Thrombomodulin is a glycoprotein that can bind to thrombin and activate protein C, thus mitigating the effects of cytokines produced by inflammatory and immunological processes. The molecule exerts a protective function on endothelial cells.

Thrombomodulin is cleaved to its soluble form by neutrophil elastase and by other substances produced during acute and chronic inflammatory responses, immunologic reactions and complement activation.

ELISA technique yields normal serum levels of 3.1 ± 1.3 ng/ml; in males these levels are higher; TM levels also rise during menopause. Other circumstances associated with an increase of serum TM levels are smoking, disseminated intravascular coagulation (DIC), cardiac surgery, atherosclerosis, ARDS, liver cirrhosis, diabetes mellitus, cerebral and myocardial infarction, and multiple sclerosis.

Serum levels of TM represent an useful prognostic index, because they are associated with an increase in mortality rate, or however a progression of the underlying pathological condition.

Key Words:
- Thrombomodulin, Thrombin, Protein C, Inflammation, Cytokines.

Introduction

Thrombomodulin is a membrane glycoprotein expressed on endothelial cells. Thrombomodulin is present in the body in two forms; the first type has a higher molecular weight, and it is bound to the cytoplasmic membrane of endothelial cells; the second form has a lower molecular weight and represents the soluble or plasmatic form. The concentration of TM bound to the cell surface is regulated by genetic factors. The presence of sequence mutations of the gene or mutations of the regulatory sequences of TM transcription give place to the production of a molecule with impaired function or its lower expression. The concentration of serum TM levels varies accordingly.

The proteinaceous component of the TM molecule has multiple moieties, each formed by about forty amino acid residues, called domains, that influence the tertiary structure and are responsible for the specificity of action of the glycoprotein. Different domains have different functions. These amino acid sequences can act as cofactors in the activation of protein C and have an anticoagulant effect by binding to thrombin.

Thrombomodulin can stimulate endothelial cell growth. This characteristic of TM depends from the molecular substrate of the sequences, known as EGF-like (epidermal growth factor-like).

The binding of thrombin with thrombomodulin activates protein C and the thrombin-thrombomodulin complex functions as an anticoagulant and anti-inflammatory stimulus. The reaction is regulated by the concentration of surface TM and also by the function of protein C, and is conditioned by genetic mutations that can alter the anticoagulant efficacy. Many factors such as cytokines (IL-1, TNF) and neutrophils can have a regulatory role on TM activity. Cytokines reduce surface TM expression and cleave the molecule when activation of inflammatory processes takes place. Neutrophils, on the other hand, influence TM activity by the enzyme elastase.
Molecular structure

Thrombomodulin is a surface glycoprotein of endothelial cells, whose gene, localized on chromosome 20 does not contain introns. Thrombomodulin is found in the body in two forms with different molecular weight. The heavy form weighs 150 kDa and the light form 69 kDa.

The domains with epidermal growth factor-like structure play an important role as cofactors. These domains are formed by sequences of about forty amino acids, with six cysteine residues that form three disulphuric bonds.

The molecular sequence of thrombomodulin contains an N-terminal lectin-like element (1-154 residues), an hydrophobic region (155-222 residues), six EGF-like modules (223-462 residues), one dominium rich in Ser/Thr (463-497 residues), a trans-membrane moiety composed by 23 amino acids (498-521 residues) and a tail of 35 cytoplasmic amino acids (522-557 residues).

Thrombin binds to the EGF-like modules and this bond is strengthened by the presence of the sulphuric glycosaminoglycans of the Ser/Thr rich region.

