Physiol. Res. 57: 1-5, 2008

MINIREVIEW

Plasma Tissue Factor in Coronary Artery Disease: Further Step to the Understanding of the Basic Mechanisms of Coronary Artery Thrombosis

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Received August 17, 2006
Accepted January 2, 2007
On-line available January 2, 2007

Summary

Tissue factor is a cell surface protein that is expressed constitutively by monocytes, macrophages and fibroblasts, but also by some other cells in response to a variety of stimuli. The main function of the tissue factor is to form a complex with factor VII/VIIa that converts factors IX and X to their active forms. Tissue factor is also involved in the pathophysiology of systemic inflammatory disorders, coagulopathies, atherosclerotic disease, tumor angiogenesis and metastasis. Increased tissue factor expression either locally in the coronary plaques or systematically on circulating blood elements of patients with acute coronary syndromes may be responsible for increased thrombin generation, thus leading to platelet activation and fibrin formation. Tissue factor therefore plays a pivotal role in the initiation of thrombotic complications in patients with coronary artery disease.

Key words

Coronary artery disease • Coronary thrombosis • Tissue factor

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Tissue factor and coagulation

Tissue factor is a cell surface protein that is expressed constitutively by monocytes, macrophages and fibroblasts (Mackman *et al.* 1991, Taubman *et al.* 1993, Kamimura *et al.* 2004, Penn *et al.* 1999). Some authors also describe tissue factor expression by cardiomyocytes (Mumford and McVey 2004). Tissue factor forms a complex with factor VII/VIIa that converts factors IX and X to their active forms. The blood borne tissue factor has been discovered recently. However, TF may also be expressed in some other cell types in response to a variety of stimuli. The initiation phase of thrombus generation begins when the disruption of vessel wall or atherosclerotic plaques exposes tissue factor to circulating factor VII, thus leading to the activation of both the intrinsic and extrinsic blood coagulation cascades (Rosenberg and Aird 1999). The tissue factor also induces cell signaling by stimulating G-protein-coupled protease activated receptors (PARs). Signaling pathways initiated by both TF/VIIa complex protease activation of PARs and phosphorylation of the tissue factor cytoplasmic domain appear to regulate different cellular functions (Rao and Pendurthi 2005). In normal arteries, tissue factor expression is limited to the adventitia except for sporadic expression in the media (Wilcoxon *et al.* 1989). A small quantity of circulating tissue factor is present in both the whole blood and serum of healthy individuals (Giesen and Nemerson 2000). The tissue factor is also involved in the pathophysiology of systemic inflammatory disorders, coagulopathies, atherosclerotic disease, chronic renal failure (Zemanová *et al.* 2003), tumor angiogenesis and metastasis (Price *et al.* 2004). There is a significant positive correlation between tissue factor level and serum creatinine, glycemia, LDL cholesterol, number of cigarettes smoked per day (Sambola *et al.* 2003) and an inverse correlation

between tissue factor level and serum insulin (Zemanová *et al.* 2003).

Tissue factor in coronary artery disease

Inappropriate expression of the tissue factor may result in thrombosis contributing to acute clinical consequences of coronary artery disease. Experimental data show that atheromatous plaques contain a high concentration of tissue factor relative to surrounding tissue (Marmur *et al.* 1996). Abundant tissue factor is also found in atheromatous lesions within foamy macrophages in macrovascular disease in humans such as aortic aneurysms and carotid arteries (Wilcoxon *et al.* 1989). Ruptures of atheromatous plaques (usually multiple) expose tissue factor to circulating factor VII causing the initiation of a clot formation and may consequently lead to an abrupt vessel closure and myocardial infarction. This can be demonstrated by the examination of tissue samples obtained from patients with acute coronary syndromes, where higher levels of tissue factor are present in these lesions. In this way, the tissue factor may play not only a role in the development of acute coronary syndromes but also in the progression of coronary artery disease (Annex *et al.* 1995, Ardissino *et al.* 1997, Mann and Davies 1999, Westmuckett *et al.* 2000) *via* the tissue factor-dependent intramural fibrin deposition after plaque rupture and subsequent progression of the atherosclerotic lesion (Westmuckett *et al.* 2000). Modified ELISA (Westmuckett *et al.* 2000) and a rabbit polyclonal antibody against the solubilized tissue factor activity (Thiruvikraman *et al.* 1996) were used for *in situ* localization of the tissue factor in human atherosclerotic plaques. Quantitation of tissue factor antigen was done to test the hypothesis that thrombin generation takes place directly in the atherosclerotic lesion (Thiruvikraman *et al.* 1996). This findings support the possible role of the tissue factor-dependent coagulation pathway in the intramural fibrin deposition and the progression of the atherosclerotic lesions.

Detached endothelial cells as well as microparticles from endothelial cell monolayers express tissue factor activity and this activity is markedly inhibited by microparticle-associated tissue factor pathway inhibitor (Kushak *et al.* 2005).

