Thrombin Activatable Fibrinolysis Inhibitor (TAFI) at the Interface between Coagulation and Fibrinolysis

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\textbf{Abstract}
The thrombin-catalysed conversion of plasma fibrinogen into fibrin and the development of an insoluble fibrin clot are the final steps of the coagulation cascade during haemostasis. A delicate balance between coagulation and fibrinolysis determines the stability of the fibrin clot. Thrombin Activatable Fibrinolysis Inhibitor (TAFI) plays an important role in this process. TAFI is activated by thrombin and protects the fibrin clot against lysis. The role of TAFI in bleeding and thrombotic disorders is discussed as well as its novel emerging role in wound healing and inflammation.

\textbf{Introduction}
The fibrinolytic system is initiated after the formation of fibrin when both plasminogen and tissue-type plasminogen activator (tPA) bind to the fibrin surface to generate plasmin (Figure 1). Specific interactions of the lysine-binding sites in plasminogen and tPA with C-terminal lysine residues in partially degraded fibrin result in the formation of a ternary complex and increased catalytic efficiency of plasmin formation [1-4]. By continuous cleavage of fibrin and generation of new C-terminal lysine residues, plasmin stimulates its own formation in a positive feedback loop. This positive feedback loop is halted by the activity of activated Thrombin Activatable Fibrinolysis Inhibitor (TAFI).

Activated TAFI (TAFI\textsubscript{a}) [5], also described as plasma carboxypeptidase B, carboxypeptidase U and carboxypeptidase R [6-8], inhibits fibrinolysis by removing carboxy-terminal lysine residues from fibrin that are required for efficient plasmin formation [5,9,10]. For a more extensive review of the literature the reader is referred to Bouma et al [11].
Activation of TAFI and the Role of the Coagulation System

TAFI is activated by trypsin, plasmin, thrombin or meizothrombin [5,6,12]. Activation occurs by a single cleavage at Arg-92 resulting in the release of a glycosylated activation peptide. Activation of TAFI by thrombin is a relatively inefficient process and is stimulated about 1250 fold by the endothelial cell receptor thrombomodulin [13].

Activation of TAFI by thrombin implies a role for the coagulation system in the regulation of fibrinolysis. Coagulation is initiated upon exposure of blood to tissue factor at the site of injury (Figure 2). Tissue factor recruits factor VII(a) and activates factor X when tissue factor is abundant. Factor IX is the preferred substrate at low tissue factor concentrations, resulting in a more pronounced contribution of the tenase complex in the activation of factor X. Tissue factor pathway inhibitor (TFPI) rapidly inhibits the extrinsic pathway, however fibrin formation is induced when sufficient amounts of thrombin are generated. After clot formation, thrombin formation continues inside the fibrin clot, by thrombin-catalysed activation of factor XI and via the intrinsic pathway. A secondary burst of thrombin formation is provided by the intrinsic pathway due to the continued activation of factor XI by thrombin and the amplification power of both the tenase and the prothrombinase complex. These high concentrations of thrombin are required for the activation of TAFI and contrast the small amounts of thrombin that are sufficient for fibrin formation [14,15]. Thus, the extrinsic pathway provides the thrombin needed for formation of the fibrin clot, whereas the intrinsic pathway provides the thrombin needed for protection of the fibrin clot against fibrinolytic attack.

Inactivation of TAFI and the Role of the Anticoagulant Protein C System

The enzymatic activity of TAFIa is unstable and highly sensitive to temperature; the half-life of TAFIa increased from 10 minutes at 37°C to several hours at 22°C [14]. Proteolytic inactivation sites at Arg-302 for thrombin and Lys-327 for plasmin have been identified but mutation of these sites did not prevent the decay of TAFIa activity [16-18]. Therefore, the current consensus is that inactivation of TAFIa is caused by a conformational change [14,16-18]. Given the short half-life of TAFIa, regulation of functional activity occurs most likely at the level of activation. The profibrinolytic effects of the protein C pathway emphasize the relevance of the control of thrombin formation for the activation of TAFI [19,20]. By inactivation of the factors Va and VIIIa, activated protein C inhibits the formation of thrombin and thereby reduces the activation of TAFI.