The structure of the EGF shows two main rings of 9-15 amino acids. The N-terminal ring is formed by the amino acids located between the third and fourth cysteine that make up two disulphuric bonds between the first and the third cysteine and the second and fourth cysteine, respectively. The C-terminal ring is formed by the amino acids between the fifth and the sixth cysteine and is a simple ring with one disulphuric bond. The model considered shows that the third and the fourth cysteine, in the EGF sequences, are close enough to form a disulphuric bond. The thrombomodulin region that goes from the fifth to the sixth domain inhibits the breakdown of fibrinogen, but also plays a role as a competitive inhibitor of protein C activation, without however showing a cofactor-like activity. The fourth EGF-like domain is the one necessary for the cofactor activity. A fragment containing the fourth, fifth and sixth EGF-like moieties can solicit a structure variation at a distance of 15 Å (A ngstrom) from the active site of the thrombin molecule.

Thrombomodulin binds to thrombin by two of its structural distinctive features: the disulphuric bond (that forms a cyclic structure compressing the C-terminal ring) and the conjunction of the tail amino acids to the C-terminal ring region.

The cofactor activity of the fourth and sixth EGF-like domain depends on the presence of the molecule's fourth EGF-like domain. The fact that the fourth domain does not bind well to the thrombin molecule suggests that, although necessary for protein C activation, it is however unable to exert its action if not in conjunction with the fifth EGF-like domain. This leads to the conclusion that the main region were the thrombomodulin-thrombin bond is possible can be localized in the fifth EGF-like domain and in the region connecting the fifth to the sixth EGF-like domain. The structural characteristics of this region formed by the amino acids C409-E®426 is the cyclic structure formed by the disulphuric bond between C409 and C421 and by the bond between the amino acid tail and this ring. The ring can interact with the tail so as to determine specificity of the bond. Thrombomodulin cofactorial activity, evaluated by the quantity of protein C that can be activated by thrombin, is measured by incubation of thrombin and TM together with protein C, and than by dosage of the activated protein C. A directly proportional relationship between activated protein C and concentration of TM has been thus demonstrated. The sequence of the TM structure that depends from the O region of the sugar, where the condroitin-sulphate bond takes place, favours the inhibition of thrombin by anti-thrombin III and raises the affinity of TM for thrombin.

Genetic features

The importance of the primary, secondary and tertiary structure of TM is evident in patients with gene mutation. These subjects present an elevated risk of cardiovascular accidents. The mutation of thrombomodulin structure from 127 G (guanine) to 127 A (adenine) leads to the mutation Ala (alanine) 25 Thr (treonine) that raises the risk of myocardial infraction in men. This risk naturally increases also in association with other metabolic factors. In a study conducted in patients surviving from a myocardial
infarction, the C/T (cytosine/timine) dimorphism of the thrombomodulin gene (nucleotide 1418) appeared to be an important predictive index of early myocardial infarction. The C/T dimorphism (characterized by the substitution of the valine (Val) with alanine (A la455) in the sixth EGF-like domain of the TM) when expressed by the allele indicates a greater prevalence of myocardial infarction. The substitution of the Val with Ala 455 generates a TM molecule with an altered function and spatial conformation in the thrombin binding and protein C activating region. The mutations of the molecular structure can also influence the protein's susceptibility to proteolytic degradation and influence the serum concentration of the fragments. The allelic combination C/T, C/C and T/T in position 1418 determines a consequent reduction of the activation of protein C and a related increase of thrombin. In patients with a previous myocardial infarction the difference in Ala 455 and Val allele concentration differed significantly from that of controls: 82% for Ala and 18% for Val versus 74% and 26% respectively. However, C/T dimorphism is only one of the predisposing factors toward myocardial infarction, since this disease has a known multifactorial origin. Substitutions of single bases have been identified in others genes that codify for coagulation cascade proteins. Two of the three mutations have been identified in the region corresponding to the thrombomodulin gene. Moreover, the analysis of the reporter gene, using mutants with promoter gene deletion, demonstrated that the sequence from -33 to -70 is important for the transcription of the regulatory sequences for the transcription of the thrombomodulin gene. One of the identified mutations occurs at the end of this region close to the “TATA-box”. This mutation is more frequent in the Asiatic population. The mutation in position -9/-10 from GG to AT has been identified only in patients with myocardial infarction and in none of the controls. This mutation as well occurs in the region close to the “TATA-box”. Mutations and band shifts have been observed in the promoter region in 4.8% of patients with myocardial infarction. All these mutations occurred in fragments tm2 and tm3.

