

Role of Platelet Aggregation Inhibitors in Patients with Coronary Artery Diseases

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Abstract

Abciximab is a monoclonal antibody that is used as an intravenous treatment to prevent platelet aggregation in patients undergoing percutaneous coronary intervention. Abciximab acts by noncompetitively inhibiting the Glycoprotein IIb/IIIa receptor by binding to an allosteric site on the receptor. This inhibition prevents fibrin from binding to the Glycoprotein IIb/IIIa receptor, thus preventing the final step of the platelet aggregation pathway. Two drugs, Tirofiban and Eptifibatide, are also used as anti-platelet aggregation treatment in patients undergoing percutaneous coronary intervention and achieve similar results as Abciximab. However, the mechanism of action is different, since Tirofiban and Eptifibatide are small-molecule inhibitors that bind to the ligand binding site on the Glycoprotein IIb/IIIa receptors and serve as competitive inhibitors. This thesis discusses the biochemical interaction of platelet aggregation inhibitors within the platelet aggregation pathways of patients undergoing percutaneous coronary intervention. Specific biochemistry and clinical terms that relate to the platelet aggregation inhibitors are presented in order to fully understand the mechanism of action of the drugs.

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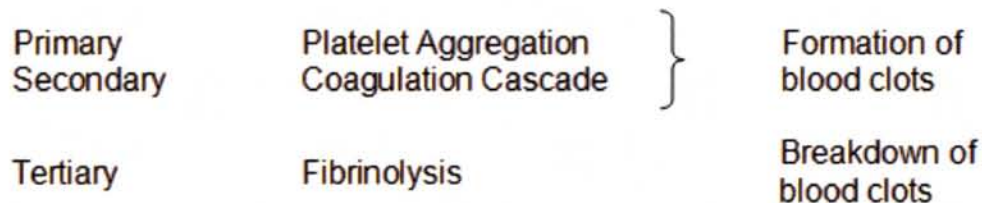
List of Abbreviations

PAI-1	Plasminogen Activator Inhibitor Type 1
GP IIb/IIIa	Glycoprotein IIb/IIIa
PCI	Percutaneous Coronary Intervention
RGD Binding Site	Arginine-Glycine-Aspartic Acid Binding Site
Da/kDa	Dalton/kiloDalton
	Unit of Atomic Mass (1 Dalton = weight of 1 hydrogen atom)
PTCA	Percutaneous Transluminal Coronary Angioplasty
CABG	Coronary Artery Bypass Graft
TF	Tissue Factor

I. Introduction

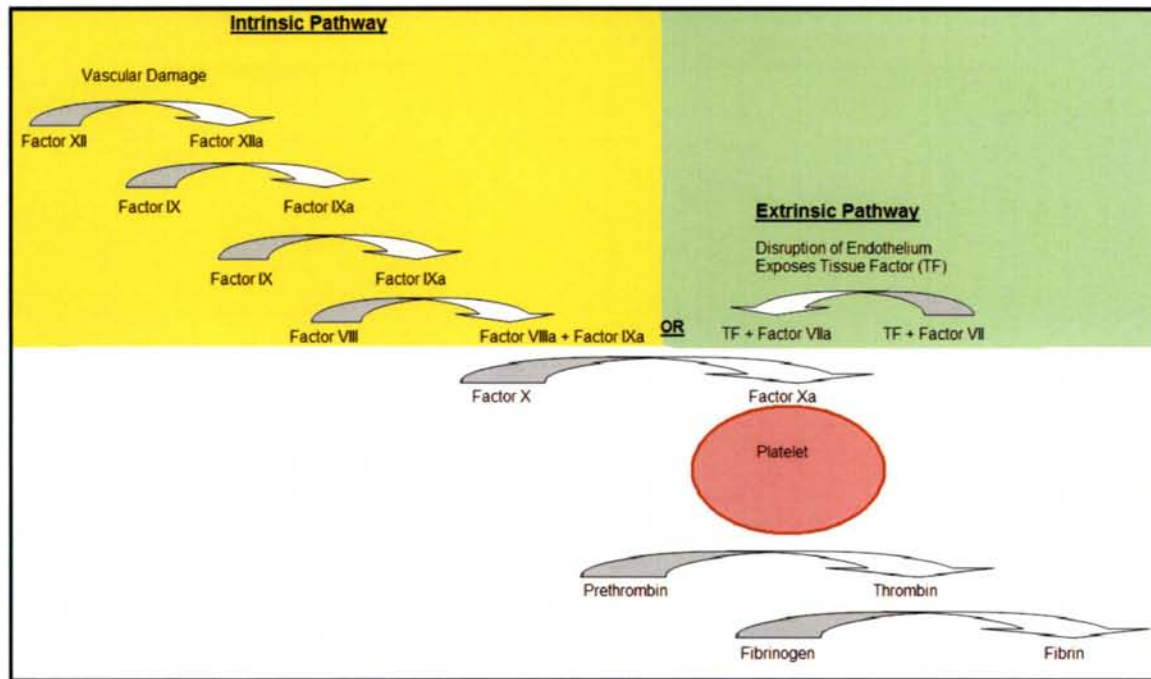
Normally, the human body is in a state of equilibrium between factors that prevent bleeding (pro-coagulant) and factors that prevent excessive blood clotting (anti-coagulant). The hemostatic system enables a body to maintain the vascular pathway by facilitating the formation and breakdown of blood clots. The formation of blood clots (thrombi) within the blood vessel can be physiological (contributing to normal bodily function) or pathological (disrupting function). Pathological thrombus formation contributes greatly to overall morbidity and mortality associated with heart diseases and is the center of ongoing research in prevention and containment.

The Hemostatic System



The blood coagulation cascade, known as secondary hemostasis, is initiated by vascular damage. Blood coagulation has two pathways, intrinsic and extrinsic. The overall goal of the cascade is to convert prothrombin into thrombin. As seen in [Figure 1](#), thrombin converts fibrinogen into fibrin. Fibrin then acts as a ligand in the platelet aggregation pathway. Fibrin forms the insoluble mesh that comprises the blood clot. The mesh is the substrate to which the platelets bind and aggregate. The extrinsic and intrinsic pathways are comprised of a cascade of reactions. The legend for [Figure 1](#) describes the cascade leading to the

Figure 1. Intrinsic and Extrinsic Coagulation Cascades



The intrinsic pathway (yellow) begins with an initial vascular damage site which activates a zymogen (inactive) factor XII into an active factor XIIa. The activated Glycoprotein factor serves as a serine protease, cleaving the downstream zymogen at specific sites in order to activate factor IX. The remaining steps of the intrinsic pathway function similarly until leading to the common pathway (white) in which factor Xa binds to platelets to convert prothrombin into thrombin. The extrinsic pathway (green) requires a tissue factor (TF) found on the subendothelium, which in turn forms a complex with inactive factor VII to form active factor VIIa. The next step, in which the TF-factor VIIa complex leads to the activation of factor X, is essential. The activated factor Xa binds to platelets and leads to a common pathway that converts prothrombin into thrombin.

Source: (Streiff 2001; Stassen, Arnout et al. 2004; Mackman, Tilley et al. 2007)

common pathway that culminates in fibrin formation (Streiff 2001; Stassen, Arnout et al. 2004; Mackman, Tilley et al. 2007).

Within the coagulation pathway, regulators, such as antithrombin, serve to inhibit the effect of the serine proteases. This regulation prevents the activation of the downstream zymogens into active factors. The procoagulant (such as the activated factors shown in Figure 1) and the anticoagulant (such as antithrombin) factors maintain the balance of the vascular system (Stassen, Arnout et al. 2004).

The tertiary component of the hemostatic system, fibrinolysis, regulates the breakdown of the blood clots. This prevents occlusion of the vascular system by the blood clots. Fibrinolysis occurs when plasminogen activators (present in the endothelium) convert plasminogen into plasmin. Plasmin is the enzyme that is responsible for the degradation of insoluble fibrin (Stassen, Arnout et al. 2004). Fibrinolysis blocks platelets from adhering by disrupting the structure of fibrin. Regulators of the fibrinolysis cycle, such as plasminogen activator inhibitor type 1 (PAI-1), α 2-antiplasmin, α 2-macroglobulin, and thrombin-activatable fibrinolysis inhibitor, control the breakdown of fibrin clots by inhibiting fibrinolysis during times of vascular injury when clotting is needed (Stassen, Arnout et al. 2004).

Platelet adhesion and aggregation, which are collectively termed primary hemostasis, are important in formation of thrombi. At sites of vessel damage, there is a propensity for platelets to aggregate and cause further arterial damage. The mechanism responsible for platelet aggregation has been studied extensively. The main components of the platelet aggregation pathway are the

platelet stimulus (an agonist), a soluble adhesive protein (such as fibrinogen, fibronectin, and the von Willebrand factor), and a membrane bound receptor on platelets (the Glycoprotein IIb/IIIa receptor) (Jackson 2007).

As Figure 2 depicts, the GP IIb/IIIa receptor is found in the ligand-unreceptive (inactive) state in resting platelets. Once an agonist, such as adenosine diphosphate, thrombin, epinephrine, etc., acts as a stimulus to initiate the platelet aggregation pathway, the GP IIb/IIIa receptor adopts a ligand-receptive (active) state. This activated state allows ligands to bind to the receptor. Once enough GPIIb/IIIa receptor ligands (such as fibrin, fibronectin, and the von Willebrand factor) have attached to the GP IIb/IIIa receptor on the platelet cell surface, the platelets are able to attach to one another creating a chain of linked platelets (Winter and Juergens 2008). This platelet aggregation and association with the fibrin mesh of the blood clot can continue until thrombus formation is complete, causing the vessel to be blocked. Although the platelet aggregation process is critical process for maintaining vascular integrity, unchecked platelet aggregation can have serious pathological consequences.

The formation of atherosclerotic plaques is a common source of vessel damage and can lead to unwarranted platelet aggregation. These plaques are thickenings of the intima, the innermost layer of the artery (Hansson and Nilsson 2008). The effects of vessel damage can be intensified when individuals add to the risks by engaging in activities that are deemed causes for heart disease. Smoking, an unbalanced diet (which can lead to high cholesterol and diabetes), and lack of physical exercise are considered to be risk factors for cardiovascular

Figure 2. Platelet Aggregation Pathway

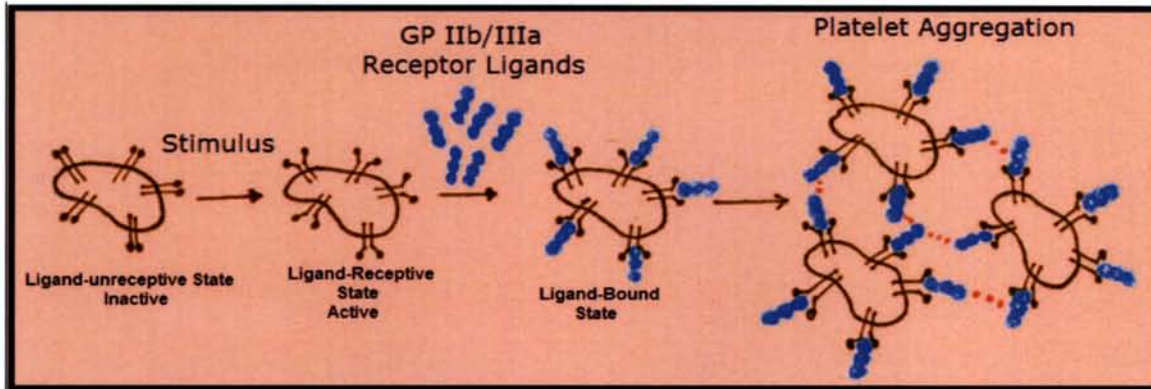


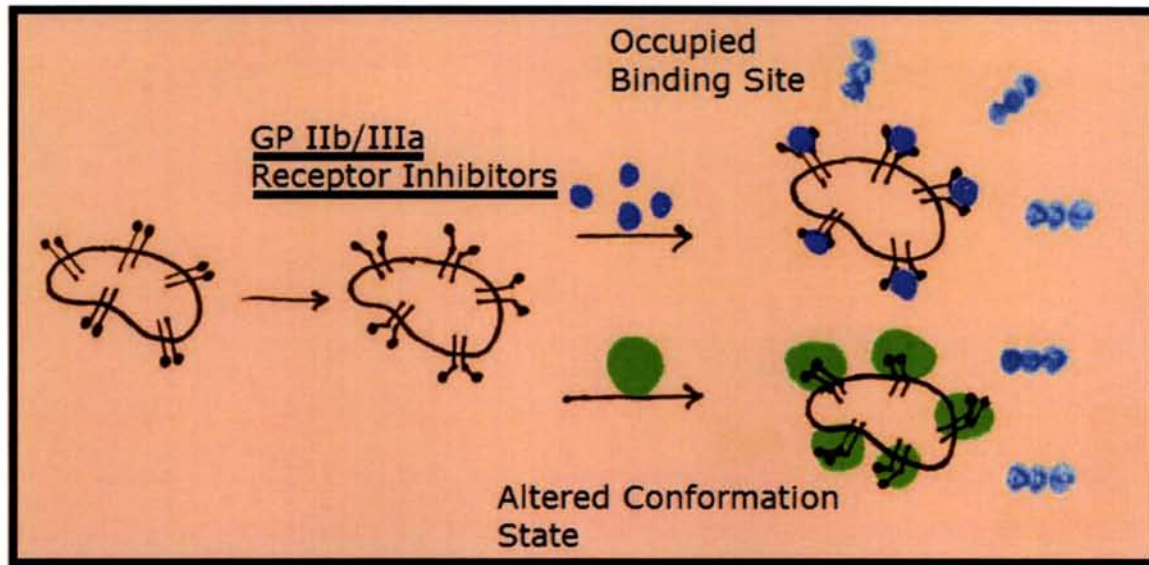
Figure 2 shows the platelet aggregation pathway. The ligand-unreceptive state, on the left, is inactive. Once an external stimulus acts on the platelets, the receptor is activated. The active state allows for ligand binding. When enough ligands have attached to the GP IIb/IIIa receptor on the platelet cell surface, the platelets are able to attach to one another.

diseases. With enough plaque formation, thrombi and arterial ruptures can create pathological problems such as myocardial infarction (heart attack) and unstable angina (chest pain) (Organization 2007).

Treatment of coronary artery diseases varies with severity. At times, individuals are required to have surgical treatment (either elective or emergent). There are many different surgical procedures that can be used to for patient management. One such procedure is Percutaneous Coronary Intervention (PCI). During a PCI procedure, occluded blood vessels are opened to restore blood flow. Since the procedure introduces damage to the vessel, the platelet aggregation pathway can be initiated. It is pertinent to use drug therapy concurrently with PCI procedures to reduce the risk of platelet aggregation. As Figure 3 shows, GP IIb/IIIa receptor antagonists serve to inhibit the binding of the GP IIb/IIIa receptor ligands by blocking or altering the active binding site on the receptor. This receptor blockade prevents fibrinogen from binding to the GP IIb/IIIa receptors, thereby preventing platelet aggregation.

This thesis will describe the biochemical interactions of platelet aggregation inhibitors within the platelet aggregation pathways in patients undergoing a PCI procedure. The paper will show the physiological responses that these drugs elicit. The paper will compare and contrast the different mechanism of action and development of the three main GP IIb/IIIa inhibitor drugs. The thesis focuses on one drug, Abciximab, and relates production and mechanism of action of Abciximab to the related drugs, Tirofiban and Eptifibatide.

