Published in final edited form as:
Curr Pharm Biotechnol. 2011 September ; 12(9): 1481–1496.

Therapeutic Modulation of Coagulation and Fibrinolysis in Acute Lung Injury and the Acute Respiratory Distress Syndrome

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Abstract

Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) are characterized by excessive intra-alveolar fibrin deposition, driven, at least in part by inflammation. The imbalance between activation of coagulation and inhibition of fibrinolysis in patients with ALI/ARDS favors fibrin formation and appears to occur both systemically and in the lung and airspace. Tissue factor (TF), a key mediator of the activation of coagulation in the lung, has been implicated in the pathogenesis of ALI/ARDS. As such, there have been numerous investigations modulating TF activity in a variety of experimental systems in order to develop new therapeutic strategies for ALI/ARDS. This review will summarize current understanding of the role of TF and other proteins of the coagulation cascade as well the fibrinolysis pathway in the development of ALI/ARDS with an emphasis on the pathways that are potential therapeutic targets. These include the TF inhibitor pathway, the protein C pathway, antithrombin, heparin, and modulation of fibrinolysis through plasminogen activator-1 (PAI-1) or plasminogen activators (PA). Although experimental studies show promising results, clinical trials to date have proven unsuccessful in improving patient outcomes. Modulation of coagulation and fibrinolysis has complex effects on both hemostasis and inflammatory pathways and further studies are needed to develop new treatment strategies for patients with ALI/ARDS.

Keywords

Tissue factor; TFPI; Protein C; PAI-1; PARs

INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are devastating illnesses marked by diffuse lung inflammation and increased vascular permeability resulting in endothelial and epithelial injury, pulmonary edema and organ dysfunction [1-4]. These syndromes result from either a direct lung injury (pneumonia, aspiration of gastric contents or inhalation injury) or from a systemic insult (sepsis, hemorrhagic shock, severe trauma, transfusion of blood products) [1]. The current therapeutic strategy to decrease ALI/ARDS-associated mortality is to utilize protective mechanical ventilation with a plateau pressure-limited low tidal volume strategy [2]. However, despite some improvement with this intervention, both morbidity and mortality remain high with mortality rates from ALI/ARDS exceeding 30% [3]. Although mechanical ventilation is a necessary and life-saving measure in patients with ARDS, mechanical ventilation itself may potentiate or aggravate lung injury

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and inflammation, referred to as ventilator-induced lung injury (VILI) [1]. Therefore, given the continued high mortality, development of new therapies for both prevention and treatment of lung injury continues to be the focus of ALI/ARDS research [4-6].

Central to the pathophysiology of ALI/ARDS is the presence of fibrin-rich exudates (hyaline membranes) in the lumen of lung alveoli due to activation of coagulation and inhibition of fibrinolysis [7] favoring fibrin formation and persistence. In the uninjured lung, low levels of TF and PAI-1 [4] prevent fibrin accumulation. However, in the lungs of patients with ALI/ARDS the balance is shifted in a procoagulant antifibrinolytic direction favoring fibrin accumulation. Despite the extensive body of literature detailing the changes in coagulation and fibrinolysis in ALI/ARDS, the direct effects of activation of coagulation on inflammatory pathways and perpetuation of lung injury are not well understood [4]. Interestingly, studies in patients with normal lungs have shown that mechanical ventilation even in the absence of lung injury can induce activation of the coagulation cascade through tissue factor (TF)-mediated events [8] suggesting that activation of intra-alveolar coagulation may be one mechanism for VILI. Highlighting our current lack of knowledge regarding the role of coagulation and fibrinolysis in ALI/ARDS is the fact that clinical studies targeting the coagulation cascade in patients with or at risk of lung injury have been largely unsuccessful [9]. Here we will review the current knowledge of the role of coagulation and fibrinolysis in ALI/ARDS and emphasize gaps in our understanding of the complex role of these pathways in human ALI/ARDS.

THE EXTRINSIC COAGULATION CASCADE

The extrinsic coagulation pathway, Fig. (**1**), is initiated by the membrane-bound protein tissue factor (TF, Coagulation Factor III, 47-kDa) which is constitutively expressed in adventitial fibroblasts within the walls of blood vessels. TF is also produced in organs that are richly vascularized such as the brain, kidney, placenta and lung, with distribution documented in numerous cell types including astrocytes, platelets, epithelial cells, endothelial cells and cardiomyocytes [10-15]. In the lung, TF is expressed by alveolar macrophages and alveolar epithelial cells [16] and *in vitro* both cell types exhibit TF activity [10].

Under normal circumstances, constitutively expressed TF in the adventitia is separated from the blood and thus from the proenzymes of the coagulation pathway [17]. Interaction of TF with the downstream factors of the coagulation cascade occurs under pathological conditions including disruption of the endothelial barrier during vascular injury, structural defects in the vascular wall, angiogenic stimulation, entry to the bloodstream of large numbers of TF-expressing cells (inflammatory leukocytes, leukemic blasts, cancer cells) [11,17-19] or release of TF-containing membrane microparticles into the bloodstream [20]. Microparticles (MPs) are submicron membrane vesicles derived from apoptotic and/or activated cells including macrophages, platelets, endothelial cells and epithelial cells [21-23]. Under these conditions, the coagulation cascade is triggered when TF binds to the circulating serine protease coagulation Factor VII (Factor VIIa) Fig. (**1**). The TF:Factor VIIa complex then activates Factor X (Factor Xa) which binds to Factor V (Factor Va) in the presence of the cofactors calcium and phospholipid membrane to form the prothrombinase complex. Factor Xa and Factor Va then activate prothrombin to thrombin. Thrombin then recruits platelets and catalyzes fibrin formation [24,25].

REGULATION OF TF EXPRESSION AND ACTIVITY

Although TF-mediated coagulation leading to fibrin formation is essential for hemostasis, wound repair, and healing, excessive fibrin deposition in the lung has been associated with

the development of pulmonary diseases, including ALI/ARDS [26]. Therefore, the regulation of TF expression is of particular importance in lung injury and inflammation. TF is an early response gene under the control of an inducible promoter. TF is up-regulated by proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFNγ), as well as lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria that stimulates innate immune responses. LPS and cytokine-induced expression are mediated by *cis*-acting regulatory elements in the promoter region of the TF gene including activating protein-1 (AP-1), specificity protein 1 (Sp-1), and nuclear factor-κB (NF-κB)-like sites [27-31]. Other transcription factors have been shown to be important in TF gene regulation in the absence of inflammation. Hypoxia-treated HeLa cells and mononuclear phagocytes increased TF expression through activation of the nuclear phosphoprotein early growth response (Egr-1). However, HeLa cells exposed to either phorbol myristate acetate (PMA) or serum had induction of TF expression through both Sp1 and Egr-1 [32,33]. Finally in human lung bronchial cells exposed to asbestos, TF gene expression was induced by NF-κB, AP-1 and Sp1 though it was determined that TF mRNA was primarily stabilized by Sp1 [34]. Thus, pathways for induction of TF gene expression appear to be specific to both cell type and stimulus.

