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CURRENT STATUS OF GLYCOPROTEIN IIB/IIIA RECEPTOR ANTAGONISTS AND EVALUATION OF THEIR MOLECULAR MODELING

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ABSTRACT: Abciximab, Eptifibatide and Tirofiban are the three main glycoprotein IIb/IIIa receptor antagonists which have played a vital role in the field of cardiac medicine. They work by blocking the final mechanism of platelet aggregation pathway. These antagonists are widely used in treating acute coronary syndrome, myocardial infarction and during percutaneous coronary intervention (PCI). In this review, we have examined the chemistry, mechanism of action and clinical uses of these glycoprotein IIb/IIIa receptor antagonists. We also tried to study the binding mode of both eptifibatide and tirofiban with the glycoprotein IIb/IIIa receptors using molecular docking software. It appears that blocking the ASP 224 may be the cause for platelet activity inhibition.

Key words: Glycoprotein IIb/IIIa; abciximab; eptifibatide; tirofiban; cardiovascular; docking.

INTRODUCTION

In a normal human system, following an injury to the blood vessel, platelets come into action by playing various roles such as platelet activation, platelet adhesion and platelet aggregation. Initially, platelets adhere to exposed sub-endothelium by the presence of von Willebrand Factor and glycoprotein receptors (IIb/IIIa and Ib/IX). This is followed by the recruitment of additional platelets to form clumps (aggregation). This leads to binding of fibrinogen to glycoprotein IIb/IIIa receptors during platelet activation and also releases adenosine diphosphate (ADP) and serotonin from their dense granules. As a final process, thrombin generation and clot formation leads to restoration of hemostasis or thrombosis (1, 2).

The therapeutic targeting of platelets is recognized as effective in the prevention and treatment of cardiovascular disease (3). Cardiovascular diseases are considered as the major cause of death in both developed and developing countries. In United Kingdom, it was estimated that the cardiovascular diseases, predominantly myocardial infarction (MI), ischaemic stroke and peripheral vascular disease, contributes to about one in three deaths in humans.

When unstable lesions are ruptured, the release of prothrombotic factors like oxidized lipids, and exposure of collagen triggers thrombosis. Thrombosis is the formation of blood clot within the blood vessel which results in occlusion of blood flow. But this normal mechanism has turned out to be a major problem leading to myocardial infarction (MI) and stroke. Thus the aetiologies of cardiovascular disorders are very complex and controversial (4).

Glycoprotein IIb/IIIa receptor antagonists

Integrins are the most abundant family of adhesive proteins which comprises mainly of glycoprotein IIb/IIIa receptors, fibronectin and vitronectin receptors. These integrins are mostly made up of heterodimers like α and β subunits (5). GP IIb/IIIa is an important member of this family of integrins which is comprised of αIIβ and β3 units, specific for platelets. It is estimated that about 50,000 to 80,000 GP IIb/IIIa receptors are present on the surface of each platelet. Thus these receptors are found to play a critical role in thrombosis (6). Glycoprotein (GP) IIb/IIIa receptor antagonists are antiplatelet drugs that reduce thrombus formation by blocking the key binding sites which are necessary in stabilizing the platelet aggregation pathway. As GP IIb/IIIa is platelet-specific, inhibition of this receptor does not affect platelet adhesion. This may lead hemostasis without any ischemic damage. This has established its broad use in clinical settings. These GP IIb/IIIa receptor antagonists have decreased the death rates in patients with acute coronary syndrome and those undergoing percutaneous coronary intervention as recorded by clinical trials (7, 8). It is reported that these inhibitors not only suppress platelet activation, but primarily acts outside the platelet by competing with the ligand binding which is important for platelet bridging and aggregate formation (9). Generally GPRAs are classified as (i) a chimeric Fab fraction of an antibody - Abciximab (ReoPro), (ii) peptide ligand - (Eptifibatide) and (iii) non peptide ligand - Tirofiban (Aggrastat) (10). Presently, these three drugs are available for intravenous use and have emerged as frontrunners in the treatment of vaso-occulusive disorders and as an adjunct in percutaneous coronary intervention. Abciximab, a monoclonal antibody is found more expensive than eptifibatide (11). Both eptifibatide and tirofiban are considered as the small molecular mass inhibitors (12). Non-peptide inhibitors are available both in parenteral and oral form. These glycoprotein IIb/IIIa receptor antagonists (GPRAs) work by the inhibiting the final mechanism of platelet aggregation pathway. It inhibits fibrinogen binding to the platelet surface receptor complex called glycoprotein IIb/IIIa receptors (GPR) which is located on the activated platelet surface. This decreases the cross-linking action of platelets, thereby inhibiting platelet aggregation (13).