Figure 3. Role of GP IIb/IIIa Antagonists in the Platelet Aggregation Pathway



GP IIb/IIIa receptor antagonists serve to inhibit the binding of the GP IIb/IIIa receptor ligands by blocking or altering the active binding site on the receptor. This receptor blockade prevents ligands from binding to the GP IIb/IIIa receptors, thereby preventing platelet aggregation.

II. The Biochemistry that Governs Platelet Aggregation

In order to fully understand the scope of this paper, it is important to delve into the biochemical mechanisms that regulate the formation and breakdown of blood clots. In addition, a focus on the platelet aggregation process and the proteins that regulate the process is also presented. Integrins are receptors on the surface of cellular structures, such as platelets. Integrins are introduced here since they play an important role in mediating platelet aggregation. Knowledge of their structure and function is important in understanding the mechanisms that the anti-platelet drugs carry out. The circulatory proteins, vitronectin and PAI-1 are discussed in Section III because of their role in the cardiovascular and circulatory system during normal physiological activity and during pathological events. The author of this thesis gained experience with these proteins during laboratory work as an undergraduate researcher.

II-A. Integrin Receptors

The discovery of integrin receptors came from the need for scientists to understand the adhesive interactions between cells and the extracellular matrix. Integrins serve as receptors on the cell membrane. These integrin structures span the entire cell membrane, forming a bidirectional control of cell adhesion. Integrins bridge intracellular molecules of the cell to that of the extracellular matrix or other cells through focal adhesion (Horwitz 1997). The binding of ligands to integrin receptors allow signals to transverse from one location to another.

II-Ai. Structure

Integrins are composed of two distinct subunits, which are designated alpha (α) and beta (β). Each subunit has a number of different variants. The combination of α and β variants create novel integrins. Some integrins are specific to which type of ligand they bind to, whereas some are recognizable by many ligand types (Horwitz 1997). Within each subunit, there is an extracellular region, a transmembrane region, and an intracellular region. These regions are outlined in [Figure 4](#). According to sequence alignment of the human integrin subunits, the transmembrane region is highly conserved in each of the α and β subunits (Lau, Kim et al. 2009). [Figure 5](#) shows the transmembrane region of the Glycoprotein IIb/IIIa receptor and is a good representation of the transmembrane region of a variety of integrins. Integrins are considered anthropomorphic molecules, since they are composed of ligand-binding heads and two long legs. Each subunit forms a leg, and both α and β subunits combine to form the ligand-binding head. The bent or non-bent configuration of the legs determine if the integrin is in a low-affinity (which is its inactive state), high-affinity (which is at its active state and ready to bind a ligand), or ligand-bound state (Mould and Humphries 2004).

The role of integrins within the body has been studied heavily. Integrins have been found to be involved in the signal transduction pathways that facilitate proliferation, platelet aggregation, embryonic differentiation, inflammation, and a host of other processes (Horwitz 1997).

Figure 4. Integrin within the Cellular and Extra-cellular Matrix

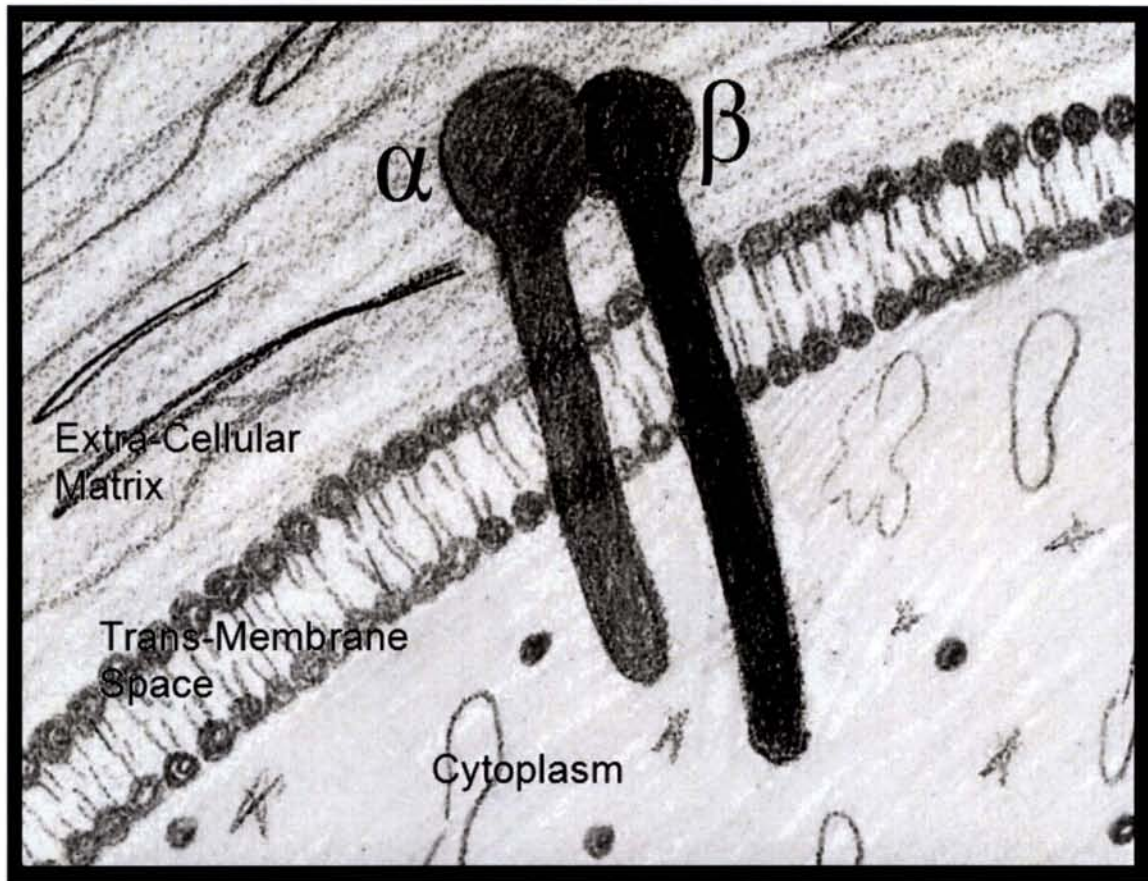


Figure 4 illustrates the placement of integrin structures within the cell.

Figure 5. Structure of the Glycoprotein IIb/IIIa (α IIb β ₃) Transmembrane Complex

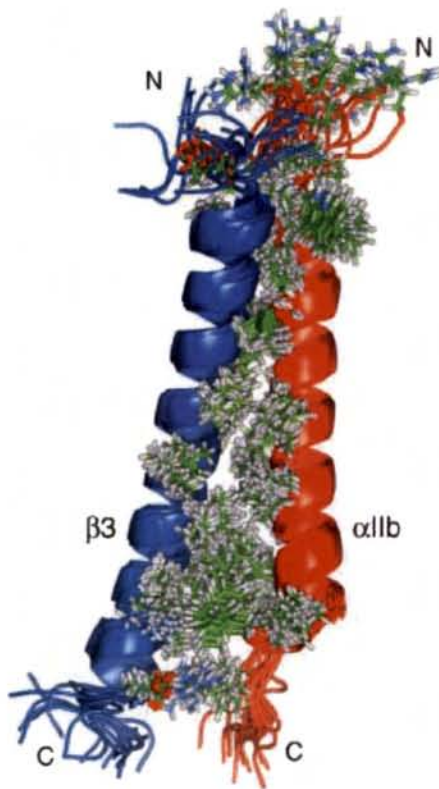


Figure 5 shows the transmembrane structure of the Glycoprotein IIb/IIIa receptor. The α subunit is colored red and the β subunit is colored blue. The N-terminal (at the top) is the portion that is exposed to the extracellular matrix and the C-terminal (at the bottom) is exposed to the cellular cytoplasm. The GP IIb/IIIa receptor transmembrane region is stabilized by glycine residues (depicted as green/gray structures in Figure 5). This stabilization is termed "glycine-packing".

Source: (Lau, Kim et al. 2009)

II-Aii. RGD Binding Site

In order to target specific integrins, researchers began to search for binding sites on the receptors. There are numerous binding sites on receptors to which ligands have a complementary structure. A known susceptible recognition site on a ligand has been identified as the RGD (arginine-glycine-aspartic acid) tripeptide binding site. It has been shown that numerous members of the integrin family, including $\alpha\text{IIb}\beta_3$ and $\alpha_v\beta_3$, recognize ligands that have the RGD motif. RGD mimics can be used to alter physiological processes by blocking the ligand-receptor interaction site (Takagi 2004; Cini, Trabocchi et al. 2009).

Though the RGD motif is recognized by many integrins, there are also other known binding sites. Even if an integrin sequence contains a RGD binding site, there are secondary binding sites that can also offer selectivity. It has been noted that the secondary sites interact with the α subunit of the integrin, whereas the RGD motif interacts with the β subunit (Takagi 2004).

II-Aiii. Glycoprotein IIb/IIIa Receptor ($\alpha\text{IIb}\beta_3$ Integrin)

The integrin $\alpha\text{IIb}\beta_3$, also known as the Glycoprotein IIb/IIIa receptor, is the primary adhesion receptor of blood platelets within the body. The GP IIb/IIIa receptor is able to control platelet aggregation by binding to fibrin or other ligands such as fibronectin and the von Willebrand factor (Lau, Kim et al. 2009). The GP IIb/IIIa receptor has an important role physiologically since it is involved in the coagulation cascade/platelet aggregation pathway that forms the necessary blood clots needed to maintain the vascular system. The GP IIb/IIIa receptor has an important role pathologically when excessive aggregation of platelets leads to

thrombus formations. These thrombus formations can lead to myocardial infarctions (heart attacks) or other adverse clinical presentations. The crystal structure of the extracellular segment of the GP IIb/IIIa receptor is shown in Figure 6.

II-Aiv. The $\alpha_v\beta_3$ Integrin

The integrin $\alpha_v\beta_3$ is highly versatile and is an important receptor in tumor angiogenesis and metastasis, inflammation, and bone resorption. The $\alpha_v\beta_3$ integrin binds ligands with promiscuity. The integrin $\alpha_v\beta_3$ is able to bind to a host of ligands including vitronectin, angiostatin, and osteopontin. The $\alpha_v\beta_3$ integrin also serves as a receptor in viral processes that are attributed to the viruses that cause foot-and-mouth disease, adenovirus, and human immunodeficiency virus. The plethora of processes associated with the $\alpha_v\beta_3$ integrin makes it an important integrin to study. Researchers have solved a crystal structure for the $\alpha_v\beta_3$ integrin. With this information, they hope to be able to further understand the disease processes that the $\alpha_v\beta_3$ integrin is associated with (Xiong, Stehle et al. 2001).

Researchers have solved a crystal structure of the 12 $\alpha_v\beta_3$ domains by growing protein crystals. These crystals were then analyzed using single isomorphous replacement-anomalous scattering and multiwave-length anomalous diffraction technology to fully solve the structure. These 12 domains, as seen in Figure 7, account for the extracellular segment of the integrin (Xiong, Stehle et al. 2001).

Figure 6. Crystal Structure of the Extracellular Domain of the GP IIb/IIIa Receptor

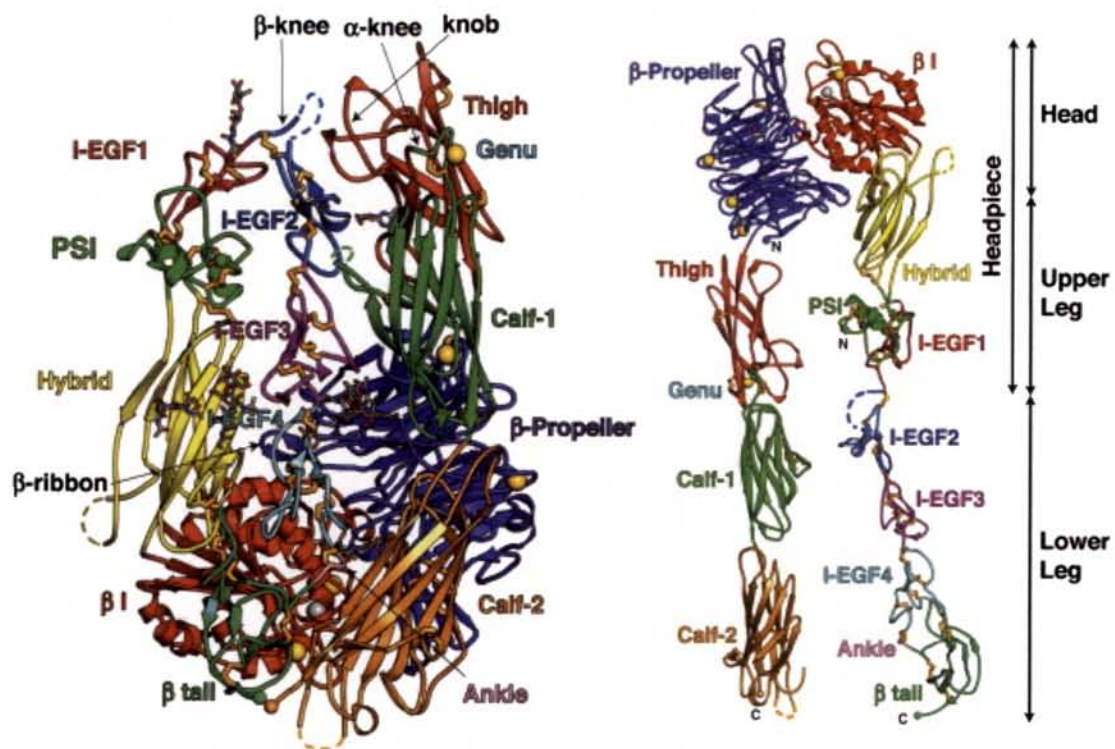


Figure 6 shows the crystal structure of the extracellular segment of the GP IIb/IIIa receptor.

