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Thrombomodulin in the treatment of atherothrombotic diseases

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TABLE OF CONTNETS

1. ABSTRACT

Thrombomodulin (TM) is a membrane-bound glycoprotein receptor for thrombin in the protein C activation pathway. Since its discovery in the 1980s as an anticoagulant protein, recent data suggest that TM plays an important role in modulating cellular proliferation, adhesion and inflammation. A soluble TM fragment has been produced with recombinant DNA technology that can bind thrombin and activate protein C. Soluble TM has received much attention because of its potential clinical applications. This article will examine recent advances in the understanding of TM's novel physiological function and review the molecular mechanisms of TM's anti-inflammatory effect. This review will also summarize TM data generated from animal studies and clinical trials to date and will focus on the role of TM in treating thrombotic, atherosclerotic and other inflammation-related diseases. These emerging data show that soluble TM is a promising agent that may provide potential therapies to modulate the pathophysiological process of atherothrombotic and other inflammatory diseases in the near future.

2. INTRODUCTION

Thrombomodulin (TM) is a vascular endothelial cell-bound glycoprotein first identified as a natural anticoagulant with its major function of thrombin binding. The association of thrombin with TM significantly inhibits thrombin's procoagulant activity and decreases the production of thrombin itself (1-3). TM also plays an important role in the protein C anticoagulation pathway. The activation of protein C is highly accelerated by the TM-thrombin complex on the endothelial surface. Activated protein C proteolytically degrades coagulation factor Va and VIIIa and further shuts down the coagulation response (1-3). Structurally, TM is a multi-domain protein that consists of an N-terminal lectin-like domain (D1), an epidermal growth factor (EGF)-like domain (D2), a highly glycosylated serine and thereonine-rich domain (D3), a transmembrane domain (D4) and a cytoplasmic domain (D5) (Figure 1). The EGF domain (D2) is the thrombin binding site that is critical for thrombin inactivation and protein C activation (4,5). Originally, TM was known as an endothelium-specific protein. However, under some circumstances, TM also appears abundantly on vascular

Table 1. Anti-inflammatory mechanisms of thrombomodulin

1. Blocks thrombin activation of protease activated receptors
2. Accelerates production of activated protein C
3. Activates thrombin activatable fibrinolysis inhibitor
4. Binds high-mobility group-B1 DNA binding protein

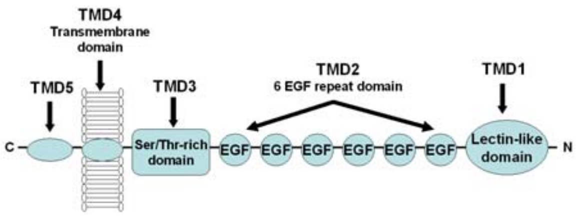

Figure. 1. Schematic representation of thrombomodulin (TM) structure. TM protein has 5 domains. The extracellular portion of TM is composed of 3 domains. From the N terminus, there is a lectin-like domain (TMD1). The second domain is a 6-repeat of epidermal growth factor (EGF)-like structure (TMD2). The third domain is a highly glycosylated serine and thereonine-rich domain (TMD3). Then, there is a transmembrane domain (TMD4) and a cytoplasmic tail (TMD5).

smooth muscle cells (6,7,8), platelets (9), monocytes (10), keratinocytes (11) and many types of cancer cells (12,13,14). The non-endothelial expression of TM implies that TM functions in physiological processes other than coagulation and hemostasis.

