its narrow therapeutic window (institution of therapy less than 3 hrs after symptom onset), and the associated increased risk of intracranial hemorrhage. The pivotal rt-PA trial that was conducted by the National Institute of Neurological Disorders and Stroke (NINDS)⁸⁶ resulted in 120 fewer deaths or disabled patients per 1000 patients treated, or a 12% overall benefit. However, the incidence of symptomatic intracranial hemorrhage increased 10-fold, from 0.6% in placebo patients to 6.4% in subjects that received rt-PA. Attempts at demonstrating benefits of initiation of fibrinolytic therapy up to six hours after symptom onset have been discouraging.⁸⁶⁻⁹¹ Thus, the narrow treatment window and significant intracranial hemorrhage risk associated with the use of rt-PA necessitates the development of safer and more efficacious reperfusion treatments that demonstrate efficacy if the therapy is initiated beyond three hours after symptom onset.

The rationale for GPIIb/IIIa antagonist therapy in ischemic stroke is derived from clinical reports with other anti-platelet agents as well as studies of abciximab in acute myocardial infarction patients. The results of two recent large, randomized trials, the International Stroke Trial³ and the Chinese Acute Stroke study⁴ suggest that platelets play an important part in the pathophysiology of acute ischemic stroke. The combined analyses of these studies revealed that administration of aspirin within 48 hours after onset of symptoms resulted in 13 fewer deaths or disabled patients per 1000 patients treated. It is currently unclear whether the therapeutic benefits of aspirin are attributed to its anti-platelet or anti-inflammatory actions, or a combination of both. However, additional support for the use of anti-platelet therapy in ischemic stroke is the demonstration that platelet ADP receptor antagonists, ticlopidine and its second generation counterpart, clopidogrel are efficacious in secondary prevention of stroke.⁹² Thus, it is feasible that a more potent anti-platelet agent, such as a GPIIb/IIIa antagonist may confer more optimal clinical benefit over partial antagonists.

The evidence indicating that abciximab may be a safer reperfusion agent than rt-PA in ischemic stroke is derived from clinical studies of the agent in patients with acute coronary syndromes. The intracranial hemorrhage rate from the coronary intervention trials with abciximab is very favorable – 0.1% or 1 per 1000 patients.⁹⁻¹² Addition-

ally, angiographic studies in acute myocardial infarction (TIMI14a and the GUSTO IV pilot trials) demonstrated that the combination of abciximab, aspirin and weight adjusted heparin reperused occluded coronary vessels.^{93,94} A composite analysis from both studies revealed abciximab resulted in a 45% patency rate and was comparable to historical reperfusion rates achieved with streptokinase. The mechanism(s) by which abciximab re-establishes flow in occluded vessels is unknown, but may be related to its ability to compete with and dissociate platelet bound fibrinogen on the activated platelet GPIIb/IIIa receptor, and by preventing platelet deposition on existing thrombi, thus allowing endogenous fibrinolysis to proceed unopposed. Abciximab has the potential to enhance thrombolysis by inhibiting release and deposition of platelet-derived plasminogen inhibitors at the site of thrombus.⁴⁴ Additionally, abciximab may further destabilize clot structure by impeding clot retraction⁹⁵ and factor XIIIa crosslinking of fibrin and plasminogen activator inhibition-1 (PAI-1) to the platelet mesh.⁹⁶

A phase I, placebo-controlled, dose escalation study of abciximab in ischemic stroke was recently completed, and the results are promising.⁹⁷ The main objective of the trial was to determine the safety of abciximab in the setting of acute ischemic stroke. Patients presenting within 24 hrs of their stroke onset were randomized to receive, (in a 3 to 1 ratio), a single, escalating dose of abciximab or placebo. Patients were stratified according to the time of stroke onset and stroke severity. The highest dose of abciximab administered was equivalent to the dose recommended for patients undergoing PCI (0.25 mg/kg bolus and a 0.125 µg/kg/min infusion for 12 hrs). No symptomatic ICH bleeds were observed in either the placebo or abciximab groups during the 3 month follow-up period. At 3 months, there was a trend towards improved functional status among abciximab patients, compared to the placebo group. Based on the results of this study, a larger, double-blind, placebo-controlled study is currently being designed to assess the efficacy of abciximab in patients with ischemic stroke. The primary objective of the study is to assess patient disability at 3 months, using a modified Rankin Scale.