Source: (Zhu, Luo et al. 2008)

Figure 7. Crystal Structure of the Extracellular Domain of the $\alpha_v\beta_3$ Integrin

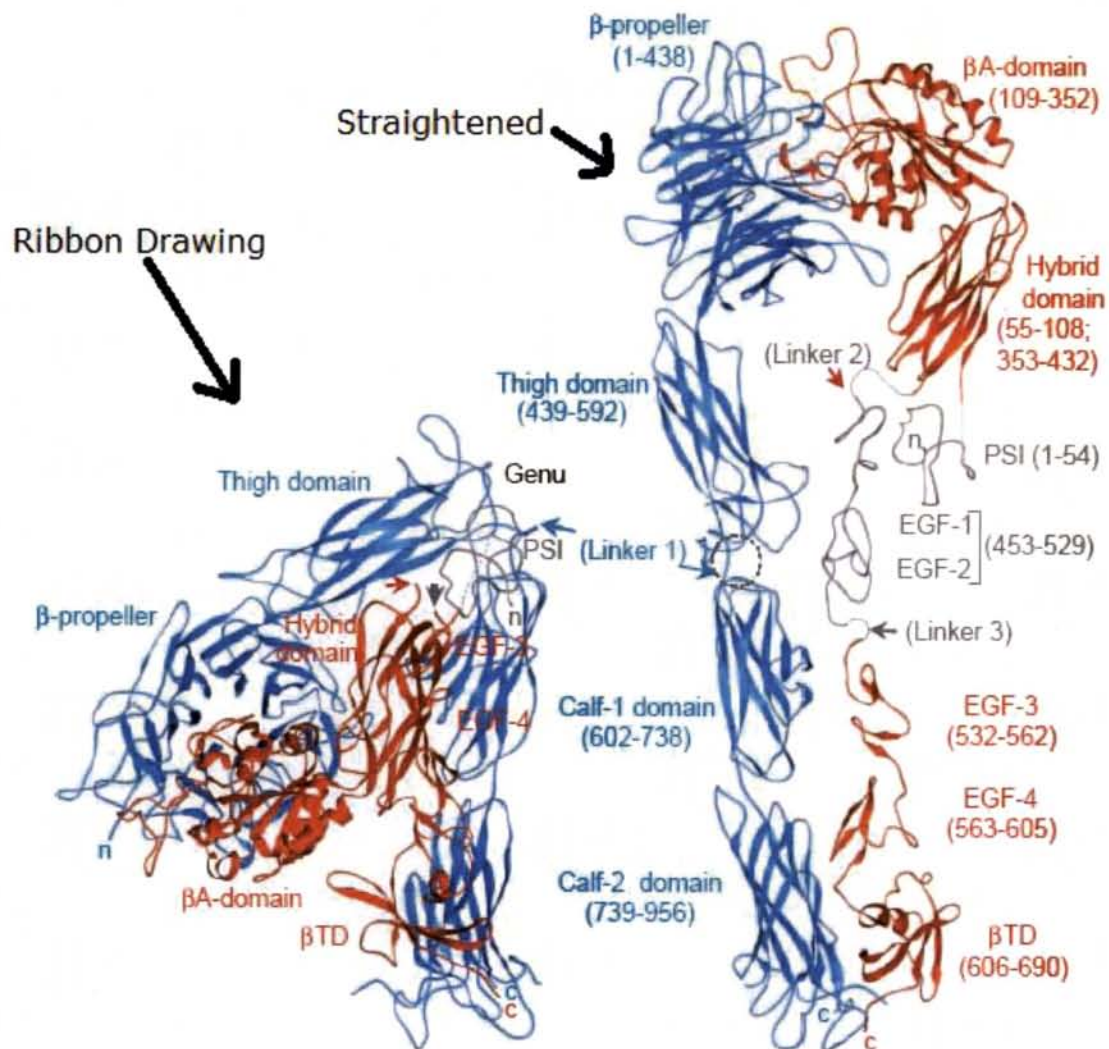


Figure 7 shows the crystal structure of the extracellular segment of the $\alpha_v\beta_3$ Integrin. The α subunit is shown in blue and the β_3 is shown in red. On the left is the ribbon drawing of the structure and on the right is the straightened.

Source: (Xiong, Stehle et al. 2001)

II-B. Circulatory Proteins: Vitronectin and PAI-1

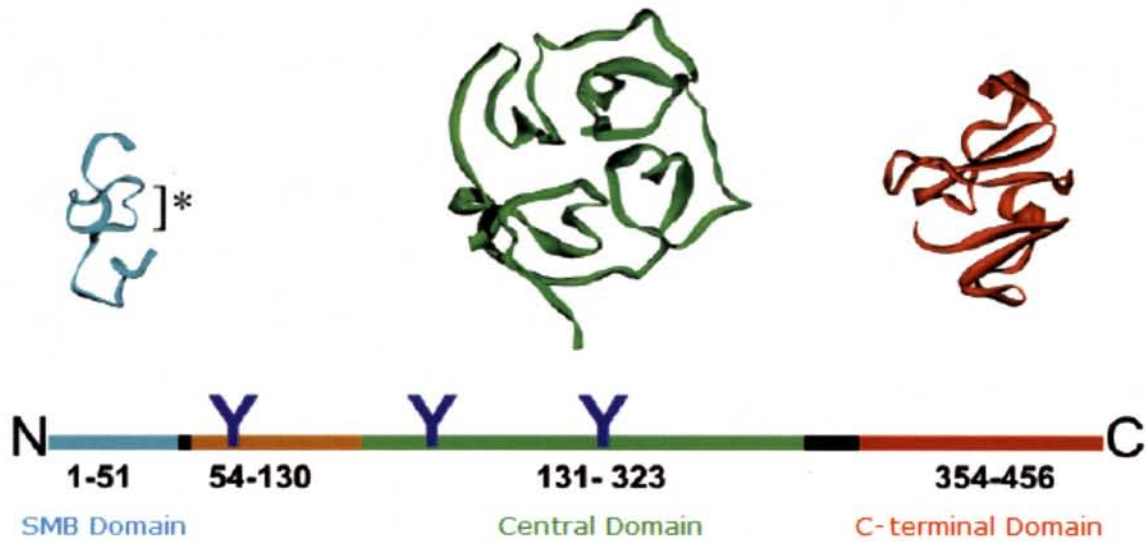
In order for the body to maintain a careful biochemical balance, there are numerous proteins that circulate. Two such proteins, Vitronectin and PAI-1, are presented here in order to provide a snapshot of the role of circulatory proteins that are involved in the hemostatic system. Vitronectin and PAI-1 are chosen for this discussion because of the personal experience with the two proteins that the author of this thesis has had during her undergraduate research.

II-Bi. *Vitronectin*

Vitronectin is a circulatory Glycoprotein that has a role in hemostasis through its regulation of blood coagulation (clotting) and fibrinolysis (break down of blood clots). The 75 kDa protein plays an important role in cell adhesion, differentiation, proliferation, and morphogenesis. Vitronectin is present in the blood plasma at a concentration of about 200-400 ug/ml and accounts for about .2-.5% of total plasma proteins (Preissner and Seiffert 1998; Ekmekci and Ekmekci 2006). Circulating levels of Vitronectin are increased 2-3 fold in pathological settings. Though Vitronectin is normally not present in most tissues, Vitronectin accumulates at sites of injury or disease in tissues (Peterson 2009).

The domains of Vitronectin, depicted in Figure 8, enable binding of Vitronectin to numerous partners. The SMB domain contains the primary binding site for PAI-1. Following the SMB domain, there is the RGD sequence that allows for binding to various integrins. The central domain could possibly contain the binding site for self-association into multimers. The C-terminal domain

Figure 8. The Domain Structure of Vitronectin



Structures for the domains of Vitronectin are shown above the linear sequence. The three domains for Vitronectin are termed the SMB domain, the central domain, and the C-terminal domain. The region between the SMB domain and the central domain, depicted brown in [Figure 8](#), is the disordered connection region. The blue "Y" structures on the linear sequence note the sites of carbohydrate attachment.

Image Source: (Lynn, Heller et al. 2005)

contains the heparin binding site and an alternate binding site for non-RGD dependent integrins (Peterson 2009).

Experimentally, it has been shown that roles of vitronectin are dependent on its conformational states. The monomeric form of vitronectin that is found in circulation is soluble and binds to many partners. The partners for the monomeric form include heparin, PAI-1, and numerous proteases. The oligomeric form of vitronectin is the tissue-associated molecule that interacts with the extracellular matrix and cell-surface receptors (such as the GP IIb/IIIa receptor, the $\alpha_v\beta_3$ integrin, and the uPA receptor) (Peterson 2009).

Vitronectin is recognized by a host of cell surface receptors, including the Glycoprotein IIb/IIIa receptor and the $\alpha_v\beta_3$ receptor. Its ability to be recognized by various agents contributes to its function and importance within the cell. Vitronectin is conserved between different species. Primary structure analysis shows an 80% similarity between human, mouse, rat, rabbit, and porcine sequences (Preissner and Seiffert 1998). The role of vitronectin in the hemostatic system and restenosis are discussed in II-Bii. and iii.

II-Bii. PAI- 1 and the Role of the PAI-1/Vitronectin Complex in the Hemostatic System

Plasminogen Activator Inhibitor - type 1 (PAI-1) is a circulatory protein involved in the regulation of vascular hemostasis. PAI-1 serves as a serine protease inhibitor that inhibits the function of plasminogen activators such as tissue plasminogen activator (tPA) and urokinase (uPA). By inhibiting tPA and uPA, PAI-1 acts to inhibit the conversion of plasminogen to plasmin. This

inhibition prevents fibrinolysis (tertiary hemostasis). Figure 9 shows the structural conformation for active and latent (in-active) PAI-1.

PAI-1 is unstable, which causes the molecule to change from active to latent form easily. Vitronectin forms complexes with PAI-1 and stabilizes PAI-1 in an active conformation. The active PAI-1/Vitronectin complex mediates stabilized binding to fibrinogen. This complex impacts the thrombin-fibrinogen interactions (Podor, Shaughnessy et al. 2000). Binding of PAI-1 to vitronectin may block vitronectin from binding to other integrins or receptors, which initiate thrombus formation. It has been shown that increased levels of PAI-1 in plasma levels are associated with increased acute coronary syndromes (Ekmekci and Ekmekci 2006). The role of the PAI-1 and the PAI-1 /Vitronectin Complex is shown in Figure 10.

Along with mediating PAI-1 binding to fibrin, it has also been established that Vitronectin interacts directly with the fibrin clots. The antithrombotic interaction is carried out along the γ -chains of fibrinogen and leads to vitronectin-mediated inhibition of thrombin-fibrinogen interactions (Podor, Campbell et al. 2002).

The role of Vitronectin in atherosclerosis is important. In the intimal thickening, there are many layers of smooth muscle cells, monocytes, T-lymphocytes, and cellular debris. Glycoproteins such as Vitronectin are found in these atherosclerosis plaques because of their function within the extracellular matrix. It has been observed that vitronectin can mediate the migration of smooth

Figure 9. Active and Latent Forms of PAI-1

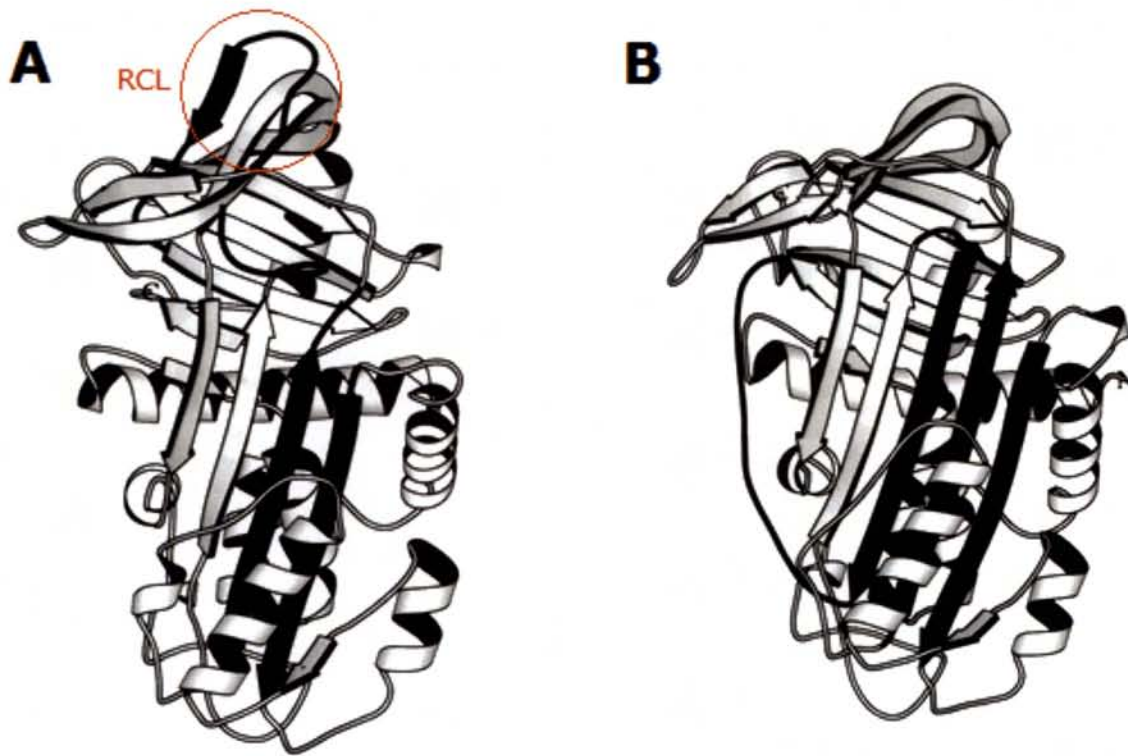
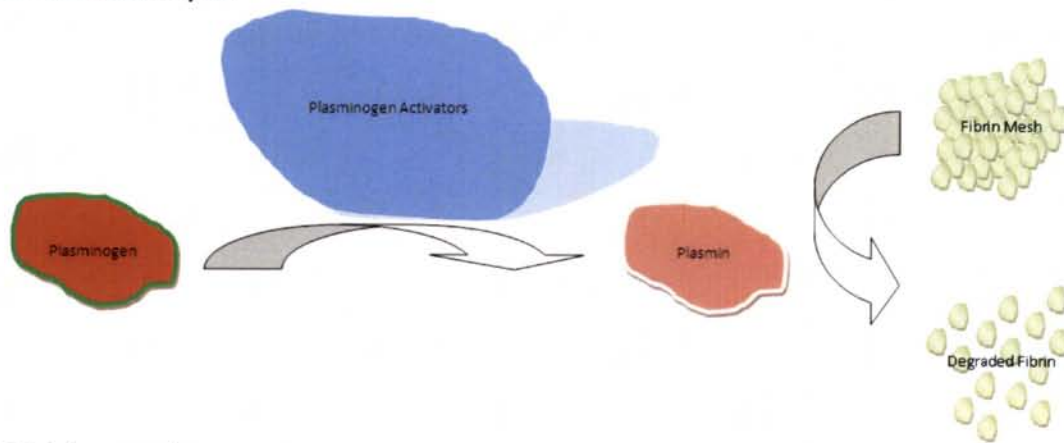


Figure 9 shows the active (A) and latent (B) forms of PAI-1. The active form has the RCL, reactive center loop, positioned in an exposed manner towards the targeted enzyme (the serine proteases).

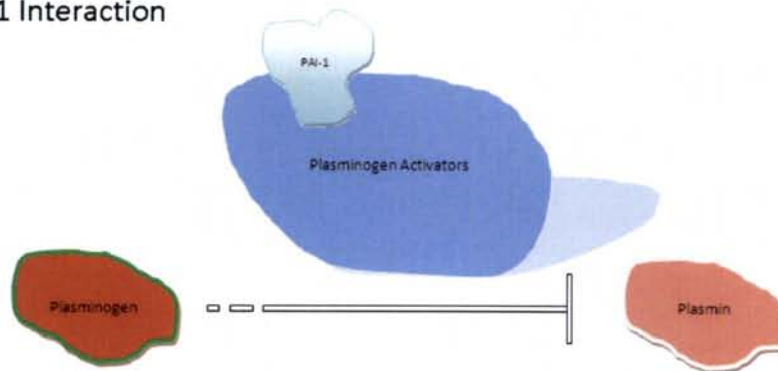
Image Source: (Stout, Graham et al. 2000)

Figure 10. Role of PAI-1 and Vitronectin in the Hemostatic System.

