TAFI: A Potential Target for Fibrinolytic Drugs

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Summary

Thrombosis is one the major cause of death worldwide. Clinicians and haematologists are confronted more and more with diagnosis and management of patients with venous and arterial thrombo-embolic phenomenon. In the recent past there have been major advancements in the field of the aetio-pathogenisis of haemostasis. Thrombosis occurs when there is an imbalance between pro-thrombotic and anti-thrombotic mechanisms. Thrombin activatable fibrinolysis inhibitor (TAFI) is a potential target to treat various thrombotic as well as metabolic disorders. TAFI is a Carboxypeptidase B like enzyme with a mol. wt. 60 kDa and it is activated by thrombin-thrombomodulin complex to activated form TAFIa which cleaves carboxyl-terminal lysine residues from partially degraded fibrin, rendering it resistant to fibrinolysis by endogenous tissue plasminogen activator (tPA). Thus inhibition of TAFI with an appropriate TAFI inhibitor will be an attractive strategy for various thrombotic as well as metabolic disorders in which levels of TAFI is highly elevated like deep vein thrombosis, angina pectoris, obesity and non insulin dependent diabetes mellitus.

Key words: Thrombosis, Bleeding disorders, thrombin activatable fibrinolysis inhibitor, potato tuber carboxypeptidase inhibitor, leech carboxypeptidase inhibitor

Introduction

Thrombosis is the pathological formation of a 'haemostatic' plug within the vasculature in the absence of bleeding [1]. An arterial thrombus is composed of so-called 'white thrombus' consisting mainly of platelets and leukocytes in the fibrin mesh. It is usually associated with atherosclerosis. Venous thrombus is composed of 'red thrombus' and consist of small white head and a large jelly red tail. Thrombus can break away, forming an embolus [2]. The imbalance in pro-thrombotic and anti-thrombotic mechanisms causes thrombosis, which is either by blood coagulation or by enhanced platelet activation or reduction in fibrinolysis activity [3].

