RECENT INSIGHTS ON BIOCHEMICAL AND MOLECULAR BASIS FOR DEVELOPING ANTIHAEMOSTATIC AGENTS: A REVIEW.

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ABSTRACT

The normal coagulation process is initiated by disruption and exposure of the subendothelial components of blood vessels. Platelets adhere to subendothelium-bound von Willebrand factor via glycoprotein (GP) Ib complex. This initial interaction per se and the release of platelet agonists transduce signals that leads to the rise in intracellular Ca^{2+} which induces shape change, prostaglandin synthesis, release of granular contents and conformational changes in platelet Gp IIb-IIIa. Gp IIb-IIIa in activated platelets binds fibrinogen and other adhesive proteins and mediates platelet cohesion the primary haemostatic plug. Furthermore, the activated platelets due to aggregation, result in the formation of fibrin (secondary hemostasis). Normally the haemostatic process plays a delicate balance between keeping the blood in the fluid state to maintain flow and rapidly forming an occluding plug following vessel injury. Thrombosis occurs because of alteration in this delicate balance. Arterial thrombosis occurs in the setting of previous vessel wall injury mostly because of atherosclerosis, while venous thrombosis occurs in areas of stasis. The recent advances in understanding of the haemostatic process have led to a better understanding of the mechanism of action of many antithrombotic drugs and identification of new targets for drug development. The molecular target of the ticlopidine has been identified. Large numbers of IIb-IIIa inhibitors have been developed. The mechanism of action of heparin has been defined at the molecular level. As a result, a synthetic pentasaccharide, based on antithrombin-binding domain of heparin, has been developed and tested successfully in clinical trials. New generation direct thrombin inhibitors are being developed. Factor Xa has a critical position at the convergence of intrinsic and extrinsic pathway. The clinical tolerability and the efficacy of low molecular weight heparins has established that inhibition of further thrombin generation, by blocking factor Xa alone can be an effective way of preventing thrombus growth without inactivating thrombin. A large number of specific factor Xa inhibitors are under development. Some of these are in preliminary clinical trials and appear to be promising. Future clinical trials will determine whether these new drugs will provide better risk-benefit ratio in treatment of thrombotic disorders. Similarly role of thrombolytics has been clearly established in many diseases including coronary artery disease.

KEY WORDS

haemostatic, platelets, thrombin, Glycoprotein IIb/IIIa, thrombolytics.

INTRODUCTION

Arterial thrombosis occurs under high flow velocity and platelets interact with aged blood vessels under high shear stress. Antiplatelet drugs are the mainstay of treatment of arterial thrombosis. Venous thrombosis occurs in areas of stasis and the activation of soluble coagulation factors plays a major role in the pathogenesis. As expected, anticoagulants rather than antiplatelets are the main in therapeutic option of

venous thrombosis. The current approach to the treatment of coronary thrombosis includes antiplatelet agents, thrombolytics and antithrombin agents. However, none of these agents can alone suppress the process of coagulation efficiently. Structural information about many enzymes involved in coagulation, and the mode of action of their inhibitors, has been elucidated in great detail like mode of action of antithrombotics such as heparin and ticlopidine has been defined at the molecular level. This review attempts to summarize the recent advances in understanding of the haemostatic process, describe newer targets for antithrombotic therapy and highlight the potential advantages of newer developing drugs over existing agents.