Normal Fibrinolysis



PAI-1 Interaction



PAI-1/Vitronectin Complex Interaction

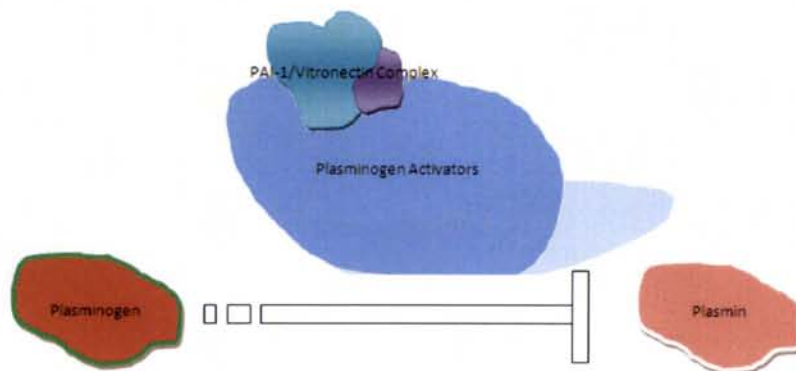


Figure 10 shows the affect of PAI-1 and the PAI-1/Vitronectin complex on fibrinolysis. Normally, Plasminogen is converted to Plasmin. Plasmin breaks down the fibrin clots. PAI-1 and the PAI-1/Vitronectin complex inhibit the fibrinolysis process. Vitronectin stabilizes PAI-1, so the complex offers greater inhibition.

muscle cells to these lesions via $\alpha_v\beta_3/\alpha_v\beta_5$ integrin interactions, contributing to thicker lesions (Ekmekci and Ekmekci 2006).

II-Biii. Role of Vitronectin in Restenosis

The role of the vitronectin receptor mediated migration of coronary smooth muscle cells into the neointima (thickened layer of arterial lining) is important in the process of restenosis. Restenosis refers to the narrowing of the blood vessels. Vitronectin binding to the $\alpha_v\beta_3$ integrin on the endothelial and smooth muscle cells enables these cells to bind to the arterial lining and cause vasoconstriction. Known antagonist treatments that are effective in inhibiting the GP IIb/IIIa receptor function are not effective for inhibiting the $\alpha_v\beta_3$ integrin from carrying out its function. However, novel treatments using biphenyls are showing promise (Urbahns, Harter et al. 2002).

III. Focus of the Research Program Led by Dr. Cynthia Peterson at University of Tennessee

In order to understand complex physiological processes, researchers from all over the world team up to understand the role of particular units, for example, proteins. In this collaborative model, each principal investigator and their corresponding research group have a vital role to play in understanding the entire process by focusing on one particular issue at a time.

III-A. Peterson Lab Group

The Peterson Lab at the University of Tennessee is one such group. This group's main focus is to understand the function and structure of vitronectin (and the proteins that vitronectin forms complexes with). Vitronectin is known to form

complexes with numerous molecules in circulation and in the extracellular matrix. One such molecule is PAI-1. As stated in a grant submitted to the National Institute of Health, a central hypothesis of the Peterson group is to show the interactions of PAI-1 and vitronectin; and, the effects of those interactions on structure, function, and tissue localization of the proteins (Peterson 2009). The Peterson group has contributed greatly to the study of the hemostatic system by determining the role that the stability of the PAI-1/Vitronectin complex has in increasing inhibition of plasminogen activators. The Peterson group has further contributed to the study of the hemostatic system by interpreting the interactions of vitronectin to fibrin clots.

Another aim of the Peterson group is to use new biochemistry technologies, such as small angle neutron scattering, high-end tandem mass spectrometry, and high-resolution cryo-electron microscopy, to further advance knowledge of complex formations. University of Tennessee's proximity and relations to Oak Ridge National Laboratory makes it feasible to study these cases in detail (Peterson 2009).

The specific focuses of study of the Peterson group are to determine the exact sites on Vitronectin that PAI-1 binds to; how binding alters their conformation; and the architecture and subunit composition of the complexes. By learning more about these topics, the group hopes to relate the findings to a whole host of physiologically important topics. Since both vitronectin and PAI-1 are immensely important in thrombus formation and atherosclerosis, the findings

of the Peterson group can contribute to researching novel prevention and containment protocols (Peterson 2009).

III-B. Undergraduate Research Experience of Dipti Chhajwani

Since 2006, the author of this senior thesis, Dipti Chhajwani, has been a part of Dr. Cynthia Peterson research group. As an undergraduate researcher, Dipti was exposed to protein biochemistry research at the start of her sophomore year. Being a part of a large scale research group, Dipti was allowed to interact with and learn from advanced biochemists. Her early exposure to scientific technique and thought process will be invaluable to her future career.

Dipti started her own research project during the summer of 2008. Since vitronectin is comprised of 459 residues (amino acids), the project goal was to isolate eight 100 amino acid peptides, (position 1 – position 100; 51-150; 101-200; 151-250; 201-300; 251-350; 301-400; 351-459). The sequences for the amino acids for the eight recombinant peptides of Vitronectin were cloned into the pET15b expression vector system. Through DNA purification and sequence analysis, Dipti ensured that the correct Vitronectin DNA was present. In order to achieve protein expression at a higher scale, it was beneficial to transform the peptides in an *E. coli* host strain, Rosetta 2(DE3). The Rosetta strain was chosen for the transformations because of the Peterson group's experience with the strain. The Rosetta strain allows for enhanced inducible expression of peptides within the pET plasmid. Once transformation was complete, Dipti began small-scale induction of the cells. However, due to complications in technique and within the cell line, expression was not achieved at a high scale.

The overall goal of the project was to isolate the eight 100 amino acid peptides to perform binding studies to test for self oligomerization of vitronectin and to study the second binding site for PAI-1.

IV. Background on Cardiovascular Diseases with a Focus on Coronary Artery Diseases

In order to understand the clinical background of patients who are given anti-platelet aggregation treatment during percutaneous coronary intervention, this thesis gives a background on Cardiovascular Diseases and Coronary Artery Diseases. Also, treatments of coronary artery disease are introduced, along with the complications that arise during/after treatment.

IV-A. Cardiovascular Diseases

Cardiovascular diseases are considered to be the number one cause of mortality globally. In the United States in 2006, 80 Million (comprising of 36.3% of the population) adults over the age of 20 had at least one form of Cardiovascular Disease (Association 2009). Cardiovascular diseases are disorders of the heart and blood vessels. There are a variety of different types of Cardiovascular Diseases, including Coronary Artery Disease, Cerebrovascular Disease, Peripheral Arterial Diseases, Rheumatic Heart Disease, and Congenital Heart Disease (Organization 2007). Table 1 gives the conditions of each of these types of cardiovascular diseases along with their clinical presentations.

The World Health Organization publishes literature yearly that focuses on prevention of cardiovascular diseases. Causes of these diseases are well known and can be preventable. The majority of causes of heart disease and stroke arise

Table 1. Types of Cardiovascular Diseases
Source: (Organization 2007; Association 2009)

	Condition/ Clinical Presentation
Coronary Heart Disease	Diseases of the blood vessels that supply the heart muscle. Examples: Myocardial Infarction (Heart Attack); Unstable Angina (Chest Pains)
Cerebrovascular Disease	Diseases of the blood vessels that supply the brain. Example: Stroke
Peripheral Arterial Disease	Diseases of the blood vessels that supply the arms and legs. Examples: Ischemia (restriction of blood supply)
Rheumatic Heart Disease	Disease that presents as damage to the heart muscle and heart valves. Caused by Streptococcal bacteria, which leads to Rheumatic Fever.
Congenital Heart Disease	Conditions or Malformations of heart structures that are existing at birth. May be caused during gestation or by genetic factors. Examples: Abnormal valves/chambers; Holes in the heart
Deep Vein Thrombosis; Pulmonary Embolism	Present as blood clots in the leg veins. These clots can cause further damage by dislodging and moving to the heart and lungs.

from modifiable risk factors. Though the process is complex and involves genetic predispositions, there are several risk factors. Modifiable risk factors such as smoking, hypertension (high blood pressure), high cholesterol, and diabetes contribute to heart disease (Organization 2007). Disease awareness plays an important role in disease prevention. Various professional organizations such as the World Health Organization and the American Heart Association have developed marketing plans to inform the public of the dangers of an unbalanced lifestyle on health (Association 2009). This was exemplified by the statistics gathered in 2006 that showed that 57% of women surveyed knew that heart disease is the leading cause of death in women. Similar surveys conducted in previous years showed that fewer women knew of the significance of heart disease. In 2003, 46% of women surveyed knew that heart disease is the leading cause of death in women. This can be further traced back to 1997 and 2000, when the figures were only 30% and 34%, respectively (Christian, Rosamond et al. 2007).

IV-B. Coronary Artery Diseases

In 2006, an estimated 16.8 million Americans suffered with Coronary Artery Diseases (Association 2009). Coronary artery disease is primarily caused by atherosclerotic lesions. These lesions are thickenings of the innermost layer of the artery, known as the intimal lining. These thickenings consist of cells, connective-tissue elements, lipids, and cellular debris. Often times, inflammatory and immune cells are found in these lesions as well as vascular endothelial and smooth muscle cells (Hansson and Nilsson 2008).

Pre-lesion materials called fatty streaks are present prior to an actual formation of an atherosclerotic lesion. These fatty streaks can eventually disappear or develop into larger units. Once an atherosclerotic lesion forms with enough depth, that it is able to block blood flow through the coronary arteries, a thrombus site is created, which can possibly rupture (Hansson and Nilsson 2008). Figure 11 shows the cross sectional view of a thrombus site that led to a myocardial infarction.

IV-Bi. Types of Coronary Artery Diseases

There are several types of coronary heart diseases. These conditions can range from mild discomfort to acute attacks that require medical attention.

Myocardial infarctions ("heart attacks") are recognized to be one of the leading causes of death and disability worldwide. Patients with coronary atherosclerosis have periods of instability and stability. During times of instability, when inflammation in the vascular walls is activated, patients are prone to develop myocardial infarctions. Myocardial infarctions are pathological conditions of damage to the cardiac muscle, which can lead to necrosis (cell death or rupture). Myocardial infarctions can be detected clinically through rise or fall of specific biomarker concentrations present in the blood stream and through electrocardiograms (Thygesen, Alpert et al. 2007).

Angina is a condition where patients experience chest pain or discomfort when there is a lack of blood flow to the heart. Patients who have severe angina, termed unstable angina, complain of pains throughout resting periods and require immediate medical attention.

Figure 11. Thrombus Formation Leading to Ruptures

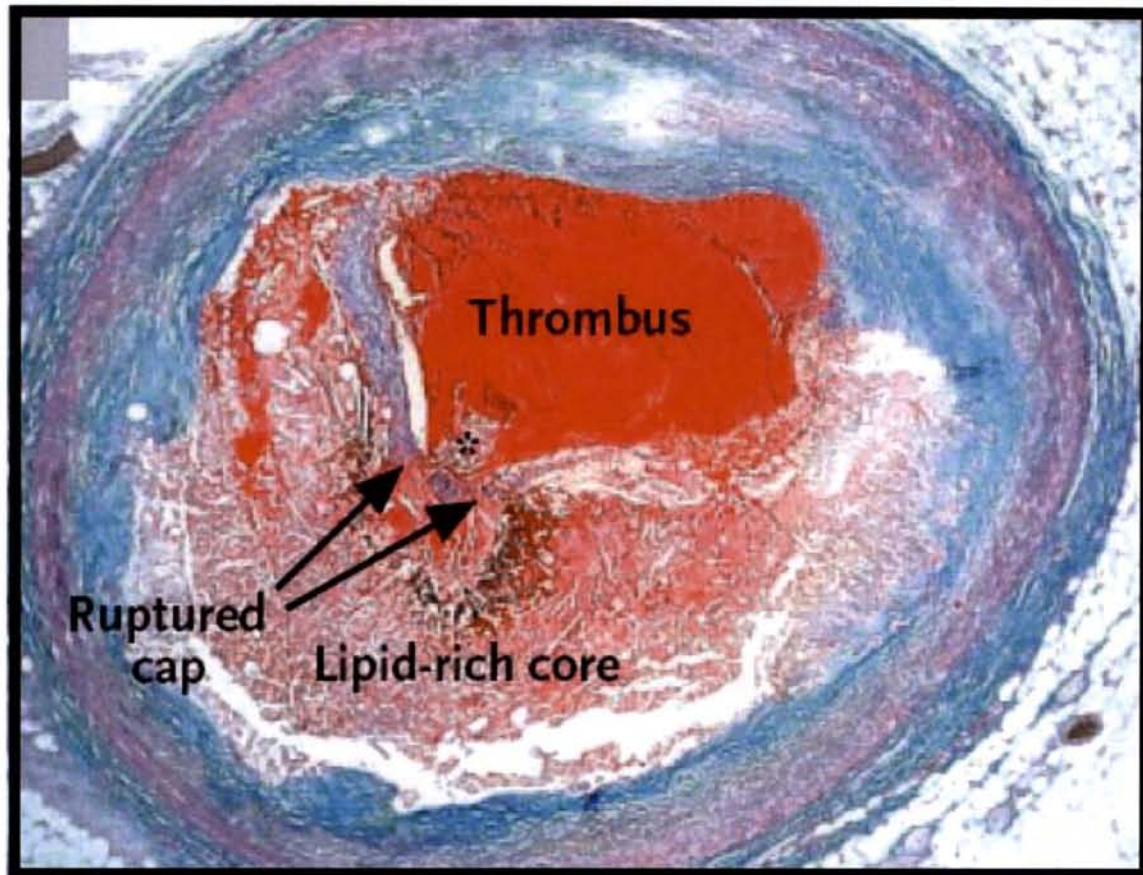


Figure 11 shows atherosclerotic lesions within a human artery. The thrombus site occludes the artery, and one can see the rupture of the thrombus between the arrows. The rupture exposes the contained debris within the thrombus to cellular environment. This shows a cross sectional view of a coronary artery of a patient who died of a myocardial infarction.

Source: Dr. Erling Falk, University of Aarhus, Aarhus, Denmark; (Hansson and Nilsson 2008)

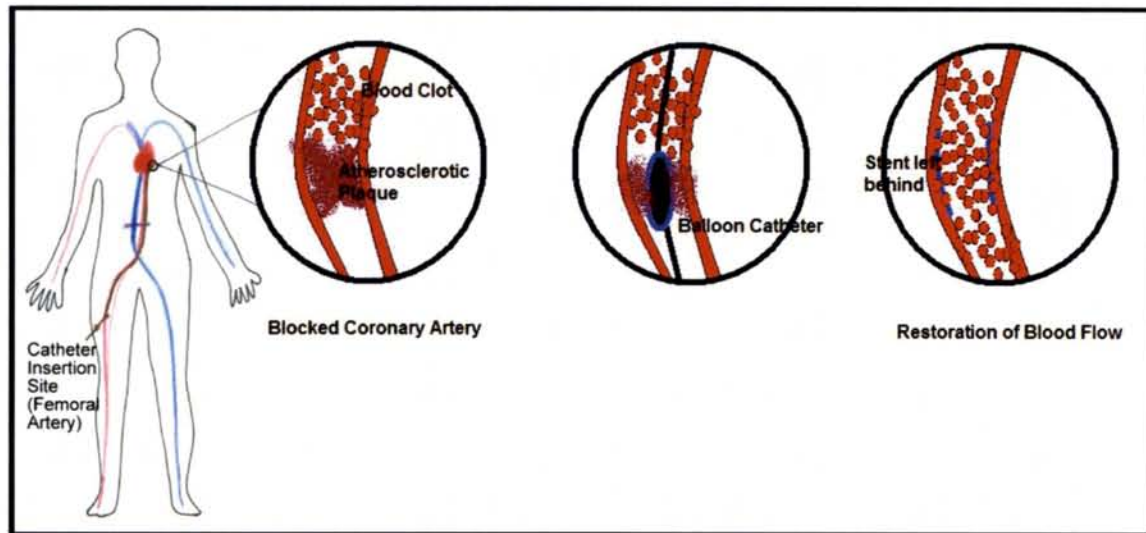
IV-Bii. Treatment

Various types of treatment for coronary artery disease have been utilized for decades. Cardiologists often choose to place patients on medications that reduce blood clot formations, such as Clopidogrel or Aspirin. If the condition worsens, then elective or emergent procedures must be carried out. There are two well known surgical procedures that are documented. Coronary-artery bypass grafting (CABG) is one such procedure. CABG is a procedure in which blood is rerouted around the blocked coronary artery by a transplanted piece of vessel, known as a graft. The CABG procedure was introduced in 1968. Through further advances in surgical techniques, CABG has grown to be one of the surgical standards for patients presenting with coronary artery disease (Serruys, Morice et al. 2009).