Although gene expression of TF is highly inducible, TF also undergoes complex post-translational modifications that regulate its procoagulant activity. Newly synthesized TF is primarily localized to the Golgi with a small fraction in early endosomes and lysosomes [35-37]. In human arterial smooth muscle cells, Schecter *et al*. showed that growth factor stimulation induced TF mRNA and protein expression with approximately 70% of TF protein found at the cell surface [38]. Interestingly, only 20% of cell surface TF was found to be biologically active; 50% was latent and the remaining 30% was intracellular [38-40]. There are several posttranscriptional and post-translational modifications that regulate cell surface TF expression and activation. In order for TF to traffic to the plasma membrane where it can interact with FVII/FVIIa, the extracellular domain must be glycosylated at three sites [41-44]. Other modifications include phosphorylation of multiple sites within the cytoplasmic domain [45], nitrosylation of specific cysteine sites or palymitoylation of the intracellular cysteine at residue 245. These post-translational modifications either target TF to lipid rafts or enhance endocytosis and thus downregulate and/or degrade the protein since TF is not recycled and returned to the cell surface [46-48].

TF procoagulant activity is also modified by a posttranslational process that regulates the activity of TF at the cell surface termed encryption/decryption [49,50]. Encryption/decryption appears primarily to be a function of membrane microenvironment. When TF is present on the plasma membrane in an encrypted form it can still bind FVII but only acts as a cytokine receptor capable of transmitting intracellular signals and cannot activate Factor X [50-52]. Decryption leads to activation of the ability to cleave Factor X and subsequent procoagulant activity, and occurs after cell exposure to various agents [53-55]. For example, TF activity increases after cell exposure to freeze-thaw cycles, calcium ionophore and phorbol myristate acetate (PMA) [56,57], stimuli that do not alter TF transcription or translation. Other factors shown to be involved in encryption/decryption include nonionic detergents, apoptosis, phosphatidyl serine exposure, lipid raft dissociation and disulfide linkage of Cysteine 186-Cysteine 209 [52,56,58,59]. Thus, regulation of gene expression, post-translational modifications and factors that regulate TF enzymatic activity may all be important in modulating TF activity in the lung.

TF-MEDIATED COAGULATION AND INFLAMMATION IN EXPERIMENTAL MODELS OF ALI/ARDS

Emerging evidence shows that there is an extensive cross-talk between inflammatory responses and coagulation pathways resulting in reciprocal modulation of these pathways in inflammatory lung diseases including ALI/ARDS [60]. In experimental studies, exposure of human lung epithelial cells to proinflammatory stimuli induced TF expression and activity [16]. In humans injected intravenously with LPS, mRNA levels of TF increased in circulating blood cells, monocyte-derived TF expression was enhanced and TF-containing MPs were released into the circulation leading to thrombin generation [61-63]. In a murine model of bleomycin-induced lung inflammation and injury, increased expression of TF, Factor X, and Factor VII was seen in the bronchial and alveolar epithelia and was associated with increased lung collagen accumulation [64].

Other studies have shown that blocking TF activity reduces inflammatory-mediated tissue injury. In a lethal sepsis model in baboons, blocking the TF:Factor VIIa complex with either a monoclonal anti-TF antibody, active-site-inactivated Factor VIIa, or the endogenous anticoagulant TF pathway inhibitor (TFPI) protected against the lethal effects of septic shock [65,66]. In addition, in rats instilled with LPS intratracheally (IT), intravenous (IV) injection of site-inactivated Factor VIIa which blocks activation of Factor X resulted in reduced intra-alveolar inflammation and fibrin deposition as well as decreased lung protein leakage and cytokine release [63,67]. Thus, experimental human and animal models have shown that inflammatory signals can upregulate TF procoagulant activity while blockade of TF can downregulate inflammation. Together, these data provide compelling evidence for a fundamental link between coagulant and inflammatory pathways.

Despite these persuasive findings, further study into molecular mechanisms involved in TF-specific events in *in vivo* models of lung injury has been challenging. Investigation into the function and modulation of procoagulant protein expression, including TF, has been hindered by the discovery that these factors are critically important in embryonic and post-embryonic survival in animal models. For example, complete tissue factor deficiency in mice results in exsanguination at midgestation between embryonic days 9.5 and 10.5 [68]. Similarly, death shortly after birth or *in utero* also occurs in mice deficient in Factor V, Factor VII, Factor X or prothrombin [69,70].

Therefore, owing to the inability to study coagulation factor null mice, a variety of novel approaches have been developed. One such strategy is to generate animals that express low levels of human tissue factor (1% compared to murine TF) or low levels of its target Factor VII (5% of wild type Factor VII) that rescue the animals from embryonic lethality but are still deficient in either TF or Factor VII. Low TF and low Factor VII mice had reduced mortality, inflammation and coagulation responses after intraperitoneal (IP) administration of LPS compared to wildtype (WT) controls confirming findings from studies utilizing a TF blockade strategy [71,72]. In addition, IP injection of LPS into mice expressing low levels of TF caused reduced coagulation, IL-6 plasma levels and mortality compared to control mice [73]. These studies and others suggest that TF and downstream members of the coagulation cascade play a role in both fibrin deposition and inflammation.

PROTEASE-ACTIVATED RECEPTORS (PARs)

One potential mechanism by which TF can influence inflammation is through its activity on protease-activated receptors (PARs). The TF:Factor VIIa complex, Factor Xa, and thrombin can all activate protease-activated receptors (PARs), members of a subfamily of related G protein coupled receptors (GPCRs) expressed ubiquitously throughout the body. PARs are

activated by cleavage of the N-terminal extracellular domain by cognate proteases. This cleavage allows the newly formed tethered ligand to bind to its receptor leading to signal transduction [74]. To date, there are four known members of the PAR family numbered 1 through 4. Thrombin acts on PAR-1, -3 and -4; the TF:Factor VIIa complex activates PAR-2 and to a lesser extent PAR-1 and Factor Xa activates PAR-1 and PAR-2 [17].

PAR signaling has been implicated in the development of both acute and chronic lung diseases through modulation of both inflammation and fibrosis. Binding of thrombin to PAR-1 enhances inflammation by upregulating inflammatory gene expression in the lung [75-77]. In addition, thrombin and Factor Xa-mediated activation of PAR-1 results in fibroblast proliferation and procollagen production [26, 78, 79]. In a model of high-tidal volume ventilation, intratracheal instillation of an agonist peptide for PAR-1 increased lung edema [80]. This effect appears to be specific to the lung since PAR-1 KO mice were not protected from endotoxin-induced systemic inflammation [73]. Conversely, PAR-1 deficient mice exposed to bleomycin had reduced lung fibrosis compared to WT mice [81]. In human studies, exposure of human lung fibroblasts to Factor Xa resulted in myofibroblast differentiation through PAR-1 and integrin $\alpha_v\beta_5$-mediated signaling [64,82]. Furthermore, immunostaining for PAR-1, α-SMA and $\alpha_v\beta_5$ was increased in the epithelium and fibrotic foci in the lungs of patients with idiopathic pulmonary fibrosis [64].

TF-mediated coagulation and signaling through PAR-2 has also been linked to inflammation, angiogenesis, tumorigenesis and atherosclerosis [50]. TF:Factor VIIa-mediated activation of PAR-2 (especially in the presence of Factor X) leads to up-regulation of the expression of adhesion molecules and subsequent microvascular inflammation [83]. In animal models of sepsis, inhibition of TF:Factor VIIa results in decreased inflammation, coagulation and increased survival [73]. Ultimately, these data indicate that PARs play a role in lung disease and may be potential targets for modulating inflammation and injury. However, current clinical trials aimed at PAR signaling are primarily focused on patients with cardiovascular disease [84]. Therefore, the effectiveness of PAR-directed therapies in modulating ALI/ARDS will require further studies.