Monoclonal antibody

Abciximab

1.1.1. Introduction

Abciximab (Reopro®, Centocor, Malvern, Pa) was the first glycoprotein IIb/IIIa receptor antagonist used in coronary angioplasty (14). The FDA has approved and licensed it as an adjunct to heparin and aspirin for use in patients with unstable angina and for those who do not respond to standard therapy when PCI is planned within 24 hours (15). Abciximab differs from tirofiban and eptifibatide in their chemical structure, binding site and pharmacokinetics.

1.1.2. Chemistry and mechanism of action

This human-murine chimeric monoclonal antibody fragment (c7E3 Fab), acts by binding non-specifically to the glycoprotein IIb/IIIa receptor [integrin α_{IIb} β_3] and it is also found to block the vitronectin receptor [integrin α (v) β_3] thereby inhibiting the thrombus (blood clot) formation. The function of integrin α_{IIb} β_3 is to mediate platelet binding, and, that of integrin α (v) β_3 is to mediate cell-to-matrix binding, cell migration and proliferation (16, 17, 18, 19). This large molecule with a molecular weight of 47.6 KD (KiloDalton) is found to have a low dissociation constant. Abciximab has a plasma half-life of about 10 minutes, and a second phase half-life of about 30 minutes. It has a strong binding affinity for glycoprotein IIb/IIIa receptors (20) and a low dissociation constant. Due to this, the speed of reversibility is found to be slow (>48 hours) due to prolonged binding to platelets. Abciximab has been known to circulate on platelets for up to 2 weeks (21).

1.1.3. Method of production

Abciximab marketed as ReoPro is the Fab section of the monoclonal antibody c7E3. An antibody is made up of two sections, Fc and Fab regions. ReoPro was derived from a chimeric antibody implying that it contains both murine variable region as well as a human constant region. This imparts properties such as low immunogenicity and a longer serum half life. This decreased the frequency of dosing. The production of abciximab is as follows: Mice are initially injected with 5×10^6 hybridoma cells. The hybridoma cells are obtained by the fusion of mouse cells with human myeloma cells. Ascites are then harvested 14-30 days after post inoculation. Antibody containing fluid is centrifuged and filtered. Ion exchange chromatography is carried out twice, followed by affinity chromatography to purify the antibodies. Pepsin is used in digesting the antibody and produced Fab and Fc fragment. Cysteine is then used to reduce the Fab fragment. Gel filtration was then carried out for final purification. Excipients such as sucrose were added and the finished product was lyophilized (22).

1.1.4. Major contraindications

One of the main adverse effects is the increased risk of bleeding, commonly, gastrointestinal haemorrhage because of its anti-platelet effects. The GUSTO IV- ACS trial revealed that patients receiving a 48 hour infusion were more prone to bleeding. The major predictors for such an event included use of low molecular weight heparin, duration of abciximab infusion, performance of CABG or PCI, advanced age and female sex. However, usage was found to be safe in subjects with non-ST-elevation acute coronary syndromes as stroke and major bleeding are rare with most events being clinically manageable or with few clinical consequences (23). It is also reported that profound thrombocytopenia occurred 7 days after the use of abciximab in the case of PCI. Recovery of the patient was uneventful after platelet infusion (24).