Clot formation activates endogenous fibrinolytic system for the formation of plasmin from plasminogen, which degrades fibrin clot and restores blood flow to the vital organs. The process of fibrinolysis removes intravascular thrombi to maintain vascular patency [4]. Clinically used drugs to restore blood flow in various diseases such as myocardial infarction are streptokinase, tissue plasminogen activator (t-PA), alteplase and reteplase [5] which have reveal many shortcomings such as enhanced risk of bleeding, GI haemorrhage and stroke. In addition, platelet rich arterial thrombi are particularly resistant to thrombolysis [6, 7]. TAFI emerges as a novel target for the treatment of thrombosis and other bleeding disorders. TAFI is a basic carboxypeptidase zymogen that can be activated by thrombin [8]. Activated TAFI (TAFIa) cleaves carboxyl-terminal lysine residues from partially degraded fibrin, rendering it resistant to fibrinolysis by endogenous tissue plasminogen activator (tPA) [9].
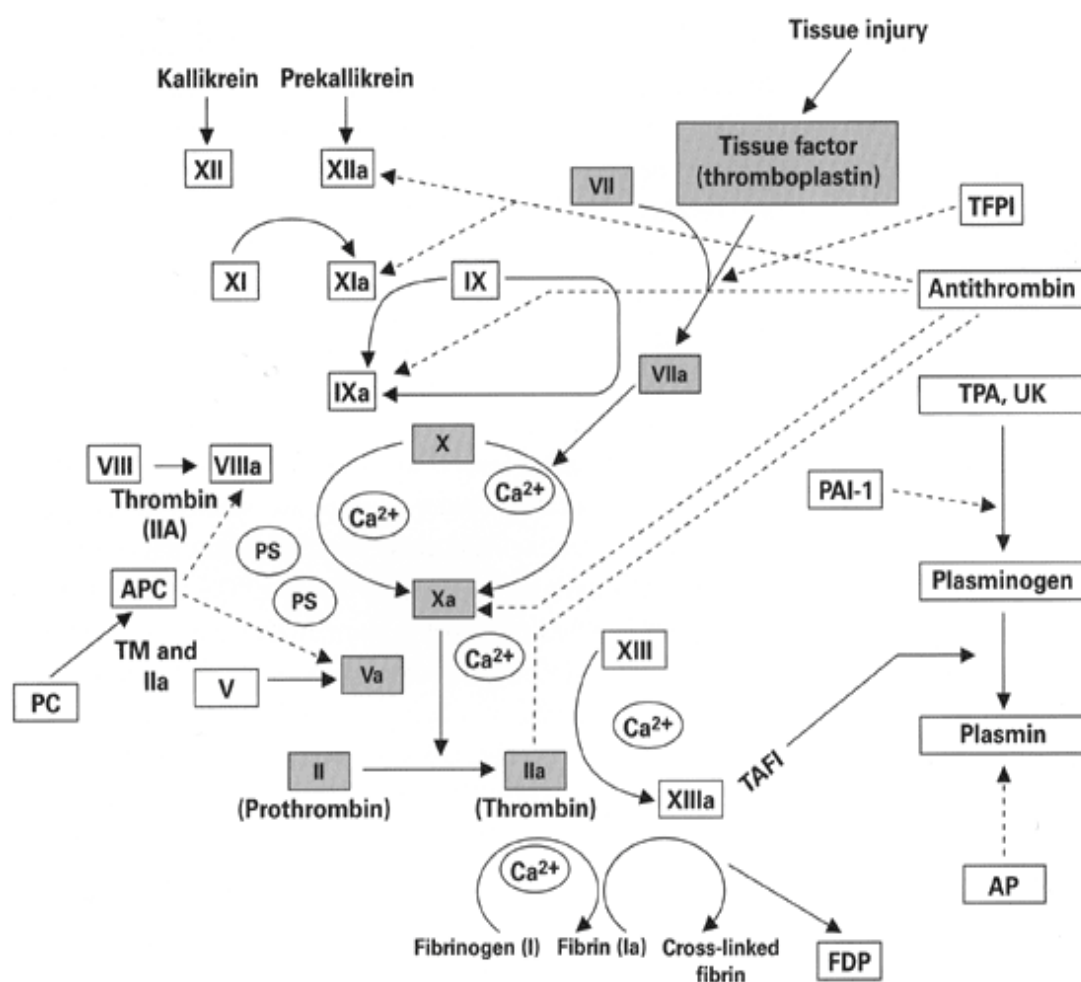

Figure: 1 Tissue factor–dependent action of recombinant factor VIIa. The tissue factor–dependent pathway is shown by gray shading. Dashed lines represent site of major inhibitors. AP, α-antiplasmin; APC, activated protein C; FDP, fibrin split products; PAI, plasminogen activator inhibitor; PC, protein C; PS, protein S; TAFI, thrombin-activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TPA, tissue plasminogen activator; UK, urokinase.