Metabolism

Thrombomodulin serum levels also depend from the degradation of cellular TM present on endothelial cells detached by natural turnover. The elimination of TM is guaranteed by renal and hepatic metabolism. Thrombomodulin levels are elevated in renal failure and this increase shows a significant correlation with creatinine serum levels. Half life of TM is ten minutes.

Serum TM may be expressed as the ratio between soluble TM/serum creatinine, to distinguish the habitual elevation of TM occurring during renal failure from that due to endothelial cell damage. Endothelial cell production of TM is up regulated mainly by cAMP, retinoic acid and IL-4 and IL-13.

Physiology of TM

Thrombomodulin production is regulated by the sequences close to its gene and depends from the vascular micro-environment. Many factors such as the presence of tPA (tissue plasminogen activator) and PAI-1 (Plaminogen activator inhibitor), inflammatory cytokines, immune reactions and coagulation cascade activation influence TM levels. An interesting observation is the sharing of the receptor site of the thrombin molecule with that of the TM molecule, through a relationship based on the concentration of TM. When TM reaches high levels it binds to thrombin with a 1:1 ratio, blocking the activation of the coagulation cascade and activating protein C. Both fibrinogen and factor VII participate in the formation of the clot. High factor VII levels favour the formation of thrombin (key enzyme for the development of the arterial thrombus) that is also a mitosis promoting factor for smooth muscle cells and fibroblasts. Thrombin is also a potent platelet activator. The platelet activating factor activates neutrophils and promotes the appearance of leucocyte adhesion molecules on the endothelial cells. Through interaction with thrombomodulin, thrombin can start an anti-coagulant cascade, by the activation of protein C. Thrombin thus seems to be able to mediate vascular responses to events that favour coagulation, anti-coagulation, inflammation and proliferation.
Two main mechanisms contribute to thrombotic disease in humans: the anticoagulant pathway of protein C and the heparin-antithrombin mechanism. These two physiological systems work together to regulate the coagulation processes. The protein C pathway inhibits the function of the regulatory proteins factor Va and factor VIIIa. The heparin-antithrombin system inhibits the coagulation proteases.

**Protein C activation pathways and clinical events associated with defects of these pathways**

The protein C pathway starts with the interaction between thrombin and thrombomodulin that transforms thrombin in an enzyme that starts an anti-coagulant response. Due to the common polymorphism of protein C, it is possible to examine the relationship between the resistance to its activated form and cardiovascular diseases. The altered function of protein C contributes to the increased risk of myocardial infarction. The activation of protein C is almost certainly due to the formation of the thrombin-TM complex. It can be assumed that low TM levels weaken the anti-coagulant response when systemic thrombin levels rise, thus promoting the formation of the clot on sites of vascular damage. It has been demonstrated that the presence of TM diminishes the thrombin’s ability of activating cells through thrombin receptors. These studies have been conducted on cells that express thrombin receptors, but not TM receptors. These cells were transfected with TM, and the response to thrombin could be attenuated according to the rise in TM concentrations. The inhibition taking place can be at least in part explained by the fact that the thrombin and the thrombomodulin receptors share some common binding sites. This demonstrates that thrombomodulin inhibits thrombin mediated cellular activation by acting on thrombin’s receptors. Keeping in mind that the same regulation also takes place on the endothelium, the reduced expression of TM, conditioned by the mutation on the 5’ region of the gene, probably can favour the activation of endothelial cells and the consequent expression of the adhesion molecules. Consequently, the loss of function of TM can contribute locally to the development of atherosclerosis and to the breaking up of the plaque. The low TM levels in patients with mutations of the 5’ region of the TM gene can generate a protein C variant that behaves less actively during transitory ischemic attacks and can consequently increase the probability of cardiovascular injury. Thrombomodulin also shows a growth factor like function. It should be remembered that the repeated EGF like sequences of the soluble TM form, corresponding to the proteolytically degraded TM, have a promoting action on mitosis of fibroblast and endothelial cell lines.