Another type of surgical procedure, PCI, was introduced in 1977 (Smith, Dove et al. 2001). It has been noted that an estimated 1.3 million PCI procedures were performed in the United States in 2006 (Association 2009). This procedure allows surgeons to place stents via catheterization at sites of thrombus formation. PCI procedures have enabled treatment of complex lesions in patients with advanced coronary artery disease, coexisting conditions, or anatomical risk factors (Smith, Dove et al. 2001). The main goal of a PCI is to open a clogged coronary artery and restore blood flow.

As depicted in Figure 12, the surgeon gains access to the heart and major blood vessels through a femoral artery in the groin or radial artery in the forearm. Once the artery is punctured using a special needle, x-ray technology allows a catheter to be threaded up into the aorta. The needle is then finally advanced to

Figure 12. Percutaneous Coronary Intervention Procedure



This illustration shows how a catheter is inserted into the femoral artery and then threaded up to the site of vascular injury. After the insertion of a stent via balloon angioplasty, restoration of blood flow occurs.

the sight of thrombus formation. Once the catheter reaches its destination, a balloon is inflated to open up the artery. Often times, a stent, which is a mesh-like metal tube, is left behind to serve as a scaffold and uphold the restoration of blood flow (Torpy, Lynm et al. 2004).

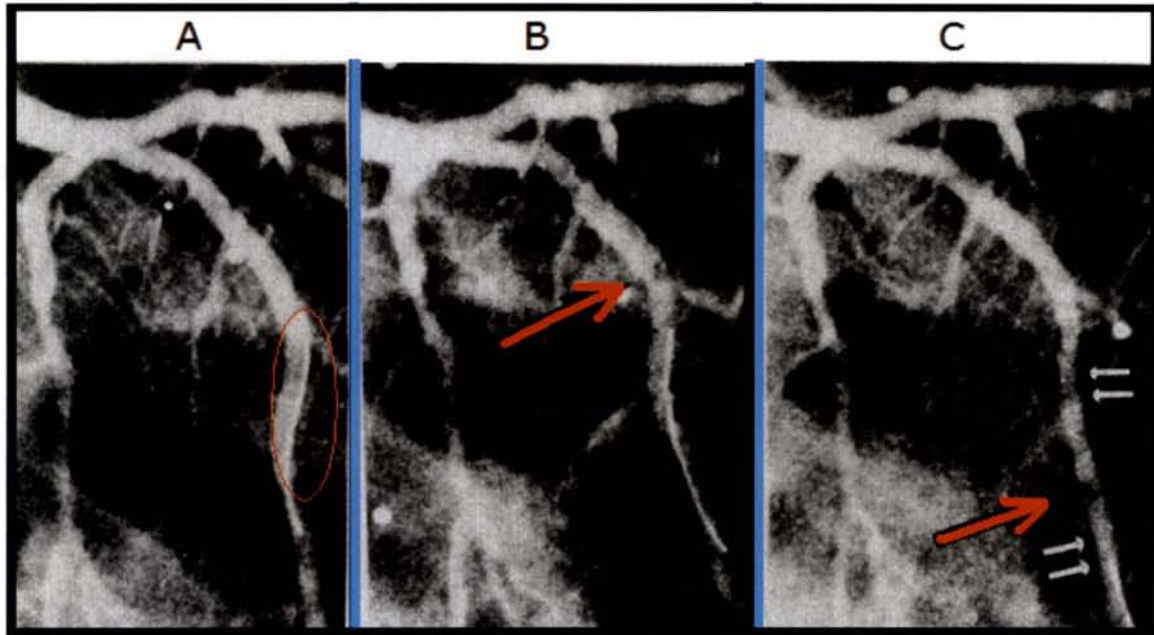
V-Biii. Complications in Treatment

A common complication arising during PCI procedures is excessive bleeding due to anti-coagulants. This problem needs to be dealt with immediately. It has been shown that increased bleeding can lead to increased mortality if correct remedies, such as blood transfusion, are not taken. The type of vascular access strategy (whether the femoral or radial artery is accessed) combined with which antithrombotic agent is used, does affect patient outcome. Combined studies are being performed to determine which methods result in the most beneficial outcome for patients (Doyle, Rihal et al. 2009).

Another complication that can arise after PCI is formation of intracoronary thrombus sites. Since a surgical procedure introduces foreign substances to a vessel wall, the damage causes the activation of the platelet aggregation pathway. As [Figure 13](#) depicts, these thrombus sites occur close to intracoronary guide wire and cause increased vasoconstriction. To combat these issues, anti-platelet agents are often used in conjunction with PCI (Zeicher, Schachinger et al. 1991).

Another complication that can arise after PCI is re-blockage of the treated blood vessel, known as restenosis. Restenosis usually occurs within six months of the PCI procedure. Restenosis occurs more often in patients who had a

Figure 13. Post Stent Placement Thrombus Formation



This angiogram shows vasoconstriction response due to thrombus formation post angioplasty. Part A shows the vessel post angioplasty, one can still see the guide wire introduced during the procedure (circled red). Part B shows the formation of a thrombus site along the side of the vessel wall, near the guide wire (denoted by the red arrow). Part C shows the vasoconstriction experienced post thrombus formation (white arrows).

Source: (Zeiher, Schachinger et al. 1991)

balloon angioplasty without stent placement. Stent placement has become the norm for angioplasty procedures. When there is restenosis post stent placement, it is referred to as in-stent restenosis. Normally, a patient grows healthy tissue inside and around the stent to create a smoother and more natural flow process for the blood. Approximately 25% of patients post stent placements have some form of scar tissue that forms around the stent. However, as Figure 14 shows, if thick scar tissue forms, the lining of the artery can become clogged and obstruct blood. If a patient presents no signs of in-stent restenosis within 12 months of a PCI procedure, the condition becomes rare. Prevention methods for restenosis are started the day of the surgery. Complications can be reduced if the surgeon places the stent appropriately via use of additional technology or expertise. Drugs and vitamins are being studied to determine their efficiency in reducing restenosis when used during PCI (Dangas and Kuepper 2002).

V. Strategies in Combating Complications from PCI Procedures

There are various classifications of drugs that serve to block receptors. The three major types are agonist, antagonist, and inverse agonist. A drug that is considered an agonist, binds to a receptor. In doing so, it alters the normal activity of the receptor. An inverse agonist serves to have an opposite effect as that of the agonist. An antagonist also binds to a receptor, but does not alter the normal activity of the receptor. An antagonist hinders the effects of agonists and inverse agonists. Receptors can be activated by their respective ligands (which serve as agonists) or by pharmaceutically produced agonists (Negus 2006).

Figure 14. In-stent Restenosis

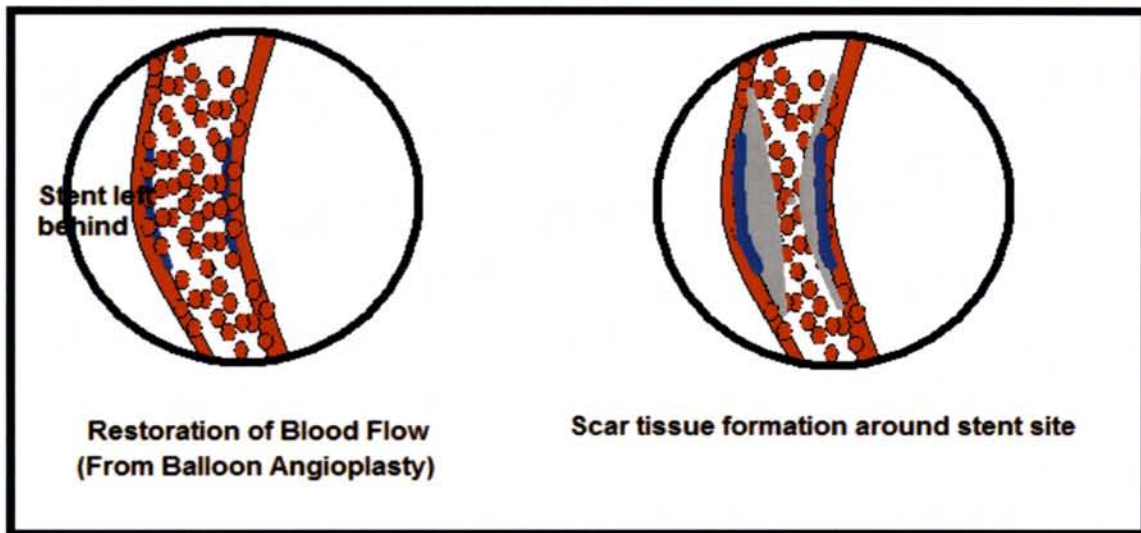
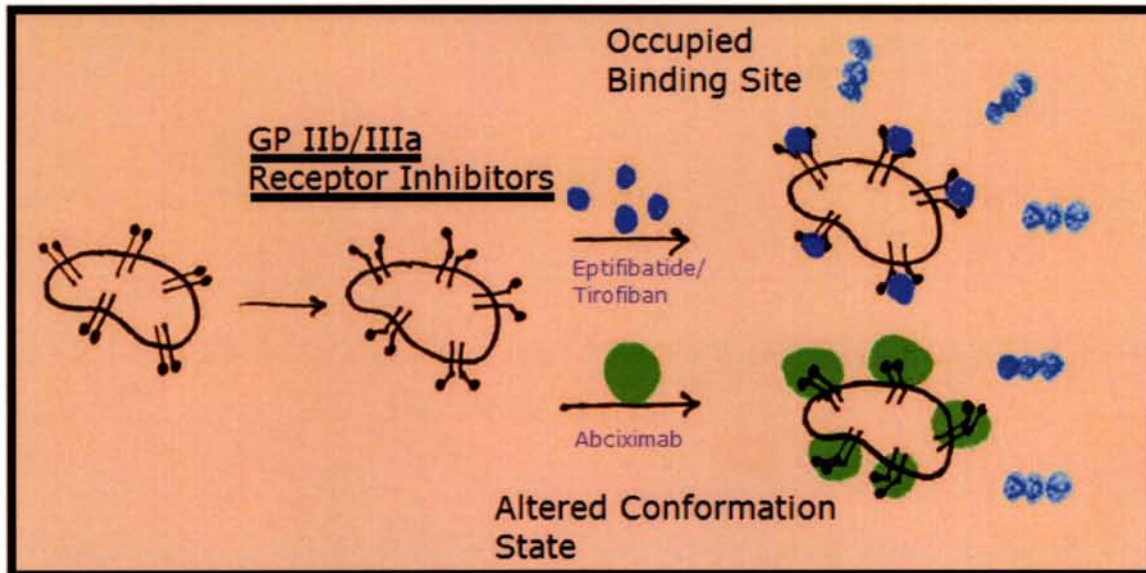


Figure 14 illustrates the formation of thick scar tissue post stent placement. This scarring blocks blood flow.

The actual site on the receptor that the drugs target is also a characteristic of the drug. Since cell surface receptors are heavily targeted by 60% of current drugs, understanding these interactions are crucial to develop effective drugs. There are two types of binding sites. One is an orthosteric site, which is the site where the actual ligand binds to the receptor. Traditionally, most drugs actively seek to bind to the orthosteric site. All types of drug types (agonist, antagonist, and inverse agonist) can recognize the domain. These drugs bind competitively and hinder the original ligand from binding to the receptor. Recent developments are showing that drugs can indeed bind at an allosteric binding site. This site is not the ligand binding site and can be located near or far from the original agonist binding site. The drugs that bind to allosteric sites can be competitive or non-competitive (Christopoulos 2002).

The Glycoprotein IIb/IIIa receptor inhibitors, Abciximab, Tirofiban, and Eptifibatide, act as antagonists to prevent fibrin from binding to the receptor. As Figure 15 shows, Abciximab binds to an allosteric site, altering the conformation of the receptor, thus preventing fibrin binding to the receptor. Eptifibatide and Tirofiban bind to the orthosteric site and directly inhibit fibrin from binding to the receptor. The remainder of this thesis presents the properties and mechanisms of the Glycoprotein IIb/IIIa receptor inhibitor drugs. Also, since Clopidogrel and Aspirin are important treatments for patients presenting with Cardiovascular Diseases, there is a brief discussion about the properties and mechanisms of the two drugs.

Figure 15. Role of Abciximab, Tirofiban, and Eptifibatide in the Platelet Aggregation Pathway



GP IIb/IIIa receptor antagonists serve to inhibit the binding of the GP IIb/IIIa receptor ligands by blocking or altering the active binding site on the receptor. Abciximab serves to alter the conformational state of the receptor. Tirofiban and Eptifibatide serve to occupy the binding site and directly inhibit ligand binding. This receptor blockade prevents ligands from binding to the GP IIb/IIIa receptors, thereby preventing platelet aggregation.

VI. Abciximab

Abciximab is sold under the trade name ReoPro. Reopro is manufactured by Centocor (a wholly own subsidiary of Johnson & Johnson) and marketed/distributed by Eli Lilly and Company. Reopro was approved by the Food and Drug Administration in 1994. In February of 1995 it was introduced to the United States as an anti-platelet drug that served as a Glycoprotein IIb/IIIa receptor inhibitor. Abciximab was introduced as a monoclonal antibody that has the ability to bind noncompetitively to GPIIb/IIIa receptors on platelets. Since the GPIIb/IIIa receptors normally bind fibrinogen, Abciximab binding to the receptor blocks the receptor-fibrinogen linkage and blocks the final pathway of platelet aggregation (1994; Tamhane and Gurm 2008).

VI-A. Monoclonal Antibody Technology

The development of monoclonal antibody technology enabled researchers to develop “magic bullets” that could target specific diseased states. These lab-made antibodies confer a protection against specific antigens. Antibodies are specific to one type of antigen, thus can offer great selectivity. These qualities are what make antibodies important in treating specific diseases (Peterson 2005).

Initial engineering of antibodies allowed researchers to direct antibodies against tumor cells of mice. Researchers were successful in inhibiting the growth of these tumors by specifically “attacking” the antigens that created the tumors. Next, it was essential to humanize these antibodies, so they could be used as treatment with humans. This was done by utilizing the mouse antibodies already

created and determining the similarities of the sequences between mouse and human antibodies. Using recombinant technology, researchers were able to create antibodies that would work within the human system (Peterson 2005).