TF-MEDIATED COAGULATION IN CLINICAL ALI/ARDS

Upregulation of TF activity in the lung has been implicated in the development of clinical ALI/ARDS. Levels of soluble TF, Factor VIIa and TF-mediated Factor X activation are increased in the bronchoalveolar fluid (BALF) of patients with ALI/ARDS Fig. (**2**) [1, 16, 85-87]. In addition, clinical investigations identified increases in BALF-derived markers of thrombin generation such as D-dimer, Factor VII and TF in patients with ALI that progressed to ARDS [1,85,88]. Blocking TF activity with a TF-specific antibody confirmed that increased alveolar pro-coagulant activity in BALF or pulmonary edema fluid from patients with ALI/ARDS was mediated by TF [4, 16]. In addition, Bastarache *et al.* showed that pulmonary edema fluid from patients with ALI/ARDS had higher concentrations of procoagulant TF-bearing MPs compared to pulmonary edema fluid from control patients with severe hydrostatic pulmonary edema; levels of these procoagulant MPs showed a trend towards association with hospital mortality [22].

Taken together, these studies suggest that increased TF-mediated procoagulant activity in the alveolar compartment is the driving force for intra-alveolar fibrin deposition in clinical ALI/ARDS. In turn, the endproducts of coagulation may contribute to lung injury and physiologic dysfunction mediated in part by PARs. In addition, the endproducts of the coagulation cascade can have independent effects on vascular and epithelial function. Both thrombin and fibrin can cause endothelial cell contraction and increased endothelial permeability [89]. In addition, fibrin deposition has been shown to inhibit surfactant

function which could contribute to atelectasis and microvascular leakage. Finally, fibrin provides a matrix for fibroblast adherence and proliferation that could promote fibrosis and inflammation [90,91]. Therefore, based on these findings, therapeutic strategies designed to modulate TF-mediated events specific to the lung may be beneficial for patients with ALI/ARDS. The next few sections will focus on the effectiveness of different anticoagulant and profibrinolytic strategies tested in experimental animal models and human trials. Findings in human trials are summarized in Table **1**.

INACTIVATED RECOMBINANT FACTOR VIIa

The TF pathway has been targeted with an inactivated form of recombinant Factor VIIa that binds TF and inhibits its activity. This compound showed protective effects against sepsis-induced lung injury in baboons [67,92,93]. However, site inactivated recombinant Factor VIIa (FFR-rFVIIa) had no beneficial effects on morbidity or patient outcomes in a phase II clinical trial in patients with ALI/ARDS [94]. In addition, certain doses of the inhibitor (4 × 400 μg/kg) were associated with increased mortality resulting in early termination of the trial. One hypothesis as to why the study did not result in beneficial outcomes is that the inhibitor may be effective only in a “very limited window of disease progression with benefit depending on the severity and nature of the underlying cause of organ failure” [94]. Ultimately, the failure of FFR-rFVIIa in human trials despite promising preclinical data is a scenario that has been repeated many times (see Table **1**) and suggests that current preclinical models do not adequately model the complex and heterogeneous clinical syndrome of ALI/ARDS.

OTHER POTENTIAL THERAPEUTIC STRATEGIES: MODULATING THE BALANCE BETWEEN COAGULANT, ANTICOAGULANT AND FIBRINOLYTIC PATHWAYS IN ALI/ARDS

Activation of the coagulation cascade is normally counterbalanced by three endogenous anticoagulant pathways: tissue factor pathway inhibitor (TFPI), the antithrombin pathway and the protein C pathway. Fibrinolysis also counterbalances fibrin deposition and is initiated by urokinase (uPA) and tissue plasminogen activator (tPA) which cleave plasminogen to activate plasmin. Plasmin in turn degrades fibrin into soluble products termed fibrin degradation products (FDPs) [95]. The central role of TF in the initiation of intra-alveolar coagulation in ALI/ARDS makes the extrinsic pathway an appealing target for therapeutic intervention.

As such, numerous studies have been done targeting downstream factors in the TF-mediated extrinsic coagulation cascade with varying results. The following sections elaborate the members of the three endogenous anticoagulant pathways and how they have been targeted in current and past therapeutic strategies in clinical ALI/ARDS.

TFPI

TF-mediated activation of Factor X is inhibited by TFPI, a Kunitz-type serine protease inhibitor and the only identified endogenous inhibitor of TF [96]. Secreted by the vascular endothelium, TFPI circulates in the blood [97-100]. TFPI inhibits activation of Factor X by binding the TF:Factor VIIa complex and thus preventing thrombin generation and fibrin deposition. Stimulation of A549 human alveolar epithelial cells with proinflammatory cytokines induced TFPI secretion into the media, indicating a potential role for the lung epithelium in modulating TF-mediated coagulation in the airspace through TFPI secretion [101]. In patients with ALI/ARDS, TFPI levels are increased in pulmonary edema fluid compared to control patients with hydrostatic pulmonary edema [101]. In addition,

Sabharwal's group found TFPI levels were increased in the BALF from patients at risk and those with ARDS compared to controls [102]. However, TF-mediated pro-coagulant activity in the pulmonary edema fluid in ARDS patients was not inhibited by endogenous TFPI despite the presence of high levels; the majority of the inhibitor was found to be in an inactive, truncated form [101]. Thus the balance between TF and TFPI in the airspace appears to be dysregulated in ALI/ARDS patients since TFPI is inactivated and unable to attenuate the deleterious effects of TF-mediated coagulation and inflammation.

As TFPI is the only endogenous inhibitor of TF activity, a recombinant form of the protein has been studied in both animals and humans as a potential therapeutic strategy. Initially promising, inhibition of TF activity either before or during early stages of ALI with recombinant TFPI was shown to be protective in several animal models of sepsis and ALI [4,65,103]. In addition, in a human study comparing high or low dose recombinant TFPI (Chiron, Emeryville, CA) to placebo after two separate intravenous administrations of LPS in normal volunteers, high dose TFPI reduced LPS-mediated increases in plasma levels of thrombin-antithrombin (TATc) complexes compared to placebo [104]. However, in a phase III trial (OPTIMIST) initiated in 2000, the same preparation of human recombinant TFPI (Tifacogin) did not improve clinical outcomes in patients with severe sepsis, many of whom (~50%) had respiratory infections and likely had ALI/ARDS, despite a previous encouraging phase II trial [105,106]. One hypothesis as to why TFPI was ineffective is that heparin coadministration in some patients modulated the activity of Tifacogin and potentially rendered the drug inactive [107]. This reasoning is based on the fact that TFPI, which has heparin-binding domains, is reported to be displaced by heparin from the endothelium and thus would not be able to come in contact with TF on the endothelium in order to inactivate it [107,108]. Other hypotheses include the possibility of insufficient dosing, differences in trial design between studies or biological activities of TFPI that are unrelated to coagulation [107].

