Antithrombotic Agents in the Management of Sepsis

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ABSTRACT

Sepsis, a systemic inflammatory syndrome, is a response to infection and when associated with multiple organ dysfunction is termed, severe sepsis. It remains a leading cause of mortality in the critically ill. The response to the invading bacteria may be considered as a balance between proinflammatory and antiinflammatory reaction. While an inadequate proinflammatory reaction and a strong antiinflammatory response could lead to overwhelming infection and death of the patient, a strong and uncontrolled proinflammatory response, manifested by the release of proinflammatory mediators may lead to microvascular thrombosis and multiple organ failure. Endotoxin triggers sepsis by releasing various mediators including tumor necrosis factor-alpha and interleukin-1(IL-1). These cytokines activate the complement and coagulation systems, release adhesion molecules, prostaglandins, leukotrienes, reactive oxygen species and nitric oxide (NO). Other mediators involved in the sepsis syndrome include IL-1, IL-6 and IL-8; arachidonic acid metabolites; platelet activating factor (PAF); histamine; bradykinin; angiotensin; complement components and vasoactive intestinal peptide. These proinflammatory responses are counteracted by IL-10. Most of the trials targeting the different mediators of proinflammatory response have failed due a lack of correct definition of sepsis. Understanding the exact pathophysiology of the disease will enable better treatment options. Targeting the coagulation system with various anticoagulant agents including antithrombin, activated protein C (APC), tissue factor pathway inhibitor (TFPI) is a rational approach. Many clinical trials have been conducted to evaluate these agents in severe sepsis. While trials on antithrombin and TFPI were not so successful, the double-blind, placebo-controlled, phase III trial of recombinant human activated protein C worldwide evaluation in severe sepsis (PROWESS) was successful, significantly decreasing mortality when compared to the placebo group. Better understanding of the pathophysiologic mechanism of severe sepsis will provide better treatment options. Combination antithrombotic therapy may provide a multipronged approach for the treatment of severe sepsis.

Key Words: Severe sepsis, Inflammatory mediators, Microvascular thrombosis, Activated protein C (APC), Thrombomodulin, Tissue factor pathway inhibitor (TFPI), Antithrombin, Thrombin activatable fibrinolytic inhibitor.

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Background

Sepsis, a major challenge in Critical Care Medicine, has been defined by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference, as a systemic inflammatory syndrome in response to infection which, when associated with acute organ dysfunction such as acute renal failure, is said to be severe[1]. Severe sepsis, a common, expensive and frequently fatal condition, with annual mortality similar to acute myocardial infarction, is especially common in the elderly and its incidence is likely to increase with the aging United States population. The increased prevalence of human immunodeficiency virus (HIV) infection contributes to this high incidence as well[2]. Based on 1995 state hospital discharge records from seven large states with population and hospital data from the US census, the Centers for Disease Control, the Health Care Financing Administration and the American Hospital association, Angus et al, identified 192.980 cases, yielding national estimates of 751.000 cases (3.0 cases per 1.000 population and 2.26 cases per 100 hospital discharges). They noted the incidence increased > 100 fold with age (0.2/1000 in children to 26.2/1000 in those > 85 years old). Mortality was 28.6%, or 215.000 deaths nationally and also increased with age, from 10% in children to 38.4% in those > 85 years old. The estimated average costs per case were $ 22100 (higher for infants, nonsurvivors, intensive care unit patients, surgical patients and patients with more organ failure) with annual total costs of $ 16.7 billion nationally[2]. Martin GS et al, evaluated sepsis trends in the US by analyzing data from the 1988 to 1998 National Hospital Discharge Survey and reported at the recent 67th annual scientific meeting of the American College of Chest Physicians that the incidence of sepsis in the US increased by 23.3% during 1988-1998. They attributed the increased incidence to the HIV epidemic and to an increase in invasive procedures. They reported that in 1988, sepsis was diagnosed in 207.9 per 100.000 hospitalized patients and increased to 256.3 cases per 100.000 hospitalized patients in 1998. The increased incidence was observed in neonates and patients over 55 years of age[3].

Severe sepsis is defined as sepsis associated with acute organ dysfunction, resulting from a generalized proinflammatory and procoagulant response to an infection and manifested by hypoperfusion and perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria or acute alteration of mental status[4]. With a mortality rate of 30-50% despite advances in critical care, it remains the leading cause of mortality in the critically ill[5-8]. Of the 750.000 cases of sepsis which occur each year in United States, at least 250.000 are fatal[9]. Approximately two-thirds of the sepsis cases occur in hospitalized patients. Although, most of the cases of sepsis are caused by gram-negative or positive bacteria, it may occur with diseases caused by fungi, *Mycobacteria, Rickettsia,* viruses or protozoans. Factors that predispose to gram-negative sepsis include diabetes mellitus, lymphoproliferative disorders, burns, cirrhosis of liver, invasive procedures or devices, drug-induced neutropenic states and asplenia. However, factors predisposing to gram-positive sepsis include vascular catheters, indwelling mechanical devices, burns and intravenous drug injections. As a complication of broad spectrum antibiotic therapy, fungal infections occur most often in immunosuppressed individuals. The increased incidence of sepsis in the United States is attributable to the aging population, increased longevity of patients with chronic infections and the relatively high frequency of sepsis in AIDS. The widespread use of antimicrobial agents, glucocorticoids, indwelling catheters, mechanical devices and mechanical ventilation are contributing factors as well.

The response to the invading microorganism can be considered as a balance between the proinflammatory and antiinflammatory reaction. A patient could die of an overwhelming infection, when the proinflammatory reaction is inadequate and the antiinflammatory response is strong. However, a strong and uncontrolled proinflammatory response, manifested by the release of proinflammatory mediators may lead to organ failure. Endotoxin present in the cell wall of gram-negative bacteria triggers sepsis by releasing various mediators such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL-1). These cytokines

activate the complement and the coagulation systems besides expressing adhesion molecules and also releases prostaglandins, leukotrienes, reactive oxygen species and nitric oxide (NO). The other mediators thought to be involved in the development of sepsis syndrome include IL-1, IL-6 and IL-8; arachidonic acid metabolites, platelet activating factor (PAF); histamine; bradykinin; angiotensin; complement components; vasoactive intestinal peptide. These proinflammatory responses are counteracted by IL-10.

Most of the trials targeting the different mediators of proinflammatory response may have failed due to the lack of a correct definition of sepsis and also due to great flucuations in the immunological status of the patient[10]. Selected targets in the treatment of sepsis include TNF-α, IL-1, endotoxin, adhesion molecules, complement system, kallikrein-kinin system, PAF, archidonic acid metabolites, NO, reactive oxygen species, inflammatory reaction & immunodepression associated with sepsis and not the least the coagulation system[10]. Several agents could be used to counteract each of these mediators. However, it would be difficult to target all the mediators at one time and on the otherhand targeting one mediator at a time will be too inadequate a treatment. It is very important to identify some of the very important mediators and then target them simulataneously or in a sequential manner. Understanding the exact pathophysiology of the disease is essential. In this review targeting of the coagulation system with various anticoagulant agents such as antithrombin, activated protein C (APC), tissue factor pathway inhibitor (TFPI), thrombin activatable fibrinolysis inhibitor, thrombomodulin etc., will be discussed at length. However, in order to provide better treatment options, targeting of the coagulation system and fibrinolytic system together with other crucial mediators of sepsis will be discussed.

Anticoagulation in Severe Sepsis

The presence of intravasculat thrombi and disseminated intravascular coagulation in humans with severe sepsis is evident that the coagulation system is activated[11,12]. This is manifested by increased levels of activated coagulation factors, tissue factor, TFPI, decreased fibrinogen, and D-dimer[11]. Contact activation of the intrinsic system of coagulation by the lipopolysaccharide in the cell walls of the bacteria and activation of the extrinsic sytem by the generation of tissue factor lead to the generation of thrombin. The thrombin can activate the thrombin activatable fibrinolytic inhibitor (TAFI) which could result in fibrinolytic deficit or the thrombin can combine with the thrombomodulin generated and form a thrombin-thrombomodulin complex which can activate protein C to APC serving as an anticoagulant. In severe sepsis, activation of the coagulation system can activate the endothelial cells resulting in the potentiation of proinflammatory responses and production of inflammatory mediators including cytokines such as TNF-α and IL-1. Thus, it appears that there is a role for different antithrombotic drugs in the treatment of severe sepsis, there is a role of different agents such antithrombin, TFPI, APC, anti-Xa inhibitors, anti-IIa inhibitors, thrombomodulin, TAFI inhibitors etc., could be useful therapeutic agents. Several studies have investigated the role of TFPI, anti-Xa inhibitors and APC. Agents to counteract increased activation of coagulation by cytokines manifested by an increased level of tissue factor have been investigated. Defective fibrinolysis manifested by an increased level of plasminogen activator inhibitor-1 (PAI-1) can be targeted for therapeutic intervention and supplementing the naturally occurring anticoagulants which are decreased such as APC, antithrombin and TFPI, will comprise an antithrombotic regimen to treat severe sepsis.

TFPI is a naturally occurring protein which can circulate freely or bound to low and high density lipoproteins which inhibits both the factor VIIa/TF complex and F Xa as it circulates[11,12]. While about 10% of TFPI is bound to the lipoproteins, 90% of it is bound to heparin-like species on the endothelial surface and is released following the adminstration of unfractionated heparin (UFH), low molecular weight heparin (LMWHs), defibrotide[13,14]. Endotoxin is known to increase the circulating levels of TFPI[11,12]. A phase II study showed clinical benefit of using TFPI infusion in patients with severe sepsis[15]. The results of a large

phase III clinical trial (OPTIMIST) of TFPI (Tifacogin-Chiron/Pharmacia Corporation) in severe sepsis indicates that tifacogin did not meet the primary endpoint of reducing 28-day all cause mortality [PRNewswire/Chiron Corporation (Nasdaq: CHIR), Emeryville, Calif., Nov 21, 2001]. The OPTIMIST trial was a prospective, double-blind, placebo-controlled trial investigating the use of tifacogin in the treatment of severe sepsis. The trial had included approximately 2000 patients from 16 countries who were randomized to receive either placebo or tifacogin. TFPI was thought to prevent multiple organ failure, a major cause of death in severe sepsis.

In animal models of severe sepsis induced by endotoxin administration, blockade of F Xa with a F Xa inhibitor, dansyl glutamyl-glycyl-arginyl chloromethyl ketone-treated-Xa, prevented disseminated intravascular coagulation (DIC) without affecting the survival rates[16]. Several clinical trials are underway evaluating the role of F Xa inhibitors in severe sepsis.

