Recombinant Thrombomodulin and Activated Protein C in the Treatment of Disseminated Intravascular Coagulation

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Introduction

The blood coagulation cascade is regulated by the luminal surface of the endothelial cell lining. Endothelial cells synthesize tissue factor pathway inhibitor (TFPI), which, in part, binds to the cell surface glycosaminoglycans and inhibits factors Xa, V, VIIa, and tissue factor. Endothelial cells also produce and exhibit thrombomodulin (TM) on their luminal surface. TM is a kind of thrombin receptor that forms a 1:1 complex with thrombin. In this complex, thrombin activates protein C (PC) more than 1,000-fold more than thrombin alone. TM then loses its procoagulant activities, which include fibrinogen clotting, activation of factors V and VIII, and platelet activation. Thus, TM converts thrombin from a procoagulant protease to an anticoagulant. Pathologic states, such as an endothelial injury or perturbation or continuous rapid coagulation cascade activation, overcomes the endothelial regulating activity, resulting in the development of intravascular coagulation and the induction of disseminated intravascular coagulation (DIC). Theoretically, then, supplementing soluble TM or activated PC (APC) to reconstitute the endothelial coagulation regulation system in the circulation and regulate pathologically-activated blood coagulation could be beneficial. In this chapter, application of soluble TM and APC in the treatment of DIC is reviewed.

Pathophysiology of Disseminated Intravascular Coagulation from the Viewpoint of Endothelial Dysfunction

Physiologically normal endothelial cells possess anticoagulant and profibrinolytic activities and prevent intravascular coagulation, including thromboembolism and DIC. These activities occur via TM-PC and the tissue plasminogen activator/plasmin system. Briefly, the endothelial cells produce membrane glycoprotein TM, which converts thrombin from a procoagulant protease to an anticoagulant. In our previous in vitro study, approximately 50,000-60,000 molecules of TM per cell are present on the cell surface. However, some studies have reported that the molecule is down-regulated by inflammatory cytokines, interleukin (IL)-1, tumor necrosis factor (TNF)-α, and endotoxin. Thus, the TM-PC system may be impaired in severe inflammatory states and Gram-negative bacterial infections. As this may partially account for the mechanism of DIC, a strategy to reconstitute the TM-PC system by providing soluble TM and/or PC could, theoretically, be beneficial in the treatment of DIC and thrombosis.

Experimental Studies of Recombinant Soluble Thrombomodulin (r-TM)

Structure and Function of Recombinant Thrombomodulin

The structure of recombinant soluble thrombomodulin (r-TM) is shown in Figure 1. We previously cloned a human TM cDNA and deduced its primary structure. Human TM is composed of five domains: an N-terminal domain (D1); a domain with six epidermal growth factor (EGF)-like structures (D2); a glycosylation site-rich domain (D3); a transmembrane domain (D4); and a cytoplasmic domain (D5). We have demonstrated that the region including the fourth, fifth, and sixth EGF-like structures of the D2 domain is the minimum region necessary for the activation of protein C and anticoagulant activity. We also constructed soluble TM, which contains D1, D2 and D3, and expressed functionally active TM in Chinese hamster ovary (CHO) cells (Fig. 1). This soluble TM has the same PC activating cofactor activity as native TM and exhibited antithrombotic activity in vitro, as described below.

Recombinant Thrombomodulin Efficiently Inhibits Clot-Bound Thrombin

Recent studies have suggested that clot-bound thrombin plays an important role in thrombus growth. It has also been suggested that active thrombin-containing microthrombi may be circulating in DIC and that this may play a role in the pathophysiology of DIC, especially the characteristic pathomechanism of “dissemination” of temporospatial activation of coagulation in the circulation. Thus, blocking and inhibition of these active thrombin on the fibrin clot provide a crucial strategy in the treatment of DIC. In this regard, we investigated the effect of r-TM on clot-induced coagulation in vitro. In these experiments, r-TM enhanced the activation of protein C by clots and attenuated clot-induced thrombin generation and fibrinopeptide
A (FPA) production in a dose-dependent manner. In addition, r-TM inhibited the regrowth of the clot in I-fibrinogen-supplemented plasma. Microthrombi prepared by thrombus homogenization also contained active thrombin, and the activity of thrombin was efficiently inhibited by r-TM.