3. ANTI-INFLAMMATORY AND OTHER FUNCTION OF TM

TM has received much attention in recent years because of its anti-inflammatory effect. There are several indirect and direct pathways by which TM can regulate the inflammatory response (Table 1). The first is that TM binding to thrombin prevents thrombin from activating the protease activated receptor on endothelium because the thrombin binding site for TM and protease activated receptor is the same (15,16). Activation of protease activated receptor on endothelial cells induces the expression of von Willebrand factor and P-selectin, promoting rolling and adhesion of leukocytes and platelets. Blocking the activation of protease activated receptor attenuates the inflammatory signaling pathways, including chemotaxis in inflammatory cells and leukocyte adhesion molecule upregulation (17). Second, TM-thrombin complex can produce activated protein C efficiently and activated protein C itself exhibits strong anti-inflammatory activity, including inhibiting leukocytes adhesion (18) and the nuclear translocation of nuclear factor-kappa B in monocytes (19). Clinically, through its potent anti-inflammatory effect, activated protein C has been used to treat septic patients (20). Third, the TM-thrombin complex can activate thrombin activatable fibrinolysis inhibitor, also known as procarboxypeptidase B (21). TM has been demonstrated to markedly accelerate (1250-fold) the activation rate of thrombin activatable fibrinolysis inhibitor, which is a very potent inhibitor of inflammatory peptide bradykinin and the anaphylatoxins C3a and C5a in acute inflammatory reaction (21,22). Through these anti-inflammatory effects, augmented inflammatory response could be observed in mice with TM deficiency. Weiler *et al.* introduced a mutation (Glu 404□Pro) into the EGF domain (D2) of the mouse TM gene and caused a 2 to 3-fold decrease in TM expression with disruption of the TM-dependent protein C activation in mutant mice (23). The mutation not only elicited a hypercoagulable state but also caused an augmented inflammatory response after endotoxin stimulation with an increased elaboration of interlukin-6 and interleukin-1 beta in mutant mice.

In addition to these indirect pathways, TM has been recently shown to possess a direct anti-inflammatory effect through its lectin-like domain (D1). Conway *et al.* (24) generated transgenic mice that lacked the N-terminal lectin-like domain (TMD1) of TM. The TMD1-deleted mice were capable of producing normal amounts of activated protein C, but had stronger inflammatory reactions after stimulation. They had more leukocyte accumulation in the lungs after lipopolysaccharide inhalation. Mortality was increased in endotoxin-induced septicemia. There was also an augmented LPS-induced elaboration of tumor necrosis factor and interleukin-1 in these mice. Moreover, *in vitro* studies showed that endothelial cells isolated from the mutant mice had increased intercellular adhesion molecule-1 expression and intercellular adhesion molecule-1 mediated leukocyte adhesion after simulation. The recombinant TMD1 also had a direct suppressive effect on the cytokine-induced polymorphonuclear leukocyte adhesion on endothelium and activation of nuclear factor kappa B. Recent studies showed that TMD1 domain can bind high-mobility group-B1 DNA binding protein which is released by necrotic cells and triggers activation of pro-inflammatory signaling pathways after engaging the receptor for advanced glycation end products (RAGE) (25). After binding high-mobility group-B1 DNA binding protein, TMD1 prevented it from engaging RAGE on endothelium and significantly reduced the subsequent proinflammatory signaling pathways (25).

Beyond the anti-thrombotic and anti-inflammatory effect, TM is also noted for its influence on cellular proliferation and adhesion. Previous studies demonstrated that recombinant human TM composed of only the EGF domain (TMD2) has a potent mitogenic activity on cultured Swiss 3T3 fibroblast cell (26) and vascular smooth muscle cells (7). However, different TM fragments have variable influence on cell behavior (27). Overexpression of TMD123 on vascular smooth muscle cell reduces the smooth muscle cell proliferation stimulated by thrombin (28). Similarly, overexpression of full-length TM in melanoma cells significantly decreases cell proliferation and tumor growth (29). A recent study also showed that recombinant soluble TMD123 treatment inhibits thrombin-induced vascular smooth muscle cell proliferation (30). These results suggest that different TM domains may have different effects on cell proliferation. TM also plays a role in cell adhesion. Melanoma cells transfected with full-length TM assumed an epithelial-like morphology that clustered closely together with strong cell-cell adhesion suggesting an involvement of TM in intercellular communication or adhesion (31). TMD1 is important in maintaining the integrity of cell–cell interactions because cells transfected with lectin domain-deleted TM dispersed as single cells in nonconfluent cell densities. In an MIN6 insulinoma cell line, TM overexpression also increased the cell's ability to firmly adhere to the basement membrane (32). These findings indicate an involvement of TM in the formation of cell adhesion complex.