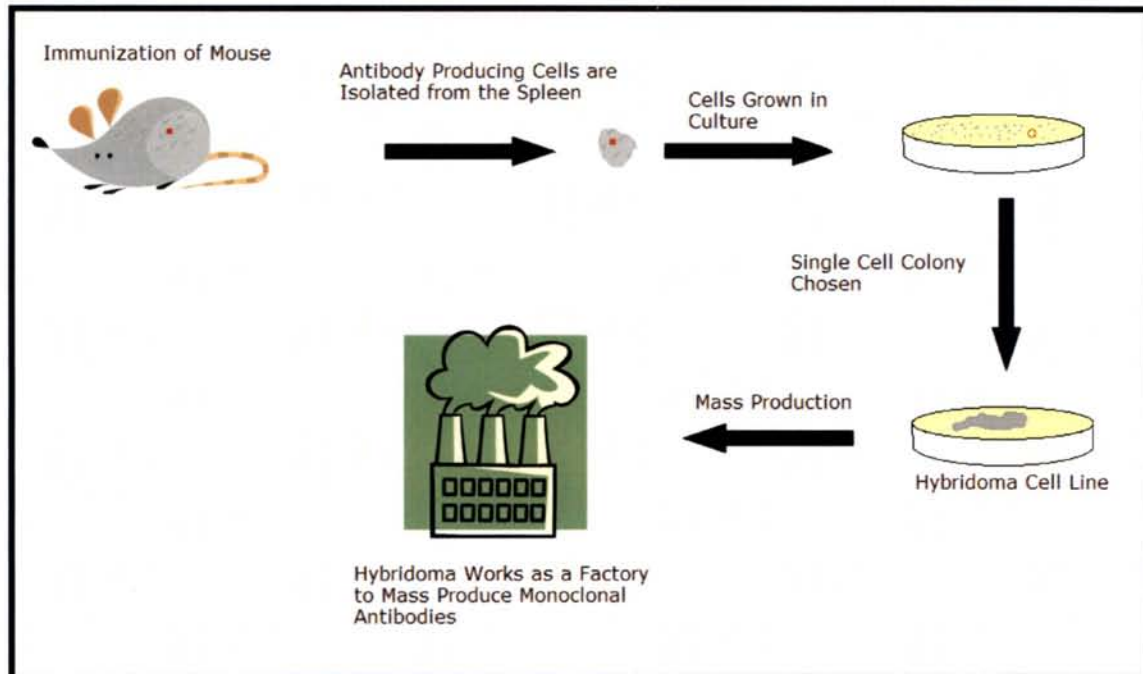
Once a particular type of antibody has been classified, a special cell line (hybridoma) is created that can produce the desired antibody. This insures that a monoclonal antibody is produced in a pure form (Peterson 2005). Figure 16 outlines monoclonal antibody production.

The production of Abciximab is carried out in a hybridoma cell. Abciximab is a part of the Fab fragment of the chimeric human-murine (mouse) monoclonal antibody 7E3. The Fab fragment is the portion of the antibody that recognizes antigens. Figure 17 outlines an antibody structure. Abciximab is produced by continuous perfusion in the cell culture. The fragment is then purified by a method of several steps that involve viral inactivation and removal procedures (2005).

VI-B. Uses

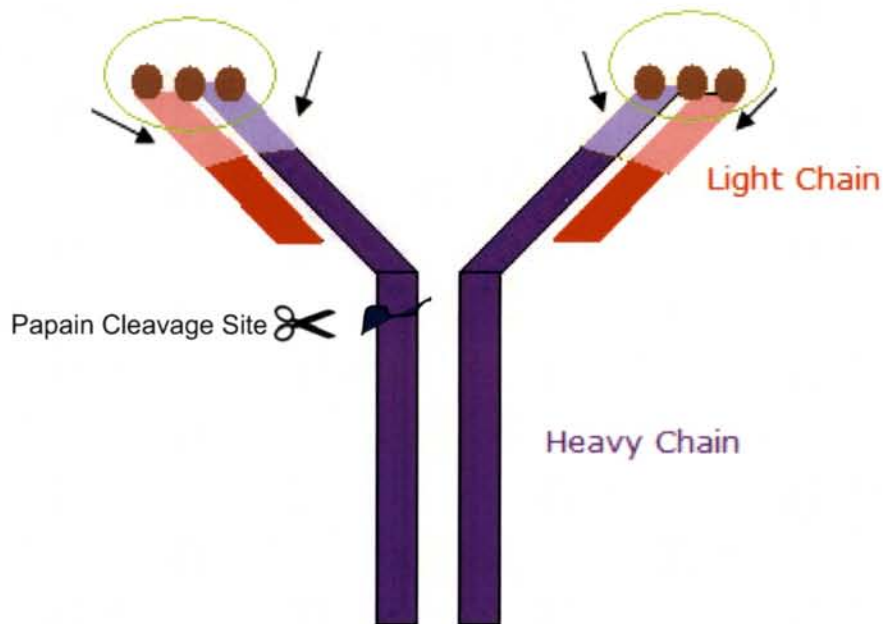
Abciximab is used in particular settings. Abciximab is the favored anti-platelet drug in high risk acute coronary syndrome patients who are under going a PCI procedure (Tamhane and Gurm 2008). Abciximab can be administered in several ways. Abciximab can be infused via intracoronary or intravenous means or it can be administered in a bolus form (all at once) along with other anticoagulant agents. In clinical settings, both models are used by using an initial bolus form and then infusion therapy concurrently with the procedure. Abciximab

Figure 16. Monoclonal Antibody Production



The figure shows the production of a murine model monoclonal antibody. First, a mouse is injected with a specific antigen, which causes the natural production of antibodies conferred against that antigen. The cells that are naturally producing the antibodies are isolated from the spleen and grown in culture. To create a pure antibody, a single cell colony is chosen and a hybridoma cell line is created. The hybridoma cell line can then mass produce the antibodies. Source: (Falkenberg 1998; Peterson 2005)

Figure 17. Antibody Structure



Antibodies are selective towards antigens on foreign particles and bacteria. As Figure 17 depicts, there several regions that make up an antibody. The dark purple and dark red regions of the heavy and light chains are similar in all antibody structures. The variable regions, in light purple and light red, are the regions which offer antigen selectivity. The antigen fragment binding sites (Fab sites) are located at the terminal end of the structure and are indicated by the green circles. The enzyme Papain digests the antibody at the site denoted by the scissors. This cleavage creates the Fab Fragment.

VII-D. Mechanism of Action

The mechanism of action for Tirofiban is different from that of Abciximab. Tirofiban serves as a highly selective antagonist for the GP IIb/IIIa receptor. Tirofiban treatment results in competitive inhibition of fibrinogen and the von Willebrand-mediated platelet aggregation on the GP IIb/IIIa receptor. Tirofiban binds to the receptor reversibly and occupies directly the binding site (the RGD recognition site, which serves as the binding site for fibrinogen/vitronectin and the von Willebrand factor). Tirofiban does not cross react with any other receptors (Winter and Juergens 2008).

The half life of Tirofiban in plasma is approximately 2 hours. Tirofiban has a shorter platelet bound half-life (10-15 seconds). Tirofiban has a lower binding affinity to the GP IIb/IIIa receptor and rapidly disassociates. Tirofiban has an equilibrium dissociation constant (K_d) of 15 nM. With a 3 fold higher K_d than Abciximab, Tirofiban binds with a lower affinity to the substrate than Abciximab. Within four hours after therapy has ended, Tirofiban is completely removed from the receptors. Tirofiban exhibits great platelet inhibitory functions. Tirofiban is 85-90% successful at inhibiting platelet aggregation by binding to the GP IIb/IIIa receptor (Winter and Juergens 2008).

VII-E. Side Effects

Adverse effects noted with Tirofiban usage are similar to the other platelet aggregation inhibitor drugs. Excess bleeding is noted as a concern and steps must be taken to prevent excessive blood loss. Unlike Abciximab, Tirofiban does not increase likelihood of Thrombocytopenia (as compared to a Placebo).

the receptor. Abciximab allows an anti-inflammatory response. Abciximab also offers long term vessel healing and stability (2005; Tamhane and Gurm 2008).

Abciximab binds to the activated Mac-1 receptor found on leukocytes. The Mac-1 receptor mediates leukocyte to platelet attachment. Abciximab serves a similar role in the Mac-1 receptor mechanism as it does with the vitronectin receptor. Abciximab blocks the Mac-1 receptor. Abciximab thereby disables the ability of the Mac-1 receptor to mediate leukocyte to platelet attachment (Tamhane and Gurm 2008).

VI-D. Clinical Trials

In order for the FDA to approve a particular drug, several clinical trials need to be completed. These trials need to test the drug in several settings versus a placebo. Abciximab has been tested in four Phase 3 clinical trials. Phase 3 clinical trials are performed on a large number of patients and used to see a definitive assessment on the effectiveness of the drug. The four trials of Abciximab were EPIC, EPILOG, CAPTURE and EPILOG Stent/EPISTENT. Table 2 summarizes the Phase 3 clinical trials, which evaluated the effect of Abciximab.

The EPIC trial conducted a controlled study in patients who had the percutaneous transluminal coronary angioplasty and were at high risk for an abrupt closure of the treated coronary vessel. There were three different groups: patients treated with Abciximab bolus plus infusion for 12 hours, patients treated with Abciximab bolus plus placebo infusion, and patients treated with placebo bolus plus placebo infusion. All groups of patients received standard heparin and

Table 2. Clinical Trials of Abciximab.

Trial	Patient Type	Methods Tested
EPIC	Patients undergoing percutaneous transluminal coronary angioplasty (PTCA) and were at risk for abrupt closure of the treated coronary vessel	1. Abciximab bolus plus (Abciximab) infusion for 12 hours; 2. Abciximab bolus plus placebo infusion for 12 hours; 3. Placebo bolus plus (placebo) infusion for 12 hours.
EPILOG	Broad population of patients undergoing PCI	1. Abciximab (both bolus and infusion) plus standard-dose heparin; 2. Abciximab (both bolus and infusion) plus low-dose heparin; 3. Placebo plus standard-dose heparin.
CAPTURE	Patients with unstable angina who have not responded well to conventional medical therapy; scheduled for PCI	1. Abciximab bolus + infusion for 18 to 24 hours before procedure; 2. Placebo bolus + infusion for 18 to 24 hours before procedure (Heparin infusion used in both)
EPILOG Stent/ EPISTENT	Broad population of patients undergoing PCI	1. Conventional PTCA with Abciximab + low dose Heparin; 2. Primary intracoronary stent placement with Abciximab + low dose heparin; 3. Primary intracoronary stent placement with Placebo + standard dose heparin

X. Aspirin

Aspirin is commonly used anti-platelet agent. Aspirin is a household drug used for a variety of ailments. The benefits of Aspirin in cardiovascular disease are important because of the ability of Aspirin to mediate platelet aggregation.

The mechanism of action of Aspirin is carried out by the ability of Aspirin to selectively and rapidly acetylate a serine residue in the in the cyclooxygenase active site for the enzyme prostaglandin-H synthase (COX) that causes irreversible inhibition. The COX enzyme is responsible for prostaglandin and thromboxane synthesis. Prostaglandin and thromboxane are involved in fat build up and clot formation. There are two isoforms of COX, known as COX-1 and COX-2. COX-1 is involved in the maintenance function of various tissues and is termed the “house-keeping” isoform. COX-2, unlike COX-1, is undetectable in normal tissues. COX-2 is referred to as the “inducible” isoform because it is expressed heavily in areas of inflammatory stimulus. By acetylating a serine residue, Aspirin is able to irreversibly inhibit COX-1 and is able to alter the enzymatic activity of COX-2. (Bjorklund, Wallander et al. 2009).

It has been found that long term aspirin treatment inhibits platelet aggregation by inhibiting the formation of a pro-aggregant prostanoid, thromboxane A₂. Thromboxane A₂ is formed in platelets through the stimulation of COX-1. By blocking formation of thromboxane A₂, Aspirin produces an inhibitory effect on clot formation and platelet aggregation. Since Aspirin is more selective for inhibiting COX-1 than COX-2, even low-dose Aspirin is effective in inhibiting platelet aggregation (Bjorklund, Wallander et al. 2009).

aspirin treatment. The results showed that with Abciximab bolus plus infusion treatment the primary endpoint (death, myocardial infarction, or urgent intervention) was reduced by 35% in the first 48 hours and lasting up to 30 days (1994; 2005).

The EPILOG trial conducted a controlled study in a broad group of patients undergoing PCI excluding patients with myocardial infarction and unstable angina who met the standards for the EPIC trial. There were three different groups: a patient group that received a placebo and standard heparin dose, a patient group that received Abciximab bolus plus infusion and standard heparin dose, and a patient group that received Abciximab bolus plus infusion and low doses of heparin. The results showed that the primary endpoint (death or myocardial infarction within 30 days) was observed less in patients undergoing Abciximab treatment. The primary endpoint was observed in 4.2% of patients undergoing Abciximab treatment with standard heparin; in 3.8% of patients undergoing Abciximab treatment with low dose heparin; and in 9.1 % of patients undergoing placebo treatment). The secondary end point (death, myocardial infarction, or repeat intervention within 6 months) was also observed less in patients undergoing Abciximab treatment. The secondary endpoint was observed in 22.3% of patients undergoing Abciximab treatment with standard heparin; in 22.8% of patients undergoing Abciximab treatment with low dose heparin; and in 25.8% undergoing placebo treatment. This study showed that Abciximab was more beneficial than a placebo treatment (1997**; 2005).

The EPISTENT trial did a control study in patients undergoing PCI. They evaluated three different treatment strategies. One group of patients underwent percutaneous transluminal coronary angioplasty (PTCA, no stent) with Abciximab plus low dose heparin treatment. Another group tested underwent primary intracoronary stent placement with Abciximab plus low dose heparin treatment. The third group underwent primary intracoronary stent placement with placebo and a standard dose of heparin treatment. The results showed that patients in both stent and non-stent studies who underwent Abciximab treatment had a reduction in primary endpoint results with relation to death, myocardial infarction or urgent intervention. Primary endpoint was observed in 5.3% of patients in the Abciximab/stent group and in 6.9% of patients in the Abciximab/PTCA group. Primary endpoint in the placebo/stent group was achieved in 10.8% of patients. The secondary end point (death, myocardial infarction, or repeat intervention within 6 months) was observed less in patients undergoing Abciximab treatment. Secondary endpoint was observed in 6.4% of patients in the Abciximab/stent group and in 9.2% of patients in the Abciximab/PTCA group. Secondary endpoint in the placebo/stent group was achieved in 12.1% of patients. This study showed that Abciximab treatment was more beneficial than a placebo treatment in both stent and non-stent utilized procedures (1998; 2005).

The CAPTURE trial conducted a controlled study in patients who presented with unstable angina that did not respond to conventional medical therapy. A PCI procedure had been planned but not performed. The trial studied cases in which the Abciximab administration started 18 to 24 hours prior to PCI

procedure and continued one hour post procedure. The results showed that the primary endpoint (death or myocardial infarction within 30 days) was observed less in patients undergoing Abciximab treatment. Primary endpoint was observed in 11.3% of patients undergoing Abciximab treatment and in 15.9% of patients undergoing placebo treatment. The secondary end point (death, myocardial infarction, or repeat intervention within 6 months) was not significantly different. Secondary endpoint was observed in 31% of patients undergoing Abciximab treatment and in 30.8% of patients undergoing placebo treatment. This study showed that Abciximab was beneficial than a placebo treatment at the initial stages in the CAPTURE trial. However, Abciximab treatment showed no difference within 6 months (1997; 2005). Though death was listed as a primary and secondary endpoint in most of the trials, high mortality rates were uncommon (2005).

VI-E. Side Effects

A majority of side effects witnessed by administration of Abciximab are related to its function as an anti-platelet agent. One of the main side effects is an increased risk of bleeding. The most common type of bleeding noticed is a gastrointestinal hemorrhage. A way to combat this issue is to use anticoagulant agents such as heparin alongside Abciximab treatment. Another rare, but very risky, side effect is thrombocytopenia, which results in dropping of blood platelet counts. Most of the observed side effects are mild and do not hinder the potential treatment. A treatment that transfuses platelets is adopted when the patient has developed Thrombocytopenia (2005).