1.1.5. Side effects

Studies revealed that contrast nephropathy and gastrointestinal bleeding is common in patients treated with Abciximab. However, this is not significant but this difference was not significant after risk adjustment or after adjusting for the propensity to receive the drug (25). A major adverse side effect of abciximab is thrombocytopenia which along with hemorrhage has been classified into four progressive groups depending on the reduction in the platelet count (26). A platelet count below 20,000/μ L is indicative of severe acute thrombocytopenia, its incidence ranging from 2.4% to 4%, according to the series. A possible mechanism involves the interaction of specific monoclonal glycoprotein IIb/IIIa antibodies or IgG antibodies against the murine sequences of activated platelets after a second administration of the drug (27, 28). Platelet transfusion and cessation of abciximab along with all other drugs with potential to cause bleeding has reduced and lessened hemorrhagic complications. Platelet count and the hematocrit is monitored until the resolution phase of upto one week (29).

1.1.6. Applications in cardiac medicine

Abciximab is used for its antiplatelet activity during PCI. Studies have revealed that on administration of abciximab, corrected TIMI frame count (CTFC) decreases significantly (30). Studies also showed that an abciximab bolus within 1 h before PCI along with a 12-h infusion reduces mortality with a hazard ratio (HR) of 0.71 compared with standard treatment without abciximab ($p = 0.003$). The absolute reduction in mortality was estimated to be 0.5% through 30 days, 0.7% through six months, 0.9% through one year and 1.8% through three years. Early MI explained 18% of the observed mortality benefit at one year. Abciximab reduces mortality related to early events as well as those undergoing PCI (31).

1.1.7. Other applications in medicine

a. A double blind, placebo controlled study was done among 70 individuals presenting 24 hours after ischemic stroke. Patients received escalating dosages of abciximab or placebo. They underwent a scheduled head CT scan as follow-up and were evaluated further for three months. The minimal residue disability was comparatively lesser in patients treated with abciximab (32).

b. Abciximab has been used to prevent rethrombosis in basilar artery after transluminal angioplasty. It has also been used as an adjunctive therapy for vertebrobasilar angioplasty. It has also been used in the rescue therapy for treatment of acute parent vessel thrombosis during Guglielmi detachable coil placement for intracranial aneurysm embolization (33).

c. Abciximab has been used in treatment acute carotid stent thrombosis. On identification of occlusion of an internal carotid artery after stent placement, IV abciximab was administered which resulted in partial resolution of the thrombus at 10 minutes and complete resolution at 20 minutes. The infusion of abciximab was maintained for 12 hours. This indicates that Abciximab has the potential to reduce complications in carotid angioplasty and stenting (34).

2. Non-peptide inhibitors - parenteral use

2.1. Tirofiban

2.1.1. Introduction

Tirofiban, N-(n-butanesulfonyl)-O-[4-(butane-4-piperidinyl)]-tyrosine hydrochloride is a non peptide drug used in treatment of vaso-occlusive disorders such as unstable angina pectoris and myocardial infarction. Tirofiban is designed for intravenous administration. Patients with ACS treated with aspirin and tirofiban without PCI had better outcomes to patients treated with UFH (Un-fractionated heparin) alone. Sole medical therapy with tirofiban appears to be inadequate. Renal failure prolongs the half-life and continues inhibition of platelet aggregation refractory to transfusions of platelets. The only option to prevent excessive hemorrhage in this condition is by extracorporeal elimination (35, 36).

2.1.2. Chemistry and mode of action

Tirofiban (Aggrastat®) is a small non-peptide antagonist belonging to the group of GP IIb/IIIa receptor. It works by inhibiting platelet aggregation by competitively binding to the glycoprotein GPIIb/IIIa receptor on the surface of activated platelets thus preventing the binding of fibrinogen. Tirofiban is found to specifically inhibit fibrinogen-dependent platelet aggregation and thereby prolongs the bleeding time of patients with acute coronary syndromes. It is short-acting and consequently both bleeding time and platelet aggregation returns back to its normal within 3–8 hours after discontinuing tirofiban infusion (36). The drug's elimination half-life was approximately about 2 hours. It is approved by FDA to be used as an adjunct to heparin and aspirin for treating patients with acute coronary syndrome and coronary angioplasty.