Discovery of TAFI

TAFI is a 60-kDa glycoprotein with carboxypeptidase like activity. Due to its instability, Hendriks et al. named it as carboxypeptidase U (U referring to unstable) [10], Campbell et al. named it arginine carboxypeptidase (carboxypeptidase R) [11]. Meanwhile a novel protein with a high affinity for plasminogen was isolated, cloned and shown to have high-sequence homology to that of pancreatic pro-carboxypeptidase B. Characterization of TAFI showed identity between carboxypeptidase U and plasma pro-carboxypeptidase B [12]. After purification and characterization Bazjar et al., found that this protein inhibited fibrinolysis and named as 'thrombin actvatable fibrinolysis inhibitor [13, 14].
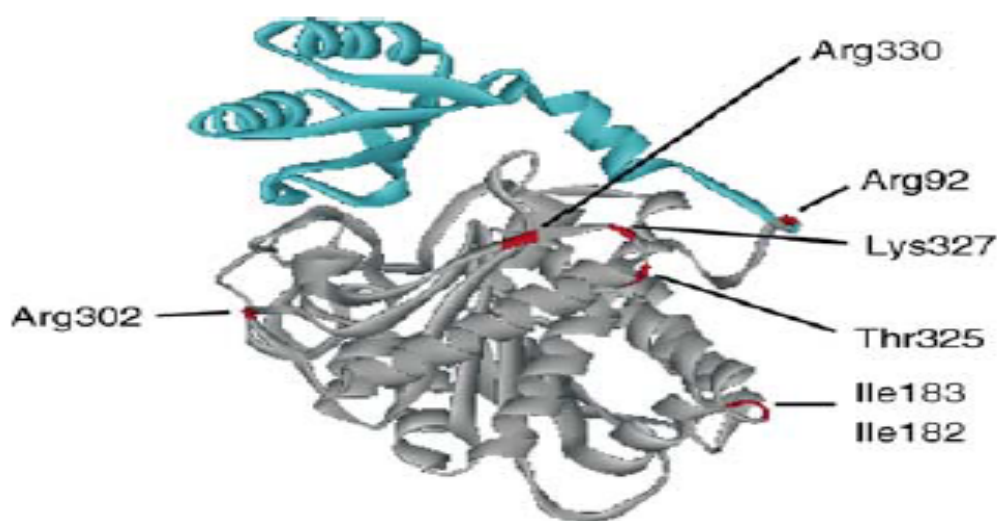

Figure: 2 This new member of the pancreatic procarboxypeptidase family.Allowed homology modelling of TAFI, thrombin-activatable fibrinolysis inhibitor.

Molecular Biology and Activation of TAFI

Human TAFI is a single chain 60-kDa protein secreted by the liver and found in plasma [15]. TAFI is N-glycosylated, and the attached glycans account for 20% of the overall size of the protein. All five potential N-linked glycosylation sites are occupied, and four of these are located on the activation peptide and one on the enzyme moiety [16]. The protein belongs to the metallocarboxypeptidase family and an important regulator of fibrinolysis that act by removing surface-exposed C-terminal Lys from partially degraded fibrin clots, thus reducing the number of plasminogen-binding sites. TAFI inhibits fibrinolysis when activated by thrombin which cleaves an Arg92-Ala93 bond to form an activation peptide of 19 kDa and activated TAFI (TAFIa) of 36 kDa [17]. Isolated thrombin poorly activates TAFI (kcat=0.021 s-1, Km=0.5-2.1 µM) but this process becomes much more effective in the presence of the endothelial membrane-bound thrombin cofactor called thrombomodulin. This cofactor protein present on vascular endothelial cells inhibits the procoagulant functions of thrombin and enhances thrombin-catalyzed activation of anticoagulant protein C. Thrombomodulin also accelerates the proteolytic activation of a plasma pro-carboxypeptidase TAFI [18]. The rate of TAFI activation by the thrombin-thrombomodulin complex exceeds that of thrombin alone by 1250-fold. This is predominantly due to an increase in the catalytic constant (kcat=0.4-1.2