APC is a natural anticoagulant that plays a key role in the regulation of blood coagulation by selectively degrading coagulation F Va and F VIIIa eventually inhibiting thrombin generation[17]. Protein C is one of the vitamin K-dependent plasma proteins that is activated by the thrombin-thrombomodulin complex on the surface of intact endothelial cells. The anticoagulant effect of APC is enhanced by a cofactor, protein S, another vitamin K-dependent plasma protein[18]. An endothelial cell protein C receptor has been identified[19]. Both the plasma derived and recombinant forms of protein C are now available[20]. APC is formed when protein C is cleaved by thrombin[15,21]. Serum APC levels are decreased in children and adults with severe Meningococcemia and purpura fulminans[22,23]. Based on these findings several nonrandomized trials have been conducted, where infusions of protein C at a dose of 50-100 IU/kg every 6 hours were given to adults and children with severe meningococcal disease and purpura fulminans[21,23-25]. These studies demonstrated that protein C infusion normalized protein C levels, increased fibrinogen levels and resolved the DIC. However, differences in mortality could not be demonstrated in these small nonrandomized studies with low power[21,23-25]. A phase II trial on APC in sepsis did not show improved survival with APC[15]. A phase III clinical trial on APC was recently stopped after enrollment of 1500 patients because of efficacy associated with APC therapy[26]. Such promising results ensure that infusions of APC will in the future become part of a standard therapy of severe sepsis[27].

ENDOGENOUS ANTICOAGULANTS IN INFLAMMATION

Natural anticoagulants such as antithrombin, APC and TFPI can modulate the coagulation induced increases in the mediators of the inflammatory response. These natural anticoagulants, besides inhibiting activated coagulation factors, can also interact with the cells that generate antiinflammatory substances[28]. It has been demonstrated that the generation of thrombin, F Xa and the tissue factor-F VIIa complex can augment acute inflammatory responses. These responses could be due to activation of the protease activated receptors on the endothelium leading to expression of adhesion molecules and platelet activating factor which facilitates leukocyte activation[28]. Besides, TAFI has been recently identified in platelets and could be secreted upon stimulation of the platelets (Mosnier, Buijtenhuis, Marx, Meijers, Bouma, 2000, sumitted for publication). It has also been recently reported that the regulation of fibrinolysis in plasma by TAFI and protein C is dependent on the concentration of thrombomodulin[29]. Systemic deposition of fibrin leading to impaired organ perfusion thereby contributing to multiple organ failure is a hallmark of severe sepsis. Since all the three major natural anticoagulant pathways are defective in severe sepsis and DIC, steps to restore these pathways by administering these anticoagulants, coagulation inhibitor concentrates or recombinant anticoagulant factors could markedly improve survival and reduce the rate of multiple organ failures[30]. A clear understanding of the role of TFPI, TAFI, APC, thrombomodulin and interaction of TFPI in modulation of TAFI or vice versa should help untangle the complex pathophysiology of severe sepsis[31]. Furthermore, antithrombin has been shown in vitro to not only increase prostacyclin

responses but also to inhibit endotoxin induced calcium fluxes in monocytes, and, to inhibit nuclear translocation of NFkB, an important step in the generation of inflammatory response. These natural anticoagulants in some animal models have been shown to inhibit endotoxin/*Escherichia coli*-mediated leukocyte activation and to diminish elaboration of TNF-α, IL-6 and IL-8. While the phase III clinical trial of TFPI (OPTIMIST) failed, APC has shown promising results in a recently completed phase III clinical trial, trials with antithrombin were not successful[32,33]. Severe sepsis has a complex pathophysiologic mechanism, the successes and failures of each of these trials have to be critically evaluated. Until an ideal treatment regimen for the management of patients with severe sepsis is established, each anticoagulant, even those that have been shown to be unsuccessful in clinical trials have to be critically evaluated in combination with other active agents. Each agent will be discussed below to evaluate its true potential either alone or in combination with other agents.

Coagulation leads to fibrin deposition and platelet activation, eventually contributing to activation of the leukocytes. Leukocytes are found in high numbers in venous thrombi. The leukocytes and activated platelets can form rosettes mediated by P-selectin expression on the activated platelets[34,35]. Prevention of this interaction between inflammatory cells and platelets resulted in inhibition of both arterial and venous thrombosis in animal models[36,37]. Activation of the endothelium due to thrombin results in increased leukocyte adhesion due to P and E-selectin expression[34,38]. Thrombin is an agonist for the formation of PAF and the adherent neutrophils on the endothelium are vulnerable to the action by PAF, resulting in the release of proteases and oxidants which might increase the damage to the endothelium by various proteases and oxidants[39]. Furthermore, the F VIIa-TF complex and F Xa have been shown to activate cells through protease activated receptors thereby generating cellular responses similar to those initiated by thrombin activation of protease receptor 1. Thrombin also activates TAFI. Thus, inhibition of targets such as F VIIa, F Xa or thrombin may suppress the inflammatory response which plays a key role in the pathophysiology of severe sepsis. Since F VIIa and F Xa can also activate the cells in the absence of thrombin, it might be better to inhibit the coagulation cascade at the top in order to limit the coagulation mediated inflammatory response[28]. Natural anticoagulants have shown in animal models of sepsis that they not only inhibit the inflammatory response but also show anticoagulant activities[40-42]. Inhibition of the functions of these natural anticoagulants can potentially activate the coagulation as well as inflammatory responses[43]. It is crucial to know whether or there are any independent effects of these natural anticoagulants on the inhibition of inflammatory responses other than those of coagulation-mediated cellular activation.

ANTITHROMBIN

Antithrombin levels in sepsis are seen to decrease by 50% of normal[44]. The fact that antithrombin can protect healthy animals from the adverse effects of bacterial infusion prompted the protocol for replacement therapy[41]. A large phase III clinical trial failed to demonstrate the beneficial effects of antithrombin. Evaluation of this trial to determine reasons for failure is necessary. Antithrombin binds to heparin-like proteoglycans on the endothelial cell surface not only facilitates inhibition of thrombin, but has also been reported to induce prostacyclin formation[45,46]. The reasons of failure of this phase III clinical trial could be the inadequate dosage of antithrombin which does not form prostacyclin in adequate amounts as higher doses are required for prostacyclin synthesis. Secondly, perhaps there was saturation of the antithrombin binding to heparin-like proteoglycans on the endothelial cell surface. Thirdly, perhaps the other plasma proteins might have minimized the protective and beneficial effects of antithrombin. In a large clinical trial antithrombin should again be evaluated in combination with other natural anticoagulants. Based on this concept of combination therapy it is necessary to understand the mechanism of action of antithrombin to evaluate its true potential. Antithrombin inactivates not only thrombin but also F IXa, F Xa and F VIIa bound to tissue factor. Heparin-like proteoglycans present on the endothelial cell surface help acce-

lerate these reactions. At high doses, antithrombin may prevent coagulation-mediated activation of cells thereby limiting expression of adhesion molecules, cytokines and PAF[41]. Antithrombin can cause inhibition of leukocyte adhesion and alterations in vascular permeability[47-49]. Since bacterial toxins compete with heparin-like proteoglycans on the endothelial cell surface for binding to antithrombin, it could be used to modulate the sepsis response[50]. Antithrombin given to baboons challenged with *E. coli,* were found to have significantly decreased levels of IL-6 and IL-8 and IL-10[51]. Antithrombin (AT) being the main inhibitor of thrombin and F X, but other serine proteinases including F IX, F XI, F XII, plasma kallikrein, uPA, tPA and plasmin are also inactivated by AT[52]. Low AT levels in septic shock are predictive of a fatal outcome[53]. Low AT could result from consumption, degradation of elastase released from neutrophils and extravascular leakage due to increased vascular permeability[54]. Baudo et al., recently concluded from a double-blind, randomized, multicenter study of 120 patients receiving ATIII or placebo, that AT reduces mortality only in a subgroup of septic shock patients[55]. Inthorn et al, reported that prolonged treatment with AT minimizes the systemic inflammatory response resulting in decrease of IL-6 in patients with severe sepsis. Giudici et al, reported on the results of a double-blind placebo-controlled study and concluded that within 30 days of treatment with AT, an increased survival of patients suffering from severe sepsis was noticed[56].

TISSUE FACTOR PATHWAY INHIBITOR

TFPI is a proteinase inhibitor containing three kunitz type domains[57]. The first domain combines with F VIIa and inhibits it. The second kunitz type domain combines with F Xa and inhibits it. The function of the this domain is not completely understood. In normal conditions, TFPI is expression is restricted to megakaryocytes, to small capillary endothelium and to macrophages. TFPI blood levels are reported to increase during inflammation[58]. Despite a modest increase of TFPI in sepsis, a significantly higher concentration (perhaps 10 fold) of TFPI is needed to inhibit the uncontrolled activation of the extrinsic pathway of coagulation[59]. In rabbit and baboon sepsis models, different variants of recombinant TFPI demonstrated increased survival[60-63]. However, in pigs, although there was an attenuation of the response TNF-α and IL-8, there was no significant increase in the survival[64]. Park et al. have shown that TFPI binds to endotoxin, thereby depressing the cellular responses to bacterial cell wall and other components[65]. Although, in humans, TFPI infusion resulted in attenuation of thrombin generation, the initiation of fibrinolysis and release of cytokines, TNF-α and IL-6 were not affected[66]. The TFPI: Xa: VIIa: TF quarternary inhibitory complex blocks protease activated receptors 2[67]. Cell activation by TF-VIIa complex can trigger a) upregulation of EGR-1, b) activation of the mitogen-activated protein kinase, c) in vitro intracellular calcium flux d) in vivo expression of reactive species and adherent molecules[68-71]. The exact mechanism of action of TFPI is still to be learned. Phase III trials were not successful.

ACTIVATED PROTEIN C (APC)

The protein C pathway prevents microvascular thrombosis and neonatal purpura fulminans is reversed by the administration of purified protein C[72,73]. Thrombin binds to thrombomodulin (TM) on the vascular endothelium and results in higher concentration of TM especially in the microcirculation where protein C activation takes place[74,75]. Protein C activation is enhanced by its binding to the endothelial cell protein receptor (EPCR)[76-79]. It has been shown earlier that inhibition of protein C binding to EPCR results in a 90% decrease in the ability of thrombin to activate protein C as a response to infusion of thrombin[42]. The protein C and APC bind to EPCR. The APC as long as it is bound to soluble EPCR is not an anticoagulant since EPCR blocks APC binding to lipid surfaces and perhaps also due to change of specificity of APC[80,81]. APC after dissociating from EPCR, binds to protein S and this complex inactivates F Va and F VIIa. F V serves as an additional cofactor in the inactivation of F VIIIa by APC[82].