Recombinant Thrombomodulin Recycling Model: Hypothesis of Kinetics Among Recombinant Thrombomodulin, Protein C, Thrombin, and Antithrombin III

Previously, we examined the kinetics of r-TM in the presence of thrombin, PC, and antithrombin III in vitro and proposed a hypothetical model among these molecules (Fig. 2).12 We reported data suggesting that r-TM rapidly forms reversible complexes with thrombin, resulting in the activation of PC. Then, the r-TM bound to thrombin releases from the complex and delivers the thrombin to antithrombin III. Finally, free r-TM is recycled and recaptured by another thrombin molecule (Fig. 2). The rather long half-life of r-TM in vivo, as described below,13 could be explained by this in vitro based hypothetical model.

The Antithrombotic Effect of Recombinant Thrombomodulin

We previously demonstrated the antithrombotic effect of r-TM on thrombin-induced thromboembolism in mice.9 Thrombin injection caused fatal acute thromboembolism in the lung with fibrin deposition in the large and small vessels. Pretreatment of mice with r-TM rescued the mice with remarkable improvement in histological findings.

Next, we examined the effect of r-TM on endotoxin-induced DIC.14 Continuous intravenous infusion of endotoxin into rats caused reductions in the number of platelets, decreased fibrinogen, and increased fibrinogen/fibrin degradation products (FDP) and fibrin deposition in the glomerulus, suggesting the development of DIC. In these experiments, r-TM administration improved these abnormal parameters and diminished the glomerular fibrin disposition. Although heparin displayed effects similar to r-TM, the activated partial thromboplastin time (aPTT) was much more prolonged in heparin-treated rats than that of r-TM.

Next, we investigated the effect of r-TM on tissue factor-induced DIC in rats15 and monkeys.16 Extended infusion of TF induced typical DIC with decreases in platelet and fibrinogen and increases in FDP. Pretreatment or co-infusion of r-TM blocked changes of these DIC-parameters without prolongation of aPTT. Heparin, which is a potent anti-DIC drug, also inhibited these changes with extra prolongation of aPTT and PT. Thus, these results suggest that r-TM prevents DIC with less bleeding tendency than heparin.

Heparin, used worldwide to prevent DIC, is antithrombin III-dependent, and the therapeutic efficacy markedly decreases in pathologic conditions with depleted plasma antithrombin III. However, antithrombin III is consumed and, as a result, antithrombin III levels are decreased in most patients with DIC. Based on this observation, we investigated the effects of r-TM in rats with immune-depleted antithrombin III levels and compared these effects with those associated with heparin.17 Antithrombin III-depleted rats were prepared by injecting rats with anti-rat antithrombin III polyclonal antibody. When infused with tissue factor, the rats developed characteristic DIC. At doses of r-TM and heparin that were equally effective at inhibiting the decrease in platelet count and fibrinogen level in control rats treated with tissue factor, only r-TM remained effective in preventing DIC in the antithrombin III-depleted rat model. Therefore, r-TM effectively inhibits coagulation independent of antithrombin III levels, in contrast to heparin, which depends on the antithrombin III level. These results suggest that r-TM may be effective in treating DIC in patients with decreased antithrombin III levels.

The Effect of Recombinant Thrombomodulin on Disseminated Intravascular Coagulation in Humans

As described above, a potent effect of r-TM on thromboembolism was demonstrated. As a result, r-TM was studied in humans with DIC. First, the pharmacokinetic parameters were determined by intravenously infusing 0.03 mg, 0.1 mg, or 0.3 mg r-TM into healthy volunteers after informed consent was obtained. When 0.3 mg of r-TM was administered as a bolus intravenous injection, the mean elimination half-life was approximately 20 hours. No abnormal findings or laboratory tests, including blood pressure, heart rate, electrocardiogram, body temperature, hematology, blood chemistry, and urinalysis, were observed.13,18

Based on these data, we are now conducting a nationwide trial of the effect of r-TM on DIC in Japan. We have finished the open trial and obtained good dose-response effect of r-TM in patients with DIC. A double-blind study to evaluate the effect of r-TM in DIC is now being designed.
Activated Protein C

Protein C is a vitamin K-dependent anticoagulant protease that is activated by thrombin and TM complex. Activated PC (APC) efficiently inactivates activated coagulation factors Va and VIIIa and acts as a component of natural anticoagulant. It has been reported that PC is consumed and decreased in the plasma of patients with DIC. Moreover, it has been described that the inflammatory cytokines, IL-1 and TNF, and endotoxin down-regulate TM expression. This may lead to a marked decrease in the capacity to generate APC and maintain anticoagulant homeostasis within the circulation. Thus, administration of APC, and subsequent inhibition of activated coagulation, is reasonable. Plasma-derived APC has been shown to prevent thrombus formation in various animal models and be useful in the treatment of DIC. Taylor et al described that APC prevented E. coli-induced organ damage and coagulopathy in baboons, resulting in a reduction in the mortality rate.

