

Theme Issue Article

Factor VII-activating protease (FSAP): Vascular functions and role in atherosclerosis

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Summary

FSAP is a plasma serine protease for which a potential role in the regulation of coagulation and fibrinolysis is postulated, based on its property to activate factor VII (FVII) as well as pro-urokinase (uPA). In clinical studies, the G534E single nucleotide polymorphism (Marburg I) of FSAP has been linked to late complications of atherothrombosis and is associated with a low proteolytic activity, particularly, towards pro-uPA. This has stimulated much interest in a search for additional functions of FSAP in the cardiovascular system. FSAP is a potent inhibitor of vascular smooth muscle cell proliferation and migration *in vitro* and local application of FSAP (but not Marburg I variant) in animal models re-

duces neointima formation. This is due to a reduced proteolytic activity of the variant isoform towards platelet derived growth factor-BB, a key mediator of neointima development. Moreover, appreciable quantities of FSAP are localized to unstable atherosclerotic plaques and may contribute to plaque instability. These data indicate that the cellular regulatory effects of FSAP may be more important than its influence on haemostasis. In this review the contribution of FSAP to vascular fibroproliferative inflammatory diseases in the context of pericellular proteolysis of the extracellular matrix, growth factor activity and haemostasis will be highlighted.

Keywords

Atherosclerosis, restenosis, blood coagulation, fibrinolysis, serine protease, growth factors, cell proliferation

Thromb Haemost 2008; 99: 286–289

Factor VII-activating protease (FSAP): the basic molecular facts

FSAP is predominantly produced as an inactivate zymogen in the liver and circulates in plasma at a concentration of 12 µg/ml. Despite its restricted organ/tissue expression, FSAP is widely distributed in several tissues, particularly under disease conditions. FSAP is composed of various structural modules, including three epidermal-growth-factor (EGF)-like domains, a kringle domain and a serine protease domain, exhibiting high homology to urokinase (uPA), plasminogen or hepatocyte growth factor-activator (HGF-A) (1, 2) (Fig. 1). If homology is used as a predictor of function, then this would indicate that FSAP has a role in fibrinolysis and the regulation of growth factor activity. The protein was originally isolated based on its ability to bind to hyaluronic acid (1), and this property to interact with negatively charged polyanions was extended to heparin (3, 4) and nucleic acids, particularly RNA (5, 6). FSAP binding to

these polyanions results in the activation of the single-chain inactive zymogen to the two-chain active enzyme that exhibits autocatalytic activity (3, 4, 7–9). FSAP might also be activated, to a certain extent, by uPA (3). Following autocatalytic activation there is rapid auto-proteolysis of FSAP which makes the isolated protein rather unstable in solution (4, 6, 8). This property of FSAP was used to define the nature of its degradation products and the regions responsible for binding to heparin and RNA. Multiple domains of FSAP contribute to polyanion binding, whereby the EGF-3 domain (containing a cluster of positively charged amino acids) plays an important role (6). In spite of the availability of such data the nature of the physiologically relevant activator of FSAP remains elusive.

Once activated, FSAP can be rapidly inhibited by serine protease inhibitors such as α 1 proteinase inhibitor, α 2-plasmin inhibitor, antithrombin, C1 inhibitor (4, 7, 10, 11), as well as plasminogen activator inhibitor-1 (PAI-1) (12) and protease nexin-1 (13). Complex formation of FSAP with inhibitors results in rapid internalization of the complexes by low-density lipoprotein re-

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Received October 30, 2007
Accepted after minor revision December 22, 2007
Prepublished online January 11, 2008
doi:10.1160/TH07-10-0640

ceptor-related protein (LRP) via receptor-mediated endocytosis (13). It is likely that contact with polyanions does not only promote FSAP (auto-) activation, but also subsequent neutralization by serine protease inhibitors and the internalization of these complexes and their degradation. Hence, specific surface-dependent mechanisms in conjunction with destabilization or injury of tissue exist to activate and deactivate FSAP indicative of a tightly regulated system.

