

Thrombin Activatable Fibrinolysis Inhibitor (TAFI): A Molecular Link Between Coagulation and Fibrinolysis

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SUMMARY

Although the maintenance of precise balance between coagulation and fibrinolysis is of utmost importance for normal haemostasis, until recently these two systems were considered as completely separate mechanisms involved in the process of formation and dissolution of blood clot. Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently described attenuator of the fibrinolytic rate and is considered to be the molecular link between coagulation and fibrinolysis. TAFI circulates in plasma as an inactive precursor and its conversion in active enzyme (TAFIa) occurs by the action of thrombin or plasmin, but most efficiently by thrombin in the presence of its cofactor thrombomodulin. Once generated, TAFI down-regulates fibrinolysis by removing C-terminal lysine residues from partially degraded fibrin; thereby preventing the upregulation of plasminogen binding and activation. Because TAFI is activated by thrombin on one side, and acts as the attenuator of fibrinolysis on another side, it enables fine synchronization between these two systems. The antifibrinolytic function of TAFI mostly depends on TAFI concentration, the rate of its activation and the half-life of TAFIa in plasma. Changes in thrombin generation can have a profound effect on the rate of TAFI activation, and consequently on the rate of fibrinolysis. Therefore, it has been hypothesized that increased thrombin generation seen in thrombophilia patients may enhance TAFI activation, leading to a hypofibrinolytic state, which may further contribute to the thrombotic tendency. However, the results of several studies, in which relation between TAFI level and the occurrence of thromboembolic complications in carriers of hereditary thrombophilia have been investigated, were not consistent.

Keywords: TAFI; fibrinolysis; hereditary thrombophilia

INTRODUCTION

Activation of coagulation system at the site of blood vessel injury leads to the generation of thrombin and conversion of fibrinogen to fibrin which forms fibrin clot and prevents blood loss from damaged vessels. If coagulation system is inappropriately over-activated, deposition of fibrin may occur even in intact vessels leading to thrombosis. Contrary, the fibrinolytic system has ability to dissolve fibrin mesh; either it is formed during normal haemostasis or in the process of thrombosis [1].

Although the normal function of both systems is essential for the maintenance of blood in fluid state and for the prevention of bleeding, until recently blood coagulation and fibrinolysis were considered as completely separate systems. This understanding has changed substantially after the recent discovery of a proenzyme in plasma (procarboxypeptidase U), which, after being activated by thrombin-thrombomodulin complex (IIa-TM complex), exerts a potent antifibrinolytic activity, and for that reasons was also named thrombin activatable fibrinolysis inhibitor (TAFI). Thus, the activation of coagulation system leads to fibrin formation, and at the same time, through activation of TAFI, to the protection of formed clot (or thrombus) from early lysis. It has become clear that the TAFI system provides an explicit molecular link between the coagulation and the fibrinolytic cascade which enables fine synchronization of these two processes. The discovery of this link gives opportu-

nity for better understanding of the pathogenesis of both bleeding and thromboembolic disorders.

DISCOVERY AND PROPERTIES OF TAFI

Hendriks et al. [2] first reported in 1988 that during the formation of serum from blood (during coagulation) a novel unstable basic carboxypeptidase activity is generated from an inactive precursor circulating in blood. This enzyme was named carboxypeptidase U (CPU), where "U" stands for "unstable". Eaton et al. [3] provided the first clue of the important role of CPU in fibrinolysis in 1991, and this group first purified protein, isolated cDNA and deduced amino acid sequence. Independently, Bajzar et al. [4] discovered in 1995 that the antifibrinolytic activity of thrombin was due to the activation of a proenzyme, which they called thrombin activatable fibrinolysis inhibitor (TAFI). Subsequent investigations and amino acid sequencing have demonstrated that proCPU and TAFI are the same protein.

TAFI is synthesized in the liver as a propeptide consisting of 432 amino acids, which includes 22 amino acids signal peptide, a 92 amino acids activation peptide and a 309 amino acids catalytic domain. After the separation of signal peptide in N-terminal end during the secretion of propeptide, the remained protein consisted of 401 amino acids and with the molecular mass of 60 kDa on SDS page (proCPU) [3]. A single cleavage at Arg92 removes the heavily

glycosylated activation peptide and liberates the 35-kDa thermo labile catalytic unit-CPU [5]. The molecular mass of the active enzyme is below the glomerular filtration limit, but it might be retained in the circulation by binding to the α_2 -macroglobulin or pregnancy zone protein. It has been reported that TAFI is also synthesized by megakaryocytes and present intracellularly in α -granule-like structures in platelets (estimated concentration is about 50 ng/ $\times 10^9$ platelets) [6].