In vivo Role of TAFI in Fibrinolysis

In vivo evidence for a role of the intrinsic pathway of coagulation and TAFI in fibrinolysis was obtained in an experimental thrombosis model [21]. Incorporation of anti-factor XI antibodies or a specific TAFIa inhibitor in jugular vein thrombi resulted in an almost two-fold increase in endogenous thrombolysis [21]. Several studies showed that TAFI plays an important role in the susceptibility of a clot for lysis. Inhibition of TAFI by specific inhibitors enhanced the tPA induced lysis of a thrombus in rabbit thrombolysis models [22-24].
Three independent groups investigated the physiological role of TAFI in knockout mice [22-24]. TAFI deficient mice had normal embryonic development, were fertile and had a normal life expectancy. No signs of bleeding or other phenotypic abnormality were observed. Several acute challenges were tested to provoke a phenotype: models for venous and arterial thrombosis, thrombin-induced acute thromboembolism, endotoxin-induced disseminated intravascular coagulation, tail bleeding and kaolin-induced writhing response. None of these models showed a difference between knockout and control animals [25,26,28]. Only after backcrossing TAFI deficient mice to a heterozygous plasminogen background, a role for TAFI in models of pulmonary embolism and peritoneal inflammation could be demonstrated [27]. This indicates that TAFI can modulate the in vivo functions of plasmin(ogen) in fibrinolysis but that redundancy in the regulation of the fibrinolytic system may mask the phenotype of TAFI deficient mice.

**Role of TAFI in Bleeding Disorders**

The role of the coagulation system in the generation of thrombin required for TAFI activation implies that perturbation of thrombin generation will result in clots that are relatively unprotected against lysis. Thus, defects in activation of TAFI might contribute to the severity of bleeding disorders. Such a mechanism might help to explain the bleeding disorder of factor XI deficient patients. These patients are prone to bleeding from tissues with a high local fibrinolytic activity (urinary tract, nose, oral cavity, tonsils) [29,30], and it is at these sites that the down regulation of fibrinolysis is not provided for by the factor XI dependent generation of thrombin and TAFI activation. Similarly, defective activation of TAFI might also contribute to the severity of the bleeding disorder in factor VIII and IX deficiency (Haemophilia A and B) [31,32].
Role of TAFI in Thrombotic Disorders

On the other hand, an increased rate of thrombin formation and inappropriate protection of fibrin clots might lead to thrombotic disorders. Thus, increased activation of TAFI might exacerbate a prothrombotic disposition. Such a mechanism might help to explain the increased risks for venous thrombosis associated with high levels of intrinsic coagulation factors. In recent years, high levels of factor VIII, IX and XI have been associated with an approximately two-fold increased risk for venous thrombosis [33-35].

Elevated TAFI levels in vitro correlated well with increased TAFIa activity and increased anti-fibrinolytic function, indicating that TAFI plasma levels are an important factor in the rate of TAFI activation [36]. In the Leiden Thrombophilia Study (LETS) increased plasma TAFI antigen levels were associated with an approximately two-fold increased risk for venous thrombosis [37]. In addition, the relative risk for recurrent venous thromboembolism was two fold higher in patients with high TAFI levels compared to patients with low TAFI levels and three times higher in patients with high TAFI and high factor XI levels [38]. Similarly, a higher risk for recurrent venous thromboembolism was found in patients with both high TAFI and High factor VIII. Furthermore, plasma levels of TAFI were significantly higher in men with stable angina pectoris than in the healthy age-matched men [39]. These studies support the concept of a link between fibrinolysis and coagulation as outlined above.

In contrast to venous thrombosis, unexpected results are reported for the role of TAFI in myocardial infarction. One study reported that patients with a recent myocardial infarction presented lower values of TAFI antigen and that elevated TAFI levels were actually protective against myocardial infarction [40,41]. Another study reported that inherited Factor XI deficiency conferred no protection against acute myocardial infarction [42]. These two studies seem to contradict the previous established relationship between coagulation and fibrinolysis and the role of TAFI in the development of venous thrombosis, but one has to keep in mind the differences in pathophysiology of venous- vs. arterial thrombosis. The novel emerging activities of TAFIa in inflammation, tissue remodelling and other processes might help us understand the apparent discrepant role of TAFI in myocardial infarction. After all, inflammatory processes play a major role in the development of arteriosclerosis and arterial thrombosis [43].

An Emerging Role for TAFI in Inflammation

As the list of substrates for TAFIa is growing, it becomes clear that besides regulation of fibrinolysis, TAFI might also play a potential important role in processes such as blood pressure regulation, inflammation and wound healing (Figure 2). In fact, Campbell et al were the first to identify "TAFI" (which they named carboxypeptidase R) as the protein responsible for the difference in inactivation of bradykinin in plasma and serum [44,45]. Bradykinin, cleaved from high molecular weight (HMW)-kininogen through a series of reaction involving the activation of factor XII and prekallikrein, is one of the important mediators of inflammation as it can cause all four classic signs of inflammation, that is, swelling, heat, redness and pain [46]. TAFIa converts bradykinin into inactive des-Arg9-bradykinin (Figure 3) and the catalytic efficiency of des-Arg9-bradykinin formation by TAFIa was approximately 10 fold higher than by carboxypeptidase N [47]. An indication of the physiological relevance came from the observation that activation of endogenous TAFI in mice by anticoagulant thrombin (E229K) effectively blocked bradykinin-induced hypotension in wild-type mice but not in TAFI-deficient mice [47]. Furthermore, a single nucleotide polymorphism (1040C->T) resulting in an amino acid change at TAFI residue 325 (Ile->Thr325) was associated with lower diastolic blood pressure [48]. The half-life of Thr325-TAFIa is about half that of Ile325-TAFIa [49], and it is tempting to
speculate that diminished TAFIa activity due to the shorter half-life of Thr325-TAFI plays a role in the lower diastolic blood pressure.