ANTITHROMBIN

Antithrombin (AT) is an endogenous protease inhibitor that binds to proteoglycans and glycosaminoglycans (heparins and heparin sulfates) on the cell surface of endothelial cells. After binding to the endothelial cell surface, AT neutralizes thrombin and several other proteinases of the coagulation pathway. The importance of AT in modulating coagulapathy in ALI was demonstrated in rats exposed to endotoxin intravenously where intravenous administration of high-dose AT prevented endotoxin-mediated inflammation, lung vascular injury and coagulation abnormalities including reduced platelet count and plasma fibrinogen levels [109]. In other models of lung injury, intravenous AT has also been demonstrated to decrease vascular injury as well as vascular permeability [110-112]. Finally, in patients with sepsis, low plasma levels of AT have been shown to be associated with the development of ALI/ARDS [110, 113, 114].

However, in a phase III trial of AT in 2314 patients with severe sepsis (KyberSept) AT failed to improve patient mortality. At 28 days in the antithrombin III treatment group overall mortality was 38.9% versus 38.7% in the placebo group. Coadministration of heparin may have inhibited the efficacy of the AT, similar to the effects observed in the clinical trials of TFPI [115]. An additional hypothesis as to why the recombinant AT protein was effective in animal models of sepsis but not in humans involves species differences in metabolism. Thus, alternative dosing strategies may be necessary in future human studies [106]. Therefore, although AT might still have potential as a therapeutic strategy in ALI/ARDS, more studies to determine appropriate dose and efficacy without heparin need to be done in addition to the effects of AT in patients with ALI/ARDS.

HEPARIN

With regards to heparin, studies have demonstrated that these glycosaminoglycans possess anti-inflammatory effects in addition to anticoagulant properties [1,116,117]. Specifically, both endogenous heparin, found in the mucopolysaccharide-containing fractions of the lung [118] and as a normal constituent of blood [119], and exogenous heparin act as anticoagulants which prevent the formation of clots and extension of existing clots. In a model of allergen-induced eosinophil recruitment, exogenous heparin administration inhibited eosinophil infiltration into the lungs of sensitized guinea pigs [120]. Wang *et al.* demonstrated in rabbits that LPS-induced inflammatory cell lung recruitment as well as serum cytokine markers were reduced with IV injection of either heparin or low-molecular weight heparin (LMWH) [121]. LMWH also attenuated lung injury in LPS-induced models in rats or sheep [122,123]. Finally, a recent study in a rat model of ALI demonstrated animals exposed to heparin had reduced endotoxin-mediated injury compared to controls [122, 124].

In clinical trials, Dixon *et al.* studied the effects of repeated doses of nebulized heparin over the course of the trial in treating mechanically ventilated patients with ALI [125]. This phase I trial showed longer activated partial thromboplastin time (APTT) levels, from 40 seconds at baseline to 69 seconds post heparin, at higher doses without any adverse effects. The authors suggested the longer APTT could have been a result of the route of administration used or due to the repeated dosing protocol, although there were only a small number of patients (16) in this study [125]. Given the relatively small number of experimental and clinical studies, it is still unclear whether heparin administration has therapeutic potential in clinical ALI/ARDS.

THE PROTEIN C PATHWAY

Protein C is an endogenous anticoagulant that circulates as an inactive zymogen synthesized by the liver. Protein C is cleaved in the presence of thrombin to activated protein C (APC) [126]. APC generation is accelerated by two cell surface receptors, thrombomodulin (TM) and the endothelial cell protein C receptor (EPCR). TM is a transmembrane glycoprotein that binds thrombin while EPCR, another transmembrane protein, binds protein C and presents it to the TM-thrombin complex for activation [127]. The plasma glycoprotein Protein S (ProS) acts as a cofactor for APC, enhancing APC activity by several-fold [128]. First described as a potent anticoagulant factor, APC and ProS inactivate the coagulation factors Factor Va, Factor VIIIa and may also enhance fibrinolytic activity by inactivating plasminogen activating inhibitor-1 (PAI-1) [126,129-132]. In addition to anticoagulation, APC suppresses cytokine production, inhibits leukocyte attachment to the endothelium and inhibits p53-mediated apoptosis [4,133-135]. Finally, APC has been shown to decrease inflammatory mediator-induced TF expression in leukocytes through an EPCR-dependent mechanism [129,136,137].

Various studies have focused on the endothelium as the primary site for activation of protein C. However, lung epithelial cells *in vitro* have also been shown to modulate the protein C pathway [4]. Wang and colleagues showed that cultured lung epithelial cells could activate protein C at levels similar to cultured human umbilical vein endothelial cells [138]. Other studies analyzing sputum from asthma patients and cultured human epithelial cells showed that lung epithelial cells express mRNA for protein C, TM and EPCR and that expression of these genes is downregulated in the presence of inflammatory mediators [139]. Interestingly, the presence of thrombin increases protein C activation in bronchial airway-derived cells, again demonstrating the ability of lung cells to activate protein C [139]. In addition, Wang *et al.* demonstrated that exposure of A549 lung epithelial cells to inflammatory stimuli resulted

in shedding of TM and EPCR into the medium and a reduced capacity to activate protein C [138]. These findings are concordant with other studies demonstrating that inflammatory mediators such as IL-1β, TNF-α and LPS that play a role in the development of sepsis and ALI/ARDS, also lead to shedding of both TM and EPCR from the endothelial cell surface [140,141]. Taken together, these findings suggest that downregulation of the protein C pathway both systemically and in the injured alveolus could play a role in modulating intra-alveolar fibrin deposition.

In animal models of LPS-induced lung injury, inhalation of APC resulted in a dose-dependent decrease in BALF-derived coagulation and inflammation that correlated with improved lung function [142]. Furthermore, in a rat model of hyperoxia-induced injury, IP injection of APC attenuated lung injury, apoptotic activity in the epithelium and cytokine levels associated with hyperoxia [143]. Interestingly, in another rat model of intestinal ischemia-reperfusion that leads to indirect lung injury from systemic inflammatory mediators, recombinant APC attenuated ischemia-induced lung neutrophil recruitment and activation compared to saline-infused controls [144]. In another study, exposure of rats to IV-LPS resulted in increased pulmonary vascular injury measured by wet to dry ratios and tumor necrosis factor-α (TNF-α) levels which were attenuated after treatment with IV-APC [145]. However, in a rat model of LPS-mediated lung injury, administration of nebulized APC attenuated only pulmonary coagulopathy as measured by BAL-derived thrombin-antithrombin (TATc) levels and fibrin degradation products (FDPs) without altering inflammatory endpoints [146]. These findings suggest that modulation of LPS-mediated lung injury by APC depends on the route of exposure and more studies need to be conducted to further clarify the mechanisms of protection by APC.

Clinical studies of the protein C pathway have focused primarily on patients with severe sepsis. Patients with severe sepsis have reduced plasma levels of protein C that are associated with an increased propensity for developing ARDS, an increased need for mechanical ventilation and higher mortality [147]. The ability to activate protein C also has been shown to vary amongst patients with severe sepsis. Despite having similar levels of the zymogen protein C in the plasma, patients who survive had higher plasma levels of activated protein C [148]. In an attempt to understand the mechanism of lower levels of activated protein C in sepsis patients, levels of soluble thombomodulin and EPCR have been measured in plasma. Sepsis patients have higher soluble EPCR and TM levels, consistent with shedding of TM and EPCR from the endothelial cell membrane into the circulation and loss of the ability of these cells to activate protein C [149]. In numerous studies involving sepsis patients, circulating TM levels in the blood have been associated with multiple organ failure including cardiovascular, respiratory, neurologic, hematologic, renal and hepatic failure [4,149-151]. These findings suggest that increased circulating levels of these proteins reflect loss of an important biologic function (the ability to activate protein C) which may contribute to the imbalance between coagulation and anticoagulation.