2.1.3. Major side effects and contraindications

Tirofiban is a well tolerated glycoprotein IIb/IIIa antagonist. The Sant'ANna Tirofiban Safety study (SANTISS) assessed the combination of bleeding and access site in hospital complications (primary end-point) in patients undergoing percutaneous coronary intervention (PCI). Patients on oral single anti-aggregating drug (AAD) who received, just prior to PCI, high-dose tirofiban and a second oral antiplatelet agent with those who were already on an oral double AAD regimen and did not receive tirofiban. Tirofiban did not increase risks of complications (37). However, a 50% dosage reduction is required if creatinine clearance is <30ml/min and is contraindicated if serum creatinine is >2.5mg/dl (38).

2.1.4. Applications in cardiac medicine

a. Clinical trials have reported that, when tirofiban is used in combination with heparin and aspirin, it reduced the risk of ischaemic complications in patients with unstable angina/non-Q-wave myocardial infarction (MI) and also in high-risk patients undergoing percutaneous revascularization. The intracoronary mode of administration has been found to be superior to the intravenous route in patients undergoing PCI and experienced better outcomes in the former (39).

b. Treatment with tirofiban reduces concentration of white blood choline (WBCHO), a marker reflective of coronary plaque instability and platelet activation (40). It had improved outcome in patients with acute coronary syndrome. Patients have safely undergone CABG after treatment with tirofiban hydrochloride. It had no adverse clinical effects but showed only minor post-operative bleeding (41).

c. Direct PCI is the generally accepted strategy used in acute ST-segment myocardial infarction. A pilot trial carried out with combination fibrinolysis showed positive outcome. A larger trial was carried out with 151 patients on combination fibrinolysis of alteplase and tirofiban *versus* 162 patients on tirofiban before invasive approach including PCI. A TIMI 2 or 3 flow in the infarct-related vessel could be demonstrated in 87% of this combination fibrinolysis group, indicating the efficacy of tirofiban when used in combination with altepase (42).

2.1.5. Other applications in medicine

a. During endovascular embolization of intracranial aneurysm, one of the serious problem associated with it is thromboembolism. An intra-arterial infusion of tirofiban is found to be a very safe and effective method to encounter this without any subsequent hemorrhagic problems (43).

b. After the initial success in patients with acute coronary syndrome, glycoprotein IIb/IIIa receptor antagonists showed promising results for acute ischemic stroke (AIS) which is one of the major causes of morbidity worldwide. Tirofiban appears to be safe and effective in initial trials but further trials should be done (44).

c. Squamous cell carcinomas account for over 90% of oral cancers. A study was done to explore the association of cell adhesion domain Col15 of collagen XVII with malignant migration seen in squamous cell carcinoma of the tongue. In the invasive areas of epithelial tumors, collagen XVII and its ligands are upregulated. Results indicated that migration promoting effects of Col15 were mediated by $\alpha5\beta1$ and $\alpha\gamma$ integrins. A possible explanation could be that SCC and melanoma cells, express the platelet-specific $\alpha IIbIIIa$ integrin in order to bind to collagen XVII. A 2.5 µg/ml concentration of tirofiban was used to carry out cell transmigration assay. Tirofiban blocked Col15 induced transmigration by 35% ($P \leq 0.05$), hence displaying the potential in anti- cancer therapeutics (45).

2.2. Lamifiban

It is a non-peptide glycoprotein IIb/IIIa inhibitor with a half-life of about 2-2.5 hours. A bolus dose of 300 µg is given followed by 1µg/min infusion over 72-120 hrs for ACS. The outcome of PARAGON trial using low dose lamifiban and heparin was positive with a significant reduction seen in MI and death (16). It is reported that usage of lamifiban reduces adverse ischemic events by 6 months in the case of unstable angina and non–Q-wave myocardial infarction (46).

3. Oral Non-peptide glycoprotein IIb/IIIa inhibitors

3.1. Xemilofiban

Oral glycoprotein inhibitors have the advantages of easier administration and compliance. Their efficacy was tested with clinical trials. A double-blind trial was carried out with 7232 patients who received oral xemilofiban or placebo 30 to 90 minutes before undergoing percutaneous coronary revascularization. The two primary composite end points were death, nonfatal myocardial infarction, or urgent revascularization at 182 days, and the death or nonfatal myocardial infarction at 182 days. The incidence of death or myocardial infarction was similar in all three groups with no significant reduction in the end points. Additionally, bleeding was increased in the patients receiving xemilofiban on a dose dependant basis (47).