s-1). It is positively activated by trypsin and plasmin. The efficiency of TAFI activation by plasmin (kcat=0.00044 s-1;Km=55 nM) is 8 times lower than by thrombin, but increases in the presence of heparin and endothelial glycosaminoglycans to become about 10% of that of the thrombin-thrombomodulin complex [17].
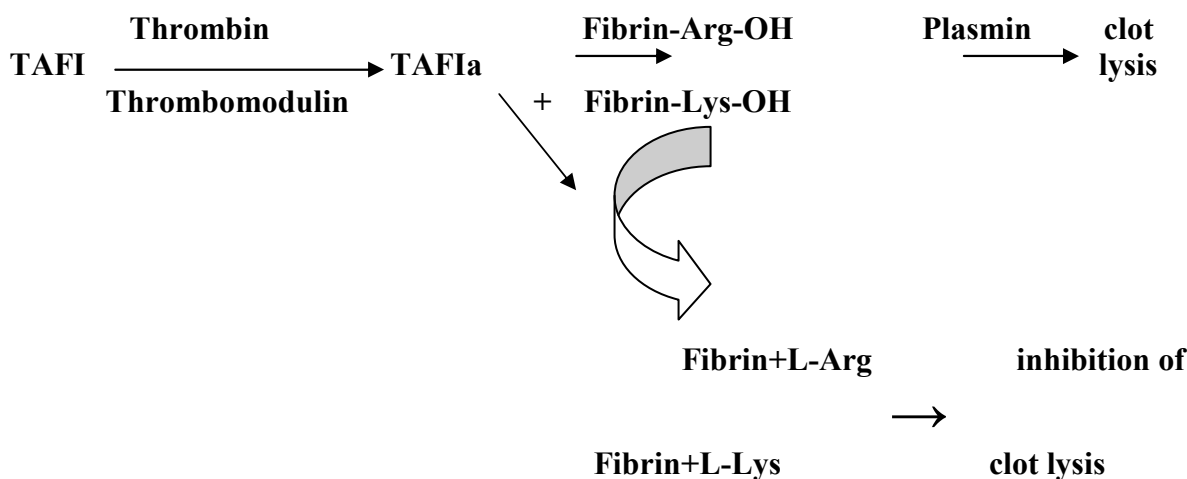
Figure: 3 The fibrinolytic system and inhibition by TAFIa.

Thrombin (plasmin, trypsin) cleavage of the zymogen releases, a 92 amino acid N-terminal activation peptide containing 4 N-linked glycosylation sites (N22, N51, N63, N86) and the proposed plasminogen recognition site. The 309 amino acid C-terminal (Mol wt. = 35,783) catalytic domain (TAFIa, pCPB) displays the properties of a basic carboxypeptidase, hydrolyzing lysine and arginine from the C-terminal position of polypeptides [19].
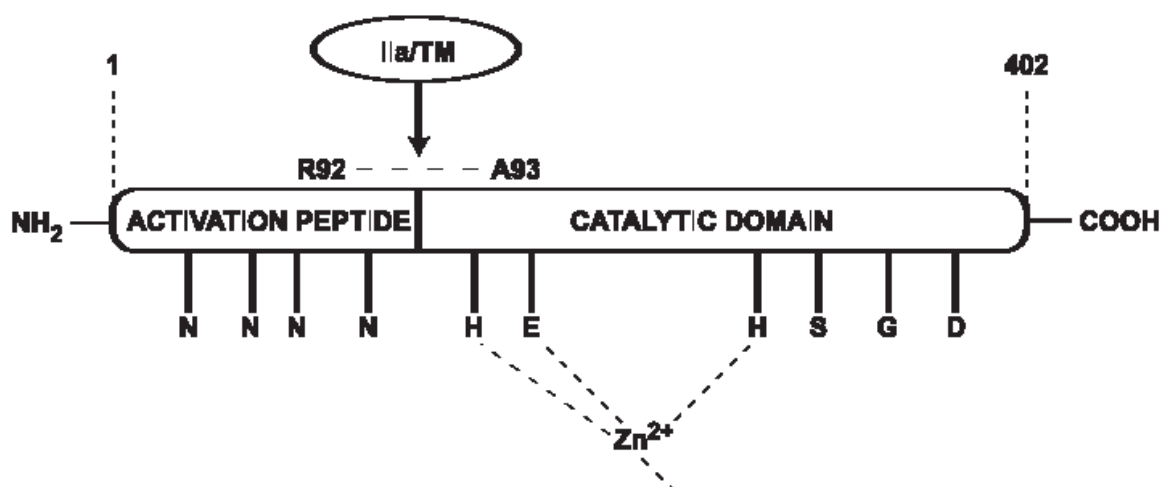
Figure 4. Domain structure of TAFI. The N-linked glycosylation sites (N22, N51, N63, N86) are represented by N. The active sites Zn^{+2} and residues (S299, G336, D344) involved in substrate binding are shown.