FSAP in thrombosis and haemostasis

The high homology of plasma haemostasis factors with FSAP led to a search for its functions in coagulation and fibrinolysis (14), whereby factor VII (15) and pro-uPA (11) were identified as potential FSAP substrates. However, the activation of the contact or the extrinsic pathway of blood coagulation was not associated with any conversion of single-chain to two-chain FSAP (3). Although exogenously added FSAP did shorten plasma recalcification times and pro-thrombin times, there were no changes in activated partial thromboplastin times (14). This indicates an action of FSAP on the extrinsic rather than the intrinsic pathway of blood coagulation (14). Similarly, pro-uPA is activated by exogenous FSAP and fibrinolysis in whole blood is induced (15). However, the stimulation of fibrinolysis by addition of pro-uPA is not associated with the activation of endogenous FSAP (3). Since the extra-vascular concentration of uPA is very high as compared to its almost undetectable intra-vascular levels, it is likely that FSAP activation of pro-uPA (and subsequent plasminogen generation) is more relevant for the regulation of extra-vascular cellular processes such as extracellular matrix turnover or cell migration. Critical experiments with regard to FSAP-dependent factor VII or pro-uPA activation (by using e.g. blocking antibodies or FSAP-deficient plasma) are required to further consolidate the character of FSAP as a haemostasis protein (Fig. 2).

Fibrinogen was also found to be a substrate of FSAP, but this proteolytic cleavage does neither influence the formation nor affect the dissolution of clots (16). High-molecular-weight kininogen appears to be another haemostasis-relevant substrate of FSAP giving rise to low-molecular-weight kininogen and the production of bradykinin with potent vasodilating activity (17). This reaction of FSAP may alter local vascular permeability and blood pressure and thereby could indirectly influence haemostasis (18). It seems that exogenously applied FSAP does participate in many reactions relevant for haemostasis, but the role of endogenous FSAP (possibly controlled by serine protease inhibitors) in these processes remains to be demonstrated.

FSAP polymorphisms and cardiovascular disease

Two prominent single nucleotide polymorphisms (SNP) exist in the FSAP gene, G534E and E393Q, that each result in an exchange of a single amino acid in the protease domain (19) (Fig. 1). About 5% of the Caucasian population are carriers of each SNP. The G534E polymorphism (designated Marburg I) is a weak activator of pro-uPA, but its ability to activate factor VII is unchanged (19). Hence, the presence of this genotype may shift

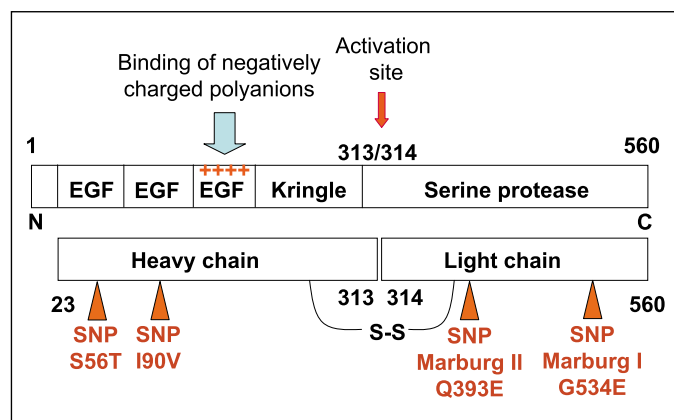


Figure 1: Structural features of FSAP. A schematic representation of single-chain FSAP (top) and two-chain FSAP (bottom) indicating the EGF, kringle and the serine protease domains. A major cluster of positively charged amino acids is involved in polyanion binding. Cleavage at residues 313/314 leads to activation of the zymogen into the active two-chain enzyme. The four single nucleotide polymorphisms (SNPs) that lead to an amino acid exchange are also indicated of which the Marburg I SNP is likely of high functional significance.

the activity profile of FSAP towards more “prothrombotic” properties. Moreover, the Marburg I SNP was found to be strongly linked to late complications of carotid stenosis, indicating that FSAP may play a direct role in atherosclerosis (20). Subsequently, it was shown that this SNP is also a risk factor for cardiovascular disease in general (21). The Marburg I SNP was also stated to be associated with venous thromboembolism (22), but this conclusion remains controversial (23, 24), and requires further patient studies in defined populations. The E393Q polymorphism (Marburg II) is not associated with altered enzymatic activity or any other changes in FSAP function (19).