**Physiopathological characteristics**

In endothelial injury determinism, a great importance must be attributed to the components that modulate TM expression and consequently influence protein C, S5 and the processes associated to these proteins (inflammation, immune responses). Protein C plays the fundamental role in endothelial injury physiopathology by blocking the inflammatory reactions and the injury secondary to liberation of inflammatory peptides during coagulation, fibrinolysis and complement activation. Naturally, in this process thrombomodulin plays a fundamental role due to its ability to activate protein C. Above protein C, we can find the regulation of cell surface TM expression that is influenced by other substances. Different peptides, such as bradykinine, complement factor C3a, fibrinogen degradation products (PDF) function as stimuli for inflammatory cell (neutrophils, monocytes, macrophages) production of cytokines. Cytokines such as IL-1 and TNF down regulate the expression of TM, in particular by acting on the membrane form. A iso TNF, endotoxins such as lipopolysaccharide (LPS), neutrophil elastase, oxygen free radicals, hydrogen peroxide and anaphilotoxin are capable of reducing TM activity by influencing the “cleavage” of the extracytoplasmatic part.

The cytokines that down regulate TM concentration on endothelial cells also influence protein C. In this situation an increase of t-PA and PAI.1 takes place.

For what pertains to the interaction of endothelium with the cellular component of blood, it must be remembered that activated
neutrophils can liberate TM from endothelial cells. The oxidation of TM methionine residues by neutrophil activation products can block the cofactor-like activity6. The activated neutrophils desensitise TM anticoagulant activity by their products elastase, cathepsin G, H2O2, and exercise a proteolytic activity that can cause detachment of endothelial cells.

**Trombomodulin serum levels and dosages**

Different pathological situations increase soluble circulating TM. The endothelial cells more exposed to hemodynamic turbulence (such as those on the bifurcation of major arteries) liberate a great quantity of TM10. Thrombomodulin levels range from 3 to 300 ng/ml. It is believed that normal levels are 3.1 ± 1.3 ng/ml, with slightly higher levels in males2. It seems that in the women TM levels rise during menopause. Women with surgical induced menopause have soluble TM levels well above normal. After six weeks of hormone replacement therapy a significant reduction of TM levels takes place16. Thrombomodulin levels vary according to race and Blacks seem to have lower levels17.

Thrombomodulin levels are usually measured with ELISA method. During the study of the different weight circulating fragments, it was realized that the lighter fragments (M.W. 69 kDa) were the extracellular domain of the TM, while the heavier fragments (M.W. about 150 kDa) are formed by TM tied to the plasma membrane and its intracytoplasmatic component3. Other studies conducted with the same immunoenzymatic method have shown a variation of these two components in different diseases (disseminated intravascular coagulation (DIC), pulmonary thromboembolism, adult respiratory distress syndrome, acute renal and chronic renal failure)18. Thrombomodulin levels also vary in moderate alcohol drinkers, because alcohol concentration is inversely correlated with the soluble form of TM17. A positive significant correlation has been found between TM levels and the number of years of tobacco smoking19. Vascular smooth muscle cells and some tumor cells produce TM both in vivo and in vitro. Besides endothelium and lymphatic vascular cells, TM is produced also by trophoblasts, leptomeninges, mesothelium and mesangium10.

Moreover, a significant difference in soluble TM levels has been demonstrated in the different blood groups (A B O)20. Thrombomodulin concentration is lower in group 0 and A subjects and higher in group B subjects. In A B subjects TM levels are intermediate between those of group A and group B.