4. TM TREATMENT OF THROMBOTIC DISEASE

Because TM extracellular moiety (TMD123) is responsible for thrombin inhibition and protein C activation, soluble TMD123 has been produced with recombinant DNA technology and used as a new anticoagulant to treat thrombotic disease. Soluble TMD123 possesses the ability to attenuate the generation of thrombin and enhance the activation of protein C, resulting in decreased thrombus growth *in vitro* (33). In animal studies, it has a long half-life of 22 hours in monkey and is effective in decreasing thrombin generation and thrombus formation in mouse (34), rat (35), monkey (36) and baboon (37). The safety of injecting soluble TMD123 in humans was first proven in a small-scale study (38). Subsequently, in a phase I study of the recombinant human soluble TMD123, subcutaneous injection of a single dose of TM yielded a plasma half life of 48 to 72 hours in human (39). After administrating a single dose of 0.45 mg/kg TM subcutaneously, effective antithrombotic TM plasma concentration can be achieved for more than 6 days. The effective concentration can persist for 12 days if 2 subcutaneous soluble TMD123 doses (0.3 mg/kg) are given 5 days apart (39). There was no major bleeding episode. The effect of recombinant human soluble TMD123 on venous thromboembolism prophylaxis was evaluated in 312 patients following total hip replacement in a dose-finding study (40). Soluble TMD123 (0.3 mg/kg), administered subcutaneously 2-4 hours after surgery and followed by the same dose on day 6, was associated with a venous thromboembolism rate of 3.4% and an incidence of major bleeding of 1.4%. A single 0.45 mg/kg dose following surgery demonstrated increased efficacy with a venous thromboembolism rate of 0.9% but also an increased incidence of 6.3% bleeding complication (40). Soluble TMD123 is highly effective in thromboembolism prophylaxis and is comparable to other anticoagulants. Disseminated intravascular coagulation (DIC) is a life-threatening disorder usually associated with severe infection and malignancy. It causes widespread thrombus formation and coagulopathy. Recently, recombinant human soluble TMD123 was evaluated for efficacy and safety in the treatment of DIC (41). Patients were randomized to receive intravenous 0.06 mg/kg soluble TMD123 once daily or 8 U/kg/hr heparin sodium 24 hours for 6 days. The TM group demonstrated a significantly higher resolving rate of DIC symptoms (66.1% vs. 49.9%; 95% confidence interval 3.3-29.1). Patients in the TM group also showed a more marked improvement in the clinical course (41). These preliminary study results show that recombinant human soluble TMD123 is effective and safe in preventing and treating thrombotic diseases. Further clinical trials are warranted to investigate the role of TM in the treatment of other thrombotic diseases.

5. TM TREATMENT OF ATHEROSCLEROSIS

Atherosclerosis is an inflammatory disease that involves not only cholesterol deposition but also leukocyte adhesion and recruitment, smooth muscle cell proliferation and migration (42). Decreased endothelial TM expression was found in coronary atherosclerotic specimen from human autopsy study (43). TM gene polymorphism causing decreased endothelial TM expression was also associated with coronary and carotid atherosclerosis (44,45). Because TMD123 possesses anti-thrombotic, anti-proliferatory and anti-inflammatory properties, it was tested for atherosclerosis and restenosis treatment. Complete human TM cDNA was cloned into the adenoviral vector and delivered to balloon-injuried rabbit femoral arteries (46). Increased TM expression in the arterial wall significantly reduced the infiltration of inflammatory cells in the arterial wall and thrombus formation in the arterial lumen after balloon injury. The therapy decreased the neointima formation in rabbit injuried femoral artery after 4 weeks. Later, with the same animal model, recombinant soluble TMD123 was administered intravenously into rabbits undergoing femoral artery balloon injury (47). Perioperative systemic infusion of TM (145 μg/kg) significantly reduced the intimal hyperplasia at 2 and 4 weeks after balloon injury. TM infusion also inhibited arterial thrombus formation and neutrophil infiltration in the injuried arterial wall. Recently, we tested the effect of soluble TMD123 on neointima formation in a mouse carotid ligation model (48). Four weeks after carotid ligation to interrupt the blood flow, significant neointima could be observed without much thrombus formation. Recombinant TMD123 (145 μg/kg) was administered intravenously as a bolus injection immediately before and after carotid ligation. The severity of neointima formation was significantly reduced at 4 weeks in mice receiving TMD123 treatment compared with the saline injection, resulting in an altered arterial remodeling process after carotid ligation. The results of these studies clearly demonstrated that TM might effectively inhibit neointima formation in the artery after arterial balloon injury or ligation model. Soluble TMD123 may have a wide therapeutic role in preventing atherosclerosis and restenosis after percutaneous coronary intervention.