Fewer studies have focused on the protein C pathway in patients with ALI/ARDS. Lower levels of protein C have been measured in the plasma of patients with ALI/ARDS compared to normal controls in a single center study [152]. In a larger multicenter study, Ware *et al.* reported that lower protein C was an independent predictor of mortality and was modulated by ventilator strategy in a study of patients with ALI/ARDS enrolled in a multicenter clinical trial [153]. In addition, patients with ALI/ARDS have higher levels of TM in the pulmonary edema fluid compared to critically ill control patients and levels were associated with worse clinical outcomes [152]. Therefore, similar to patients with sepsis, patients with ALI/ARDS have an imbalance between APC and protein C levels resulting in a decreased ability to downregulate lung fibrin deposition that appears to be strongly associated with adverse outcomes.

In experimental studies, recombinant APC treatment reduced endotoxin-induced mortality in a baboon model of sepsis [154]. Recombinant activated protein C was tested in a phase III trial (PROWESS) in 1690 patients with severe sepsis. Analysis of the patients' baseline characteristics showed that although the incidence of ALI/ARDS was not reported, a number of patients (approximately 20% in each treatment group) had chronic lung disease such as chronic obstructive pulmonary disease (COPD) and 50% had acute lung infections [155]. In this trial, drotecogin alfa (a recombinant form of human APC) resulted in significantly improved mortality compared to placebo [124,156]. Specifically, the reduction in relative risk of death was 19.4% and the absolute reduction in mortality at 28 days was 6.1% [156]. Interestingly, it was determined in a retrospective study that in the group of patients enrolled in PROWESS who were likely to have had ALI/ARDS, the time to resolve respiratory dysfunction was significantly shorter in patients treated with rhAPC compared to placebo-treated controls [4,157]. Based on findings from the PROWESS trial, the Food and Drug Administration (FDA) approved the use of recombinant human APC (rhAPC) in the treatment of severe sepsis if the APACHE II score was greater than 25 [126,158].

In subsequent years there have been additional trials involving rhAPC. Once again, these studies did not report the incidence of ALI/ARDS but included numerous patients requiring mechanical ventilation suggesting there was a substantial number of patients with ALI/ARDS. These studies included the ENHANCE trial, a single-arm, open-label trial of rhAPC that yielded similar results to the PROWESS study with the suggestion that patients treated earlier with the drug had better outcomes [159]. Another study, the ADDRESS trial was stopped after enrollment of 2640 patients due to the fact that there were no differences in outcome in severe sepsis patients with low risk of death receiving drug compared to placebo controls [160,161]. Finally a phase II trial (RE-SPOND) investigated the value of variable duration of treatment with rhAPC in septic patients directed by the levels of endogenous protein C as some patients in the clinical trials still had low levels of protein C despite treatment with rhAPC. The results of this trial have not yet been published [126,162]. However, in a very small phase II randomized placebo-controlled trial involving nonseptic patients with ALI, APC treatment did not alter the number of ventilator-free days or mortality compared to placebo controls [163]. These findings suggest that rhAPC is a viable option to treat patients with ALI/ARDS due to severe sepsis.

Protein S

Protein S acts as a cofactor for APC, enhancing APC activity by several-fold [126]. Investigations focusing on members of the Protein C pathway led by Takagi *et al.* demonstrated the potential for Protein S in ameliorating ALI in a mouse model of LPS-mediated lung injury. IP injection of Protein S alone or in combination with APC followed by IT instillation of LPS resulted in decreased injury represented by a reduction in lung cytokines and chemokines. However, protein S did not alter TATc measurements, indicating it was modulating LPS-mediated inflammation but not coagulation [128]. In addition, exposure of A549 cells to protein S also inhibited LPS-induced expression of cytokines, again indicating a role for protein S in modulating inflammatory processes involved in lung injury [128]. Although not yet studied after lung injury is established, these data show the potential for investigating Protein S as another possible therapeutic strategy for patients with ALI/ARDS.

Thrombomodulin

Thrombomodulin (TM), the transmembrane protein responsible for binding thrombin and activating protein C, has also been investigated in modulating inflammation and acute lung injury. Patients with sepsis and ALI/ARDS have elevated plasma levels of soluble TM that are associated with worse clinical outcomes and multiple organ dysfunction [4,149-151].

Soluble TM is thought to be a less effective inhibitor of platelet and fibrinogen activation compared to full-length TM [164]. Therefore, investigators have utilized modulated forms of TM in experimental and clinical studies.

Hagiwara *et al.* showed in a rat model of sepsis that rats treated with IV recombinant TM and then concurrently injected with IV LPS had reduced inflammation and ALI compared to saline-treated, LPS-injected rats [165]. In other models of LPS-induced sepsis, administration of a recombinant soluble or recombinant human TM reduced fibrin deposition, inflammation and edema [122,166]. However, the mechanisms of the protective effects of TM may not be primarily related to modulation of coagulation. Recent studies have shown the N-terminal C-type lectin-like domain (LLD) of TM, which has no effects on coagulation, has anti-inflammatory properties. Specifically, the LLD acts to inhibit inflammatory cell adhesion, aids cell survival, suppresses activation of mitogen-activated protein (MAP) kinase and NF-κB pathways and inhibits complement activation [167-169]. In a model of ischemia-perfusion lung injury, mice lacking the LLD had augmented inflammation as evidenced by an increase in pro-inflammatory mediators and cells in bronchoalveolar lavage compared to wildtype mice [169]. Pretreatment of wildtype mice with recombinant LLD from TM reduced inflammation induced by ischemia-reperfusion [169].

Recently, a corporation in Japan (Asahi Kasei) developed a drug consisting of soluble recombinant human TM labeled ART-123 with the intention of treating patients with thromboembolism and blood clotting disorders, such as disseminated intravascular coagulation (DIC), a condition known to complicate infections such as sepsis [170,171]. ART-123, which consists of the active, extracellular domain of TM and has the same protein C activating cofactor activity as full-length native TM, has been shown to contain both thrombin inhibiting and protein C inducing properties [170,171]. It has since been studied in numerous clinical trials. In a phase I study involving healthy volunteers, the pharmacokinetics and pharmacodynamics were determined [171]. A phase II study demonstrated good dose-response effects in patients with DIC [172]. Finally, in a phase III trial comparing low-dose heparin to ART-123 therapy in patients with DIC, DIC was resolved in 66.1% of the ART-123 group compared to 49.9% of the heparin group [172]. Based on these findings, ART-123 is approved to treat patients in Japan with DIC. Currently, the only experimental study published so far utilizing ART-123 demonstrated that ART-123 decreased LPS-induced mortality, liver dysfunction and inflammation in rats [173]. Future studies of ART-123 in both experimental models of ALI/ARDS as well as in clinical studies will be of great interest.