Pathophysiology

Vascular injury disrupts the endothelial cell continuity

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and exposes the subendothelial components that are recognized by receptors on the platelet surface. Platelets adhere to the exposed matrix interacting between the platelet surface glycoprotein Ib-IX- V complex and the von Willebrand factor (vWf) in the subendothelium. Platelet adhesion *per se* and the release of mediators such as adenosine diphosphate (ADP) from platelets at the site of vascular injury facilitates signal transduction that leads to a rise in cytosolic calcium (Ca^{2+}). The rise is linked to change in platelet shape, prostaglandin (PG) synthesis and secretion and the activation of glycoprotein IIb/IIIa complex, and the development of platelet procoagulant activity. Glycoprotein IIb/IIIa is a major heterodimeric membrane protein belonging to the integrin super family. Glycoprotein IIb/IIIa on resting platelets is unable to bind soluble ligands. After platelet activation, the glycoprotein undergoes a conformational change and becomes competent for ligand binding. Activated glycoprotein IIb/IIIa binds to a number of adhesion molecules such as vWf, collagen, vitronectin and fibronectin present in the subendothelial matrix, leading to stable platelet adhesion. The glycoprotein IIb/IIIa complex promotes thrombus growth by mediating platelet aggregation. Activation induced binding of fibrinogen to glycoprotein IIb/IIIa is the primary mechanism of platelet aggregation. Fibrinogen cross-links two glycoprotein IIb/IIIa molecules on adjacent platelets. Many platelet agonists (ADP, epinephrine, platelet-activating factor, thrombin and PGs) interact with their platelet receptors that are coupled to G-proteins leading to the activation of phospholipase C β phosphatidyl-inositol breakdown. Inositol triphosphate induces cytosolic Ca^{2+} release from the dense tubular system while diacylglycerol activates protein kinase C. As yet it is not clear how the activation of this enzyme contributes to platelet aggregation, even though a number of substrates have been identified. Recently, signal transduction pathways utilizing protein tyrosine kinases and phospholipase C, independent of G-protein, have been elucidated for collagen, immune complexes and possibly for shear stress. Additional platelets are recruited to the growing haemostatic plug by the release reaction and PG synthesis. In the release reaction, the platelet granules discharge their contents to the exterior via the open canalicular system, increasing the local levels of ADP and other haemostatic factors. The increased cytosolic Ca^{2+} stimulates membrane phospholipase A $_2$, liberating arachidonic acid from membrane phospholipids. Cyclooxygenase-1 exerts arachidonic acid to cyclic endoperoxides, which are converted to thromboxane A $_2$ by thromboxane synthase. Thromboxane A $_2$ is a very potent platelet agonist and vasoconstrictor. Both ADP release and PG synthesis play an important role in consolidating the initial haemostatic plug. The rise in cytosolic Ca^{2+} also activates the enzyme scramblase allowing the movement of anionic phospholipid from

the inner to the outer leaflet of the cell membrane bilayer. This anionic phospholipid surface provides a binding site for enzymes and cofactors of the coagulation system, facilitating efficient generation of thrombin and formation of fibrin. Activation of coagulation cascade is triggered by exposure of blood to tissue factor. Tissue factor binds factor, which circulates in low levels in the blood, and this interaction converts more factor VII to VIIa autocatalytically. More importantly, the tissue factor- VIIa complex activates factor X and factor IX. Factor IXa, in the presence of factor VIIIa, catalyzes the generation of more Xa, thus amplifying this pathway. Furthermore, factor Xa can also activate factor VII to VIIa. Tissue factor pathway inhibitor (TFPI), a multivalent plasma protease inhibitor, inhibits activated factor Xa, in a factor VIIa-dependent manner providing a feedback inhibition of the e factor pathway. Factor Xa, in the presence of Va and Ca^{2+} , catalyzes the conversion of prothrombin to thrombin on the anionic phospholipid surface. Thrombin, in turn, converts fibrinogen to fibrin in a multi-step reaction that eventually leads to formation of cross-linked and insoluble networks of strands. In addition, thrombin activates coagulation factors V, VIII and IX. Thrombin is also a key mediator of platelet activation, release reaction and aggregation. Its action on platelets produces a highly efficient catalytic surface for further generation of thrombin. Thrombosis occurs because of inappropriate activation of the haemostatic process in the setting of vessel wall injury. The major difference between arterial and venous thrombosis are the underlying rheological factors.

Pharmacotherapy

The following categories of drugs are used in management of thrombosis.

I. Antiplatelet Agents

Other than aspirin, newer categories of drugs available in market are

A) Aspirin and agents acting on cyclooxygenase (COX) enzyme pathway

Aspirin inhibits COX enzyme responsible for production of proaggregatory prostaglandins like thromboxane A $_2$, PGI $_2$. In spite of the short half-life of aspirin, antiaggregatory effect remains for 10 days due to long regeneration time of COX enzyme. However, aspirin does not affect other pathways of platelet activation like thrombin, ADP and collagen. The antiaggregatory dosage of aspirin varies from 75-350 mg/day. The adverse effects are hemorrhage, hypersensitivity and skin rashes, alopecia and purpura.

B) Dipyridamole

It inhibits phosphodiesterase enzyme which prevents the inactivation of cyclic AMP, the levels of which are

increased during injury. However, other mechanisms have been proposed for its action like stimulating prostacyclin release and inhibiting thromboxanes A_2 formation. Overall effect is reduced platelet aggregation and adhesion. The adverse effects are nausea, vomiting, abdominal pain, neutropenia, rarely cholestatic jaundice and blood dyscrasia.