The proCPU gene, denoted the CPB2 gene maps to chromosome 13q14.11, contains eleven exons, and spans approximately 48 kb of genomic DNA [7]. Several single-nucleotide polymorphisms (SNPs) were identified in the 5' flanking region and in the codon region of TAFI gene. From the 6 SNPs in the coding region, only 505A/G and 1040C/T result in the amino acid substitution.

A wide range of variation with up to a ten-fold difference in TAFI plasma concentration between individuals has been reported, and it has been suggested that TAFI levels are likely to be under genetic control supported by the fact that environmental factors poorly explain TAFI level variability [8].

It has been found that concentration of TAFI correlates with the concentrations of acute phase reactants such as the C-reactive protein and haptoglobin in humans, indicating that TAFI gene expression may be under the influence of inflammatory stimuli such as cytokines and glucocorticoid hormones. Although it has been shown that other hormones may also influence the level of different haemostatic variables in humans, their effect on TAFI activity or antigen concentration is less known [9].

ACTIVATION AND ANTIFIBRINOLYTIC ACTIVITY OF TAFI

It has been shown that thrombin, plasmin, trypsin and neutrophil elastase can catalyze a single proteolytic cleavage at the Arg92-Ala93 bond, resulting in TAFI activation. All of these substances are relatively weak activators of TAFI, but in the presence of soluble or cellular form of the endothelial cell receptor thrombomodulin, the catalytic efficacy of thrombin as the activator of TAFI can be enhanced for about 1250 times. Because TM so potentially stimulates TAFI activation by Ila, the Ila-TM complex is thought to be physiological activator [10]. Thrombomodulin plays a dual role in TAFI activation. On one hand, it makes Ila more effective activator of TAFI, thereby downregulating fibrinolysis; on the other hand it increases the efficiency of the Ila mediated protein C activation. The generated protein C downregulates the coagulation cascade leading to less Ila generation and subsequently less TAFIa generation. The net effect seems to depend on the TM concentration and the presence of other cofactors and inhibitors. Such role of TM clearly illustrates a complex interplay between the different components of coagulation and fibrinolytic system. The physiological role of plasmin in the regulation of TAFI activation is not clear. Since Ila generation usually precedes plasmin formation, the importance of plasmin-mediated TAFI activation is very limited. A significant acti-

vation of TAFI by plasmin could not be excluded in situations when a high concentration of plasmin is generated, such as during thrombolytic therapy.

In contrast to the majority of coagulation and fibrinolytic enzymes being downregulated by protease inhibitors such as antithrombin or antiplasmin, there is no known physiologic inactivator for TAFIa. Instead, TAFIa spontaneously loses its activity over time [11]. Naturally occurring polymorphism at the position 325 in the TAFI gene has a significant effect on the stability of the active enzyme. TAFIa variant with isoleucine (Ile) at the position 325 is twice as stable as variant with threonine (Thr) at this position (15 min versus 8 min at body temperature). For that reason the antifibrinolytic activity of Ile325Ile TAFIa variant is for almost 60% higher than of Thr325Thr variant [12]. The prevalence of individuals with Ile325Ile variant of TAFI in general population is about 10%, but it seems that it is ethnically dependent.

TAFI exerts antifibrinolytic effect by multiple mechanisms. By its function, TAFIa is a carboxypeptidase B-like enzyme, meaning that it is capable to cleave basic amino acids such as arginine and lysine from the carboxyl termini of selected peptides or proteins. In response to the formation of fibrin, vasculature releases tPA which catalyses the activation of plasminogen to plasmin. In this reaction fibrin serves as a template to bind both tPA and plasminogen, and enhances generation of plasmin for approximately 500 times. Plasminogen binds to fibrin through carboxy-terminal lysine residues in the fibrin molecule. Once formed, plasmin begins to digest the clot by catalysing cleavages after selected arginine and lysine residues, exposing new carboxy-terminal lysine residues in fibrin mesh that provide additional binding sites for plasminogen. In that way, positive feedback mechanism in the activation of plasmin is established that may result in a complete lysis of fibrin mesh. TAFIa interferes with this positive feedback by removing the newly exposed carboxy-terminal arginine and lysine residues as they appear in fibrin [13].