IMPAIRED FIBRINOLYSIS IN ALI/ARDS

Fibrin deposition is normally balanced by endogenous fibrin degradation pathways. Fibrin degradation is modulated by plasminogen (PA) and plasminogen activator inhibitors (PAI-1 and PAI-2) which govern the conversion of plasminogen to plasmin, a fibrinolytic enzyme [4]. Urokinase-type plasminogen activator (u-PA), a cell surface protein which functions to activate fibrinolysis at the tissue level and tissue-type plasminogen activator (t-PA), a soluble protein, which activates intravascular fibrinolysis [4,174,175] are two plasminogen activators inhibited by PAI-1 and PAI-2 with PAI-1 being the principal endogenous fibrinolytic inhibitor in humans. Inhibition of fibrin degradation pathways has been demonstrated in patients with ALI/ARDS [110].

In the lung there are many cellular sources of plasminogen activators and inhibitors. Alveolar macrophages that are unstimulated maintain a pro-fibrinolytic state which shifts with stimulation to an anti-fibrinolytic state [4]. For example, LPS treatment of human

alveolar macrophages increases PAI-1 activity and inhibits fibrin degradation while the lungs of patients with ARDS have constitutively increased mRNA expression of PAI-1 [176,177]. In mice genetically deficient in PAI-1, bleomycin exposure resulted in a decreased fibroproliferative response concomitant with enhanced fibrinolytic activity while PAI-1 overexpression led to enhanced fibroproliferation [26,178].

In addition to alveolar macrophages, lung microvascular endothelial cells also produce and secrete PA and PAI-1 [179,180]. These cells behave similarly to alveolar macrophages in that pro-inflammatory mediator stimulation results in a shift towards an enhanced anti-fibrinolytic state as is evidenced by a study analyzing isolated endothelial cells from ARDS patients in which cells constitutively expressed higher levels of PAI-1 compared to endothelial cells isolated from healthy controls [180].

Lung epithelial cells also express PA and PAI-1 and are therefore able to modulate fibrinolysis. In mice expressing an inducible lung-specific u-PA within the epithelium, increased expression and activity in the lungs and lavage fluid led to attenuation of bleomycin-induced lung collagen deposition, accelerated fibrin clearance and reduced mortality [181]. In primary rat epithelial cells isolates, PA and PAI-1 expression increased over time as the cells differentiated from type II to type I cells [4,182,183]. Exposure of rat lung epithelial cells to inflammatory mediators such as LPS or TNF-α increased PAI-1 and u-PA expression demonstrating that during inflammatory diseases the epithelium can modulate the fibrinolytic axis [7,182,184,185].

However, there have been fewer studies of PA and PAI-1 in human lung epithelial cells. In A459 cells, exposure to TNF-α and IL-1β increased u-PA mRNA and protein without altering PAI-1 expression [4,186]. However, recently in a model of cigarette-smoke and LPS-induced injury, human epithelial cells showed increased expression of PAI-1 compared to controls. This led to increased inflammatory mediator production that was attenuated after siRNA-mediated downregulation of PAI-1 expression [187]. Lung epithelial expression of u-PA, u-PAR and PAI-1 is regulated by specific, newly recognized posttranscriptional mechanisms that control expression of these proteins at the level of mRNA stability [188]. This suggests that modulation of the expression of both fibrinolytic and anti-fibrinolytic mediators is stimulant-specific.

There are relatively few clinical studies of fibrinolysis in ALI/ARDS. Idell *et al.* showed decreased fibrinolytic activity in the BAL fluid of ARDS patients compared with normal, control subjects Fig. (**3**) [87]. In other studies, PAI-1 levels in plasma and edema fluid were shown to be higher in comparison to control patients with pulmonary edema due to hydrostatic causes [189]. Similar results in which PAI-1 levels are higher in the plasma of ARDS patients than controls have also been shown in other clinical studies [153,190,191]. Studies of genetic heterogeneity in ARDS demonstrated that in two populations of patients at risk (meningococcal septicemia and severe trauma) for developing ALI/ARDS that polymorphisms in the promoter region of PAI-1 were associated with developing ALI/ARDS [192]. Specifically, increased disease severity and impaired fibrinolytic ability in patients with meningococcal disease and severe trauma have been associated with single base pair insertion/deletion promoter polymorphisms [4,193-196]. More work needs to be done to fully characterize the fibrinolytic pathway and its modulation in patients with ALI/ARDS as potential therapies could also be directed at this pathway.

There have been relatively few experimental and clinical studies on the role of fibrinolytic agents in modulating lung inflammation and injury and results of these studies have been mixed. In a pig model of lung injury induced by trauma, intravenous administration of u-PA or t-PA was protective [197] whereas in a model of LPS-induced lung inflammation, u-PA

has been shown to potentiate PMN recruitment. This effect was demonstrated to be specifically mediated through the u-PA kringle domain (KD) which was shown to be largely localized to alveolar epithelial cells [198]. In these experiments, antibodies against the KD reduced LPS-induced inflammation indicating a possible role for neutralization of u-PA activity as a therapy in inflammatory lung diseases [198]. Furthermore, in mice genetically deficient in u-PA or uPA-R, immune complex (IC)-mediated pulmonary inflammation was attenuated compared to WT mice [199]. However, other models of lung injury have shown attenuation of bleomycin-induced fibrosis after administration of u-PA, either by inhalation, instillation or injection [200-202]. Finally, a phase I clinical trial demonstrated the ability of plasminogen activators (u-PA and t-PA) to improve lung function as evidenced by increased arterial blood oxygen levels in a small group of ARDS patients [203]. Perhaps u-PA and other plasminogen activators modulate lung inflammation and injury differentially depending on the underlying cause of lung injury or the route of administration of the drug. Thus, more trials in targeted patient populations will be necessary to investigate efficacy and safety in treating ALI/ARDS patients.

With regards to PAI-1 inhibitors, currently there are only a few described and none are in clinical use [204]. Amongst these are a variety of antibodies that block PAI-1 activity *in vivo* [205]. In addition, peptides, low molecular weight inhibitors and antisense oligonucleotides are also available to inhibit PAI-1 synthesis and activity [205]. In a rabbit sepsis model, a monoclonal antibody to PAI-1 attenuated the dramatic and sustained increase in plasma PAI-1 activity after infusion of LPS [206]. In a rat model of arterial thrombosis, utilization of a Fab-fragment that inhibits PAI-1 activity resulted in reduced thrombus size and increased the rate of perfusion, thus partly restoring blood flow [207]. Finally, Izuhara *et al.* recently identified new orally active molecules that inhibited PAI-1 activity and enhanced fibrinolysis in a rat model of arteriovenous shunt and a mouse model of bleomycin-induced lung injury [204]. Although these studies are promising, inhibitors of PAI-1 have yet to be tested in patients with ALI/ARDS. Therefore, the development of pharmacologically active PAI-1 inhibitors and evaluation of their efficacy in animal models and eventually in humans is essential.