In an effort to unravel the mechanism by which FSAP-Marburg I differs from the wild-type, we have isolated this isoform and characterized its biochemical properties. This variant had a five-fold lower enzymatic activity towards all the substrates tested, but its ability to bind to heparin was unchanged (25). In view of the overwhelming difference in enzymatic activities between wild-type and Marburg I FSAP, the earlier suggestion that the variant form can activate factor VII equally well as the wild-type form (19) needs to be re-examined. In various population studies a few subjects homozygous for Marburg I FSAP have been identified, although they do not have any overt symptoms or conditions that can be related to this SNP (unpublished observations). The decreased enzymatic activity as well as the linkage of the Marburg I SNP with late complications of carotid stenosis indicates that FSAP may be involved in the pathogenesis of atherosclerosis. In a comprehensive study the functional differences between isoforms of FSAP were compared in a wire-induced arterial stenosis model in mice, whereby wild-type FSAP but not the Marburg I variant significantly prevented neointima formation (25). Utilizing a fast and reliable method based on real-time PCR to detect the Marburg I SNP (26), large patient studies are required to better define the role of FSAP in vascular diseases.

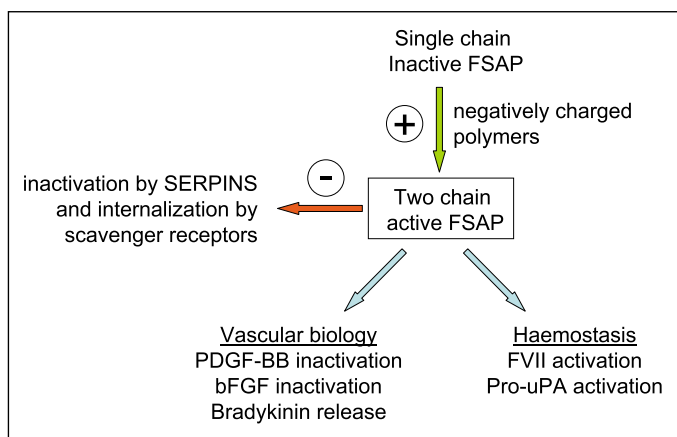


Figure 2: Multiple activities of FSAP in the vascular system. The inactive circulating zymogen is activated by polyanions and the active protease can be inhibited by serine protease inhibitors and the resulting complexes are internalized via scavenger receptors. The active form of FSAP may influence the haemostasis system through activation of pro-urokinase or factor VII or it may exert effects on the vasculature through PDGF-BB and/or bFGF inactivation as well as bradykinin release.

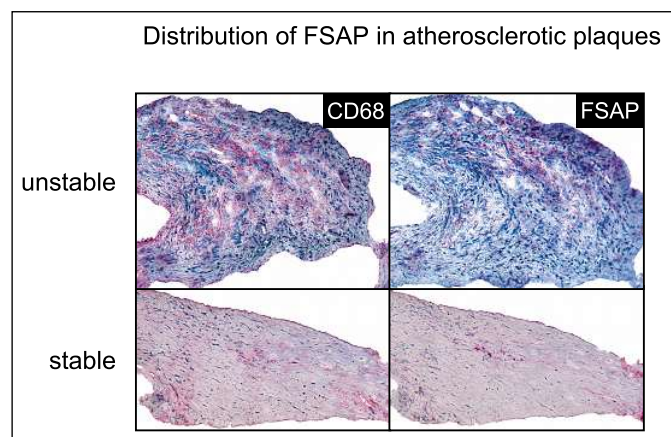


Figure 3: Distribution of FSAP in atherosclerotic plaques. FSAP staining (pink colour) in unstable (top panels) and stable plaques (bottom panels) is depicted. Specific FSAP staining is compared to the distribution of CD68, a macrophage marker. FSAP accumulates to a larger extent in unstable plaques compared to stable plaques and there is co-localization with macrophages. Some extracellular matrix associated staining is also apparent.

FSAP and atherosclerotic plaques

If FSAP is involved in atherosclerosis it is imperative to know its distribution in atherosclerotic plaques and diseased vessels. Immunolocalization studies indicated that FSAP was not present in normal vessels but substantial immunostaining was found particularly in association with unstable atherosclerotic plaques (27, 28) (Fig. 3). The distribution of (intra- and extracellular) FSAP was quite similar to that of uPA and was found predominantly in association with macrophages and, to a much lesser degree, with vascular smooth muscle cells. Moreover, isolated peripheral blood monocytes/macrophages, but not other cells of the vascular wall, were found to express FSAP mRNA, which was up-regulated by pro-inflammatory mediators. Not only protein but also FSAP mRNA was localized in atherosclerotic plaques with elevated levels, particularly, in unstable plaques (27). This is indicative for *in situ* expression and protein deposition of FSAP from the circulation. Based on its molecular characteristics, FSAP might thereby become concentrated onto negatively charged molecules such as heparin sulphate proteoglycans, hyaluronic acid or nucleic acids at these sites, become (auto-) activated and partially complexed by inhibitors followed by rapid internalization via LRP (13). A portion of the immunostained FSAP might represent such complexes.