Pathophysiological role of TAFI

Thrombin activatable fibrinolysis inhibitor (TAFI) binds to active sites on plasminogen and prevents plasminogen effects on fibrin, thus inhibiting fibrinolysis. TAFI has multiple biological effects that include platelet activation, cytomodulation, pro-inflammatory mediation, leukocyte and integrin modulation, pro-thrombotic effect, and endothelial modulation [20].

A] Bleeding disorders

TAFI is identified as the main intermediate between coagulation and fibrinolysis [21] TAFI affects the clot dissolution through a threshold-dependent mechanism: as long as the TAFIa concentration remains above the threshold, TAFIa prevents the progression of lysis into the propagation phase [22, 23]. TAFI plays a important role in bleeding disorders indicating the absence of factor XI in bleeding disorder patients. These patients are prone to bleeding from tissues with a high local fibrinolytic activity (urinary tract, nose, oral cavity, tonsils) [24, 25]. Similarly, defective activation of TAFI might also contribute to the severity of the bleeding disorder (Haemophilia A and B) [26, 27]. High concentration of TAFI is observed in patients with of angina pectoris. While in case of myocardial infarction, totally opposite results were found [28, 29].

B] Diabetes

Increase in the level of TAFI in type -2 diabetes mellitus leads to the reduction in fibrinolytic activity and enhancement of coagulation system. High incidence of premature macro- and microangiopathy and increased morbidity and mortality observed in diabetic patients [30-34]. Hypo-fibrinolysis with hyper-coagulable states frequently occurs in conditions associated with insulin resistance in diabetic patients [35, 36]. Insulin resistance is a pluri-metabolic syndrome characterized by the presence of obesity, arterial hypertension, glucose intolerance, and biochemical abnormalities such as hyper-insulinemia, hyper-trigliceridemia, and decreased high density lipoprotein cholesterol (HDLC) [36]. Hypo-fibrinolysis is a common finding in patients with diabetes mellitus and a risk factor for diabetic nephropathy. Increased TAFI in diabetes patient leads to the endothelial cell injury. Inhibition of TAFI prevents the allied complication with the diabetes mellitus patient **[**36].

C] Obesity

Obesity and its associated metabolic complications can impair the physiologic regulation of fibrinolysis, leading to thrombosis [37]. Hemostatic abnormalities including TAFI alterations represent a link between obesity and vascular thrombosis. Effective interventions should be considered in improving the obesity-associated prothrombotic risk profile. A study on obese and diabetic and non-obese and non-diabetic human volunteers showed high level of TAFI in plasma of obese persons as comparative to non-obese one and the increase of TAFI antigen and activity in the plasma was independent of age, BMI, glucose, cholesterol, triglycerides, insulin and leptin concentrations in the plasma. Orilistat treatment significantly reduced the level of TAFI in obese volunteer. Selective TAFI inhibitor enhances the therapeutic paradigm in obesity [38].

D] Inflammation

Previous studies have established the potential role of TAFI in inflammation. In fact, Campbell et.al. have reported that "TAFI" (which they named carboxypeptidase R) as the protein responsible for the difference in inactivation of bradykinin in plasma and serum [39, 40]. Bradykinin, cleaved from high molecular weight (HMW)-kininogen through a series of reactions involving the activation of factor XII and prekallikrein, is one of the important mediators of inflammation characterized by swelling, heat, redness and pain. The anaphylatoxins, C3a and C5a, are important inflammatory mediators and potent leukocyte chemotractants. TAFIa inactivates both C3a and C5a by hydrolysis of their C-terminal Arg, thereby reducing their pro-inflammatory effects [41, 42]. TAFIa efficiently reduced C5a-induced activation of neutrophils in-vitro. Carboxypeptidase N is traditionally regarded as the physiological anaphylatoxin inhibitor [41, 42]. These results suggest that TAFI could be a novel target for the inhibition of inflammation by inactivation of C5a. [43]