Chastain et al.,⁹⁸ evaluated the effect of abciximab in patients undergoing cerebral vascular stenting. Abciximab was used either prophylactically or as emergency bail-out following distal atherosclerotic debris embolization. The mean percent vessel stenosis prior to PCI was $75.9 \pm 17.4\%$ and was reduced to $6.2 \pm 9.1\%$ after PCI. There were no thromboembolic complications. Hemorrhagic complications occurred in two patients, one resulting in death from an occult berry aneurysm. With the exception of one patient who experienced a minor stroke, each of the patients in whom abciximab was used for bailout following distal debris embolism recovered completely.

Table 2. Overview of TIMI14 and SPEED Trials

	TIMI 14*	Speed/Gusto IV AMI Pilot**
Criteria	Age 18 to 75 Symptoms ≤ 12 hrs	≥ 18 years old Symptoms ≤ 6 hrs
Control Arm(s)	Accelerated t-PA or 10 U + 10 U Reteplase	Standard Reteplase (10 U + 10 U)
Combination Therapies Studied	t-PA, SK, and Reteplase Abciximab*** Heparin 60 U/kg and 30 U/kg	Reteplase + Abciximab*** Heparin 60 U/kg and 40 U/kg
Primary Endpoint(s)	TIMI grade 3 flow at 90 minutes	TIMI grade 3 flow at 60 and 90 minutes
PCI	Rescue only	"Encouraged" -Facilitated PCI

*Ref 94. **Ref 93. ***bolus 0.25 mg/kg; infusion 0.125 µg/kg/min H 12 hrs

Management of AMI with Fibrinolytics and GP IIb/IIIa Receptor Inhibitors

We are now entering a new age in the treatment of acute myocardial infarction (AMI). The past decade has seen great emphasis on the use of fibrinolytic agents in the treatment of AMI. Early success with first generation fibrinolytic agents (e.g., streptokinase) lead to the development of second (e.g., alteplase) and third generation fibrinolytic agents (e.g., reteplase, tenecteplase, lanoteplase) with the hope that altering certain characteristics of the plasminogen activator (e.g., fibrin affinity, fibrin specificity, PAI-1 inhibition, etc) would lead to improvements in TIMI 3 (normal coronary) flow rates and improved survival benefit. Unfortunately, while the objective of achieving bolus administration was achieved, these efforts were not successful in increasing survival. Three large (approximately 16,000 patients each) clinical trials⁹⁹⁻¹⁰¹ all failed to demonstrate a survival benefit of one agent over another.

Mechanistic evaluation now suggests an explanation for these clinical findings. Fibrinolytics act only on the fibrin mesh that aggregates platelets, red cells, and thrombin together. They do not address the ongoing stimulation of platelets, the release of clot-bound thrombin during fibrinolysis, new thrombin generation, or the initiation of the thrombotic cascade that proceeds on the platelet surface. Not only are activated platelets exposed following fibrinolysis, but released thrombin is a potent activator of platelets and studies have demonstrated a paradoxical activation of platelets following fibrinolysis. Furthermore, fibrinolysis may cause athero-embolization, a process that can activate platelets and lead to obstruction of the microvasculature in

the myocardium. Thus, to optimize vessel patency, minimize the possibility of reocclusion (which occurs in 15% to 20% of cases following fibrinolysis), and protect/improve flow into the microcirculation, platelet inhibition during fibrinolysis would be desirable. Recently completed Phase II clinical trials provide evidence that the addition of the platelet inhibitor abciximab to the fibrinolytic agents reteplase or alteplase provides earlier and more complete reperfusion, reduction or elimination of cyclic flow variation and reocclusion, and improvements in microvascular obstruction.

Early trial data supported the concept of the benefits of combining platelet blockade with fibrinolysis. The ISIS-II trial demonstrated

an additive effect when the platelet inhibitor aspirin was combined with streptokinase. When used in combination, the two agents provided a 42% reduction in mortality versus placebo.¹⁰² However, as previously noted, drugs targeted at blocking only one pathway of platelet activation achieve only partial platelet inhibition because of the numerous, redundant pathways involved in platelet function. The more potent GPIIb/IIIa receptor inhibitors prevent aggregation regardless of the pathway of activation. In fact, several studies were undertaken in the early 1990's to examine the benefit of GP IIb/IIIa blockade with of fibrinolytics.¹⁰³⁻¹⁰⁵ While successful in demonstrating more rapid and complete clot lysis, these studies suffered from increased bleeding rates presumably due to the use of full dose lytic, high dose heparin, and non-optimal access site management.