It therefore slows down the process of fibrinolysis by eliminating the positive feedback steps in plasminogen activation. It has been also demonstrated that TAFIa exerts antifibrinolytic activity by modulating the inhibition of plasmin by antiplasmin.

When the clot lysis time is measured *in vitro* at various input concentrations of TAFIa, it increases at low concentrations and eventually appears to reach a plateau. Typically, by the maximal TAFIa activity, the clot lysis time in the plateau is three to four times longer than that observed in the absence of TAFIa [14].

Half-maximal prolongation of lysis time is usually achieved by the concentration of TAFIa which is only approximately 1% of the plasma concentration of the zymogen (TAFI). Therefore, although TAFIa is not capable to completely eliminate fibrinolysis, it is very potent in that regard, because only a small fraction of available proenzyme (TAFI) needs to be activated to have a significant antifibrinolytic effect. The antifibrinolytic effect of TAFI depends on the initial concentration of proenzyme (TAFI), the rate of TAFIa generation and half-life of TAFIa. A more stable variant of TAFIa (Ile325Ile) remains for longer time above key

threshold concentration for the inhibition of fibrinolysis than less stable variant (Thr325Thr), and provides more potent antifibrinolytic effect. If TAFIa were to be generated acutely, and then decay, the effect would be to delay fibrinolysis for some time depending of TAFIa concentration and its half-life. This situation may occur as a physiological event after acute injury of vasculature where the main physiological role of TAFI would be to prevent early fibrinolysis and premature degradation of protective fibrin clot. However, if TAFIa were to be generated chronically such that it were replenished over time, a situation might exist whereby fibrinolysis would be eliminated so long as the coagulant stimulus were present and TAFIa concentration remained above key threshold. Theoretically, a permanent TAFIa generation may occur in individuals with chronic activation of coagulation system and blood hypercoagulability.

TAFI AS A RISK FACTOR FOR VASCULAR DISEASE

It has been demonstrated that individuals with a higher concentration of proenzyme (TAFI) generate more enzyme (TAFIa) by the same procoagulant stimulus, because K_m for TAFI activation by thrombin or thrombin-thrombomodulin is considerably above the plasma concentration of TAFI [10]. Therefore, in keeping with the role of TAFI as an antifibrinolytic factor, several clinical studies have shown that high plasma concentrations of TAFI are a risk factor for various thrombotic disorders. Contrary, low TAFI concentration or decreased TAFIa generation, because of insufficient thrombin production, such as in patients with haemophilia, may give rise to enhanced fibrinolysis, premature fibrin clot lysis and bleeding diathesis. Van Tilburg and co-workers demonstrated that TAFI level above the 90th percentile of control population conferred an almost 2-fold increased risk for venous thrombosis compared to TAFI concentrations below these values [15]. A synergic effect on the risk of combined elevated TAFI levels and elevated factor VIII levels was observed, in keeping with a role for the intrinsic pathway of coagulation in activation of TAFI [16]. Eichinger et al. [17] followed 600 patients with first episode of venous thrombosis and demonstrated that high TAFI levels were associated with a 2-fold higher risk for recurrence of venous thromboembolism. Hereditary thrombophilia is characterized by chronically increased thrombin generation which may resonate in the degree of clot protection via the activation of TAFI [18, 19]. Most common hereditary thrombophilias in Caucasians but also in Serbian population are caused by the presence of the factor V Leiden or FII 20210A mutation [20]. It has been hypothesized that thrombotic tendency observed in carriers of hereditary thrombophilia relate not only to increased fibrin deposition, but also to attenuation of fibrinolysis because activation of TAFI/TAFIa system through increased