E] Inflammatory Bowel Disease

In both, Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel disease (IBD), an increased risk of thrombotic events have been demonstrated. The fibrinolytic system has been widely investigated in IBD. Most of the available data report an imbalance in fibrinolytic capacity with a tendency toward a hypofibrinolytic state. Plasma thrombin-activatable fibrinolysis inhibitor is fundamental inhibitor of the fibrinolytic process and is considered to be acute-phase reactants. Recent studies have shown an imbalance of thombin-activatable fibrinolysis inhibitor, suggesting that these molecules might contribute to thrombo-embolic events in both forms of IBD [44].

F] Cardiovascular disorders

High levels of TAFI has been observed in various CVS disorders, specially in coronary artery disease. Increased TAFI level associated with hypofibrinolysis increases the chances of various thrombotic abnormalities [45]. Ischemic stroke is a highly heterogeneous disorder and previous study shows that plasma TAFI was elevated in ischemic stroke [46].

G] Lung disease

Intra-alveolar activation of the coagulation system due to reduced fibrinolytic function plays a critical role in the pathogenesis of interstitial lung disease. TAFI plays an important role in patients with interstitial lung disease [47]. Decreased fibrinolytic function favors the development of pulmonary fibrosis. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a strong suppressor of fibrinolysis, but its role in lung fibrosis is unknown. [48]. Patients with lung injury including those with idiopathic pulmonary fibrosis have increased intra alveolar levels of TAFI, the abnormality which has been linked to decreased plasminogen activator activity in the lung [47]. In conclusion, the anti-fibrinolytic activity of TAFI is relevant and may play a role in the pathogenesis of lung fibrosis.

Inhibition of TAFI

The most important technique of enzyme inhibition is either binding of the enzyme to an inhibitor or proteolytic degradation of the enzyme (often by its activator). TAFIa binds to the well-known plasma inhibitor alpha-2 macroglobulin without any loss of activity. It is thought that, in this case, the binding of TAFIa to alpha-2 macroglobulin prevents rapid clearance from blood by the kidneys and prolongs TAFIs lifetime in the bloodstream. Otherwise, TAFIa half life is very less and is quickly eliminated by the kidneys due to its small molecular weight and lack of carbohydrate components [49].TAFIa is extremely unstable at 37°C with rapid loss of carboxypeptidase activity. TAFIa was found to be stable at 0°C. Proteolysis at Arg-302 leads to a decrease in TAFIa activity and the formation of inactive fragments with molecular masses of 25 and 11 kDa. Thrombin, plasmin and trypsin only degrade TAFIa. It is believed that the inactivation of TAFIa is due to conformational rearrangements in the TAFIa molecule, and that these conformational changes make TAFIa more susceptible for further proteolysis [50]. Various inhibitors of TAFIa are available and initial stage of research is being carried out to evaluate its activity.

1] Potato tuber carboxypeptidase inhibitor (PTCI)

A 39-amino acid protein PTCI is a naturally occurring molecule in potatoes that can form complexes with several metallo-carboxypeptidases and inhibiting them in a strong competitive way with a Ki in the nanomolar range [51]. This 39 amino acid peptide is a specific inhibitor of both the carboxypeptidase A and B family of proteases [52, 53]. It has been suggested that PTCI potentiates fibrinolysis by inhibiting TAFIa-dependent removal of C-terminal lysine residues exposed on fibrin partially degraded by plasmin [54, 55, 56] These C-terminal lysine residues are considered to be potent cofactors in tPA-mediated activation of plasminogen [57, 58]. These C-terminal lysines increase the efficacy of tPA to activate plasminogen and induce thrombolysis by facilitating both colocalization of the components and stabilization of the ternary complex comprising tPA-fibrin-plasminogen [59]. PTCI has been to possess potential therapeutic application in thrombotic diseases and bleeding disorder.