**TM levels during DIC**

During sepsis with DIC, the high TM levels seem to participate in the development of MOF (multiple organ failure)21. Patients with higher TM levels during DIC have a higher mortality rate compared to patients who survived.

**TM levels during cardiac surgery**

Thrombomodulin levels rise in patients undergoing cardiac surgery with variations related to age, continuity of the disease and complexity of the surgical procedure22.

**TM levels during myocardial infarction**

Gene mutations of TM or alterations of the regulatory sequences for gene expression favour myocardial infarction4. The presence of these alterations causes the production of TM with impaired function that at the same serum levels, presents an inferior anticoagulant power23. However, soluble TM levels decrease because of the cytokine mediated inhibition of the mechanisms responsible for TM production, and conversely, rise again after 24-48 hours from thrombolysis2.

**TM levels during atherosclerosis**

Atherosclerosis is associated with high soluble TM levels24. Soluble TM levels rise in patients with localized atherosclerotic disease (carotid artery disease, iliac or femoral artery disease) with a significant further elevation in patients with multivascular disease (contemporary presence of carotid disease, coronary disease and iliac or femoral disease). Soluble TM levels are thus correlated with the extension of the vascular pathology21. It has also been demonstrated that TM mRNA present in monocyct cells positively correlates to low density lipoproteins (LDL) concentration25.
**TM levels in ARDS**

Only a significant TM elevation (> 100 ng/ml) is able to predict an increased incidence of ARDS in patients at risk of this complication. Moreover, TM levels have been demonstrated to be very high in bronchoalveolar lavage fluid in ARDS patients.

**TM levels in liver cirrhosis**

Patients with alcoholic cirrhosis present the highest TM levels, comparable to those in patients with chronic active hepatitis B or C. In hepatic cirrhosis the high weight forms are significantly reduced, with an increase of the low weight molecules. It is interesting to note that in acute hepatic failure the different TM forms rise in a similar fashion.

**TM levels in diabetes mellitus**

Elevated serum glucose levels can cause endothelial cell damage. Thrombomodulin rises in adult diabetic patients and also in patients with juvenile diabetes, also in absence of vascular disease.

Thrombomodulin levels are in direct relation with proteinuria. In patients with NIDDM TM levels are higher in those with macroalbuminuria compared to those with microalbuminuria.

Serum levels of TM correlate with the duration of the disease both in patients with type I and type II diabetes mellitus.

Serum TM levels are increased in patients with a growing number of complications (nephropathy, retinopathy, arterial occlusive disease, neuropathy, hypertension). Moreover, progression of diabetic retinopathy can be predicted by monitoring of serum TM levels that rise with the progression of the disease.

Elevated TM serum and urinary levels in diabetic patients are predictive of an early evolution towards diabetic nephropathy.

**TM levels in cerebral vascular disease**

The presence of a silent lacunar infarction represents a risk factor for successive evolution towards a clinically evident ischemic cerebral infarction. In the patients with symptomatic and silent lacunar infarction together with an elevation of fibrinogen levels, a major activation of factor VII and thrombin is also present, as measured by the concentration of factor 1 + 2, proportional to thrombin concentration. With the increasing number of lacunae, we assist to a parallel rise of the major parameters and risk factors of coagulation activation, among those soluble TM levels. Moreover, it should be remembered that Lp(a) levels, considered to be a reliable marker of atherosclerosis progression, show a significant positive relation with TM levels (p < 0.05).

**TM levels in multiple sclerosis**

Thrombomodulin levels are significantly elevated in patients with clinical recurrences of multiple sclerosis and it has been shown that steroid therapy can reduce them.

In conclusion, soluble thrombomodulin serum levels are a valid prognostic index during many diseases. Thrombomodulin levels can be interpreted as a marker of disease progression both in acute and chronic pathological conditions. Higher serum TM levels are associated with greater disease severity and progression probability indicating a progressive evolution of inflammatory and immunological mechanisms.

**References**

Clinical importance of thrombomodulin serum levels


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