The localization of FSAP in unstable atherosclerotic plaques together with the association of Marburg I FSAP as a risk factor for carotid stenosis indicates that FSAP is involved in the regulation of plaque stability or rupture. Unstable plaques are prone to rupture and the culprits in this process are proteases released from macrophages (29). FSAP could contribute to this proteolytic “soup” and promote plaque rupture. Elegant as it may sound, this theory does not explain why carriers of the Marburg I SNP, with lower proteolytic activity, are more prone to atherosclerosis. An alternative explanation with regard to smooth muscle cell coverage will be discussed below. Further insight

into the role of FSAP in atherosclerosis can be obtained through an analysis of FSAP in mouse models of atherosclerosis and also through analysis of the distribution of Marburg I FSAP. Measurements of circulating FSAP isoforms and complexes in patients with acute coronary syndrome can also shed more light on the role of FSAP in cardiovascular disease.

Cellular activities of FSAP on vascular cells

The high structural homology of FSAP to HGF-A suggests a possible functional overlap, but our preliminary studies excluded the possibility that FSAP activates HGF (our unpublished observations). In contrast, FSAP was identified as potent regulator of smooth muscle cell proliferation and migration (28), specifically through inhibition of platelet derived growth factor-BB (PDGF-BB) but not that of other growth factors. FSAP binds to PDGF-BB with high affinity and cleaves PDGF-BB in a region that is crucial for receptor binding and activation (30). This activity is significantly enhanced in the presence of heparin (28) or nucleic acids (30), but lost upon inhibition of FSAP enzymatic activity (12, 13, 25, 28, 30). A similar mechanism was proposed for FSAP-mediated inactivation and inhibition of basic fibroblast growth factor (bFGF)-dependent endothelial cell proliferation (31, 32). Conversely, FSAP can release bFGF bound to heparan sulphate proteoglycans on the cell surface that in turn can activate cells, resulting in opposing effects of FSAP on bFGF actions (33). In our studies FSAP had no influence on bFGF in relation to smooth muscle cells, indicating that the bFGF-FSAP interaction may not be relevant for all cell types (25).

As expected, the aforementioned PDGF-directed anti-proliferative function of FSAP could not be duplicated by enzymatically inactivated FSAP or the naturally occurring Marburg I isoform, both of which did not influence neointima formation in animal models (25). These results indicate that FSAP may serve as “protective” factor in preventing hyper-proliferation in the

vessel wall. This interpretation has certain implications for the prediction and treatment of restenosis. In atherosclerotic plaques, a lower extent of smooth muscle cell proliferation leads to a thin cap structure and unstable plaques, whereas hyper-proliferation results in vessel occlusion. In this scenario, the Marburg I variant would be associated with enhanced smooth muscle activation and this in turn may be responsible for the late complications of atherosclerosis observed in population studies (20).

Conclusions and perspectives

In vascular lesions, FSAP may regulate coagulation and fibrinolysis through its actions on factor VII and pro-uPA. By virtue of activation of pro-uPA with subsequent generation of plasmin and matrix metalloproteases, FSAP can also influence extracellular matrix degradation. Regulation of PDGF and bFGF by proteolytic degradation may be another function of FSAP. Through these diverse mechanisms FSAP could influence vascular remo-

deling or the development of vascular lesions. Although we restricted the discussion to fibro-proliferative diseases in the vasculature, similar mechanisms are at work in fibrotic or remodeling processes in other organs such as the lung (34).

Based on these considerations, therapeutic application of FSAP may represent a novel approach to prevent vascular proliferative disease such as restenosis. The information that the Marburg I isoform of FSAP exhibits altered proteolytic degradation of PDGF-BB provides a direct mechanistic link to vascular lesion formation in patients that harbor this polymorphism. These results provide a clear rationale for using the Marburg I-FSAP as a diagnostic parameter to predict the development of post-angioplasty restenosis. The current understanding is that FSAP is likely to be a vascular cell regulatory factor, and further investigations with the help of specific FSAP inhibitors and transgenic/knock-out animals will help to decipher the role of FSAP in haemostasis and in cardiovascular diseases in general.

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