In patients observed within EPIC, EPILOG, and CAPTURE trials, several minor but uncomfortable side effects were noticed. Adverse events that occurred in more than 10% of patients include hypertension (14.4% vs. 10.3% in placebo patients); nausea (13.6% vs. 11.5% in placebo patients; and back pain (17.6% vs. 13.7% in placebo patients) (2005).

VII. Tirofiban

Tirofiban, a Glycoprotein IIb/IIIa inhibitor, is sold under the trade name Aggrastat. Aggrastat is manufactured/distributed/marketed by Merck Sharp and Dohme. Tirofiban is considered a small-molecule inhibitor with a molecular weight of 495 Da. Tirofiban is a tyrosine derived, non-peptide molecule that has a high specificity to the GP IIb/IIIa receptor integrin (Winter and Juergens 2008).

VII-A. Small Molecule Inhibitors

Being able to control protein to protein interactions is important in pharmacology and in patient management. Antibody technology (as seen with Abciximab) is very useful. Antibodies provide specificity to the target and are stable in human serum. However, Antibodies are difficult to manufacture (since they rely on living systems to be produced). Antibodies are also not cell membrane permeable (because of the massive size). Small-molecule inhibitors are providing means to alter the course of existing protein to protein interactions. Once the targeted protein is understood, with both its structural and biophysical properties studied, manufacturing of a particular small-molecule inhibitor as a therapeutic agent becomes possible. Fortunately, the GP IIb/IIIa receptor is a

highly studied protein, enabling synthesis of small-molecule inhibitors of the GPIIb/IIIa receptor (Arkin and Wells 2004).

VII-B. Development

Development of Tirofiban is different from Abciximab since Tirofiban is a small molecule inhibitor. Tirofiban is derived from the RGD motif sequence that binds to the Glycoprotein IIb/IIIa receptor. This sequence is also present in fibrinogen, von Willebrand factor and vitronectin. Initially, production of Tirofiban was difficult due to enzymatic breakdown of peptide bonds within the molecule, which resulted in an unstable molecule. After further research, Tirofiban was synthesized using D-amino acids instead of α -amino acids. These conformational changes made the molecule stronger. The new molecule lacked the vulnerable peptide bonds enabling the molecule to stay in circulation longer (Winter and Juergens 2008).

VII-C. Uses

Tirofiban is used in highly specific clinical settings. Similarly to Abciximab, Tirofiban is used in patients with a ST-Segmented Elevation Myocardial Infarction (STEMI). STEMI is an acute obstruction in an epicardial artery. Tirofiban is also used in non-ST-segment elevation acute coronary syndromes. The surgical procedure, PCI, is used as a management protocol to restore function in patients who suffer from STEMI and non-ST segment elevation acute coronary syndromes. Tirofiban is used in conjunction with aspirin and heparin. It has been found that Tirofiban in a high-weight adjusted dose is most beneficial to patients (van 't Hof and Valgimigli 2009).

is also accompanied by a low-dose weight-adjusted heparin infusion (2005; Tamhane and Gurm 2008).

VI-C. Mechanism of Action

Abciximab is a large 47615 Dalton (48 kDa) molecule. Abciximab blocks access of ligands to the RGD-sequence binding site and secondary fibrinogen binding site on the GPIIb/IIIa receptor. Abciximab does not directly bind to the RGD binding site; however, it acts as a noncompetitive inhibitor of the GP IIb/IIIa receptor by binding to an allosteric site (Tamhane and Gurm 2008). Abciximab has an equilibrium dissociation constant (K_d) of 5 nM. A low K_d indicates that the molecule binds with high affinity to the substrate. This high affinity allows it to remain bound to the receptor with a half-life of 4 hours (for the platelet bound drug) and disassociates slowly. The plasma half life is 10-30 minutes (for unbound drug). The biological half life (amount of time the half of the drug is removed from the entire system) is 12-24 hours (Kam and Egan 2002). With appropriate dosage, Abciximab can achieve a greater than 80% inhibition of the GP IIb/IIIa receptor population (2005; Tamhane and Gurm 2008).

Unlike the other GP IIb/IIIa receptor inhibitors (Tirofiban and Eptifibatide), Abciximab can cross react with other receptor integrins not related to the platelet aggregation pathway (Winter and Juergens 2008). Abciximab binds to the vitronectin receptor $\alpha_v\beta_3$, which is located on the surface of endothelial and vascular smooth muscle cells. The vitronectin receptor has roles in smooth muscle migration and endothelial cell attachment to platelets. Abciximab blocks

VIII. Eptifibatide

Eptifibatide, a Glycoprotein IIb/IIIa inhibitor, is sold under the trade name Integrilin. Integrilin is manufactured/distributed by COR Therapeutics. Eptifibatide was produced after Abciximab. Similarly to Tirofiban, Eptifibatide was designed to reduce the unintended complications arising from Abciximab. The main goal of Eptifibatide synthesis was to produce an agent that could rapidly disassociate from platelet bound GP IIb/IIIa receptor. Eptifibatide is an agent that is reversible and lowers the risk of bleeding. Eptifibatide is used in similar PCI cases as Abciximab and Tirofiban (Scarborough 1999).

VIII-A. Development

The development of a novel GP IIb/IIIa inhibitor drug began with researching a wide range of snake viper venoms. These venoms contain compounds known as disintegrins. Disintegrins are small proteins that serve as antithrombotics and inhibit platelet aggregation. These small molecules contain the RGD amino acid sequence. However, it was noted that these RGD containing molecules also were able to cross react with other integrins (such as the $\alpha_v\beta_3$ integrin). After continual research, venom from the *Sistrurus barbouri* pigmy rattlesnake, known as barbourin, a 73- amino acid disintegrin, was found to contain a KGD (lysine- glycine- aspartate) sequence. This sequence allowed the small molecule to interact with the GP IIb/IIIa receptor but not other integrins. Barbourin provided a template for the construction of a synthetic peptide that could be used clinically. Eptifibatide, a heptapeptide (7 peptides) cyclized by a disulfide bridge, was created. Eptifibatide proves to be a potent, specific inhibitor

with high affinity for the GP IIb/IIIa receptor. Eptifibatide contains the modified KGD peptide recognition (Scarborough 1999).

VIII-B. Mechanism of Action

The mechanism of action of Eptifibatide is similar to Tirofiban. Eptifibatide acts as a small molecule inhibitor to block the binding of fibrinogen and the von Willebrand factor to the GP IIb/IIIa receptor on activated platelets. Effects of Eptifibatide on inhibition of platelet aggregation can be seen within 15 minutes of drug administration. Eptifibatide has a short half life and rate of elimination from circulation in plasma of 1-2 hours. Eptifibatide has an equilibrium dissociation constant (K_d) of 120 nM. With A significantly higher K_d than Abciximab, Eptifibatide binds with a much lower affinity to the substrate than Abciximab. Eptifibatide is able to act as a reversible agent (Scarborough 1999). The biological half-life of Eptifibatide is 2-4 hours (Kam and Egan 2002).

VIII-C. Side Effects

Adverse effects, such as bleeding, are reduced because of the reversibility and short platelet bound life of Eptifibatide. Also, thrombocytopenia is not observed in greater proportions in patients undergoing Eptifibatide treatment vs. Placebo treatment (Scarborough 1999).

IX. Clopidogrel

Clopidogrel is an oral agent with inhibitory properties of platelet aggregation. Clopidogrel is used in patients who present with cardiovascular risks and multiple co-morbid conditions such as stroke, myocardial infarction, atherosclerosis (and other forms of coronary artery disease), peripheral vascular

disease, and cerebrovascular diseases. Clopidogrel is manufactured and marketed by three pharmaceutical companies. Clopidogrel was developed by Sanofi Research teams in 1998. The well known trade name Plavix is marketed by Bristol-Myers Squibb and Sanofi-Aventis. The trade name Clopilet is marketed by Sun Pharmaceuticals. The trade name Ceruvin is marketed by Ranbaxy Laboratories (Mullangi and Srinivas 2009).

The mechanism of action of Clopidogrel is to selectively bind to the platelet surface on the adenylate cyclase coupled ADP receptors to block one of the starting pathways of platelet aggregation. Clopidogrel allows for an irreversible blockade of the ADP receptor, $P2Y_{12}$. The $P2Y_{12}$ receptor is important in platelet aggregation and in the cross linking of platelets by fibrin. The clopidogrel molecule blocking the $P2Y_{12}$ receptor inhibits the preliminary activation of the Glycoprotein IIb/IIIa pathway. In order for Clopidogrel to be activated, the drug requires catalyzation by the cytochrome P450 isozymes (Mullangi and Srinivas 2009).

Clopidogrel is used as preventive medicine. Clopidogrel is taken daily by at-risk patients who are taking remedies to reduce their risk of thrombus formation leading to blocked arteries. For patients who are taking Clopidogrel as a type of preventative treatment, the recommended dosage is 75 mg/day. The inhibitory effects can be noticed after 2 hours of initial treatment and can last 3-7 days after oral treatment. If the medication is taken continually then the effects are longer lasting (Mullangi and Srinivas 2009).

Patients who are at risk for cardiovascular disease are usually also at risk for gastrointestinal complications. Consideration needs to be made during administration to account for the gastrointestinal adverse effects that are well documented for Aspirin treatment. In efforts to mediate these complications, health care providers should develop a plan to reduce dosage and length of treatment. Health care providers should also avoid prescribing drugs with similar adverse effects (Bjorklund, Wallander et al. 2009).

XI. Drug Comparisons

When considering which drug therapy to use in patients undergoing PCI health care providers have a selection of GPI drugs that they can choose. Abciximab is a chimeric monoclonal antibody. Tirofiban is a nonpeptide tyrosine derivative. Eptifibatide is a cyclic heptapeptide. Each treatment varies in molecular structure, active half-life, plasma half-life, receptor binding affinity, method and duration of administration, and cost. Several studies have been conducted to determine end-point outcome of patients undergoing the above mentioned drugs. Table 3 outlines the differential properties of the three GPI drugs.

XI-Ai. Abciximab/Tirofiban – source: (Gowda, Vacek et al. 2003)

A study conducted at the Mid America Heart Institute in Kansas City, MO observed 228 patients (114 that received Abciximab and 114 that received Tirofiban) who underwent treatment for unstable angina or myocardial infarction between 1/98 and 12/99. Clinical parameters, such as equipment, techniques, and decision for revascularization, were made by the attending physician.

Table 3. Properties of GP IIb/IIIa Receptor Inhibitors

	Abciximab	Tirofiban	Eptifibatide
Molecule Type	Monoclonal Antibody; Fab fragment of Antibody	Small-molecule inhibitor; nonpeptide	Small-molecule inhibitor; peptide
Size	47615 Da	495 Da	832 Da
Platelet Bound Half life	4 hours	10-15 seconds	Similar to Tirofiban
Plasma Half- Life	10-15 minutes	2 hours	1-2 hours
Biological Half-Life	12-24 hours	2-4 hours	2-4 hours
Mechanism of receptor binding	Noncompetitive inhibition; binds to allosteric site; with rapid onset; high receptor affinity; slow disassociation	Competitive inhibition; reversibly binds to orthosteric site; rapid onset; low receptor affinity; rapid disassociation	Competitive inhibition; reversibly binds to orthosteric site; rapid onset; low receptor affinity; rapid disassociation
Equilibrium dissociation constant (K_d)	5 nM	15 nM	120 nM
Cost (Approximate)	\$1500/ 12 hour dose	\$500/ 24 hour dose	\$500/ 12 hour dose

In order to have a statistically valid test, it was pertinent to have non-significance in variation between the Abciximab and Tirofiban group in the baseline characteristics of: age, gender, ejection fraction. It was also pertinent to insure that the past medical histories of the patients were similar in relation to diabetes, congestive heart failure, hypertension, atrial fibrillation, cardiomyopathy, end stage renal disease, hyperlipidemia, occurrence of a cerebrovascular accident, occurrence of a transient ischemic attack, peptic ulcer disease, or occurrence of prior coronary artery bypass graft surgery/percutaneous transluminal coronary angioplasty. Smoking history, which was one baseline factor, was significantly different between the patients who where administered Tirofiban (34 smokers out of 114) and those that were administered Abciximab (21 smokers out of 114). Distribution of diseased vessels and which vessel was revascularized was similar in both groups. Table 4 shows which type of interventional treatment the patients underwent.

The results showed that no intracranial bleeds occurred in either group. Length of stay was also similar to both groups (5 days for Abciximab and 4.2 days for Tirofiban). Post procedure complications such as death, myocardial infarction, urgent revascularization, a cerebral vascular accident, transient ischemic attack, occurrence of thrombocytopenia, GI bleed, or need for immediate blood transfusion occurred at with a non-significant difference in both groups. Table 5 shows the immediate post procedure complications observed. There was no significance in difference between Tirofiban treatment and Abciximab treatments immediately post procedure.

Table 4. Type of Interventional Treatment in Mid America Heart Institute Study

Type of Treatment	Tirofiban (N=114)	Abciximab (N=114)
None	9.6%	2.6%
PTCA	1.8%	3.5%
PTCA with Stent	79.8%	92.1%
CABG	9.6%	2.6%

Table 5. Immediate Post Procedure Complications in Mid America Heart Institute Study

Immediate Complication	Tirofiban (N=114)	Abciximab (N=114)
Death	1.8%	1.8%
Myocardial Infarction	1.8%	0
Urgent Revascularization	4.4%	.9%
Cerebral Vascular Accident/ Transient Ischemic Attack	0	.9%
Access site bleed	18%	17%
Gastrointestinal Bleed	5.3%	2.6%
Required Blood Transfusion	1.8%	4.4%
Thrombocytopenia	.9%	1.8%

**p Value = Non-significant for all data points

The results from the one year follow up data showed an event-free survival (no death, myocardial infarction, or repeat-revascularization) trend favoring Tirofiban. There was a 68% event-free survival in patients with Tirofiban treatment. There was a 55% event-free survival in patients with Abciximab treatment. However, the p-value showed non-significance with the data points comparing Tirofiban and Abciximab in event-free survival one year post procedure. Table 6 shows several factors studied at the one-year follow up data.

The study also considered whether the intervention was performed in an elective or emergent manner. Emergent intervention was described as having a procedure performed immediately after a patient made a trip to the emergency department for an acute attack. All other patients were considered elective.

There was non-significance in the difference between which treatment (Tirofiban or Abciximab) was used between elective or emergent patients. The results showed that patients who were administered Abciximab in an emergent setting had a greater chance of no-repeat-revascularization than the patients who were administered Tirofiban. 100% of patients administered Abciximab were free from repeat-revascularization versus 81% of the Tirofiban group. The results showed that patients who were administered Tirofiban in an elective setting were offered a greater chance of no-repeat-revascularization than the patients who were administered Abciximab. 93% of patients administered Tirofiban were free from repeat-revascularization vs. 77% of the Abciximab group. The cost of each administration varies greatly. Abciximab is approximately \$1500 for a 12-hour dose and Tirofiban is approximately \$500 for a 24-hour dose.