It is very important to understand the exact mechanism of how APC inhibits inflammation. Endotoxin interacts with CD14 facilitating signaling

through the toll receptors and generating several signals which activates the cell. APC in complex with EPCR and interacting with a cell surface receptor generates signals which block calcium influx into the cell and translocation of nuclear factor kB (NFkB). APC bound to EPCR can undergo nuclear translocation and can modulate gene expression profiles, to enable the cell to facilitate inflammatory responses[42]. Experiments in animals have demonstrated that thrombin infusion protected the animals from *E. coli* infusion as a result of thrombin activating the protein C[83]. However, in baboons, direct infusion of APC, protected them from the lethal effects of *E. coli* infusion[84]. However, when protein C, protein S and EPCR were blocked, the infusion of *E. coli* infusion became a lethal event[84-86]. In several rodent models of sepsis, APC reduced not only IL-6, IL-8 but also decreasing the levels of TNF-α in circulation and tissues[87-90]. Hancock et al found a binding site for APC on monocytes and after binding it blocks the rise of intracellular calcium and other responses[91]. Inhibition of the signaling response was consistent of protein S. APC can block endotoxin-induced NFkB nuclear translocation[92]. Since elevation in adhesion molecules and generation of inflammatory cytokines often require NFkB nuclear translocation, its blockade by APC administered to endotoxin treated animals resulted in inhibition of TNF expression and decrease in leukocyte activation. Recently it has also been established that APC can prevent endotoxin-induced expression of tissue factor on monocytic cell lines in an EPCR dependent manner[93]. While protein C has no biological activity, APC has shown to be antithrombotic, profibrinolytic and antiinflammatory[94-96]. Protein C, the inactive precursor of vitamin K-dependent serine protease APC, circulates in healthy adults at a concentration of approximately 4000-5000 ng/mL (@ 70.000 pM), whereas the circulating concentration of APC is approximately 1-3 ng/mL (@ 35 pM)[97-99]. Hence, protein C is normally circulating in the body approximately 2000 fold higher than APC. The circulatory half-life of protein C in humans is about 10 hours while that of plasma derived APC or recombinantly produced APC is only about 20 minutes[100-103]. The decreased half-life of APC is as a result of inhibition of APC by several plasma serine protease inhibitors such as, α-1 antitrypsin, α-2 antiplasmin and PAI-1[104-106].

Assay Methods for Protein C and APC
Commercial kits to measure antigenic levels (using plasma prepared from anticoagulated blood samples) and functional activity of protein C are available (using citrated plasma samples). In both of these assays, protein C has to be converted to APC with snake venom, and the activity of APC is measured by either an activated aPTT-based or an amidolytic-based assay. The protein C functional activity measurements using citrated plasma can be performed in automated coagulation instruments. Considerable data has accumulated on the levels of protein C due to ready availability of these assay methods and instruments[102,103,107-109]. However, no commercial kits are available for measurement of APC levels, despite publication of several methods[98,99,110,111]. These methods involve several steps requiring several hours to several weeks to perform with no commercial supply of the reagents. For direct quantitative measurements of APC levels, since APC is irreversibly inactivated by several plasma serine proteases, blood samples are collected with the reversible inhibitor of APC, benzamidine in addition to citrate. The blood samples are immediately centrifuged to collect plasma which is frozen at -70°C. In the presence of benzamidine, APC is immunocaptured with a monoclonal antibody that blocks its active site. The excess of plasma and benzamidine are removed and the amount of APC is measured by its ability to hydrolyze the chromogenic peptide substrate (amidolytic activity) generating a yellow color. Bauer et al and Espana et al have developed methods which can quantitate the levels of APC indirectly[110,111].

Esmon reported that in the absence of disease with normal functioning endothelium, the conversion of protein C to APC by thrombin-thrombomodulin is dependent on the circulating levels of protein C[112]. Thrombin generation alters the relationship between protein C and APC plasma levels. Hanson et al reported that infusion of low concentrations of thrombin in healthy

baboons increased their APC levels from a baseline of 5 ng/mL to 250 to 500 ng/mL (representing 5000% to 10.000% increase) while endogenous protein C levels decreased by only 15% to 30%[113]. Likewise, prothrombotic states such as aging, F V Leiden, and localized vessel occlusion, where there is increased generation of thrombin, without generalized endothelial dysfunction, a less than two fold increase of APC from normal baseline was observed[110,114,115]. There was a positive correlation between the increase of APC with markers of thrombin generation such as prothrombin fragment F1.2, Thrombin-antithrombin (TAT) complexes or fibrinogen fragment A[111,116-118].

In severe sepsis, the generalized systemic response as a result of infection includes, activation of inflammatory pathways, activation of coagulation pathway, impairment of fibrinolytic pathway and the interaction of coagulation and inflammatory response, leading to generalized systemic endothelial dysfunction, microvascular thrombosis and multiple organ failure [119-121]. In severe sepsis, 80% of the patients have protein C levels which are below the normal limits[122-125]. Low protein C levels in sepsis relates to poor prognosis[103,109,126]. It was thought that low protein C levels in severe sepsis were due to increased conversion of protein C to APC which has a much shorter circulatory half-life, leading to consumption of protein C. It was also assumed that if circulating levels of protein C were restored to normal by infusion of exogenous protein C, there could be a reduction of morbidity and mortality from sepsis. This formed the basis of a number of clinical trials and an ongoing small, placebo-controlled trial of severe sepsis, where exogenous protein C was administered to restore the normal circulating levels of protein C, with the assumption that the vasculature in patients with severe sepsis could adequately convert protein C to APC[127-139]. Only recently, the data started emerging on the levels of APC in experimental animals and humans which provides rationale to support the hypothesis of protein C replacement therapy. Recent data show quite the opposite that treatment with protein C may not be appropriate in patients with severe sepsis. Taylor et al, recently, measured the levels of endogenous APC in baboons administered with colony forming units of *E.coli* intraperitoneally[140]. Some baboons recovered completely, some sustained illness for 2 weeks and some died within 48 hours of administration of *E. coli.* While there was a 50% reduction in protein C levels, there was a maximum of four-fold increase in the levels of APC. There was no correlation between the decrease of protein C levels, increase of APC levels and increase of the thrombin generation markers such as TAT. On the contrary, healthy baboons infused with low doses of thrombin showed a persistent 50-100-fold increase in endogenous APC over the baseline with a 15 to 30% decrease in endogenous protein C levels.

In phase II and phase III clinical trials of severe sepsis with drotrecogin alfa (activated), a recombinant human APC, endogenous APC levels in the placebo group during the first 2-4 days of the study and majority of placebo-treated patients with severe sepsis, did not increase above the baseline level of 5 ng/mL, while the remaining placebo-treated patients had levels between 5-20 ng/mL[122]. In the double-blind, placebo-controlled phase III clinical trial of recombinant human activated protein C Worldwide Evaluation in Severe Sepsis (PROWESS), the levels of APC in the placebo-treated arm was similar to the placebo-treated arm of the phase II study[124]. In the drotrecogin alfa treatment group with infusion for 96 hours, patients showed a 20-fold increase in the APC levels over the baseline and a significantly decreased mortality when compared to the placebo group was observed[124].

ROLE of THROMBOMODULIN in SEVERE SEPSIS

It has been shown in in vitro experiments that endotoxin and TNF-α can decrease endothelial surface thrombomodulin by decreasing synthesis or increased degradation [141-143]. Endothelial surface thrombomodulin may be cleaved and released in the circulation is soluble thrombomodulin[144]. Boffa et al reported on correlation between increase in circulatory soluble thrombomodulin in diseases with endothelial dysfunction[145]. Although elevation of soluble thrombomodulin has been demonstrated in animal models of sepsis

and also in patients with sepsis by Dhainaut JF (unpublished), the reduction in endothelial surface thrombomodulin in animal models of sepsis has not been demonstrated[140,146-153]. The EPCR augments the conversion of protein C to APC via thrombin-thrombomodulin as shown in in vitro experiments. Endothelial surface levels of thrombomodulin and EPCR were reduced in skin biopsy samples from in a majority of patients with meningococcal septicaemia. This suggests that in patients with severe sepsis, there is insufficient endothelial surface thrombomodulin and EPCR for conversion of protein C to APC[154]. Faust SN and colleagues are evaluating the endogenous levels of APC in meningococcemic patients treated with protein C[155]. This study would establish whether or not the decrease in endothelial surface thrombomodulin and EPCR results in impairment of conversion of protein C to APC.

INTERACTIVE ROLE of THROMBIN-THROMBOMODULIN COMPLEX, TFPI, PROTEIN C, APC, TAFI and FIBRINOLYTIC PATHWAYS in SEVERE SEPSIS

Tissue factor induced thrombin generation is downregulated by TFPI and the functional protein C pathway[156]. Thrombin-TM complex links coagulation with the fibrinolysis by thrombin activatable fibrinolysis inhibitor (TAFI)[157,158]. In severe septic state increased thrombin and reduced APC inhibition of thrombin generation, leading to increased thrombin levels promote TAFI activity thereby inhibiting fibrinolysis[159]. When the TM levels are increased the TAFI activity is reduced and when the TM levels are decreased the TAFI activity is promoted and there is more fibrinolytic deficit[160]. Protein C also combines with PAI-1 to prevent inhibition of fibrinolysis. In sepsis there is reduced protein C/APC activity. That formed the basis of administering protein C to patients with meningococcal septicaemia where changes in TM and EPCR results in purpura fulminans[161-163]. Maruyama demonstrated in rodent and primate models of TF-induced DIC that recombinant soluble TM may prevent DIC even when the AT levels are low[164].

TAFI is a carboxypeptidase, an enzyme that hydrolyzes C-terminal peptide bonds, and upon activation by thrombin, downregulates fibrinolysis. The carboxypeptidase activity is not present in plasma but appears only after clotting of blood. TAFI is an inactive zymogenic form of carboxypeptidase and the active enzyme is designated as TAFIa. TAFI is synthesized in the liver and circulates in the plasma at a concentration of 4-15 µg/mL[165,166]. Activation of TAFI by trypsin, plasmin, thrombin or meizothrombin occurs by a cleavage at Arg-92. Although, sufficient proof is lacking TAFI is suggested to circulate in a complex with plasminogen[167]. Activation of TAFI reduces the affinity for Glu- and Lys-plasminogen by approximately 10-fold[168]. It has also been observed that a2-antiplasmin and e-amino caproic acid reduced TAFI binding to plasminogen[168]. TAFIa is also inhibited by EDTA, 2-mercaptoethanol, peptide inhibitor from a leech, Hirudo medicinalis[169-171]. TAFIa because of its molecular mass could easily be eliminated however, it remains in the circulation in a noncovalent complex with α-2 macroglobulin[172]. The half-life of TAFIa is 10 minutes and increases with decreasing temperatures and is stable at 0°C[170]. Its stability is achieved also by e-amino caproic acid and heparin[168,173,174]. TAFIa including its mutant form are inactivated by thrombin-thrombomodulin[174]. TAFI expression is influenced by inflammatory response in the body. The human TAFI cDNA has been isolated and characterized[175]. TAFI was also expressed upon stimulation of the platelets (Mosnier, Buijtenhuis, Marx, Meijers, Bouma, 2000, Submitted for publication). TAFI might protect the clot in early stages by increased levels of TAFI during platelet plug formation. TAFIa inhibits fibrinolysis by cleaving carboxy-terminal lysine residues from fibrin, limiting the formation of plasmin[176-178]. While Eaton et al identified TAFI as a contaminant during the purification of α2-antiplasmin, Bajzar et al isolated the protein from plasma in search of something which provides an explanation for the profibrinolytic effect of APC[175,176]. The role of TAFI in fibrinolysis involves activation of TAFI to TAFIa by plasmin and inactivation of plasmin by TAFIa and inactivation of TAFIa by plasmin[173,178].