Apart from the role, which the tissue factor has in the acute coronary syndromes, tissue factor expression has a potential role in vascular remodeling after coronary angioplasty. Cell culture studies have demonstrated that the TF/VIIa complex is critical for smooth muscle cell migration (Siegbahn *et al.* 2000). Furthermore, TF/VIIa-mediated smooth muscle cell migration can be inhibited by the overexpression of the tissue factor pathway inhibitor. The links between lipoproteins and tissue factor expression, as well as tissue factor and vascular remodeling (Singh *et al.* 2001) suggest a potential mechanism for the surprisingly early benefits of high-dose statin therapy in patients with acute coronary syndrome. Alternatively, statin therapy could be beneficial by decreasing CRP levels in these patients, because C-reactive protein has been shown to induce tissue factor expression in monocytes (Cermak *et al.* 1993).

Heparin treatment is associated with a decrease in tissue factor plasma levels and monocyte procoagulant activity (Gori *et al.* 1999) as well as with increased plasma level of the tissue factor pathway inhibitor.

Arnaud *et al.* (2000) investigated whether individual differences in tissue factor gene expression could predispose subjects to thrombosis. They sequenced the 5' domain of the gene and found six different polymorphisms. Genotyping of patients with myocardial infarction in a case-control study involving 2354 subjects showed no association between the polymorphisms and nonfatal coronary thrombosis (Arnaud *et al.* 2000).

Many studies demonstrated elevated tissue factor plasma levels in patients with myocardial infarction (Saigo *et al.* 2001), stable angina (Kim *et al.* 2000), unstable angina and an increased risk for unfavorable outcomes in patients with unstable angina and raised tissue factor levels. Other authors found only a non-significant trend to raised plasma levels of tissue factor in patients with acute myocardial infarction and unstable angina pectoris as compared to patients with stable coronary artery disease and normal subjects (Malý *et al.* 2003). Tissue factor could be useful for the evaluation of the effect of cardiovascular risk intervention, but results in this field are still controversial (Lim *et al.* 2004). Can tissue factor also be used as a prognostic marker in patients with cardiovascular disease? Seljeflot *et al.* (2003) followed patients after myocardial infarction for 4 years. Patients, who had suffered an endpoint event (reinfarction or stroke), had significantly higher tissue factor levels as compared to those who did not. Other authors (Sambola *et al.* 2003, Lim *et al.* 2004) reported higher plasma levels of tissue factor in patients with diabetes than in control subjects. They suggest that higher levels of tissue factor may be the mechanism responsible

for the increased thrombotic complications associated with the presence of other cardiovascular risk factors. No significant differences were found between diabetics and non-diabetics in patients with chronic renal failure (Zemanová *et al.* 2003).

Diagnostic tests for plasma tissue factor

The plasma level of tissue factor may be determined by means of the commercially available Tissue Factor ELISA Kit. The kit employs a murine anti-human tissue factor monoclonal antibody for antigen capture. Plasma samples are incubated in microtest wells precoated with capture antibody. Once captured, the tissue factor is detected using a biotinylated antibody fragment that specifically recognizes the bound tissue factor. The subsequent binding of horseradish peroxidase conjugated streptavidin completes the formation of the antibody-enzyme detection complex. Quantitative data are obtained by measuring the solution absorbance at 450 nm and relating it to the standard curve. The detection limit of this assay is about 10 pg/ml.

Tissue factor may circulate in the blood incorporated in pro-coagulant microparticles shedded as membrane vesicles (Mallat *et al.* 1999). Moreover, a form of human tissue factor generated by alternative splicing has been identified (Bogdanov *et al.* 2003). Alternatively spliced human tissue factor is soluble, circulates in the blood, exhibits pro-coagulant activity when exposed to phospholipids, and is incorporated into thrombi and thus may contribute to thrombus growth (Bogdanov *et al.* 2003). Plasma tissue factor activity not associated with cells or microparticles has also been studied. Tissue factor-dependent generation of factor Xa on cryosections was used to assess the functional activity of the tissue factor (Thiruvikraman *et al.* 1996).

To employ tissue factor as a useful marker of atherothrombosis, a very sensitive diagnostic test for its detection and quantitation is needed. High-affinity antibodies are employed in the improved version of ELISA diagnostic tests for tissue factor plasma level assessment (Chen *et al.* 2005). The new assays provide higher sensitivity with much lower detection limit than currently available diagnostic sets. Data obtained by these assays also show markedly reduced individual variation. Higher sensitivity in tissue factor detection is of critical value especially in the group of patients with normal or slightly elevated tissue factor levels and opens a new field for research. It is also very important to detect accurately also low levels of tissue factor in order to use tissue factor as a potentially useful tool for risk stratification in patients with cardiovascular disease.

However, the question how to interpret an elevated tissue factor levels still remains unanswered. Additional investigations will be necessary for better acceptance of the tissue factor as an independent biomarker for cardiovascular risk that we can incorporate into decision-making algorithms. We should not ignore the limited specificity with elevated tissue factor levels in a variety of different clinical situations. Larger scale clinical studies should confirm the independent significance of the tissue factor.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This paper was supported by the Research Grant IGA NR/8131-3 and NR/9176-3.

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