CONCLUSION

ALI and ARDS are characterized by profound imbalances between coagulation and fibrinolysis. Fibrin deposition in the alveolar spaces is a hallmark of this clinical syndrome that most likely results from inflammation-induced activation of the coagulation cascade and impairment of fibrinolysis. However, the exact mechanisms regulating intra-alveolar fibrin deposition remain unclear. Potential therapeutic strategies could be aimed at reducing TF activity, enhancing fibrinolysis through the use of various recombinant proteins, and reducing PAR signaling to limit inflammation. In spite of improved outcomes in experimental animal studies with these strategies, there have been few successes in clinical trials. Reasons may include heterogeneous patient populations, insufficient understanding of drug activity, interactions and metabolism in humans, and need for further optimization of timing of therapeutic intervention, doses and duration of therapy. In addition, adverse effects in humans, which were not observed in experimental models, have also contributed to limited success with these strategies. Furthermore, lack of understanding of the complex role of coagulation and fibrinolysis in the pathogenesis of ALI/ARDS may also contribute to the limited accomplishments in affecting patient outcomes. In addition, many of the published clinical studies have been conducted in patients with sepsis, and have not been targeted specifically at ALI/ARDS. A better understanding of the impact of potential therapies aimed at coagulation and fibrinolysis on disease progression through both experimental and human studies may ultimately lead to new therapies for acute lung injury.

Acknowledgments

Funded by: American Heart Association Established Investigator Award to LBW, NIH K08 grant HL090785 to JAB and NHLBI T32 HL087738 to SCS.

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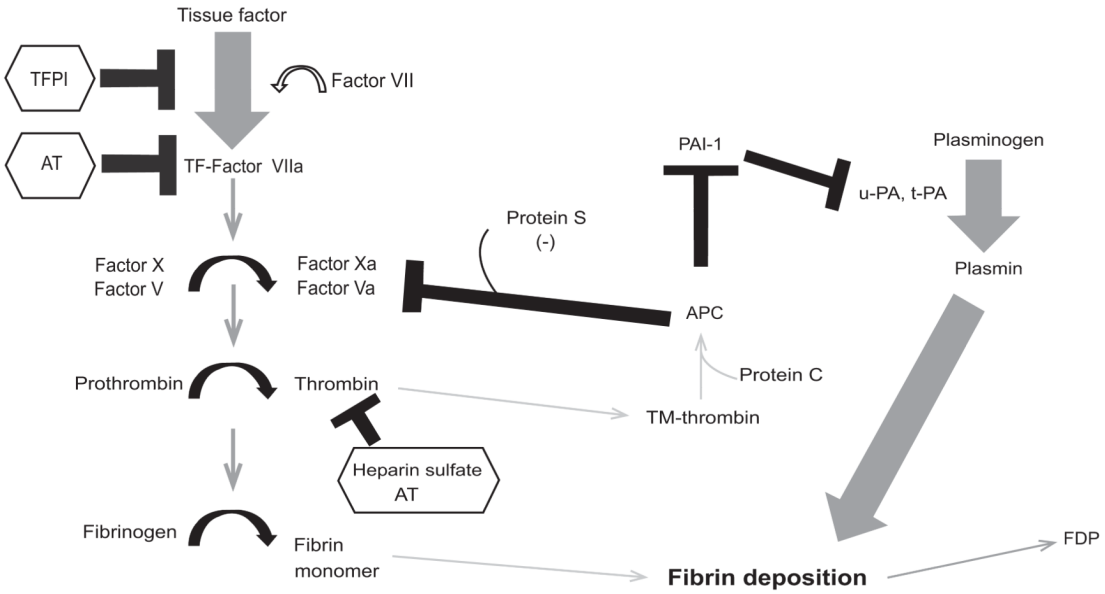

Fig. (1).

The extrinsic coagulation pathway, the anticoagulant pathway and the fibrinolytic pathway. Activation of TF results in activation of coagulation factors and generation of thrombin from prothrombin ultimately leading to fibrin formation and deposition. The endogenous inhibitor TFPI limits the action of TF. AT degrades Factor Xa and thrombin while heparin sulfate inhibits thrombin-mediated activation of fibrinogen. The anticoagulant mediator Protein C is activated by thrombin binding to TM to generate APC which, along with Protein S, degrades Factor Va. APC also limits PAI-1 activity which inhibits the fibrinolytic mediators u-PA and t-PA from generating plasmin which degrades fibrin into FDPs. TF, tissue factor; TFPI, tissue factor pathway inhibitor; Factor VIIa, activated Factor VII; Factor Xa, activated Factor X; Factor Va; activated Factor V; AT, antithrombin; TM, thrombomodulin; APC, activated protein C; PAI-1, plasminogen activator inhibitor-1; u-PA, urokinase plasminogen activator; t-PA, tissue plasminogen activator; FDP, fibrin degradation product.

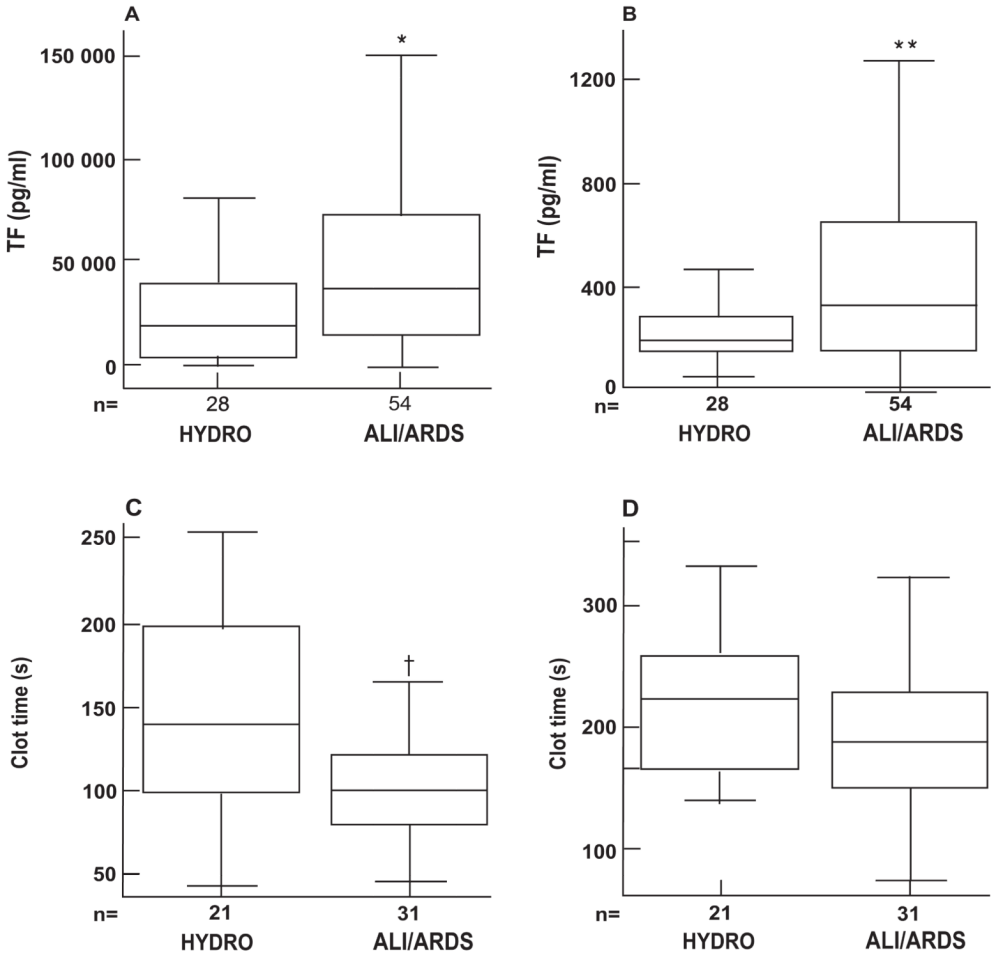

Fig. (2).