Table 6. One Year Post Procedure Complications in Mid America Heart Institute Study

Post One year Complication	Tirofiban (N=114)	Abciximab (N=114)
Death	1.8%	3.6%
Myocardial Infarction	1.8%	0
Revascularization	9.6%	13%
In-stent Restenosis (at prior damage site)	4.3%	8.7%
Different Lesion	.9%	3.6%
Hospitalization – Cardiovascular Issues (p Value = .01)	8.7%	21.9%
Hospitalization- non Cardiovascular Issues	15.8%	7.9%
Composite of Death, Myocardial Infarction, or Cardiovascular Issues Hospitalization (p Value = .02)	12.2%	25.4%

**p Value = Non-significant for all data points (except those noted)

The study concluded that the outcome of each drug therapy was not significantly different. The authors of the Kansas City study believe that Tirofiban was preferred for initial administration for patients presenting with acute coronary syndromes. The authors believed that Abciximab was preferred by intervention cardiologists in catheterization laboratory use.

XI-Aii. Abciximab/Tirofiban – TARGET trial (Topol, Moliterno et al. 2001)

The TARGET trial was conducted in 149 hospitals in 18 countries with 4809 patients. The trial considered patients who were administered Tirofiban or Abciximab before undergoing PCI with stent treatment. The primary end point was death, nonfatal myocardial infarction, or urgent target-vessel revascularization within 30 days.

The results showed that the primary endpoint occurred more in the Tirofiban group (7.6% of the total 2398 patients experienced one of the primary end point events) than the Abciximab group (6% of the total 2411 patients experienced one of the primary end point events). However, Abciximab therapy resulted in higher minor bleeding episodes (4.3% vs. 1.8% with Tirofiban therapy, p value- significant at .05 confidence level at 95% significance level). Thus it was shown that Tirofiban offered less protection from major ischemic events than Abciximab did in a 30 day period.

XI-B. Abciximab/Eptifibatide– EVA-AMI Trial (Zeymer and Wienbergen 2007)

The EVA-AMI trial was conducted with 427 random STEMI patients who underwent PCI with Eptifibatide or Abciximab treatment. It was shown that eptifibatide was found to be non-inferior in comparison with Abciximab when

looking at the primary endpoint of complete ST resolution 1 hour after the PCI procedure. Also, no significance was found in differences in in-hospital mortality or major bleeding events.

XI-C. Abciximab/Eptifibatide/Tirofiban – (De Luca, Ucci et al. 2009)

A group of physicians from Italy conducted a meta analysis of the randomized trials. The physicians considered trials that compared Abciximab treatment against the small molecule inhibitors (Eptifibatide and Tirofiban) in primary angioplasty procedures conducted on patients who presented with ST-segment elevated myocardial infarction. This meta analysis aimed to study the outcomes following the differential treatments. They screened 2297 patient files from MEDLINE and CENTRAL (electronic databases) and in scientific journals (Circulation, Journal of the American College of Cardiology, European Heart Journal, and American Journal of Cardiology) listed from 01/99 to 10/08. They deemed the primary endpoint to be mortality within 30 days. They deemed the secondary endpoint to be reinfarction within 30 days, thrombolysis in myocardial infarction (TIMI) within 30 days (ability to restore flow to grade 3), or ST-segment resolution.

The study found 1082 patients undergoing Abciximab versus 1115 undergoing small molecule inhibitor treatment,. It was found that in analysis of both the primary and secondary end points, Abciximab offered similar results to the small molecule inhibitors. The results have been summarized from the study in Table 7. The study also did several statistical tests to validate the results from the different trials.

Table 7. Outcomes from Meta Analysis of Abciximab and Small Molecule Inhibitors (Tirofiban/Eptifibatide)

	Abciximab N= 1082	Tirofiban/Eptifibatide N= 1115
Primary Endpoint Mortality within 30 days	2.2%	2.0%
Secondary Endpoint Reinfarction	1.2%	1.2%
Secondary Endpoint TIMI	89.8%	89.1%
Secondary Endpoint ST-segment resolution	67.8%	68.2%
Major Bleeding Complications	1.3%	1.9%

XII. Discussion

In order to fully understand a physiological process, there are many biochemical and clinical aspects to be considered. This thesis presents the clinical portion of the hemostatic system by discussing cardiovascular diseases, specifically coronary artery diseases and how the potential treatments affect individuals. This thesis also discusses the biochemical interactions of the hemostatic system during platelet aggregation, coagulation, and fibrinolysis that ultimately cause the clinical manifestations.

When choosing the best course of treatment for a patient, a physician has several choices. As seen by the studies presented in this thesis, often times the choices vary only slightly in outcome. In April 2009, the author of this thesis conducted an interview with a local Knoxville physician, Dr. Stuart Bresee. Dr. Bresee serves as the Chief of Cardiology at the University of Tennessee Graduate School of Medicine. He is also on staff at the UT Medical Center University Cardiology group. As a Cardiologist, Dr. Bresee was able to offer tremendous amounts of clinical insight concerning the GP IIb/IIIa inhibitor drugs.

Dr. Bresee believed Abciximab to be a prime contender for one of the most effective drugs of its kind. He noted that nationally, Abciximab is used over other similar drugs. However, at his Knoxville practice, Eptifibatide is used more often due to cost. He stated that the clinical trials that he and his colleagues had studied indicated that Abciximab and Eptifibatide provoke similar results. Thus, to be more cost efficient, University Cardiology mainly uses Eptifibatide. The side effects of any of the anti-platelet drugs that Dr. Bresee encounters are minimal

and the risk of the patient potentially displaying signs of the side effects are not solely enough reason to prevent administration of the drugs.

XIII. Conclusion

According to the trials presented, it was noted that Abciximab does not provide superior treatment for patients undergoing percutaneous coronary intervention over its small molecule inhibitor counterparts, Tirofiban and Eptifibatide. Thus, physician preference and exposure to treatments highly influences which treatment type is actually used.

The study of pharmacology is continually evolving and producing novel substances that are more effective than their predecessors. The future of research in anti-platelet treatment relies on advanced findings, both clinical and biochemical. In order to combat some of the clinical side effects of the existing drugs, researchers are continually searching for novel GP IIb/IIIa receptor inhibitor drugs. Progress is being made towards finding effective inhibitors that have a lower affinity for the receptor. This lower affinity could result in reduced bleeding complications. Clinical trials that consider dosage and duration of treatment are providing valuable insight into finding the most effective treatment for patients.

Preventative measures to combat cardiovascular diseases need to be taken in order to solve the underlying problem. Risk factors such as smoking, hypertension, and obesity are modifiable. Groups such as the World Health Organization and the American Heart Association are educating citizens of the dangers of unhealthy lifestyles. However, these organizations should also take

part in the actual progress towards changing modifiable risk factors. Hopefully, with continual education and outlets for action, individuals will chose to modify their lifestyle.

Works Cited

- (1994). "Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. The EPIC Investigation." N Engl J Med **330**(14): 956-961.
- (1997). "Randomised placebo-controlled trial of abciximab before and during coronary intervention in refractory unstable angina: the CAPTURE Study." Lancet **349**(9063): 1429-1435.
- (1997**). "Platelet glycoprotein IIb/IIIa receptor blockade and low-dose heparin during percutaneous coronary revascularization. The EPILOG Investigators." N Engl J Med **336**(24): 1689-1696.
- (1998). "Randomised placebo-controlled and balloon-angioplasty-controlled trial to assess safety of coronary stenting with use of platelet glycoprotein-IIb/IIIa blockade." Lancet **352**(9122): 87-92.
- (2005). ReoPro: Drug Information Sheet, Centocor.
- Arkin, M. R. and J. A. Wells (2004). "Small-molecule inhibitors of protein-protein interactions: progressing towards the dream." Nat Rev Drug Discov **3**(4): 301-317.
- Association, A. H. (2009). Heart Disease and Stroke Statistics - 2009 Update. A. H. Association. Dallas, TX, American Heart Association.
- Bjorklund, L., M. A. Wallander, et al. (2009). "Aspirin in cardiology--benefits and risks." Int J Clin Pract **63**(3): 468-477.
- Christian, A. H., W. Rosamond, et al. (2007). "Nine-year trends and racial and ethnic disparities in women's awareness of heart disease and stroke: an American Heart Association national study." J Womens Health (Larchmt) **16**(1): 68-81.
- Christopoulos, A. (2002). "Allosteric binding sites on cell-surface receptors: novel targets for drug discovery." Nat Rev Drug Discov **1**(3): 198-210.
- Cini, N., A. Trabocchi, et al. (2009). "Morpholine-based RGD-cyclopentapeptides as alphavbeta3/alphavbeta5 integrin ligands: role of configuration towards receptor binding affinity." Bioorg Med Chem **17**(4): 1542-1549.
- Dangas, G. and F. Kuepper (2002). "Cardiology patient page. Restenosis: repeat narrowing of a coronary artery: prevention and treatment." Circulation **105**(22): 2586-2587.
- De Luca, G., G. Ucci, et al. (2009). "Benefits from small molecule administration as compared with abciximab among patients with ST-segment elevation myocardial infarction treated with primary angioplasty: a meta-analysis." J Am Coll Cardiol **53**(18): 1668-1673.
- Doyle, B. J., C. S. Rihal, et al. (2009). "Bleeding, blood transfusion, and increased mortality after percutaneous coronary intervention: implications for contemporary practice." J Am Coll Cardiol **53**(22): 2019-2027.
- Ekmekci, O. B. and H. Ekmekci (2006). "Vitronectin in atherosclerotic disease." Clin Chim Acta **368**(1-2): 77-83.
- Falkenberg, F. W. (1998). "Monoclonal antibody production: problems and solutions." Res Immunol **149**(6): 542-547.

- Gowda, M. S., J. L. Vacek, et al. (2003). "Differential benefits and outcomes of tirofiban vs abciximab for acute coronary syndromes in current clinical practice." Angiology **54**(2): 211-218.
- Hansson, G. K. and J. Nilsson (2008). "Introduction: atherosclerosis as inflammation: a controversial concept becomes accepted." J Intern Med **263**(5): 462-463.
- Horwitz, A. F. (1997). "Integrins and health." Sci Am **276**(5): 68-75.
- Jackson, S. P. (2007). "The growing complexity of platelet aggregation." Blood **109**(12): 5087-5095.
- Kam, P. C. and M. K. Egan (2002). "Platelet glycoprotein IIb/IIIa antagonists: pharmacology and clinical developments." Anesthesiology **96**(5): 1237-1249.
- Lau, T. L., C. Kim, et al. (2009). "The structure of the integrin α IIb β 3 transmembrane complex explains integrin transmembrane signalling." EMBO J **28**(9): 1351-1361.
- Lynn, G. W., W. T. Heller, et al. (2005). "A model for the three-dimensional structure of human plasma vitronectin from small-angle scattering measurements." Biochemistry **44**(2): 565-574.
- Mackman, N., R. E. Tilley, et al. (2007). "Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis." Arterioscler Thromb Vasc Biol **27**(8): 1687-1693.
- Mould, A. P. and M. J. Humphries (2004). "Cell biology: adhesion articulated." Nature **432**(7013): 27-28.
- Mullangi, R. and N. R. Srinivas (2009). "Clopidogrel: review of bioanalytical methods, pharmacokinetics/pharmacodynamics, and update on recent trends in drug-drug interaction studies." Biomed Chromatogr **23**(1): 26-41.
- Negus, S. S. (2006). "Some implications of receptor theory for in vivo assessment of agonists, antagonists and inverse agonists." Biochem Pharmacol **71**(12): 1663-1670.
- Organization, W. H. (2007). Cardiovascular Diseases Fact Sheet N317, World Health Organization.
- Peterson, C. B. (2009). NIH Grant.
- Peterson, N. C. (2005). "Advances in monoclonal antibody technology: genetic engineering of mice, cells, and immunoglobulins." ILAR J **46**(3): 314-319.
- Podor, T. J., S. Campbell, et al. (2002). "Incorporation of vitronectin into fibrin clots. Evidence for a binding interaction between vitronectin and gamma A/gamma' fibrinogen." J Biol Chem **277**(9): 7520-7528.
- Podor, T. J., S. G. Shaughnessy, et al. (2000). "New insights into the size and stoichiometry of the plasminogen activator inhibitor type-1.vitronectin complex." J Biol Chem **275**(33): 25402-25410.
- Preissner, K. T. and D. Seiffert (1998). "Role of vitronectin and its receptors in haemostasis and vascular remodeling." Thromb Res **89**(1): 1-21.
- Scarborough, R. M. (1999). "Development of eptifibatide." Am Heart J **138**(6 Pt 1): 1093-1104.
- Serruys, P. W., M. C. Morice, et al. (2009). "Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease." N Engl J Med **360**(10): 961-972.

- Smith, S. C., Jr., J. T. Dove, et al. (2001). "ACC/AHA guidelines for percutaneous coronary intervention (revision of the 1993 PTCA guidelines)-executive summary: a report of the American College of Cardiology/American Heart Association task force on practice guidelines (Committee to revise the 1993 guidelines for percutaneous transluminal coronary angioplasty) endorsed by the Society for Cardiac Angiography and Interventions." Circulation **103**(24): 3019-3041.
- Stassen, J. M., J. Arnout, et al. (2004). "The hemostatic system." Curr Med Chem **11**(17): 2245-2260.
- Stout, T. J., H. Graham, et al. (2000). "Structures of active and latent PAI-1: a possible stabilizing role for chloride ions." Biochemistry **39**(29): 8460-8469.
- Streiff, M. D., Chi Van (2001). *Coagulation Cascade Interactive Animation*. M. Linkinhoker., Johns Hopkins University.
- Takagi, J. (2004). "Structural basis for ligand recognition by RGD (Arg-Gly-Asp)-dependent integrins." Biochem Soc Trans **32**(Pt3): 403-406.
- Tamhane, U. U. and H. S. Gurm (2008). "The chimeric monoclonal antibody abciximab: a systematic review of its safety in contemporary practice." Expert Opin Drug Saf **7**(6): 809-819.
- Thygesen, K., J. S. Alpert, et al. (2007). "Universal definition of myocardial infarction." Circulation **116**(22): 2634-2653.
- Topol, E. J., D. J. Moliterno, et al. (2001). "Comparison of two platelet glycoprotein IIb/IIIa inhibitors, tirofiban and abciximab, for the prevention of ischemic events with percutaneous coronary revascularization." N Engl J Med **344**(25): 1888-1894.
- Torpy, J. M., C. Lynm, et al. (2004). "JAMA patient page. Percutaneous coronary intervention." JAMA **291**(6): 778.
- Urbahns, K., M. Harter, et al. (2002). "Biphenyls as potent vitronectin receptor antagonists." Bioorg Med Chem Lett **12**(2): 205-208.
- van 't Hof, A. W. and M. Valgimigli (2009). "Defining the role of platelet glycoprotein receptor inhibitors in STEMI: focus on tirofiban." Drugs **69**(1): 85-100.
- Winter, J. P. and C. P. Juergens (2008). "The role of tirofiban in the management of coronary artery disease." Cardiovasc Hematol Disord Drug Targets **8**(2): 138-146.
- Xiong, J. P., T. Stehle, et al. (2001). "Crystal structure of the extracellular segment of integrin alpha Vbeta3." Science **294**(5541): 339-345.
- Zeiher, A. M., V. Schachinger, et al. (1991). "Intracoronary thrombus formation causes focal vasoconstriction of epicardial arteries in patients with coronary artery disease." Circulation **83**(5): 1519-1525.
- Zeymer, U. and H. Wienbergen (2007). "A review of clinical trials with eptifibatide in cardiology." Cardiovasc Drug Rev **25**(4): 301-315.
- Zhu, J., B. H. Luo, et al. (2008). "Structure of a complete integrin ectodomain in a physiologic resting state and activation and deactivation by applied forces." Mol Cell **32**(6): 849-861.