6. TM TREATMENT OF OTHER INFLAMMATION-RELATED DISEASES

Because TM has been proven to possess indirect and direct anti-inflammatory effects, recombinant soluble TMD123 was used to treat a variety of inflammatory diseases. One complication related to sepsis is altered pulmonary vascular permeability and adult respiratory distress syndrome, in which inflammation plays a major pathophysiological role. Pretreatment of rats with intravenous soluble TM (1 mg/kg) prevented endotoxin-induced coagulation abnormalities. It also significantly reduced endotoxin-induced pulmonary leukocyte infiltration and attenuated the increased pulmonary vascular permeability, resulting in less pulmonary edema (49). Experimental glomerulonephritis with extensive glomerular injury could be induced in rats by simultaneous injection of lipopolysaccharide and anti-glomerular basement membrane antibody (50). Intravenous injection of recombinant soluble TMD123 (1-3 mg/kg) in rats with glomerulonephritis not only reduced glomerular thrombus formation but also decreased glomerular neutrophil infiltration. Intravenous heparin infusion in the same rats showed similar anti-thrombotic effects but had no influence on neutrophil infiltration. TM treatment significantly reduced mortality rate of rats suffering from induced glomerular injury. TM was also used in the treatment of trauma-related inflammation. Crush syndrome is an extensive muscle crush injury resulting from trauma or accident. It causes hypovolemic shock, hyperkalemia and acute renal failure. Systemic inflammatory response was noted in crush syndrome. In addition to the traditional volume resuscitation, combined treatment with recombinant soluble TMD123 (3 mg/kg) significantly decreased the serum interleukin-6 level in a rat crush injury model (51). The mortality rate of rats with crush injury was also reduced after TM treatment. Soluble TM treatment also reduced the inflammatory response in spinal cord injury. Recombinant soluble TMD123 (1 mg/kg) was administered intravenously in a compression spinal cord injury model in rats (52). Pretreatment of rats with soluble TM significantly reduced the spinal cord tissue level and mRNA expression of tumor necrosis factor-alpha. Infiltration of leukocytes in the damaged spinal cord was also inhibited. Rats receiving TM treatment had a better recovery of motor disturbance. These findings suggest that soluble TM treatment prevents compression trauma-induced spinal cord injury by inhibiting leukocyte accumulation and reducing the expression of tumor necrosis factor-alpha. Recently, TM was found to have a direct suppressive effect on inflammatory response through its lectin-like domain (TMD1) by inhibiting high-mobility group-B1 DNA binding protein; therefore, TMD1 was evaluated to treat inflammatory arthritis (53). Mice lacking the TMD1 domain exhibit a more severe arthritis severity score after stimulation. Intravenous injection of TMD1 naked DNA significantly increased the serum level of soluble TMD1 protein. Mice receiving the TMD1 DNA delivery were significantly protected from induced inflammatory arthritis. TMD1 protein was purified and injected intraperitoneally into mice. These mice also demonstrated less severe symptoms of arthritis. In the joints of mice with induced arthritis, there was increased monocyte infiltration and the high-mobility group-B1 DNA binding protein was highly expressed in the nuclei and cytoplasm of synovial monocytes. Treatment of mice with TMD1 not only markedly attenuated monocyte infiltration but also diminished monocyte expression of high-mobility group-B1 DNA binding protein. Since TMD1 itself possesses an anti-inflammatory activity without anticoagulant effect, TMD1 is a promising and effective therapeutic agent in arthritis and other inflammatory diseases.

7. SUMMARY

TM-activated protein C pathway is a potent anticoagulation mechanism that regulates and prevents thrombus formation. Two decades after the anticoagulant role of TM was first demonstrated, much has been learned about its molecular mechanisms and physiological roles in cellular proliferation, adhesion and inflammation. In particular, the discovery of TM greatly enhanced our understanding of the cross talk between coagulation and inflammation. Recent studies have explored the potential therapeutic use of soluble TM fragment in the prevention and treatment of diseases and demonstrated its safety and efficacy in treating venous thrombotic disorder. Recent advances may help in the development of new therapeutic agents to manipulate the TM and activated protein C pathway in order to ameliorate or prevent atherothrombotic and other inflammation-related diseases.

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