C) Agents interfering with ADP receptor activity

The various categories of drugs are

a) Agents blocking ADP induced platelet aggregation

ADP is responsible for not only proaggregatory agent but also potentiates the proaggregatory actions of all other agents. There are two types of G-protein coupled receptors involved in platelet activation(6). The $P2Y_1$ receptor increases cytosolic Ca^{2+} and activates phospholipase C and mediates change in platelet shape and initial aggregation in response to ADP but $P2Y_{12}$ receptor is coupled to inhibition of adenylyl cyclase and mediates amplification of the initial aggregation response and thrombus stability. The drugs are clopidogrel and ticlodipine. These thienopyridine derivatives irreversibly block ADP induced platelet aggregation with impairment in aggregatory response to thrombin, collagen, fibrinogen and Von willebrand factor(7). Adverse effects are bone marrow suppression especially leucopenia with ticlodipine. Recent trials have advocated the efficacy of combination of aspirin and clopidogrel in coronary disorders.

b) Glycoprotein Ib Inhibitors:

Glycoprotein Ib mediates the initial contact between platelet and vessel wall. This interaction is enhanced under high shear stress similar to that seen in coronary arteries and is an important target for the inhibition of arterial thrombosis. The domain in vWf that interacts with platelet glycoprotein Ib has been identified as the A1 domain (8). This peptide is being developed as an anti thrombotic drug.

c) Aurin Tricarboxylic Acid

Aurin tricarboxylic acid (ATA) is a polycarboxylated compound which binds to high molecular weight multimers of vWf effectively, preventing binding of vWf to platelet membrane glycoprotein Ib. By this mechanism, ATA inhibits platelet adhesion to subendothelium and shear-induced platelet aggregation. In animal models, ATA prolongs the bleeding time, protects against thrombosis and enhances the thrombolytic effect of alteplase(9). ATA has clinical potential for inhibiting platelet aggregation.

d) Prostaglandin Analogs

Thromboxane synthase is an enzyme responsible for platelets aggregation and selective inhibitors against

this enzyme have been developed. However, result with thromboxane synthase inhibitor were disappointing probably because cyclic endoperoxides, the precursor thromboxane A_2 is a potent agonist of platelet aggregation. Another area of development is the synthesis of selective inhibitors of the thromboxane receptor. A hybrid compound, ridogrel, possessing inhibitory action against thromboxane synthase and thromboxane receptor has been developed. This compound has been tested clinically and has been shown to improve the outcome in-patient's with acute myocardial infarction, who were also treated with alteplase and heparin. There have been several attempts to develop PGI_2 analogous such as iloprost as antithrombotic agents (10). However, its therapeutic use has been limited by adverse events such as severe hypotension, and receptor desensitization. Beraprost is new, stable, orally active PGI_2 analog with antiplatelet and vasodilating properties. Beraprost has been clinically tested in patients with peripheral vascular disease. Alprostadiol, an analog of eprostenoil, inhibits platelet reactivity and induces vasodilatation and has been undergoing clinical trial, in patient's with pulmonary hypertension (12).

e) Platelet Glycoprotein IIb/IIIa Complex Inhibitors.

Several classes of compound have been developed which binds to GP IIb/IIIa receptors. These receptors antagonists prevent fibrinogen binding and platelet aggregation induced by all known platelet activators especially thrombin and collagen. Thus, glycoprotein IIb/IIIa inhibitors have a clear advantage over other conventional agents, such as aspirin and ticlopidine, whose inhibitory effects can be bypassed by agonists coupled to receptors that directly activate phospholipase C. However, the potential limitations include absence of an effect on platelet adhesion, release reaction and platelet procoagulant activity.

Abciximab: Abciximab is derived from a murine monoclonal antibody that binds glycoprotein IIb/IIIa and prevents adhesive ligands from binding to the activated receptor(11). It is a potent inhibitor of platelet aggregation. In combination with percutaneous coronary intervention it is effective in reducing the incidence of subsequent myocardial infarction, urgent target vessel revascularization, and death in patients at risk of recurrent coronary ischemia. In therapeutics, abciximab has been used in a variety of clinical circumstances such as saphenous vein grafting, acute myocardial infarction and stroke. The limitation of abciximab includes rare but serious bleeding, absence of an effective antidote and the high cost.