The anaphylatoxins, C3a and C5a, are important inflammatory mediators and potent leukocyte chemotactants. TAFIa inactivates both C3a and C5a by hydrolysis of their C-terminal Arg, thereby reducing their proinflammatory effects [47,50]. TAFIa efficiently reduced C5a-induced activation of neutrophils in vitro and although carboxypeptidase N is traditionally regarded as the physiological anaphylatoxin inhibitor, inactivation of C5a by TAFIa was approximately 10 fold more efficient than by carboxypeptidase N [47,50]. These results suggest that TAFI could play a role in inhibition of inflammation by inactivation of C5a, the most potent of the complement-derived anaphylatoxins.

TAFI can regulate inflammation and tissue repair also by an entirely different mechanism that involves osteopontin [47]. Osteopontin is an RGD-containing phosphoprotein with adhesive and cell-signaling functions involved in inflammatory responses [51]. Osteopontin circulates as a soluble proinflammatory cytokinin and is present as an extracellular matrix component in mineralized tissues and in the subendothelial matrix of blood vessels involved in atherosclerosis. Cleavage of osteopontin by thrombin generates an N-terminal fragment that exposes a cryptic integrin-binding motif (SVVYGLR) on its C-terminus allowing the interaction to cells bearing specific integrins [52,53]. TAFIa effectively removed the C-terminal Arg of thrombin-cleaved osteopontin and inhibited adhesion to Jurkat cells [47]. Mice lacking a functional osteopontin gene were shown to have an altered wound healing indicating a role of osteopontin in structural remodeling and resolution of dermal wounds [54]. Similarly, TAFI deficient mice showed delayed and altered wound healing with disturbed keratinocyte migration [55]. During normal wound healing, the keratinocytes migrate over the extracellular matrix from one wound edge to the opposite edge. In TAFI deficient mice, the keratinocytes migrate mostly down into the dermal layer indicating a disruption of functional extracellular matrix. Identical results were obtained in fibrinogen and plasminogen knock out mice supporting the importance of the fibrinolytic system in the wound healing process [56,57]. Healing of colonic anastomoses, which is independent of keratinocyte migration but dependent on matrix remodeling and angiogenesis, was also impaired in TAFI deficient mice as reflected by decreased strength of the tissue at the site of the suture. These results indicate that TAFI plays a role in modulating cell migration and/or invasion independent of cell type and that TAFI appears to play a role in tissue repair.

TAFI may also be important for the anti-inflammatory effects of recombinant soluble thrombomodulin. In a thrombotic glomerulonephritis model, rats treated with LPS and an antibody against glomerular basement membrane developed first cellular infiltration in glomeruli followed by severe glomerular injury with massive thrombotic reaction. Injection of recombinant human soluble thrombomodulin prevented these changes and rescued the rat. Administration of recombinant human soluble thrombomodulin resulted in a decreased infiltration of leukocytes and higher levels of TAFIa. Administration of CPI increased the infiltration of leukocytes and neutrophils again, suggesting that anti-inflammatory activity of thrombomodulin functions at least in part via TAFI activation [58]. These results strongly suggest that besides having a role in the regulation of fibrinolysis, TAFI may also have an important function in the regulation of inflammation.

**Conclusion**

The relationship between coagulation and fibrinolysis determines the role of TAFI in the development of venous thrombosis. In arterial thrombosis the inhibitory effect of TAFI on inflammation may play an additional role. In such a mechanism high TAFI levels are protective against arteri thrombosis by inactivation of inflammatory mediators such as bradykinin and C5a. Factor XI deficiency does not confer protection against acute myocardial infarction and we hypothesize that as a result of the impaired activation of TAFI, factor XI deficient patients miss the benefits of TAFI's anti-inflammatory activity.

**References**

18 Marx PF, Dawson PE, Bouma BN, Meijers JC. A arginine specific carboxypeptidase generated in blood during coagulation or inflammation which is unrelated to carboxypeptidase N or its subunits. Biochem Biophys Res Commun 1989;162:933-939.


31 Broge JG Jr, Hijguchi DA. Coagulation-dependent inhibition of fibrinolysis: Role of carboxypeptidase-U and the premature lysis of clots from meizothrombin and Meizothrombin(desF1).


44 Campbell W, Okada H. An arginine specific carboxypeptidase generated in blood during coagulation or inflammation which is unrelated to carboxypeptidase N or its subunits. Biochem Biophys Res commun 1989;162:923-927.


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51 Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival: J Clin Invest 2001;107:1055-1061.