The safety and efficacy of combination therapy using full dose abciximab with reduced-dose lytic agent (reteplase, alteplase, or streptokinase) has recently been evaluated in two Phase II clinical trials, TIMI 14, and the pilot phase of the GUSTO IV AMI trial, SPEED (Table 2; ref 93,94). In the Strategies for Patency Enhancement in the Emergency Department (SPEED) trial, the pilot for the GUSTO-IV AMI study, abciximab administration alone was compared with combination therapy consisting of abciximab and various doses of reteplase.⁹³ The combination of full dose abciximab and half-dose (5 units + 5 units) reteplase resulted in accelerated optimal TIMI 3 flow rates, compared to full-dose lytic alone control (Figure 1). Reduced dose heparin, while marginally safer in terms of bleeding rates, led to reduced TIMI-3 flow rates. Control arms included abciximab for the dose finding phase of the trial (demonstrating the dethrombosis effect

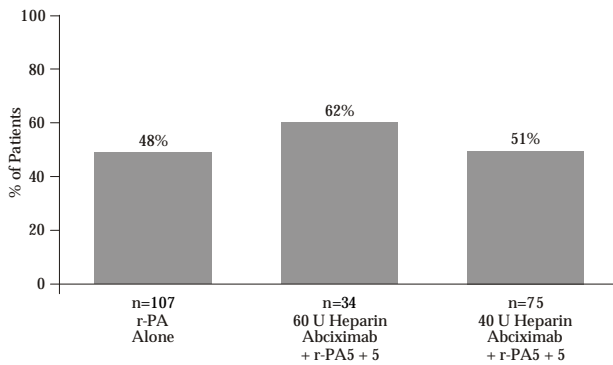


Figure 1. Angiographic core laboratory TIMI 3 flow rates at 60 min following r-PA alone (10 U + 10U), abciximab (0.25 mg/kg bolus and 0.125 mg/kg/min infusion for 12 hours) and combined lower dose r-PA and abciximab with variable heparin dosing. A significant trend towards increased TIMI 3 flow was observed with combined abciximab and half-dose r-PA. (Adapted with permission from Ohman et al., ref 93).

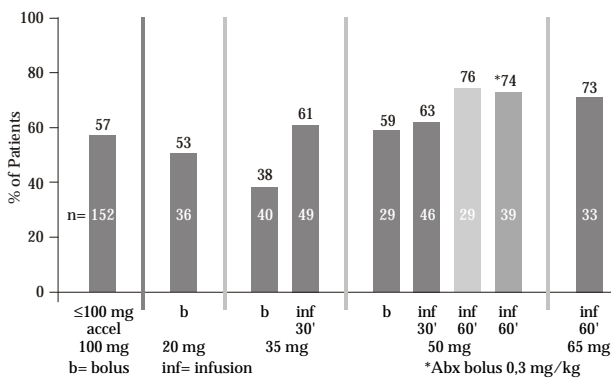


Figure 2. Angiographic core laboratory TIMI 3 flow from the dose-finding phase of the TIMI 14 trial. Variable flow rates are achieved between the different study agent protocols. A trend towards increased TIMI 3 flow at 90 min in patients receiving standard dose of abciximab and 50 mg t-PA was observed, compared to 100 mg t-PA alone. (Adapted with permission from Antman et al., ref 94).

of abciximab alone) and standard reteplase for the dose confirmation phase of the trial.

In TIMI-14 the combination of full-dose abciximab and reduced dose lytics were tested using safety and TIMI-3 flow at 90 minutes as the primary endpoints. A variety of doses of alteplase lead to the interesting observation that bolus administration was not as effective as bolus plus infusion. Optimal TIMI-3 flow rates were observed with a 15 mg bolus followed by a 35 mg infusion over 60 minutes (Figure 2).

In the reteplase phase, optimal TIMI-3 flow rates at 90 minutes were observed with half dose reteplase given as two 5 U boluses 30 minutes apart. Unusually high TIMI-3 flow rates were observed in the control (lytic alone)

arm of this trial and especially in the reteplase control arm; this was found to be due to a disproportionate number of non-anterior myocardial infarctions as well as an earlier time to treatment in this control arm of the trial. Given the wide confidence intervals (due to the limited numbers of patients in each arm), these and other Phase II combination therapy data are most objectively viewed in terms of the trends observed with different lytic doses. The optimal doses of lytic (reteplase 5 U + 5 U) and heparin (60 U/kg bolus followed by 7 U/kg/hr) with full dose abciximab are being tested in the 16,600 patient Phase III GUSTO IV AMI trial which is presently underway to test the key clinical benefit (ie, mortality at 30 days) of combination therapy.