Eptifibatide: Eptifibatide, a disulfide-linked cyclic peptide, is a short acting and reversible inhibitor of platelet aggregation(11). Eptifibatide contains a lysine-glycine-aspartic acid sequence similar to that found in the snake venom, barbourin. This sequence

selectively blocks the platelet glycoprotein IIb/IIIa complex without affecting the functions of other integrins. The potential benefits of eptifibatide include its ability to bind reversibly, an advantage in treating patients at high risk for bleeding. Eptifibatide has been evaluated in acute coronary syndromes, extra corporeal bypass and following percutaneous transluminal coronary angioplasty (PTCA)(12).

Nonpeptide Platelet Glycoprotein IIb/IIIa Receptor Antagonists

Nonpeptide platelet glycoprotein IIb/IIIa antagonists were developed following the discovery of disintegrins, potent peptides from vipers, which bind to platelet glycoprotein IIb/IIIa complex with a high affinity. Based on these structural data, tirofiban, a tyrosine analog, was developed. Tirofiban has been tested in several clinical trials in the treatment of non- ST -elevation myocardial ischemia (11). It has reduced the death rate, new myocardial ischemia, or refractory ischemia during the 48-hour infusion period largely as a result of reduction in recurrent ischemia. Further, patients who received the combination of tirofiban and heparin had reduced 30-day mortality and recurrent ischemia. Several other oral compounds were developed for clinical use including lamifiban, xemilofiban and fradafiban(11). However, despite the potential advantage of sustained glycoprotein IIb/IIIa blockade and the ease of oral administration, initial results with oral agents were disappointing and these agents had an adverse impact on myocardial infarction and death(13).

II. Anticoagulants

Thrombin formed *in vivo* has potent anticoagulant functions. Thrombin formed in the vascular bed binds to the endothelial cell surface protein thrombomodulin. Thrombomodulin-bound thrombin has no procoagulant activity, but it rapidly activates protein C, a vitamin K-dependent protein, to a serine protease. Activated protein C then cleaves factors Va and VIIIa by limited proteolysis, thereby inactivating their activity and blocking their binding to factors IXa and Xa. Protein C has an activator for its proteolysis, protein S, a nonenzymatic and vitamin K-dependent protein. Furthermore, thrombomodulin-bound thrombin does not activate platelets. The regulation of blood coagulation is a balance between these two opposing effects of thrombin. Newer anticoagulants are being developed that enhance the anticoagulant functions of thrombin. The various types of thrombin inhibitors are

a) Direct Thrombin Inhibitors

During fibrin generation thrombin is incorporated into the clot and fibrin bound thrombin is protected from its natural anticoagulant, antithrombin(14). Fibrin-bound thrombin is thought to play a major role in the

propagation of the thrombus by promoting continued local fibrin formation and by recruiting additional platelets to the thrombus. Experimental studies in animal models have suggested that direct thrombin inhibitors can inhibit fibrin-bound thrombin, are more potent anticoagulants than heparin. The various inhibitors are

Hirudins, a family of 7000-dalton proteins found in the salivary glands of the medicinal leech, *Hirudo medicinalis*, are the most potent specific thrombin inhibitors known(15,16). Hirudins interact irreversibly with the active site of thrombin's catalytic triad residues. One recombinant hirudin, lepirudin, has been studied in several clinical studies in patients with arterial and venous thrombosis. Desirudin was better than heparin in the prevention of venous thromboembolism in patients undergoing hip replacement. When given in higher dosages with thrombolytic therapy hirudins were associated with intracranial bleeding. Subsequent trials have used lower dosages of hirudins in patients receiving thrombolytic therapy and the results showed that the efficacy of hirudins was comparable to heparin.

Hirulogs: Hirulogs are groups of peptides derived from hirudins(17). This design of these peptides is based on the structural model of hirudin-thrombin interaction. In randomized studies in patients with coronary artery disease, bivalirudin decreased angioplasty-associated acute ischaemic complications(18). Compared with hirudin, hirulog has a shorter half - life. Hirulog has been studied in several clinical trials in patients with unstable angina pectoris, following PTCA and following knee and hip surgery. In combination with streptokinase infusion, hirulog was as effective as heparin, but associated with lesser bleeding complications, in patients with acute myocardial infarction.