The role of TAFI in inflammatory disease was suggested when it was identifies as an acute phase reactant[179,180]. Thrombin at high concentrations not only increase TAFI generation but also increased formation of TAFIa. Increased TAFI levels during inflammation results in increased prothrombotic and antifibrinolytic state seen in DIC. Increased levels of soluble TM seen in DIC stimulate the TAFI activation[181]. Hackeng et al demonstrated the endotoxin-induced downregulation of protein C mRNA[182]. Endotoxin also downregulates protein C antigen, which is decreased progressively during early stages of DIC in humans suggesting decreased inhibition of TAFI activation by protein C[165,183]. Increased TAFI levels during inflammation can either inactivate the inflammatory mediators such as C3a and C5a in order to reduce susceptibility to septic shock or cause increased inhibition of fibrinolysis as DIC progresses. Further studies are needed to demonstrate which mechanism functions at which particular time. The role of antithrombin drugs needs to be established as they could cause decreased TAFI levels resulting in decreased inhibition of fibrinolysis. Besides the antithrombin drugs, the role of F Xa inhibitors in inhibiting F Xa at a higher step in the coagulation cascade so as to block the generation of thrombin have to be evaluated.

ROLE of OTHER ANTICOAGULANTS in SEVERE SEPSIS

As discussed earlier, severe sepsis is manifested by microvascular thrombosis resulting in multiple organ failure. This is a serious challenge to a coagulationist despite the availability of scores of antithrombotic agents to prevent the formation of new clots; antiplatelet agents to counteract future platelet plug formation and thrombolytic drugs to lyse the prevalent clots. Although clinical trials have been performed on very select antithrombotic agents such as antithrombin, TFPI, thrombomodulin and APC, each antithrombotic, antiplatelet and thrombolytic agent has a potential role to play in the management of severe sepsis. Each class of antithrombotic, antiplatelet and thrombolytic agents have certain unique advantages over other classes of respective kinds of agents. For the drugs which are already approved by FDA, the physician has to carefully select among the different kinds of antithrombotic drugs available. Carefully conducted clinical trials for the other emerging antithrombotic drugs would help evaluate their potential in the management of severe sepsis. Different classes of antithrombotic agents will be discussed for they definitely have a potential to be used in severe sepsis, to intervene and inhibit various steps of the coagulation cascade. Further understanding of the pathophysiology of severe sepsis will unfold the need for specific kinds of antithrombotic, antiplatelet or antiinflammatory and thrombolytic agents.

NEW ANTICOAGULANT DRUGS

Inhibition of thrombogenesis is focused on inhibiting thrombin, preventing thrombin generation or inhibiting initiation of coagulation. Drug-development strategies involve inactivation of targeted coagulation factors such as thrombin, F Xa, F IXa, F VIIa/TF complex and enhancing endogenous anticoagulant pathways or promoting fibrinolysis. Thrombin generation is important in arterial and thrombotic disorders such as acute coronary syndromes. It is reported that tissue factor mRNA within atherosclerotic plaques is increased compared with the normal arterial wall and is upregulated after vascular injury[184,185]. Thrombin generation is a trigger for thrombus initiation and further growth. Teitl and Rosenberg have earlier reported that arterial thrombi express F Xa and F Va activity which is protected from inhibition mediated by antithrombin-dependent mechanisms[186]. In most pathologic conditions endogenous thrombin-mediated platelet activation precedes prothrombinase complex assembly as reported by Miletich et al[187]. Thus inhibition of thrombin and its generation is one of the key targets for development of new anticoagulant drugs (Table 1). Various anticoagulant drugs in different phases of clinical development are mentioned in Tables 2-6. The scope of the new anticoagulant drugs and their characteristics are mentioned in Tables 7-14.

THROMBIN INHIBITORS

Thrombin can be inhibited indirectly by endogenous antithrombin or heparin cofactor II or directly by drugs that bind to thrombin thereby preventing its interaction with substrates.

INDIRECT THROMBIN INHIBITORS

Heparin, the most widely used intravenous and subcutaneous anticoagulant is a glycosaminoglycan composed of a mixture of polysaccharide (14-100 disaccharide units) and has the mean molecular weight of 15.000 daltons. It combines with antithrombin (AT) causing a conformational change in its active center, and accelerates the formation of thrombin-AT complexes several thousandfold. Once thrombin is neutralized, heparin is released from the complex and combines with another antithrombin molecule. Heparin with more than 24 disaccharide units inhibits thrombin through the interaction with AT III and with heparin co F II. However, heparins with fewer than 18 disaccharide units cannot adequately bind thrombin and antithrombin simultaneously. The major limitations of unfractionated heparin (UFH) besides bleeding, osteoporosis and alopecia include the following.

1. Heparin is an indirect thrombin inhibitor and requires antithrombin for its action.

2. Heparin's anticoagulant kinetics is initially nonlinear because of binding to different receptors and plasma proteins; the dose of heparin to saturate these recptors varies among the individuals.

3. Patients may show heparin resistance because of limited antithrombin levels or availability. The platelet F IV released from activated platelets interferes with the binding of heparin to antithrombin.

4. Heparin cannot inactivate clot-bound thrombin.

5. F Xa in the prothrombinase complex is also inaccessible to heparin for neutralization.

6. Heparin can induce platelet aggregation, possible by generating thromboxane A2 and by potentiating platelet response to adenosine diphosphate (ADP) and epinephrine.

7. Heparin-induced thrombocytopenia (HIT) and thrombosis syndrome (HITTS) develop in some patients.

8. Heparin has a circadian anticoagulant effect-this has not been confirmed.

9. Heparin has a narrow therapeutic window and laboratory monitoring is required.

The major thrust of these limitations of UFH led to the development of LMWHs and other drugs. There are several advantages of LMWHs over UFH including better bioavailability through subcutaneous administration, lower incidence of HIT or HITTS, predictable anticoagulant response, and higher antithrombotic but lower hemorrhagic potential.

The LMWHs, obtained through chemical or enzymatic depolymerization of the benzylic esters of porcine intestinal mucosal heparin have a partial effect on thrombin but mainly inhibit F Xa. Heparins augment the activity of AT and neutralizes the activated forms of coagulation F X, F II, F XII, F XI, F IX and TF-VIIa complex. Despite the limitations of UFH, it continues to be used since its complete potential is still to be unravelled. UFH and LMWHs are used for the prophylaxis and treatment of venous thrombosis and as adjuncts to antiplatelet drugs and thrombolytic drugs for the treatment of Acute Coronary Syndrome (ACS). Since monitoring of LMWHs is not considered necessary, they may be used for out-of-hospital treatment[188]. At prophylactic dosages, LMWHs cannot be monitored by the aPTT or any other clotting test. At higher dosages of LMWHs the activated clotting time (ACT) is sensitive. The amidolytic AXa assay has sufficient sensitivity but is available for research and in specialized coagulation laboratories.

LMWHs are gradually replacing UFH for the treatment of venous thrombosis. LMWHs are indicated for:

1. For prevention of DVT which may lead to pulmonary embolism.

2. In patients undergoing hip replacement surgery, during and following hospitalization.

3. In patients undergoing knee replacement surgery.

4. In patients undergoing abdominal surgery who are at risk for thromboembolic complications.

5. For inpatient treatment of acute DVT with or

without pulmonary embolism, when administered in conjunction with warfarin sodium.

6. For the outpatient treatment of acute DVT without pulmonary embolism when administered in conjunction with warfarin sodium.

7. For the prevention of ischemic complications of unstable angina and non-Q-wave myocardial infarction, when concurrently administered with aspirin[188].

Recent drug delivery systems have made it possible to give UFH and LMWHs orally by utilizing synthetic amino acids such as sodium N-(8[2-hydroxybenzoyl]amino) caprylate (SNAC) which facilitates heparin absorption by the gut[189]. Following phase I and phase II studies, phase III studies are now underway to compare SNAC/heparin with LMWH for thromboprophylaxis in patients undergoing elective hip or knee arthroplasty[190,191].

Dermatan sulfate is a glycosaminoglycan (GAG) that acts as an antiticoagulant by activa-

Table 1. Anticoagulant drugs launched

Agent	Site	Company	Status
Enoxaparin	Xa, IIa	Aventis, USA	Launched
Fraxiparin	Xa, IIa	Sanofi, France	Launched
Dalteparin	Xa, IIa	Pharmacia, Sweden	Launched
Heparin (Novo)	Xa, IIa	Novo Nordisc, DM	Launched
Heparin (Opocrin)	Xa, IIa	Opocrin, Italy	Launched
Reviparin	Xa, IIa	Knoll, Germany	Launched
Oversulfatd LMWH	Xa, IIa	Iketon, Pharm, Italy	Launched
AT Green cross	IIa	Green Cross, Japan	Launched
Antithrombin (Kabi)	IIa	Pharmacia, Sweden	Launched
Argatroban	IIa	Mitsubishi, Kasei, Japan	Launched
CGP 16056		Ciba-Geigy, Swiss	Launched
Hirudin Hoechst	IIa	Hoechst, Germany	Launched
Bivalirudin	IIa	Medicines Co, USA	Launched
Mesoglicano		Mediolanum, Italy	Launched

Table 2. Anticoagulant drugs in phase III clinical trials

Agent	Site	Company	Status
H376/95	IIa		Phase III
Dermatan SO4, (OP)	IIa	Opocrin, Italy	Phase III
Antithrombin, CSL	IIa	CSL, Australia	Phase III
Hirudin, Ciba-Geigy	IIa	Ciba-Geigy, Swiss	Phase III
TFPI	VIIa/TF		Phase III
APC	Va,VIIIa		Phase III
Pentasaccharide	Xa	Sanofi, France	Phase III

Table 3. Anticoagulant drugs in phase II clinical trials

Agent	Site	Company	Status
DX9065a	Xa	Daiichi, Japan	Phase II
Inogatran	IIa	Astra, Sweden	Phase II
ART-123		Asahi Chemical, Japan	Phase II
MB-015		Moichida, Japan	Phase II
RO-46-6240		Roche, Switzerland	Phase II
NAP-c2	IIa		Phase II
SNAC-heparin (solid formation)		Emisphere	Phase II
SNAD-heparin (liquid formulation)		Emisphere	

Table 4. Anticoagulant drugs in phase I clinical trials

Agent	Site	Company	Status
HV-1		Japan Energy, Japan	Phase I
CX-397		Japan Energy, Japan	Phase I