2] 2-guanidinoethylmercaptosuccinate (GEMSA)

This arginine analog, 2-guanidinoethylmercaptosuccinate (GEMSA), not only inhibits TAFIa but also slows the spontaneous inactivation of the enzyme, thus reducing the activity of TAFIa, while extending its apparent half-life [60]. The magnitude of the effect depends on the concentration of TAFIa, the concentration of the inhibitor and the potency of the inhibitor. A previous study shows the paradoxical effects of GEMSA and PTCI on t-PA induced fibrinolysis [60, 61].

3] Leech carboxy peptidase inhibitor (LCI)

The first metallocarboxypeptidase inhibitor found in leeches, is leech carboxypeptidase inhibitor (LCI) [62]. LCI is a cysteine rich polypeptide of 66 residues that behaves as a tight binding and competitive inhibitor of different types of pancreatic-like carboxypeptidases (A1, A2, B, and plasma carboxypeptidase B) with equilibrium

dissociation constants Ki of 0.1– 0.4 [60]. The lowest Ki value of LCI is shown for plasma carboxypeptidase B, an enzyme also known as thrombin activable fibrinolysis inhibitor (TAFI), that proteolytically removes C-terminal residues from fibrin and down-regulates plasminogen activation, leading to an attenuation of fibrinolysis [63, 64]. The unstructured C-terminal tail of LCI interacts with the carboxypeptidase in a substrate like manner, similar to the potato carboxypeptidase inhibitor (PCI). No sequential homology is observed between PCI and LCI except for the C-terminal tail; however, both proteins show the structural feature of being stabilized by disulfide bridges [65].

Conclusions

TAFI has multiple biological effects that include platelet activation, cytomodulation, proinflammatory mediation, leukocyte and integrin modulation, prothrombotic effect, and endothelial modulation. Hence, TAFI can be an important target for various thrombotic disorders associated with diabetes, obesity and other heart related disorders. Its plasma level can be decreased by various inhibitors and these inhibitors can be used as important drug molecules against such disorders and can be used in combination with other thrombolytic drugs. Role of TAFI in coagulation and fibrinolytic system as well as other various pathophysiological disorders making it an attractive target for treatment associated with thrombosis, diabetes, obesity and other various pathophysiological disorders. Its role as a lysine-specific carboxypeptidase and its participation in the regulation of fibrinolysis as a fibrinolysis inhibitor suggests that a high level of TAFI in blood would cause thrombophilia, while a low level of TAFI would cause haemorrhage. The analysis of clinical data has revealed a correlation between elevated TAFI levels in blood and hyper-coagulation disorders. High plasma levels of TAFI were observed in angiographically-confirmed coronary artery disease. Venous TAFI levels tend to be higher in patients with coronary artery disease as compared to controls. An elevated TAFI concentration in blood is considered to be a risk factor for venous thrombosis, and high TAFI levels are associated with a 2-fold higher risk of recurrence of venous thrombosis when compared to patients with lower TAFI levels. These findings make TAFI an important diagnostic parameter for evaluating cardiovascular diseases. The level of TAFI was found to correlate with the level of C-reactive protein, which points to a role in inflammation. The fact that inflammation is accompanied by local or systemic hypercoagulation confirms this suggestion. A deficiency of TAFI might contribute to the severity of bleeding disorders in haemophilias A and B. TAFI is an attractive target and inhibition of TAFI with suitable inhibitor may be valuable to correct various thrombotic and metabolic disorders. Novel research being carried out for the evaluation of various TAFI inhibitors including PTCI, GEMSA and many more.

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