Argatroban: Argatroban, a synthetic small molecular weight (500D) direct thrombin inhibitor, is based on the structure of the amino acid arginine(19). It was specifically designed to bind to the catalytic site of thrombin. Argatroban can rapidly and completely inhibit thrombin present at the thrombotic site. It has comparable *in vitro* inhibitory potency for soluble and fibrin-bound thrombin. *In vitro*, argatroban has also been shown to inhibit the formation of factor XIII-mediated fibrin cross-linking and thrombin-induced platelet aggregation. These features of argatroban led to the possibility that it will have a therapeutic advantage over other antithrombins such as heparin or hirudin. Argatroban has been evaluated in a number of clinical trials and it was as effective as heparin in reducing the incidence of myocardial infarction after PTCA and thrombolysis(20). Argatroban is considered to be a promising therapeutic agent because of its selectivity, rapid interaction and reversibility.

Other Direct Thrombin Inhibitors: Melagatran is a competitive and rapidly acting low molecular weight inhibitor of thrombin(21). Ximelagatran, an orally administered prodrug of melagatran, is an uncharged lipophilic drug with little intrinsic activity against thrombin. However, after absorption from the gastrointestinal tract it undergoes biotransformation to the active form. This drug is currently being evaluated in clinical trials. A large number of thrombin inhibitors are being developed and some of them, such as inogatran(22) have undergone clinical trials.

Current Status: Despite their theoretical advantage over heparin in selectively inhibiting clot-bound thrombin, clinical studies with direct thrombin inhibitors have not shown a significant benefit over heparin therapy to date. However, they are useful in patients with heparin-induced thrombocytopenia. Whether direct thrombin inhibitors will play a major role in the management of acute coronary syndromes will be a subject of continued research in coming years.

b) Antithrombin-Dependent Thrombin Inhibitors

Low Molecular Weight Heparins (LMWH)

Heparin acts by binding to antithrombin III, now called antithrombin. Antithrombin inactivates thrombin and other serine proteases forming a complex that traps their catalytic site in an irreversible form(23). Heparin binds to antithrombin III and accelerates this interaction (1000-fold) by altering the serine protease reactive site on antithrombin III. Once the stable complex is formed heparin dissociates from the complex and binds to other antithrombin III. Studies have shown less bleeding complications with LMWH than with UFH. Whether this is because of its better bioavailability or increased anti-factor Xa to antithrombin activity ratio, is still being debated. LMWHs are replacing regular UFH for most clinical indications. However, despite favorable pharmacokinetics and simplicity of administration, there is no convincing clinical evidence of lesser bleeding complications with LMWH compared with UFH.

Danaparoid Sodium

Danaparoid sodium is a heparinoid derived from porcine intestinal mucosa(24). It is a sulfated glycosaminoglycan preparation. Danaparoid sodium inactivates factor Xa, and to a much lesser extent thrombin, by acting on antithrombin III. Its anticoagulant profile is characterized by a very high ratio of anti-factor Xa activity to antithrombin activity (20: 1). In contrast to the platelet-activating activity of heparin, danaparoid sodium does not inhibit platelet function in vivo or in vitro. Because of low immunological cross-reactivity with heparin, danaparoid sodium has been used in patients who develop heparin-induced thrombocytopenia. However, it has limitations including a long

biological half-life and absence of an effective antidote. Other heparinoids such as dermatan sulfate and heparan sulfate have also shown promising anticoagulant activity in animal models.

Factor Xa Inhibitors

It propagates thrombus by binding factor Va on platelet surface, and thus forming a potent prothrombinase complex. Various animals and clinical studies have established that factor Xa inhibition provides an effective anticoagulant effects(25). A large number of specific factor Xa inhibitors are under development.

Fondaparinux sodium

The minimum sequence in heparin required for binding to antithrombin III has been identified as a pentasaccharide sequence(23). The detailed elucidation of this interaction led to the development of more selective antithrombin III-dependent factor Xa inhibitors. Fondaparinux sodium, the first compound of this class, is a synthetic pentasaccharide based on antithrombin- binding domain of heparin. It binds antithrombin III to induce a conformational change that potentiates the inactivation of factor Xa, with no effect on the rate of thrombin inactivation. It has predictable pharmacokinetics and pharmacodynamic profiles when administered subcutaneously and intravenously. Recent clinical trials suggest that it has the potential to improve significantly the risk-benefit ratio in the prevention of deep vein thrombosis compared with UFH(26).

Direct Factor Xa Inhibitors

Several synthetic nonpeptide inhibitors of factor Xa have been developed and shown to have anticoagulant effects in animal models of thrombosis. DX-9065 is one such compound that is orally active.