Table 5. Anticoagulant drugs in preclinical stage

Agent	Site	Company	Status
GM-1630		Ligand Pharm'l US	Preclinical
GS-522		Gilead Sciences US	Preclinical
LEX-026		Lexin Pharm'l US	Preclinical
Antithrombin Genzy	IIa	Hoechst, Germany	Preclinical
Bacithrocin A		Roche, Switzerland	Preclinical
LY-293435		Lilly, US	Preclinical
Hirutonins Biochem		Biochem Phm, CAN	Preclinical
Thrombin Inhibitors		Pentapharm, Swiss	Preclinical
SDZ-MTH-958		Novartis, Switzerland	Preclinical
C 186-65		COR Therap'cs US	Preclinical
Corthrombin compd		CORVAS, US	Preclinical
Heparin Oral PD	Xa, IIa	Pharm'cal Disco, US	Preclinical
CVS-995		CORVAS, US	Preclinical

Table 6. Anticoagulant drugs-registered or preregistered

Agent	Site	Company	Status
Antithrombin Bayer	IIa	Bayer, Germany	Registered
Dermatan Sulfate Mediolanum	IIa	Mediolanum, Italy	Preregistered

Table 7. Scope of new anticoagulant drugs

Heparin related drugs
Low molecular weight heparins
Medium molecular weight heparins
High molecular weight heparins
Chemically modified heparins
Dermatans
Heparans
Semisynthetic heparin derivatives (Suleparoid)
Chemically synthesized antithrombotic oligosaccharides
Sulfated dextrans
Synthetic hypersulfated compounds
Polyanoinic agents
Marine polysaccharides
Antiplatelet drugs
Ticlopidine & related antiplatelet drugs
Platelet & related phosphodiesterase inhibitors
Prostanoid modulators (Iloprost)
Eicosanoid & related drugs
w-3 fatty acids & fish oil related products
Antibodies targeting membrane glycoproteins
Peptides and proteins modulating platelet function
Endothelial modulators
Nucleic acid derivatives (Defibrotide)
Sulfomucopolysaccharide mixtures
1-Deamino-8-D-arginine vasopressin (DDAVP) and related peptides
Growth factor-related peptides
Protein digests
Vitamins
Viscosity modulators
Synthetic and natural polymers
Pentoxifyline
Venoms (defibrinating agents)
Polyelectrolytes
Biotechnology-based products
Tissue type plasminogen activator & mutant
Hirudins, mutants and fragments
Activated protein C
Thrombomodulin-thrombin complex
Biotechnology Based Proteins
Antithrombin III
Antithrombin III-heparin complexes
Recombinant heparin cofactor II
Glycoprotein targeting proteins & peptides
Protease-specific inhibitors
Recombinant TFPI
Peptides and related antithrombotic peptides
Hirulogs
D-Me-Phe-Pro-Argderived antithrombotics
Argatroban
Inogatran
Borohydride derivatives
Synthetic inhibitors of thrombin
Peptide inhibitors
Heterocyclic conjugates
Nucleic acid derivatives (Defibrotide)
Others
Recombinant inhibitors of thrombin
Hirudin and related proteins
Site-specific proteins
Others
Polytherapy
Heparin and antiplatelet drugs
Coumadin and antiplatelet drugs
Thrombolytic agents and heparin
Thrombolytic agents and antiplatelet drugs
Recombinant drugs and conjugates
Thrombolytic agents and hirudin
Hirudin & Glycoprotein-targeting antibodies
Thrombolytic agents, hirudin and other thrombin inhibitors
Newer drug-delivery systems and formulations
Target-specific antithrombotic drugs (antibody-directed)
Catheters and devices capable of targeted Drug delivery

Table 8. Three generations of thrombolytic agents

First generation
Streptokinase
Urokinase
Second generation
Recombinant tissue plasminogen activator (rtPA, alteplase, Duteplase)
Anisoylated plasminogen streptokinase activator complex (APSAC, Anistreplase).
Single-chain Urokinase type plasminogen activator (scu-PA, prourokinase)
Third generation
Vampire bat salivary plasminogen activator
Reteplase (rPA)
TNK-tPA
Tenecteplase
Lanoteplase (n-PA)
Staphylokinase
Recombinant glycosylated plasminogen activator
Thrombolytic drugs under development
Antibody-targeting thrombolytic agents
Polyethylene glycol-coupled thrombolytic agents
Mutants and variants of plasminogen activator
Recombinant chimeric plasminogen activator (Fibrolase)

Table 9. Characteristics of first generation thrombolytics

Characteristics	Streptokinase	Urokinase
Source	Gr C Streptococci	Recombinant, human fetal kidney
Molecular Weight (Kd)	47	35-55
Immunogenecity	Yes	No
Mode of action	Forms an activator complex	Direct
Plasma half-life (min)	18-23	14-20
Metabolism	Hepatic	Hepatic
Dose	1.5 million Units	3 million Units
Cost per dose	$300	$2000

ting heparin cofactor II has been reported to be more effective than low-dose heparin for thromboprophylaxis in cancer patients[192,193]. The developmental status of glycosaminoglycan derived drugs are mentioned in Table 15. Currently several dermatan sulfates are under development for the prophylaxis of thromboembolism. While similar in structure to heparin, these agents do not produce any effects on platelets. Furthermore, they are poorly absorbed after subcutaneous administration, although recently, some LMW dermatans are produced which unlike dermatan sul-

Table 10. Characteristics of second generation thrombolytics

Character	APSAC	RtPA	Scu-PA(saruplase)
Source	Gr C Streptococci plasminogen anisoylated	Recombinant human	Prodrug from a naturally occuring physiologic protease
Molecular Weight (Kd)	131	63-70	49
Immunogenecity	Yes	No	No
Mode of action	Direct	Direct	Direct
Fibrin specificity	+	++	+
Plasma half-life (min)	70-120	4-6	9
Metabolism	Hepatic	Hepatic	Hepatic
Dose	30 units IV over 2-5 minutes	15 mg bolus + 90 min infusion	20 mg bolus + 60 mg infusion for 1 hour.
Cost per dose	$2400	$2200	$2100

Table 11. Characteristics of third generation drugs

Characteristic	r-PA	n-PA	TNK-tPA	Vampire bat PA	Staphylokinase
Source	Recombinant, human mutant type PA	Chinese Hamster ovary cells	Variant of tPA-rearranging gene sequence	Saliva of Desmodus rotundus	PA of bacterial origin-strains of *Staphylococcus aureus*
MW (Kd)	39	39	39	52	15.5
Immunogenecity	No	?	No	Yes	Yes
Mode of action	Direct	Direct	Direct	Indirect	Indirect
Fibrin specificity	Yes	+	+++	+++	+++
Plasma half-life (min)	14	37	20	170	6
Metabolism	Renal	Hepatic	Hepatic	Hepatic	Hepatic
Dose	20 million Units	120.000 U/kg single bolus	0.5 mg single bolus	0.5 mg single bolus	1.5 mg + 15 mg double bolus over 30 minutes

fate (Organon) are absorbed subcutaneously. Heparan sulfates have been developed as prophylactic antithrombotic agents. These agents are homogeneous and contain other chondroitin sulfates. They bind to AT and HCII but to a lesser degree than heparin, and they are weakly anticoagulant. Thus, large doses of heparan are needed for effective antithrombotic treatment. Depolymerized heparans have better bioavailability than the native heparans. A synthetic hypersulfated lactobionic acid amide, aprosulate (Luitpold) has been developed for prophylactic antithrombotic use. This agent produces its action via heparin cofactor II and by inhibiting protease generation. The bioavailability of this agent is better than that of dermatan and heparan sulfates. However, this product exhibits teratogenic potential and clinical trials have therefore been suspended.

A semi-synthetic sulfated pentomannan derivative PI-88, phosphomannopentaose sulfate (Progen Industries, Brisbane, Australia) has been shown to exhibit anticoagulant activity via heparin

Table 12. Classification of GPIIb/IIIa inhibitors

Structure	Receptor binding	Genetic name	Trade name	Company
Monoclonal antibody	Binds to GPIIb/IIIa receptor and inhibits binding of large adhesive ligands by steric blockade	Abcixmab	ReoPro	Centocor, Lilly
Peptide receptor antagonist	Competitive antagonist; binds specifically to the fibrinogen binding site	Eptifibatide	Integrelin	COR Therapeutics/ Scherring-Plough
Nonpeptide receptor antagonist/ peptidomimetic	Competitive antagonist; binds specifically to the fibrinogen binding site	Tirofiban	Aggrastat	Merck & Co.
Oral GPIIb/IIIa agents/ Peptidomimetic prodrugs	Competitive antagonist; bind specifically to the fibrinogen binding site	Lamifiban Xemillofiban Sibrafiban Orbofiban Lotrafiban RPR-109891 Roxifiban Lefradafiban		Roche Searle Roche/genentec Searle SK Beacham Aventis DuPont BoehIngelheim

Table 13a. Molecular and chemical characteristics of various LMWHs

LMWH	Characteristics
Enoxaparin nus	Presence of 4,5 unsaturated uronic acid at nonreducing termi-
Nadroparin	Presence of 2,5-anhydro-D-mannose at reducing terminus
Certoparin	Presence of 2,5-anhydr-D-mannose at reducing terminus
Dalteparin	Presence of 2,5-anhydr-D mannose at reducing terminus
Tinzaparin	Presence of 4,5 unsaturated uronic acid at nonreducing
Reviparin	Presence of 2,5-anhydro-D-mannose at reducing terminus
Ardeparin	Labile glycosidic bonds

cofactor II activation and TFPI release. This agent is being developed as a potential antitumor agent, having the important property of simultaneously being potent inhibitors of in vitro angiogenesis and heparanase activity. PI-88 inhibited the primary tumor growth of the highly invasive rat mammary adenocarcinoma by approximately 50%, inhibited metastases by approximately 40% and reduced the vascularity of tumors by approximately 30%. This agent is undergoing phase II clinical trials.

Many other glycosminoglycans are being developed for the prophylaxis of thromboembolism. Some of these represent mixtures of GAGs with varying molecular weight profiles. Noteworthy are Intimitan, Lomoparan and suleparoide which are depolymerized heparan preparations. These agents exert their antithrombotic actions via unknown mechanisms but are clinically very effective drugs. Additional synthetic heparinomimetics include synthetic oligosaccharides with high affinity to AT. More recently, mixed inhibitors of F Xa and F IIa have also been developed.

There have been significant developments in

Table 13b. A comparison of LMWH preparation

Agent	Axa/IIa ratio	USP U/mg
Enoxaparin	3.8	35
Nadroparin	2.3	34
Certoparin	2.3	46
Dalteparin	2.8	53
Tinzaparin	1.9	49
Reviparin	3.4	32
Ardeparin	2.0	51

Each LMWH is different and cannot be used interchangeably. The reasons why different LMWHs are not interchangeable are as follows:

1. Due to manufacturing procedures, different products have different physical and chemical compositions. This translates into the differences in biologic actions.