Okajima and coworkers have been investigating the effect of APC on various pathologic states. They showed that APC attenuated endotoxin-induced pulmonary vascular injury. They also presented data indicating that DIP-APC, which has no protease activity, prevented neither the lipopolysaccharide (LPS)-induced pulmonary leukocyte accumulation nor the subsequent pulmonary injury, suggesting that the inhibitory effect of APC on leukocyte activation may be mediated by its serine protease activity. This suggested that APC inhibits pulmonary vascular injury by inhibiting activated leukocytes and not by its anticoagulant activity. It has also been reported that APC prevents the lethal effects of shock in primate and rodent models. This beneficial action of APC may be mediated through the inhibitory effects of cytokine production by macrophages, as APC injection markedly suppresses the peak blood levels of TNF-α in rats challenged with high doses of LPS, and anti-TNF-α mAb therapy suppresses many of the toxic features of endotoxemia. Salem et al reported that APC inhibited the production of TNF-α and prevented down-regulation of membrane CD11b, CD14, and CD18, but had no effect on the up-regulation of major histocompatibility complex (MHC) class II, intercellular adhesion molecule (ICAM)-1, or IL-2 receptor in human mononuclear phagocytic cells. Thus, they concluded that APC inhibits host cytokine production but maintains macrophage responses associated with adhesion, phagocytosis, and killing of Gram-negative bacteria. This proposed action of APC may be a logical and potent adjunctive therapy in the treatment of selected inflammatory diseases involving macrophage activation and host cytokine overproduction. Grinnell et al demonstrated that APC inhibits E-selectin-mediated cell adhesions through carbohydrate moieties of APC. Thus, APC inhibits not only the coagulation cascade through degradation of factors Va and VIIIa, but also cytokine production in macrophages. However, the anti-inflammatory action of APC on cytokine production remains to be elucidated.

Conclusion

Thrombin plays a crucial role in the thromboembolic state and in the development of DIC through its effects on fibrin formation, platelet activation, and activation of coagulation factors V, VIII, XI, and XIII. Moreover, thrombin induces various kinds of cellular responses to vessel-wall cells, including endothelial cells, inflammatory cells, and neuronal cells. This wide variety of cellular responses to thrombin is mediated by thrombin receptor activation. Four types of thrombin receptor isoforms have been identified so far, and these are called protease-activated receptors (PAR). The firstly discovered receptor is called PAR-1. We previously showed that thrombin activates PAR-1 and results in the activation of NF-κB in vascular smooth muscle and endothelial cells. NF-κB activation induces the expression of inflammatory cytokines (IL-1, IL-6 and IL-8), factors in the coagulation/fibrinolytic system (tissue factor, PAI-1, tissue factor), cell adhesion molecules (P-selectin, ICAM-1 and VCAM-1), oncogenes (c-myc, c-fos, and c-jun).

These facets of thrombin action may also play a role in the pathophysiology of DIC. Thus, the goals of the therapeutic strategy for DIC are as follows: to inhibit thrombin generation, neutralize thrombin, block the thrombin/thrombin receptor signaling pathway, or inhibit NF-κB activation (Fig. 3). From this viewpoint, unfractionated and low molecular weight heparins,
as described above, inhibit thrombin generation and neutralize thrombin, while antithrombin III neutralizes thrombin. Since r-TM converts thrombin from a procoagulant protease to an anticoagulant, it may be a new kind of potent anti-DIC agent. This is partly suggested by our experimental studies.

APC inhibits thrombin generation through inactivation of activated factors V and VIII. However, in addition to the anticoagulant activity, APC has been suggested to possess anti-inflammatory action. The pathophysiology of DIC is not only limited to the intravascular activation of the coagulation cascade, but it is also accompanied by an inflammatory process, including the production of increased levels of cytokines (IL-1, IL-6, IL-8 and TNF-α) and leukocyte activation. Therefore, if APC has anti-inflammatory activity, APC may be a potent anti-DIC medicine.

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References