Tissue Factor as a Target

The realization that tissue factor may be the sole initiator of the coagulation cascade in vivo led to concept of targeting tissue factor. Recombinant TFPI has shown an antithrombotic effect in animal models(29). High affinity antibodies to tissue factor, capable of blocking its proteolytic activity, have been developed(27). Another approach is the use of active site-blocked factor VIIa, which acts as a competitive inhibitor of enzymatically active VIIa(28).

III) Thrombolytic Agents

Five plasminogen activators are available: streptokinase, anistreplase, saruplase, urokinase and alteplase.

Streptokinase and Anistreplase

Streptokinase is produced by several strains of b

haemolytic streptococci. It consists of single polypeptide chain with 414 aminoacids. Anistreplase (anisolated plasminogen-streptokinase complex) was developed for controlling the enzymatic activity of plasminogen-streptokinase complex

Mechanism of action : streptokinase activates plasminogen to plasmin indirectly, following three step mechanism. In the first step, streptokinase forms a complex with plasminogen which undergoes a conformational change resulting in exposure of active site in plasminogen moiety. In the second, this active site catalyses the activation of plasminogen to plasmin. In the third, plasminogen-streptokinase molecules are converted to plasminogen-streptokinase complex. Anistreplase binds to fibrin via the lysine binding sites of plasminogen. Deacylation of anistreplase uncovers the catalytic in the complex converting plasminogen to plasmin.

Pharmacokinetics The half-life of streptokinase is approximately 20 minutes. After streptokinase infusion the antibody titers rise rapidly to 50 to 100 times the preinfusion values and remain high for 4 to 6 months. However the previous circulating antibodies in patients with history of sore throat may inactivates the streptokinase and thus increasing the dose. Anistreplase has shown first order deacylation half-life of about 105 minutes in humans(30).

Therapeutic dose The initial dose of streptokinase is determined by streptokinase resistance test i.e. dose required to lyse a clot induced in patient plasma in a given time or giving standard dose ranging from 500,000 to 750,000 U over a period of 10 to 30 minutes followed by maintenance of 100,000 U hourly for 1 or more days. However a high dose regimen Of 1.5 MU over 15 to 20 minutes is now routinely used in patients with acute myocardial infarction. The dose of anistreplase in acute myocardial infarction is 30 U (1.25 MU = 30 mg) given as bolus dose slowly(31).

Adverse Effects Due to antigenic nature of streptokinase serious anaphylactic reactions (urticaria, bronchospasm, angio-oedema) can occur. Shivering, fever, rashes may occur in 10 percent of patients. Bleeding is the most serious side effect and minor bleeding may occur in 3-4 percent of patients. The incidence of cerebral bleeding is reported to be 0.1-0.5 percent.

Alteplase (Tissue- Type Plasminogen Activator)

It is a recombinant human tissue type plasminogen activator (rt-PA) composed of one polypeptide chain containing 527 aminoacids.

Mechanism of action t-PA is a poor enzyme in absence of fibrin. In fibrin presence the enhanced activation rate of plasminogen by two fold is seen. This allows efficient activation on fibrin clot of plasminogen.

Pharmacokinetics: In animal species t-PA disappearances was found to be rapid (initial half life of 1-4 min) and probably being metabolized via liver. Following infusion the half-life in healthy volunteers and in patients was 6 min with terminal elimination phase half-life of 64 min.

Clinical use: The recommended dose of alteplase is 100 mg for AMI, administered as 60 mg in first hour of which 6 to 10 mg is administered as a bolus over first 10 minutes. The next 20 mg is given over second hours, and 20 mg over third hour. Recently it was proposed to give total dose of 100 mg starting with a bolus of 15 mg then 50 mg in next 30 minutes and remaining 35 mg in next 60 minutes.

Tolerability: alteplase does not appear to be immunogenic and no serious life threatening allergic reactions has been reported. However intracerebral bleeding is still a major cause of concern.

CONCLUSION

Recent advances in our understanding of thrombus formation, coagulation reaction and platelet function at the molecular level have propelled the development of new drugs aimed at more specific targets. Furthermore, gene-knockout models have identified a role of previously unrecognized molecules such as P-selectin, CD40 and Gas6 in the haemostatic process. These advances in molecular medicine will undoubtedly lead to development of newer drugs. Clinical trials will determine whether these new drugs will be well tolerated and more effective than currently available antithrombotic therapy.

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