2. The amount of pharmacologically active (chemically active) material varies from product to product.

3. Clinical trials for specific indications on each product are carried out at optimised dosages for each product. Thus, a specific dosage used for individual products must be used.

4. Each drug is classified by the USFDA as a distinct drug and cannot be interchanged.

Table 14. Direct factor Xa inhibitors

Agent	Company	Chemical	Source	Status
ANTISTATIN	Merck Sharp & Dohme	Mexican leech protein (119 amino acids)	Recombinant	Suspended
YAGIN	Bio-Technology General	Medicinal leech protein (85 amino acids)	Animal derived	Not reported
TAP	Merck Sharp & Dohme	Tick protein (60 amino acids)	Recombinant	Preclinical
NAP-5	CORVAS	Hookworm protein	Recombinant	Preclinical
TFPI	Searle/Chiron	Human protein	Recombinant	Clinical
DX-9065a	Daiichi	Propanoic acid derivative	Synthetic	Phase II
SEL-2711	Selectide	Pentapeptide produced by combinational chemistry	Synthetic	Preclinical
YM-60828	Yamanouchi		Synthetic	Preclinical
BX-807834	Berlex	Peptidomimetic	Synthetic	Preclinical
KFA-1411	Kissei	Peptidomimetic	Synthetic	Preclinical
RPR-120844	Aventis (RPR)	Peptidomimetic	Synthetic	Preclinical
INDIRECT Xa INHIBITOR				
SR-90107	Sanofi	Oligosaccharide; requires binding to AT	Synthetic	Phase III

the area of nonheparin GAGs-derived products as antithrombotic drugs. It is no longer believed that a sulfomucopolysaccharide of natural origin must exhibit some interaction with AT to have effective antithrombotic properties. Several agents without this interaction produce therapeutic effects on the blood and vascular system[194-195]. Several mammalian GAG-derived drugs are currently being used in European countries as antithrombotic, antilipemic and antiatherosclerotic agents[196]. These agents represent mixtures of native sulfomucopolysaccharides or its derivatives obtained by depolymerization and/or fractionation. With an increase in knowledge of their structure and functional activity, preliminary pharmacological studies were carried out to determine the proper indications for individual drugs. The GAG-derived drugs are generally used as antithrombotic agents, however, several other indications such as atherosclerosis, stroke, hyperlipidemia and senile dementia are now being considered. A list of GAG-derived antithrombotic drugs are mentioned in Table 15. SP-54 (Hemoclar; Bene Chemical), a hypersulfated pentosan polysulfate with structural and functional characteristics similar to other sulfated GGS, is a plant (beech tree) product.

Danaparoid sodium is a depolymerized mixture of heparans, dermatans and other chondroitin sulfates and is undergoing clinical trials for the prophylaxis of DVT after general and orthopedic surgery. This agent is also being used in the prevention of ischemic complications associated with stroke. It is claimed to have a better safety/efficacy ratio than heparin, such that it produces minimal antihemostatic effects at antithrombotic doses[197]. MF-701 (Mediolanum Laboratories) is a heterogeneous mixture of dermatan sulfate of mammalian mucosal origin. Currently, it is being developed for prophylaxis against DVT after general and orthopedic surgery. Since the bioavailability of this agent via subcutaneous administration is rather limited, it is being administered intramuscularly and several clinical trials are ongoing with this agent. Suleparoide is a widely used semi-synthetic GAG that has been used for the prophylaxis of both arterial and venous thrombosis. OP-435 (Opocrin Laboratories) is extracted from bovine mucosa and is being developed for prophylactic antithrombotic usage in patients undergoing general surgery. There have been concerns over the safety and efficacy of the higher molecular weight dermatans such as MF-701 and OP-435 and as a result low molecular weight dermatan preparations have been introduced. One such preparation is Desmin (Alfa wasserman), which exhibits better bioavailability, and longer duration of action than the high molecular weight dermatans, is being developed for prophylactic antithrombotic use. MPS (Luitpold) represents a mixture of mucopolysaccharides obtained from mammalian trachea for the treatment of joint diseases. Only limited data is available on the structure activity relationship of this agent. This agent may have several applications as an antithrombotic agent. Some GAGS may prove to be useful as an alternative to heparin especially in HIT and HITTS. Some of the newer indications of these agents include antiinflammatory, antiatherosclerotic, for wound healing and as a treatment of AIDS, besides other indications such as Alzheimer's disease and as a cytoprotective agent[198-202]. Heparan sulfate has been studied in DVT, chronic venous insufficiency and intermittent claudication[198,201]. A pilot study was earlier completed using dermatan sulfate in acute leukemia to control disseminated intravascular coagulation (DIC)[203].

DIRECT THROMBIN INHIBITORS

Direct thrombin inhibitors in contrast to UFH can inhibit fibrin-bound thrombin, have more predictable anticoagulant response since they do not bind to plasma proteins, and are not neutralized by platelet F IV[204-207].The developmental status of different direct thrombin inhibitors is given in Table 16.

HIRUDIN

Hirudin, a 65 amino acid polypeptide originally isolated from the parapharyngeal salivary glands of a medicinal leech, Hirudo medicinalis, is now available through recombinant DNA technology[208]. It is the most potent and specific inhibitor of thrombin known and forms a 1:1 stoichiometric complex with this enzyme which is slowly reversible[209]. A number of derivatives and re-

Table 15. Glycosaminoglycan-derived antithrombotic drugs

Drug	Composition	Status
ORG 10172	Depolymerized mixture of GAGS	Ongoing clinical trials
MF 701	Mixture of native and depolymerized dermatans	Ongoing clinical trials
Suleparoide	Semi-synthetic GAG	Available for various indications
OP 435	Mixture of dermatans	Preclinical
OP 370	LMW dermatan	Preclinical
SP 54	Hypersulfated pentosan polysulfate	Preclinical
MPS	Depolymerized hypersulfated mixture of GAGS	Developed for animal use
Sulfomucopolysaccharide mixture	Mixture of GAGS	Clinically used

combinant preparations are now available, including Hirugen, a synthetic C-terminal peptide fragment of hirudin; Hirulog (bivalirudin), a derivative of hirugen. The various recombinant preparations are, desirudin (CGP 39393); lepirudin (HBW 023, Refludan); Polyethyleneglycol-coupled hirudin (PEG-hirudin) obtained by conjugating recombinant hirudin with two molecules of PEG; and albumin r-hirudin fused molecules. Both PEG hirudin and albumin r-hirudin fused molecules are known to have longer half life compared to r-hirudin. Hirudin has a plasma half-life of 40 minutes after intravenous administration and 120 minutes after subcutaneous administration and is cleared mostly by kidneys after undergoing little hepatic metabolism[210]. Recombinant hirudin has been used for prophylaxis of thrombosis and thromboembolic complications in patients with HIT and has been approved in the USA for this specific indication[211-213]. Hirudin has also been used as an alternate to heparin in HIT patients undergoing cardiopulmonary by-pass surgery, and was found to be superior to low dose heparin subcutaneous UFH or LMWH for thromboprphylaxis in patients undergoing elective hip arthroplasty without increasing the risk of bleeding[214-217]. Recombinant hirudin is found to be more effective than heparin in patients with unstable angina and non-ST-elevation myocardial infarction, although it increased the risk of bleeding in these patients. There was no increase in life threatening bleeding complications[218,219]. Hirudin is now being considered for approval in patients with unstable angina and non-ST-elevation myocardial infarction. The advantages of hirudin over UFH are given in Table 17.

Refludan (lepirudin-rDNA for injection was successfully used for anticoagulation and effectively monitored with Ecarin Clotting Time (ECT) in patients with HIT undergoing off-pump coronary artery revascularization[220].

BIVALIRUDIN

Coupling of the peptides that mimic the carboxyterminal of hirudin to peptides that are specific for inhibition of the catalytic site of thrombin (D-Phe-Pro-Arg) has led to the development of a semi-synthetic bivalent thrombin inhibitor, bivalirudin[221]. It is a specific thrombin inhibitor by binding to both catalytic site and its anion binding exosite. Bivalirudin is a specific and direct inhibitor of free and clot-bound thrombin. The hirulog-thrombin complex is transient because thrombin, once complexed, can slowly cleave the Arg3-Pro4 bond on the amino-terminal extension. This metabolic cleavage, converting bivalirudin into a lower affinity inhibitor, contributes to its short half-life[222-224]. Bivalirudin is only 20% excreted in the urine, indicating that it is either extensively catabolized by the liver or undergoes proteolysis at other sites. Following phase III trials showing enhanced safety of bivalirudin relative to UFH in patients undergoing coronary angioplasty, it has been approved in the US for this indication[225,226]. In one pi-

lot study, angiographic patency of the culprit coronary artery lesion was assessed 90 and 120 minutes after the initiation of streptokinase and aspirin and again after 4 ± 2 days in 68 patients with AMI[227]. In this trial bivalirudin yielded higher patency rates when used in conjunction with streptokinase and aspirin in the early phase of AMI. Higher bivalirudin doses are unnecessary and may not be better than lower doses, suggesting the fact that too much thrombin inhibition may actually be harmful.

ARGATROBAN

Argatroban (Novastan) is a carboxy acid derivative, belonging to a class of peptidomimetics that also includes inogatran, efegatran and napsagatran. Argatroban has now been approved in the US as an alternate to heparin in patients with HIT. It binds covalently to the active site of thrombin[228]. Argatroban was used in one trial of 50 patients with HIT undergoing PTCA, at a dose of 350 µg/kg bolus and yielded encouraging results[229]. In the myocardial infarction with Novastan and tPA (MINT) study, low and high dose argatroban yielded 90 min TIMI grade 3 flow rates approaching 60%[230]. The rates of bleeding were similar to heparin. However, Argatroban in Acue Myocardial Infarction (ARGAMI) II trial wherein 1200 patients were randomized to receive fibrinolytic therapy to low dose or high dose argatroban or heparin alone, excessive cardiac events were noticed with low dose argatroban and this arm was dropped. The rates of mortality, recurrent MI and major bleding were no different between high dose argatroban and heparin.

H376/95

It is prodrug oral formulation of Melagatran which is currently under phase III trials for prevention and treatment of venous thrombosis. It is well absorbed from the gastrointestinal system and after rapid biotransformation is converted to an active site-directed thrombin inhibitor-melagatran[231,232].

EFEGATRAN

Efegatran sulfate (GYKI 14766), a tripeptide aldehyde (mePhe-ProArg-H), is an arginal catalytic-site inhibitor of thrombin and is a reversible, competetive, tight-binding inhibitor[233,234]. Efegatran is being evaluated in several clinical trials.