Comparison of plasma and pulmonary oedema fluid levels of tissue factor (TF) and clot time in patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) and control patients with hydrostatic pulmonary edema (HYDRO). (A, B) Boxplots of TF protein levels in (A) edema fluid and (B) plasma measured by ELISA. TF protein levels in pulmonary edema fluid were significantly higher in ALI/ARDS vs HYDRO (*p = 0.012, Mann - Whitney U test) and TF protein levels in plasma were significantly higher in ALI/ARDS vs HYDRO (**p = 0.02, Mann - Whitney U test). Note that TF levels in edema fluid (A) are more than 100 - fold higher than simultaneous levels in plasma (B) in both patient groups. (C, D) Boxplots of clot time measured by recalcification time of normal plasma mixed with pulmonary edema fluid from patients with ALI/ARDS or HYDRO in the absence (C) or presence (D) of a TF blocking antibody. Clot time was significantly longer in plasma mixed with edema fluid from patients with HYDRO vs ALI/ARDS (†p = 0.006, Mann - Whitney U test), and this difference was negated when TF activity was blocked (p = 0.095, Mann - Whitney U test). Reproduced with permission from [The alveolar epithelium can initiate the extrinsic coagulation cascade through expression of tissue factor, JA Bastarache, L Wang, T Geiser, Z Wang, K Albertine, M Matthay and LB Ware, 62(7), 608-16, 2007] BMJ Publishing Group LTD.[16]

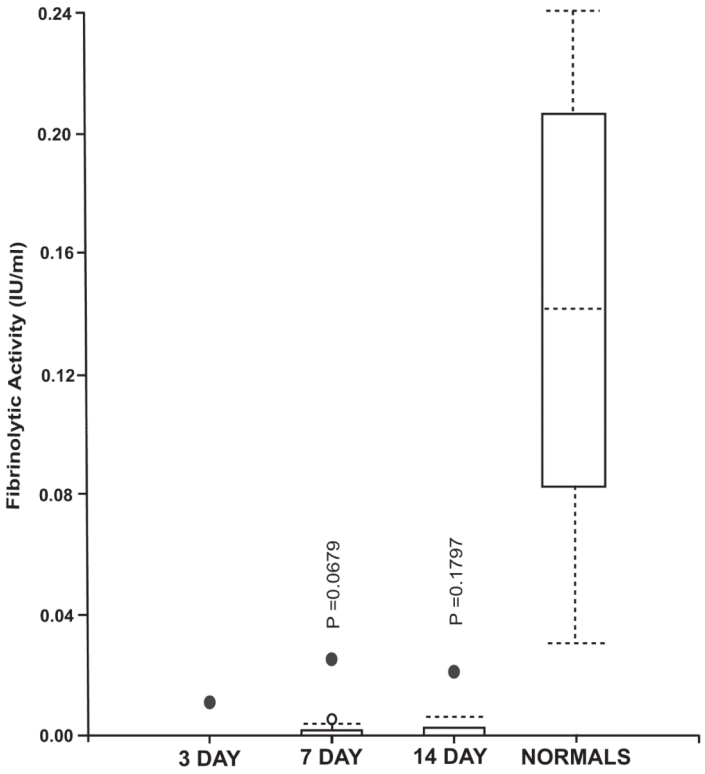

Fig. (3).

Fibrinolytic activity in human serial ARDS BAL samples. Fibrinolytic activity measured by the ^{125}I-fibrin plate assay is indicated in box plot format. Data from 7 normal control patients are shown for comparison. Reproduced with permission from Serial abnormalities of fibrin turnover in evolving adult respiratory distress syndrome, S Idell, KB Koenig, DS Fair, TR Martin, J McLarty and RJ Maunder, 261, L240-248, 1991] from AJP [87].

Table 1
Summary of Clinical Trials in Severe Sepsis and ALI/ARDS

Therapy	Study	Design	Outcome	Patient No./Type
Active site inactivated recombinant Factor VIIa (FFr-rFVIIa)	Vincent *et al.*[94]	RCT Phase II	No beneficial effects on patient morbidity or outcomes; 4 × 400 μ/kg associated with increased mortality	214/ ALI/ARDS
Recombinant TFPI	de Jonge *et al.*[104]	RCT	High dose reduced LPS-mediated TATc	16/LPS
Recombinant TFPI	Abraham *et al.*[105]	RCT Phase II	Trend toward reduction in 28-day all cause mortality	210/ Severe Sepsis
Recombinant TFPI	OPTIMIST[106,107]	RCT Phase III	No difference in overall mortality	1955/ Severe Sepsis
Nebulized heparin	Dixon *et al.*[125]	Open label Phase I	Prolonged APTT, no adverse effects	16/ ALI
Recombinant Antithrombin III	Warren *et al.*[115] (Kybersept)	RCT Phase III	No difference in 28-day mortality, high dose was associated with increased risk of hemor- rhage when administered with heparin	2314/ Severe Sepsis
Recombinant activated protein C	PROWESS[126,156]	RCT Phase III	Significant reduction in 28-day, all cause mortality; reduced hospital and 3 month mortality	1690/ Severe Sepsis
Recombinant activated protein C	ENHANCE[159]	Open label	Similar 28-day, all cause mortality compared to PROWESS; patients treated earlier had better outcomes (<24 hrs)	2434/ Severe Sepsis
Recombinant activated protein C	ADDRESS[160,161]	RCT	No difference in 28-day, all cause mortality in patients with low risk for death	2640/ Severe Sepsis
Recombinant activated protein C with dosing guided by measurement of pro- tein C levels	RESPOND[126,162]	Double blind	No published results	488/ Severe Sepsis
Recombinant activated protein C	Liu *et al.*[163]	RCT Phase II	No difference in ventilator-free days or 28- day mortality	75/ ALI without severe sepsis
Recombinant soluble thrombomodulin (ART-123) with comparison to low- dose heparin	Saito *et al.*[172]	Randomized double blind Phase III	Significantly improved DIC compared to low-dose heparin and alleviated bleeding symptoms	234/ DIC
u-PA and t-PA	Hardaway *et al.*[203]	Phase I	Improved lung function	20/ ARDS sec- ondary to trauma and/or sepsis

ADDRESS, Administration of Drotecogin alpha (activated) in early stage Severe Sepsis; APTT, activated partial thromboplastin time; AT, antithrombin; DIC, disseminated intravascular coagulation; ENHANCE, Extended Evaluation of Recombinant Human Activated Protein C; LPS, lipopolysaccharide; OPTIMIST, TFP007 OPTIMIST [Optimized Phase III Tifacogin in Multicenter International Sepsis Trial]; PROWESS, Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis; RCT, randomized controlled trial; RESPOND, Research Evaluation Serial Protein C levels in severe sepsis patients On Drotrecogin alfa (activated); t-PA; tissue plasminogen activator; TATc, thrombin-antithrombin complex; TFPI, tissue factor pathway inhibitor; u-PA, urokinase plasminogen activator.