3.2. Orbofiban

Orbofiban is an oral ethyl ester prodrug which blocks the binding of fibrinogen to the platelet GP IIb/IIIa receptor. The terminal elimination half-life of this active drug was 16 to 18 hours and excreted renally. It has higher affinity for the activated receptors which allows it to interfere with the agonists thus reducing platelet aggregation. Since the activity of platelets remains long after the patient is stabilized clinically, OPUS-TEMI trial was carried out to ascertain the benefits. It was demonstrated that patients who underwent percutaneous coronary intervention showed good benefit because of the low mortality rate when compared to those patients with acute coronary syndrome (48).

3.3. Sibrafiban

A trial was carried out to test the efficacy of the oral glycoprotein IIb/IIIa inhibitor. sibrafiban over aspirin. 9233 patients stabilized after an ACS event was randomly assigned aspirin or low-dose or high-dose sibrafiban. The primary endpoint was the composite of death, non-fatal infarction or reinfarction, or severe recurrent ischemia at 90 days. As in the former case no additional benefits were seen for secondary prevention of ischemic events and a dose related bleeding was observed (49).

3.4. Lefradafiban

Lefradafiban is the orally active prodrug of Fradafiban, a glycoprotein IIb/IIIa receptor antagonist which works specifically by inhibiting platelet release reaction (50). This prodrug is used in patients with stable angina undergoing angioplasty. A study was done to determine the dose required to block 80% of glycoprotein IIb/IIIa receptors. Percentage of fibrinogen receptor occupancy (FRO), ex vivo platelet aggregation, and plasma concentrations of fradafiban were the efficacy indices. The primary end point was the occurrence of bleeding, classified as major, minor, or insignificant according to the TIMI criteria. The results showed that bleeding increased in a dose dependant manner. It was thus concluded that a short course of lefradafiban could be used safely in doses up to 45 mg three times daily achieved stable FRO values of more than 80% in patients with stable coronary artery disease undergoing elective PTCA (51).

3.5. Roxifiban

Roxifiban (DMP754) is probably the most promising glycoprotein IIb/IIIa receptor antagonist in having completed a Phase II clinical trial in acute coronary syndromes. It is an ester pro-drug that hydrolyzes on oral administration to the active glycoprotein (GP) IIb/IIIa antagonist, XV459. A study was carried out to investigate the safety, tolerability, pharmacokinetics, and the time course of the pharmacologic response in escalating doses of roxifiban. Potent inhibition of platelet aggregation persisted over the entire dosing interval. Overall, once-daily oral administration of roxifiban was fairly well tolerated and provided sustained systemic drug exposure and pharmacologic response over the entire administration interval (52).

In a second trial, the safety, efficacy and tolerability of roxifiban was tested in patients with chronic stable angina pectoris. Pharmacodynamic response of roxifiban was dose-dependent and a consequently higher incidence of minor bleeding events. No serious adverse events, including significant major bleeding events, were associated with roxifiban treatment. Minor bleeding was reported in 5% of participants in the placebo group versus 26% in the study group. Incidence of minor bleeding associated with roxifiban was significantly greater than that with placebo. Adverse events, including gastrointestinal disorders, platelet and clotting disorders, and urinary tract disorders, were observed in 1 of 21 cases (5%) in the placebo group versus 12% in those receiving roxifiban. Reversible thrombocytopenia without other complications developed in two patients. This indicates that roxifiban is a well tolerated GP IIb/IIIa inhibitor that is clinically well tolerated in the treatment of stable angina pectoris. However, larger trials are required before wide spread use is established (53).