NAPSAGATRAN

Napsagatran (RO-46-6240) is a cyclopropyl derivative of a novel class of thrombin inhibitors and is a selective, potent, competetive and reversible inhibitor of thrombin[235]. Napsagatran is currently in phase II clinical trials for preventing pos-

Table 16. Developmental status of thrombin inhibitors

Drug	Chemical nature	Status
Hirudin (Refludan)	Recombinant protein	Alternate anticoagulant in management of HIT
Hirulog (Angiomax)	Synthetic bifunctional oligopeptide	Approved in PTCA. Several clinical trials completed & planned
Argatroban	Synthetic heterocyclic derivative	Phase II & III clinical development in USA; approved in USA.
Aptamers	DNA and RNA-derived Oligonucleotides with thrombin-binding domains	Preclinical stage; limited animal data available
Plasma-derived antithrombin	Protein and their recombinant equivalent products	Antithrombin III is currently used. HC-II is still in developmental stage
Oral thrombin inhibitor	Prodrug for the management of DVT	Phase II and III clinical trials
PI-88	Sulfated pentomannan	Phase II clinical trials.

toperative thrombosis and treating venous thrombosis.

INOGATRAN

Inogatran (H314/27) is a synthetic dipeptide which selectively, rapidly and competetively binds thrombin[236]. Further clinical development of inogatran has been stopped.

FACTOR IXa INHIBITORS

F IXa which is essential for amplification of coagulation could be inhibited either by active site F IXa inhibitors or by monoclonal antibodies directed against F IX/IXa.

Active-Site Factor IXa Blockers

The intrinsic tenase complex assembles on the surface of the activated platelets. By competing with F IXa for its incorporation in the tenase complex, the active site F IXa is blocked. The blocked F IXa inhibits clot formation in vitro and is shown to inhibit clot formation in coronary artery thrombosis in a canine model[237].

Antibodies Against Factor IX/IXa

Inhibition of F IX activation in addition to blockage of F IX activity could be achieved by monoclonal antibodies against F IX/IXa blocking F X activation by F IXa[238-240]. Antithrombotic activity in a rat model of thrombosis has been achieved utilizing a chimeric humanized derivative of this antibody[238,239].

FACTOR Xa INHIBITORS

The F Xa inhibitors could be classified as follows:

Indirect Inhibitors of Factor Xa

a. Synthetic pentasaccharide (analogue of pentasaccharide sequence of heparin UFH and LMWHs have limited ability to inhibibit platelet-bound F Xa[241-243].

Direct F Xa inhibitors: Inhibit F Xa bound to phospholipid surfaces and free F Xa[244]. A list of various direct F Xa inhibitors is given in Table 14.

a. Natural Inhibitors like Tick anticoagulant peptide (TAP) and antistatin.

b. Synthetic inhibitors like DX9065a, YM-60828, SF 303 and SK 549.

INDIRECT FACTOR Xa INHIBITORS

Pentsaccharide produces its antithrombotic

Table 17. A comparison of r-Hirudin and UFH

r-Hirudin	Unfractionated heparin
Monocomponent protein with single target (thrombin)	Polycomponent drug with multiple sites of action
Thrombin-mediated amplification of coagulation is affected only under certain conditions	Thrombin and F Xa feedback amplification of clotting is affected
No known interactions with endothelium other than blocking the thrombin-thrombomodulin-mediated activation of protein C	Significant interactions with endothelium. Both physical and biochemical modulation of endothelial function
Shorter half-life via IV route	Short half-life via IV route.
Functional bioavailability is variable and dependent on the structure of r-hirudin	Functional bioavailability is 20-30%. LMWHs are better absorbed
Endogenous factors (PF4, FVIII) do not alter its antithrombotic action	Marked modulation by the endogenous factors. Several factors may alter the anticoagulant actions
Relatively inert proteins not altered by metabolic processes	Transformed by several enzyme systems and reduces its anticoagulant actions
Information on cellular uptake and depo formation is not presently known	Significant cellular uptake and depo formation

effect via binding to antithrombin. I t has a molecular weight of 1.728 daltons. It was developed in 1983 to show that a five member heparin chain was the minimum saccharidic sequence needed for antithrombotic activity[245-247]. In various experimental models, it has been shown that inhibition of factor Xa controls excessive thrombin generation and produces antithrombotic effect with lesser bleeding risk than heparin[248-250]. Recently, in in vitro experiments, it has been shown that the anticoagulant activity of synthetic pentasaccharide can be neutralized by Heparinase, an eliminase isolated from *Flavobacterium heparinum*[251]. A synthetic pentasaccharide analogue, SANORG 34006 has also been developed which shows a longer half-life. However, SANORG 34006 was found to be resistant to heparinase I neutralization[251]. Pentasaccharide is under phase III clinical evaluation for prophylaxis of venous thrombosis, comparing LMWHs.

DIRECT INHIBITORS of FACTOR Xa

Antistatin: Antistatin, isolated and purified from the mexican leech, Haementeria officinalis, is a 119 aminoacid polypeptide with a molecular weight of 17.000 daltons[252]. It inhibits F Xa by forming a stable enzyme -inhibitor complex[253]. Since it has a potential to develop antibodies, it has been stopped for future development.

Yagin: Yagin, isolated from the medicinal leech, Hirudo medicinalis, is a 85 amino acid peptide with 50% homology with antistatin. It is a slow tightbinding inhibitor of F Xa[254].

TICK-ANTICOAGULANT PEPTIDE (TAP)

TAP is originally isolated from the tick, Ornithodorus moubata, and now manufactured through recombinant DNA technology, is a 60 amino acid peptide (6.850 daltons) with the potential of inhibiting human F Xa by its slow and tight-binding mechanism, initially forming a weaker complex and later forming a more stable enzyme complex[255,256]. TAP has been shown as an antithrombotic in experimental models of venous and arterial thrombosis and also showed favorable antiproliferative effects of smooth muscle cells in restenosis processes.

SYNTHETIC FACTOR Xa INHIBITORS

DX9065a, YM-60828, SF-303 and SK-549 are nonpeptide, low-molecular-weight, reversible inhibitors of F Xa and are effective in various animal models of thrombosis[257-260]. DX-9065a is undergoing phase II clinical evaluation in patients with unstable angina.

Factor VIIa/Tissue Factor Pathway Inhibitor

The F VIIa/TF pathway being the initial coagulation pathway, much attention has been given in blocking this pathway by developing F VIIa inhibitors and tissue factor pathway inhibitors (TFPI)[261].

TFPI

Tissue factor pathway inhibitor is a protein bound to low density lipoproteins and high density lipoproteins and as it circulates it inhibits both the VIIα-TF complex and F Xa. While about 10% of TFPI is bound to the lipoproteins, 90% of it is bound to heparin like species on the endothelial surface and is released following the administration of unfractionated heparin, LMWHs, Defibrotide and PI-88[262,263]. It has been shown that endothelial depletion of TFPI may contribute to “rebound” thrombin generation, following the sudden cessation of unfractionated heparin[264]. TFPI has shown to attenuate injury-induced neointimal hyperplasia in Pigs and also inhibits smooth muscle cell migration in vitro TFPI has also shown to attenuate the coagulopathy and improve survival in sepsis models in rabbits and baboons[265]. TFPI is now undergoing phase III clinical trials in patients with sepsis.

NAP c2 and NAP-5

These are two of the anticoagulant proteins isolated from hookworm nematode, *Ancylostoma caninum* and NAPc2 is currently undergoing phase II clinical trials for prevention of venous thrombosis in patients with elective knee arthroplasty. NAPc2 binds to a noncatalytic site on F X or F Xa and inhibit F VIIa within the F VIIa/tissue factor complex has a half-life of 50 hours following subcutaneous administration[266]. It attenuates sep-

sis-induced coagulopathy in laboratory animals. NAP-5 inhibits F Xa and the F VII/TF complex after prior binding to F Xa[267].

Other Antithrombotics Enhancing Endogenous Anticoagulant Activity

Thrombomodulin: Thrombomodulin is an endothelial cell surface protein that forms a complex with thrombin, which makes the thrombin lose its procoagulant property and activates protein C thousandfold compared to free thrombin[268-270]. APC in the presence of protein S inactivates coagulation F Va and F VIIIa[271,272]. Thrombomodulin besides playing an important role as endogenous regulator of coagulation on the surface of vascular wall, also inhibits the proteolytic action of thrombin on macromolecular substrates and inactivation of thrombin by antithrombin. Thrombomodulin is an integral membrane glycoprotein present on the vascular surface of endothelial cells of arteries, veins, capillaries and lymphatic vessels. The recombinant human oluble thrombomodulin is now available and is found to be effective in the rat model of arteriovenous shunt thrombosis and in disseminated intravascular coagulation models in mice and rats and in situations where antithrombin levels are reduced[273-276]. Parenteral administration of soluble recombinant thrombomodulin has shown antithrombotic effects without any bleeding in cancer patients[277].

Protein C: APC is a natural anticoagulant that plays a key role in the regulation of blood coagulation by selectively degrading coagulation F Va and F VIIIa eventually inhibiting thrombus generation[278]. Protein C is one of the vitamin K-dependent plasma proteins which is activated by the thrombin-thrombomodulin complex on the surface of the intact endothelial cells. The anticoagulant effect of APC is enhanced by a cofactor, Protein S, another vitaminK plasma protein[279]. An endothelial cell protein C receptor has been identified earlier[280]. The circulating plasma concentration of protein C is 4 mg/L. Both the plasma derived and recombinant forms of protein C are now available[281]. Intravenous APC has shown beneficial in the treatment of patients with sepsis-induced coagulopathy[280]. Currently, it is undergoing phase III clinical evaluations for sepsis-induced coagulopathy.

Modulation of the Endogenous Fibrinolytic Activity

TAFI: It is a latent carboxypeptidae B like enzyme that is activated by thrombin-thrombomodulin complex, and attenuates fibrinolysis by cleaving carboxy-terminal lysine residues from fibrin[282,283]. The fibrinolytic process is retarded by removal of these lysine residues which decreases the plasminogen or plasmin binding to fibrin. It has been shown in dogs and rabbits that a potato-derived carboxypeptidase B inhibitor increases tPA-induced thrombolysis[284,285].

Factor XIIIa Inhibitors

The Laki-Lorand F XIIIa, a thrombin-activated transglutaminase, crosslinks the α-and γ-chains of fibrinogen to form α-polymers and γ-dimers respectively. As the fibrin polymer is stabilized due to crosslinking, it is rendered more refractory to degradation by plasmin[286]. It is therefore thought that inhibition of F XIIIa makes the thrombus susceptible to lysis. Tridegin, a peptide isolated from the giant Amazon leech, *Haementeria ghilianii*, is a specific F XIIIa inhibitor and has shown to enhance fibrinolysis in vitro when added prior to clotting of fibrinogen[287,288]. Destabilase, a leech enzyme that hydrolyzes γ-gcrosslinks also inhibits F XIIIa action[289,290].