Facilitated Percutaneous Coronary Intervention

Percutaneous coronary intervention (PCI) appears to be effective in not only restoring perfusion but in resolving the underlying stenosis of the infarct-related artery. In a post-hoc analysis of the GUSTO III trial, a subset of patients underwent early angioplasty after failed fibrinolysis for whom complete data are available.¹⁰⁶ Death was significantly reduced in the patients who received abciximab at the time of their intervention (9.7% reduced to 3.6%, $p=0.042$). At 30 days, the composite end point of death, stroke, or reinfarction occurred in 7.3% of patients who received reteplase and abciximab and 21.4% of patients who received alteplase and abciximab ($P=0.08$; Figure 3).

These data suggest but do not prove that platelet inhibition, especially with GPIIb/IIIa antagonists, administered within 12 to 24 hours of thrombolysis may be advantageous, especially when used in conjunction with reteplase during rescue PCI.

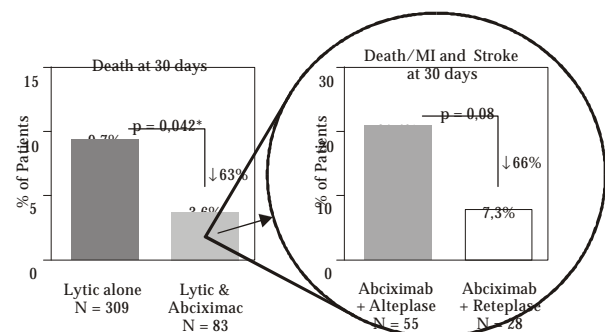


Figure 3. GUSTO III substudy data of survival benefits in patients receiving emergent abciximab therapy following thrombolytic therapy. A significant reduction in 30 day mortality was observed in patients receiving combined thrombolytic therapy. A significant trend within this small subgroup to even greater improvement was observed in patients receiving r-PA with regard to death, myocardial infarction and stroke. (Adapted with permission from Miller et al., ref 106).

Peripheral Vascular Disease

The use of abciximab in peripheral vascular disease, including intracranial interventions, carotid stenting, renal stenting, and peripheral arterial obstructive disease remains anecdotal with only case reports and small series but no controlled studies reported at this time. Abciximab has been used under two distinct but related circumstances. In the first, abciximab is used as an adjunct to lytic agents to enhance lysis of clots in extracardiac vessels.

In the second, abciximab is used as an adjunct to stenting with the goal of decreasing acute ischemic complications and possibly reducing restenosis, with the expectation of improved clinical outcomes in this difficult to treat patient population.

The rationale for the use of abciximab in these settings is clear. The underlying pathophysiology in peripheral vascular, renal, & carotid/intracranial vessel disease is atherosclerosis. As in the coronary vasculature, atherosclerotic plaques either rupture spontaneously or iatrogenically during peripheral interventions, activating both platelets and the coagulation cascade. Vascular interventions (including stenting) are a potent stimulus for platelet activation and aggregation, regardless of the location of the vessel. Finally, the incidence of diabetes is high (35–50%) in patients with peripheral vascular disease. Given the especially robust benefit of abciximab in reducing target vessel revascularization in diabetic patients undergoing coronary stent placement in the EPISTENT trial,¹⁰⁶ a similar robust benefit would be obtained from the adjunctive use of abciximab in extracardiac vascular interventions in these patients.

While the administration of abciximab in extracardiac vascular disease has not yet been formally evaluated for safety and efficacy, its use has been reported in small case series in the medical literature and it is presently being studied in a variety of controlled clinical trials and registries.

Summary

As the foregoing discussion suggests, the adjunctive use of abciximab in percutaneous intervention appears in initial reports to be safe and effective for the treatment of extracardiac vascular disease. However, the details of the safety profile and utility of abciximab in extracardiac percutaneous interventions remain undefined. Additional data on the efficacy and safety of abciximab use in these disease states awaits randomized clinical trials that are currently either in progress or in the design phase. Should these benefits be recognized, the use of abciximab will likely provide a major benefit to the care of patients with extracardiac vascular disease.

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