thrombin concentration. Recently, impaired pulmonary clot lysis in mice expressing the factor V Leiden was described, confirming the link between hereditary thrombophilia and impaired fibrinolysis [21]. Unfortunately, results of some studies investigating the relation between TAFI concentration and thrombotic tendency are not conclusive. Van Tilburg [15] did not observe the influence of TAFI level on thrombotic risk in carriers of FV Leiden. Folkeringa et al. [22] investigated the influence of TAFI level on the absolute risk of venous and arterial thrombosis in 1940 relatives in families with either deficiencies of antithrombin, protein C, or protein S, prothrombin G20210A, high FVIII levels, or hyperhomocysteinemia. The authors reported similar annual incidence of both venous and arterial thrombosis in individuals with high and in those with normal proCPU levels (adjusted relative risk for venous thrombosis 0.8; 95% CI 0.5-1.3 and for arterial thrombosis 1.4; 95% CI 0.9-2.2) [22]. Very recently, our group demonstrated a significantly increased risk of spontaneous but not of provoked venous thrombosis in carriers of FV Leiden or FII 20210A with TAFI levels above 75th percentile of control population in comparison to carriers with lower TAFI levels [23].

Several studies have suggested that TAFI levels are associated with the risk of arterial thrombosis, but results have been contradictory. Two retrospective studies, using an activity based TAFI assay have reported that TAFI plasma levels are significantly increased in individuals with myocardial infarction at a young age [24], and in individuals with stable angina pectoris [25]. In sharp contrast, in a large European multicenter case-control study, the Hypercoagulability and Impaired Fibrinolytic Function Mechanisms Predisposing to Myocardial Infarction (HIFMECH) study, a TAFI antigen value above 90th percentile was associated with a significantly lower risk of myocardial infarction (OR 0.55), indicating that elevated TAFI antigen may be protective against myocardial infarction [26]. It is difficult to find explanation for such contradictory results, but they may be related, at least partially, to different laboratory methods used for TAFI measurement in plasma.

Taken together, the data seem to agree that high plasma concentration of TAFI are a mild risk factor for venous thrombosis, although further investigations are needed to confirm the magnitude of the risk and its interaction with other prothrombotic risk factors. The relation between TAFI gene polymorphism and disease is under current investigations. Relation between the characteristics of TAFI system and arterial thrombosis is even more complex and required more studies.

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Тромбином активирани инхибитор фибринолизе (TAFI): молекуларна веза између коагулације и фибринолизе

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КРАТАК САДРЖАЈ

Иако је одржавање прецизне равнотеже између коагулације и фибринолизе у крви од критичне важности за обезбеђивање нормалне хемостазе, донедавно се сматрало да између њих нема директне повезаности и да активност једног система не утиче битније на функционално стање другог. Међутим, недавно је откривен протеин који у виду проензима циркулише у плазми и прелази у ензимску форму под дејством активаног коагулационог система, при чему доводи до снажног инхибирања фибринолизе. Тај протеин је назван „тромбином активирани инхибитор фибринолизе“ (енгл. *thrombin-activatable fibrinolysis inhibitor* – TAFI). С обзиром на то да се TAFI активира под дејством коагулационог система, а да инхибира функцију фибринолитичког система, данас се сматра да је овај протеин молекуларна веза између коагулације и фибринолизе и да омогућава фину синхронизацију активности ова два система. Трансформација TAFI у активни ензим (TAFIa) дешава се под дејством тромбина или плазмينا, али најефикасније под ути-

цајем комплекса тромбина и његовог мембранског кофактора тромбомодулина (*FIIa-TM*). TAFIa успорава фибринолизу тако што одстрањује C-терминалне лизинске остатке са делимично разграђеног фибрина, чиме спречава везивање плазминогена за фибринске нити и његову активацију у плазмин. Антифибринолитичка активност TAFI система у највећој мери зависи од концентрације проензима, степена његовог активирања под дејством комплекса *FIIa-TM* и полуживота ензима (TAFIa) у плазми. Претпоставља се да повећано стварање тромбина (или његова смањена инактивација) код особа с урођеном тромбофилијом може потенцирати перманентну активацију TAFI, а да инактивација фибринолитичког система до које на тај начин долази може додатно доприносити склоности тромбозирању. Међутим, у неколико досадашњих студија у којима је испитиван утицај особина TAFI система на појаву тромбоемболијских компликација код особа с урођеном тромбофилијом нису добијени јединствени резултати.

Кључне речи: TAFI; фибринолиза; урођена тромбофилија