PAI-1 Inhibitors

Inhibition of PAI-1 which is a major physiologic inhibitor of tPA and u-PA results in increased endogenous fibrinolytic activity. PAI-1 synthesis is decreased in vitro by lipid lowering drugs such as niacin and fibrates[291,292]. Similarly peptides that block PAI-1 activity are also identified which either prevent insertion of the reactive center loop upon cleavage by the target protease or by converting PAI-1 into a latent conformation[293,294]. Development of small molecule PAI-1 inhibitors, some of which may have antithrombotic activity in vivo may provide a more promising alternative strategy[295].

Glycoprotein IIb/IIIa Inhibitors

GPIIb/IIIa inhibitors block the final common

pathway of platelet aggregation[296]. Abciximab during percutaneous coronary interventions has reduced 30-day ischemic outcomes by approximately 35-50%[297-299]. The clinical development of peptide and peptidomimetic GPIIb/IIIa inhibitors have shown less consistent benefits[300-302]. The oral GPIIb/IIIa inhibitors have demonstrated approximately 30% increase in mortality[303]. A safe and effective level of GPIIb/IIIa inhibition by rapid platelet function testing will allow the optimization of doses in all patients. A list of GPIIb/IIIa agents are mentioned in Table 12.

The GPIIb/IIIa inhibitors can be used in combination with the thrombolytic agents in patients with acute myocardial infarction. Activase (Alteplase, recombinant) in combination with GPIIb/IIIa inhibitors or TNKase in combination with GPIIb/IIIa inhibitors can be used in patients with acute myocardial infarction.

The thrombi in the coronary arteries causinh acute myocardial infarction comprise of a platelet core in a fibrin-thrombin matrix. Following successful thrombolysis, the reocclusion is caused by excessive platelet activation which makes the thrombi difficult to lyse. In these situations, adjunctive use of thrombolytic agents with GPIIb/IIIa inhibitors will prevent platelet activation and aggregation[304]. Platelet binding to the walls of the vessel by attachment at Ia or Ib receptors on the platelet surface. Platelet-platelet binding is as a result of interaction between GPIIb/IIIa receptors involving the fibrinogen and von Willebrand factor[305]. It was demonstrated by Gold et al that the platelet Fab fragment of the murine antibody 7E3-F(ab)2 to GPIIb/IIIa binds tightly to the GPIIb/IIIa receptor and inhibited platelet aggregation[306]. In TAMI-8, a nonrandomized multicenter pilot study, 60 patients with AMI were given activase with varied abciximab dosages of 0.1 mg/kg, 0.15 mg/kg, 0.20 mg/kg and 0.25 mg/kg given at 3, 6, and 15 hours after a 100 mg dose of activase administered over 3 hours. Despite limitations of the study being small and not blinded, the safety profile was similar in the abciximab and control groups. However, in abciximab treated patients, fewer major bleeding events, decreased recurrent ischemic events and better coronary artery patency as assesed by angiography were seen. The preliminary results of an ongoing double-blind, randomized, placebo-controlled crossover trial of abciximab alone or in combination with low-dose activase in 26 patients with AMI, who presented within 6 hours of symptom onset with ST-segment elevation were initially given aspirin and heparin and then randomized to receive either abciximab 0.25 mg/kg bolus or placebo followed by an angiogram 60-90 minutes later. Patients were crossed over and given the opposite treatment. A second angiogram was taken 10 minutes later. Those patients in which TIMI grade 3 flow was not achieved were further randomized to receive activase 20 mg or placebo. A third angiogram was performed 15 minutes later. The results of the second angiogram where patients received abciximab alone, 8 patients had TIMI grade 0 flow, 5 patients had TIMI grade 1 flow, 5 patients had TIMI grade 2 flow anf 8 patients had TIMI grade 3 flow. The results of the angiogram in patients receiving activase and placebo are not yet reported[307].

Antman et al also reported the results from the dose-finding and dose-confirmation phases of TIMI-14 trial which evaluated the use of thrombolytic therapy in combination with abciximab in patients with AMI[308].

TNKase, a new genetically engineered variant of tissue plasminogen activator is produced by the recombinat DNA technology. TNkase is fibrin specific. This fibrin specificity decreases systemic activation of plasminogen and the resulting breakdown of the circulating fibrinogen when compared to a molecule lacking this feature. The ASSENT-2 was a phase III randomized trial, double-blind trial that compared TNKase with Activase. Currently, there is no published information on the safety and efficacy of TNKase in combination with GPIIb/IIIa inhibitors. Anticoagulants such as heparin and VitaminK antagonists, acetylsalicylic acid, dipyridamole and GPIIb/IIIa inhibitors may increase the risk of bleeding if administered prior to, during or after TNKase therapy.

SEVERE SEPSIS and NITRIC OXIDE

The formation of NO from the guanidine nitrogen group of L-arginine is catalyzed by nitric oxide synthases (NOSs)[309,310]. All the three iso-

forms, namely, endothelial cell NOS (ecNOS or NOS III), brain NOS (bNOS or NOS I) and inducible NOS (iNOS or NOS II) are inhibited with NG-monomethyl-L-arginine (L-NMMA). Although the iNOS is absent from mammalian cells under physiological conditions, it is induced by proinflammatory stimuli, such as bacterial lipopolysaccharide or the cytokines TNF-α, IL-1ß or IFN-c. Unlike ecNOS and bNOS, iNOS tightly binds calmodulin and hence is not regulated by intracellular calcium levels and generates large amounts of NO [311,312]. About 75% of deaths in septic shock, occurring within hours and days after the onset of shock are caused by therapy-resistant hypotension, suggesting that peripheral vascular failure is the key factor that determines the outcome[313]. The other deaths which occurs days or weeks after stabilization of blood pressure are due to multiple organ failure, most commonly the sequence of events involves adult respiratory distress syndrome (ARDS), renal and hepatic failure. Inhibitors of iNOS activity like NG-cyc lopropyl-L-arginine, NG-nitro-L-arginine, and its methyl ester, L-NAME and L-NMMA reduced the hypotension caused by endotoxin in laboratory animals, suggesting a therapeutic rationale for their use[314-316]. Reducing the enhanced generation of NO by inhibitors of the iNOS induction and inhibitors of protein kinase C (PKC) or of protein tyrosine kinase or of the activation of NFkB[317-321]. Other agents which inhibit iNOS include glucocorticoids, thrombin, or ethanol; macrophage deactivating factor and transforming growth factor-ß, platelet-derived growth factor. Endothelin-1, IL-4, IL-8, IL-10 and IL-13[312,322-326]. NFkB, under physiological conditions is held in an inactive form in the cytoplasm by the inhibitory protein IkB-α, which prevents its activation and translocation to the nucleus to induce the expression of specific genes. Activation of NFkB involves the signal-induced phosphorylation of IkB-α, resulting in its proteolytic degradation and the release of NFkB. Proteolytic degradation of IkB-α in vivo by cystein protease inhibitor calpain inhibitor I and dexamethasone resulted in attenuation of

a. The circulatory failure,

b. Multiple organ failure and

c. Induction of iNOS protein and activityin lung and liver of rats with endotoxic shock (Ruetten and Thimmermann). This suggested that prevention of the activation of NFkB by calpain inhibitor I may be useful in the therapy of circulatory shock in local or systemic inflammation[327]. Dexamethasone which is known to inhibit the endotoxin-mediated induction of iNOS in vivo and in vitro, also inhibits the action of transcription factors AP-1 and NFkB[328,329]. A recent phase III trial utilizing continuous infusion of L-NAME, a specific inhibitor of iNOS, was discontinued due to increased adverse effects such as decreased cardiac output and increased pulmonary artery pressure and increased mortality in the L-NAME group[330].

CONCLUSIONS

Sepsis continues to be a leading cause of death in the surgical intensive care unit with mortality ranging from 30-80%. Zimmerman et al found no changes in the incidence of organ failure, of multiple organ failure and of mortality from 1982-1990[331]. From Hippocrates first description in 460 A.D. of what today is called SIRS until today, a large body of knowledge has been accumulated. After 2000 years of research on sepsis, the basic principles of septic conditions are now understood[332]. It is also understood that an identifiable pathogen is not necessarily the trigger of the disease, rather the human organism himself plays a key role in the natural history of the disease. In 1990, Mitchie and Wilmore studied much about TNF-α physiology from an evolutionary point of view[333]. In 1996, R.C. Bone described SIRS as the result of dysregulation of the organisms' biological response to certain stimuli[334]. In 1996, Godin and Buchman presented the hypothesis of the multiple organ dysfunction syndrome being the consequence of biological oscillatory systems[335]. In the past decade there has been remarkable progress in the field of antithrombotic agents in the management of severe sepsis. Endothelial damage, activated leukocytes, altered platelet function and hypercoagulability lead to the development of septic organ dysfunction. Endogenous anticoagulants, including antithrombin, thrombomodulin, protein C, APC and TFPI regulate the function of vascular endothelial cells and neutrophils and also exert an antiinflammatory ef-

fect. Hence, these anticoagulants prevent the progression to severe sepsis. F VIIa, F Xa and thrombin directly activate cells, by cleavage of the cell surface protease activated receptors[336]. F Xa inhibitor DX-9065a modulated the leukocyte endothelial cell interaction in endotoxaemic rats[337]. Leukocytes play an important role in the development of sepsis-induced multiple organ dysfunction syndrome (MODS). Leukocytes also produce procoagulant and anticoagulant factors and influence the coagulation process. They also provide specific receptors that serve as direct molecular links between inflammation and coagulation. A receptor for F Xa, effector protease receptor 1 (EPR-1) was expressed on leukocytes and endothelium. Xa participates in EPR-1 related leukocyte activation and platelet and endothelial cell induced thrombin formation. EPR-1 signalling is mediated by F Xa binding and the other requiring active site. F Xa also activates cells by an EPR-1 independent fashion[338]. On the endothelium, F Xa elicits expression of IL-6, IL-8 and monocyte chemotactic protein-1 by an active-site independent reaction, independent of EPR-1[339-341]. Increased expression of E-selectin, ICAM-1, and VCAM-1 accompanies leukocyte adhesion through enzymatic activation[342]. High doses of DX 9065a appeared to be beneficial in septic MODS[337]. An early phase II trial of DX-9065a in severe sepsis is ongoing in Japan. A recent study to analyze the influence of heparins (UFH and certoparin, a LMWH) on the generation of cytokines with known antiinflammatory activities (IL-1ra, IL-6, IL-10) and of IL-12p40 from human leukocyte fractions concluded that certoparin caused a pronounced response on IL-6 generation when compared to UFH, at same concentrations[343]. IL-6 has been recently reported to be heparin-binding protein[344]. LMWHs (Dalteparin) inhibited TNF-α-induced leukocyte rolling along microvascular endothelium, explaining their antiinflammatory effects at a dose of 5000 units/kg, a relatively high dose when compared to 200 units/kg for deep vein thrombosis[345].

Footnote: This article is dedicated to Professor Orhan N. Ulutin, MD.

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