4. Peptide inhibitors

4.1. Eptifibatide

4.1.1. Introduction

Eptifibatide plays an important role in preventing blood from clotting during episodes of chest pain or a heart attack. This medicine also helps in preventing blood clots during a procedure to treat a blocked heart artery and other cardiac ailments. Eptifibatide is obtained from the protein present in the venom of southeastern pygmy rattlesnake (*Sistrurus miliarus barbouri*). Integrilin® (Eptifibatide) Injection was first launched by Cor Therapeutics Inc. and Key Pharmaceuticals Inc. in 1998. Eptifibatide (Integrilin®) belongs to the class of the so called arginin-glycin-aspartat-mimetics (RGD sequence). It is a synthetic cyclic heptapeptide containing six amino acids and one mercaptopropionyl (des-amino cysteinyl) residue. The cyclization is due to the presence of the interchain disulfide bridge formed between the cysteine residue and the mercaptopropionyl residue. Chemically it is named as *N*6- (aminoiminomethyl)-*N*2-(3-mercapto-1-oxopropyl-L-lysyl-glycyl-L-α-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide,cyclic disulfide (54, 55).

4.1.2. Chemistry and mechanism of action

Eptifibatide is one of the most commonly used anti-platelet drugs. The mechanism of action of this drug is that it prevents the binding of fibrinogen, von Willebrand Factor, and other adhesive ligands to the platelet glycoprotein IIb/IIIa receptors by reversibly binding to this complex. Thereby it inhibits the final pathway of platelet aggregation and clot formation (56). Inhibition of platelet aggregation occurs in a dose- and concentration-dependent manner. Binding to receptors is short-acting. The high affinity and high specificity of the integrin towards glycoprotein IIb/IIIa receptor is mediated by the conservative Lys substitution for Arg (KGD sequence) derived from the peptide barbourin (57). Eptifibatide has a half-life approximately of about 2.5 hours. It rapidly dissociates from its receptor (within 2-4 hours) after the cessation of the therapy (58). Eptifibatide injection is obtained as a clear, colorless, sterile and non-pyrogenic solution meant only for intravenous (IV) administration. It requires a refrigerated condition for storage. It has a chemical formula of $C_{35}H_{49}N_{11}O_9S_2$. The molecular weight of this compound was found to be 831.96 (55).

4.1.3. Biotechnological process of production

Eptifibatide is produced by Solid Phase Peptide Synthesis (SPSS) with Merrifield's protocol. The main SPSS strategies are sequential synthesis, convergent synthesis and chemical ligation. In sequential synthesis, amino acids are added stepwise until the desired peptide is achieved. Convergent synthesis involves an independent solid-phase synthesis of peptide sequences (fragments). These are then cleaved from the polymer without N-terminal (or C-terminal) and side-chain that protects group removal. The sequences are then linked by condensation in solution (mixed-phase synthesis). This solid-phase and solution-phase hybrid peptide synthesis is usually the most appropriate method to synthesize peptides that contain >50 amino acid residues. Chemoselective reactions are used to couple deprotected fragments in the process of chemical ligation (59).

4.1.4. Major contraindications

The use of eptifibatide is contraindicated in patients with impaired renal function as studies show. In one such study the pharmacokinetics and pharmacodynamics of eptifibatide was studied in 31 patients using various levels of creatinine clearance (CrCl). This was done to ascertain the appropriate dose and tolerability in patients with reduced renal function. The primary end point was the eptifibatide steady-state plasma concentration. All adverse events were recorded, with particular attention to bleeding. Patients with moderate to severe renal impairment experienced a 50% reduction in total eptifibatide clearance and a corresponding doubling of plasma eptifibatide concentration. Based on these findings, it was determined as appropriate to reduce the infusion dose by 50% as can establish the targeted plasma eptifibatide concentration in patients with impaired renal function (60).

4.1.5. Side effects

The side effects of Eptifibatide include chest pain, bradycardia, angioedema and hypotension which may occur in patients with overdose. Prolonged infusions of eptifibatide have been associated with gastrointestinal bleeding and thrombocytopenia. Clearance of Eptifibatide is delayed in renal failure and hemodialysis may be required to normalize hemostasis. Management of overdose requires discontinuation of eptifibatide, monitoring for bleeding and waiting for clearance. In cases of acute thrombocytopenia, platelet transfusion may be required to counter the effects (61).

4.1.6. Applications in cardiac medicine

a. Eptifibatide is the most effective drug in patients with non-ST-segment elevation acute coronary syndromes (NSTE ACS), acute cardiac ischemic events, unstable angina, myocardial infarction and in patients undergoing percutaneous coronary intervention, as they occur due to the common pathophysiology of occlusive intracoronary thrombosis. In these cases, Eptifibatide showed significant reduction in death and ischemic complications (62).
b. On evaluating the glycoprotein IIb/IIIa inhibitor therapy in patients with acute coronary syndrome who had congestive heart failure (CHF), it was found that in these seriously ill group, Eptifibatide had only worst outcomes compared with patients without congestive heart failure (63).
c. Eptifibatide as an adjunct to percutaneous coronary intervention (PCI) further decreases ischemic events in patients with acute coronary syndrome (ACS) and also improves their safety. Also there was no massive bleeding. Their long-term efficiency and side-effects is still under observation (64). It was reported that there was no significant change in the respiratory rate; however, the serum creatinine levels were found affected by this combination study of eptifibatide and phosphatidic acid (65).

6. Chinese traditional medicine - *Spatholobus suberectus*

Spatholobus suberectus is a vine containing red resin. The vine stem was used as a traditional Chinese and Korean medicine to increase blood circulation level in the body and also in the treatment of anaemia and thrombotic changes that occurs in the vessels. Chemical analyses have revealed the presence of flavonoids, phenolic compounds, quinones, and saponins in the vine stem. The main bioactive components are reported to be flavonoids which include formononetin, prunetin, gallocatechin, catechin, epicatechin, calycosin and genistein. Reports demonstrate that they help in the regulation of plasma lipid levels, stimulating bioactivity of hematopoetic growth factors, and also increase the cerebral blood flow (66). A similar study was carried out by a Chinese group to understand the exact mechanism of action involved. An alcohol extract of *Spatholobus suberectus* derived from the vine stem was subjected to high pressure liquid chromatography, GP IIb/IIIa assays, and platelet aggregation assays. The analyses revealed that *Spatholobus suberectus* almost inhibited the binding of fibrinogen to the glycoprotein IIb/IIIa receptor.

Reports show that mice with thromboembolism were protected from death when treated with *Spatholobus suberectus* extract in a dose dependant manner. The 200mg/kg group had protection percentage of 87.5% while the control group had 9.1%. Clotting time was not significantly affected. The effect of *Spatholobus suberectus* on collagen induced aggregation was greater than that of aspirin. It thus comes across as a potential anti-platelet agent for therapeutic purposes (67).

7. Evaluation of molecular docking studies on Eptifibatide and Tirofiban

Automated docking studies were carried out by the AutoDock 4.0.1 program, 25 using the Lamarckian Genetic Algorithm (LGA) as a search engine. The AutoDock Tools 1.4.5 {ADT} graphical interface was used to prepare the receptor and the ligands PDBQT files. The structures of the ligands Eptifibatide and Tirofiban were drawn using ChemSketch and converted to PDB format using Argus Lab as shown in (Fig. 1) and (Fig. 2). The structure of the glycoprotein IIb/IIIa receptor was retrieved from Protein Data Bank (PDB id 3FCU). Water molecules were removed. For the protein receptor and ligands Eptifibatide and Tirofiban, all hydrogens were added, Gasteiger charges were computed, and non-polar hydrogens were merged. Three-dimensional energy scoring grids of 0.375 Å resolution and 60 Å _ 60 Å _ 60 Å dimensions were computed. Docking was carried out with the default parameters for LGA. Cluster analysis was performed on the docked results using a root-mean-square (rms) tolerance of 2.0 Å. The analysis of the binding mode, the calculation of the binding energy and the prediction of the binding activity of the docked conformations were carried out using PyMol Autodock Tools plug in within PyMol software.28. Conformations with lowest inhibition constant (ki) were chosen from each simulation.

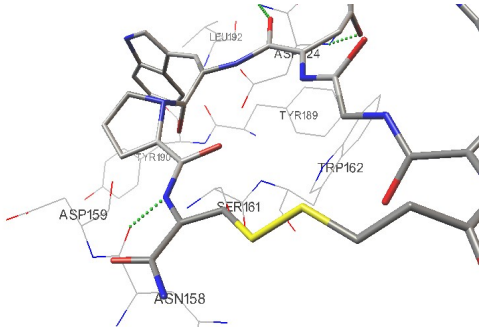

Fig. 1 : Binding mode of Eptifibatide with glycoprotein IIb/IIIa receptor in the catalytic site.

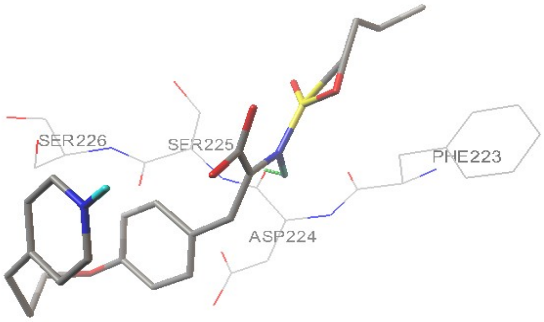

Fig. 2 : Binding mode of Tirofiban with Glycoprotein IIb/IIIa receptor in the catalytic site.

The amino acids involved in this interaction are clearly shown in Table I. ASP224 interacts with the carbonyl groups involving the carbon atoms of eptifibatide. The same atom also interacts with the amino group involving the twentieth carbon of tirofiban. This corroborative evidence could imply that blocking ASP224 inhibits the glycoprotein IIb/IIIa receptor activity and thus blocks platelet activity (68).

Table I : Possible binding energy and aminoacids involved in interaction of both Eptifibatide and Tirofiban respectively.

Sl. no	Ligand	Energies of Binding (ΔG) kcal/mol	Inhibition constant (Ki)	Length of H bond (Å)	Amino acids involved in interaction	Hydrogen bonds formed
1	Eptifibatide	−2.3	20.57mM	2.468	ARG226, GLN275, TRP235, TYR230, LEU192, SER225, SER222, PHE223,ASP224	Ep:A:MOLO:N:achain1:A:Asp224:O Ep:A:MOLO:N:achain1:A:Asp224:O
2	Eptifibatide	−4.33	674.22µM	2.916	PHE231,SER226, SER225,LEU192, ASP224,GLN275, SER226, SER225, LEU192,ASP159, ASN158,GLN275, SER225,SER226, ASN227,TRP162	Ep:A:MOLO:N:achain1:A:ASN158:O Achain1: A:ser225:N:ep:MOLO:O
3	Eptifibatide	−4.33	674.22µM	2.916	SER226,PHE231, SER225,LEU192, ASP224,TYR189, TYR190,SER161, TRP162,ASN158, ASP159	ep:A:MOLO:N1 Achain1:A:ASP224:N1 Achain1:A:SER225:N1
4	Eptifibatide	−4.8	304.24µM	2.578	ARG276, PHE223, ASP224, TRP162, SER225,SER226	ep:A:MOLO:Oachain1:A:ASP224:O
5	Tirofiban	−1.63	63.37mM	2.938	SER161, TRP162, PHE223, ASP224, SER225	Achain1:A:SER225:N:ti:A:MOLO:O
6	Tirofiban	−3.24	4.24mM	2.022	TRP235, TYR274, SER222, PHE223, SER225, ASP224, SER226,ASN227	ti:A:MOLO:H:achain2:A:TYR274:O
7	Tirofiban	−2.07	30.23mM	1.83	PHE223, ASP224, SER225,SER226	ti:A:MOLO:H:achain2:A:ASP224:OD2
8	Tirofiban	−1.77	50.62mM	2.095	ASN227, SER226, SDER225, ASP224, SER151, TRP162, ASP159, ASN158	ti:A:MOLO:H:achain2:A:ASP224:O achain2:A:SER226:OG:ti:A:MOLO:O
9	Tirofiban	−3.28	3.94mM	1.989	PHE231,SER225, ASP224,PHE225, TRP162,SER161	ti:A:MOLO:H:achain2:A:ASP224:O
10	Tirofiban	−1.54	73.84mM	2.134	SER226, SER225, PHE231, LEU192, TYR190, SER161, TRP162	ti:MOLO:H:achain2:A:SER225

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