Review

Tissue factor and coronary artery disease

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Received 11 July 2001; accepted 27 August 2001

Abstract

Plaque disruption with superimposed thrombosis is the main cause of acute coronary events such as acute myocardial infarction and unstable angina. Among other factors, tissue factor seems to play an important role determining plaque thrombogenicity. Tissue factor is a potent initiator of the coagulation cascade situated within the vessel wall and is highly exposed to the blood after plaque rupture. Several mediators involved in the process of atherosclerotic plaque formation are capable of inducing tissue factor expression in cells such as monocytes, macrophages and endothelial cells, which under normal conditions do not express tissue factor or to a limited extent only. The increased expression of tissue factor is not limited to the plaque but is also found in circulating monocytes in patients with acute coronary syndromes. In addition, studies have shown an important contribution of tissue factor in the pathogenesis of thrombosis and restenosis after balloon angioplasty. Recent basic studies focus on the therapeutic inhibition of tissue factor. Specific and non-specific inhibitors of tissue factor or the tissue factor/factor VIIa complex have been developed or identified, and have been tested in experimental studies. Clinical studies are currently being initiated. In this review, we present the current knowledge on the role of tissue factor in atherosclerosis, arterial intervention and potential pharmacological approaches, with focus on acute coronary syndromes. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Atherosclerosis; Coronary disease; Hemostasis; Thrombosis/embolism

1. Introduction

Plaque disruption with superimposed thrombosis is the main cause of acute coronary syndromes including unstable angina, myocardial infarction and sudden cardiac death [1\textendash}3]. The composition and vulnerability of atherosclerotic plaques, rather than their volume or the severity of a stenosis, seem to be the most important determinants of plaque rupture [3]. Foam cells and smooth muscle cell infiltration, and connective tissue deposition (type I\textendash}III lesions according to Stary et al. [4]) mark early lesions. As these lesions grow into ‘softer’ plaques with a high extracellular lipid and cholesterol ester content and a progressively thinner fibrous cap (types IV\textendash}V, ‘atheroma’), they become more vulnerable to disruption [1,2,4,5]. Inflammation may be the trigger for rupture of the lesion, with subsequent thrombosis [3,5\textendash}8]. Plaque content determines its thrombogenicity, and tissue factor (TF) is thought to play a key role. In this review, we outline the current understanding of the role of TF in atherosclerotic diseases with focus on acute coronary syndromes.

2. Physiology of tissue factor

TF is a 263-residue membrane-bound glycoprotein composed of a 219-residue extracellular domain, a single transmembrane sequence and a short cytoplasmic domain [9,10]. Thus, TF is a surface-bound, transmembrane glycoprotein that is normally not present in or exposed to the circulation or in contact with it. The location of TF on plasma membranes enables a nidus of coagulation to remain fixed at the site of injury, which may explain why coagulation does not disseminate from its initiation site [11]. The exposure of cell surfaces expressing TF to...
plasma proteins leads to the binding of factor VII to TF [11–13]. TF and factor VII have a high affinity for each other and neither component alone has procoagulant activity at physiological concentrations. The complex TF-factor VIIa may be activated into the complex TF-factor VIIa by free factor VIIa and/or by already formed TF-factor VIIa complexes. The complex TF-factor VIIa further activates free factor VII. These mechanisms describes the TF-mediated factor VII autoactivation. The TF-factor VIIa complex rapidly catalyzes the activation of factor X and, at a lower rate, of factor IX. Activated factor IX, in the presence of its co-factor factor VIII, cleaves additional factor X to factor Xa. Eventually, these processes lead to the generation of thrombin, which catalyzes the formation of fibrin and its polymerization to form a fibrin clot (see Fig. 1).

TF is present in vascular adventitia, organ capsules, epidermis, and mucosal epithelium [14]. Prominent expression of TF has been observed in cardiac myocytes, the cerebral cortex, renal glomeruli, and the lung [14]. TF is thus expressed at tissue barriers between the body and the environment, where it fulfills a hemostatic role in activating coagulation when the vascular integrity is disrupted.

The endogenous inhibitor of the TF-factor VIIa complex is tissue factor pathway inhibitor (TFPI). TFPI is a Kunitz-type protease inhibitor consisting of three tandem Kunitz domains [15]. TFPI inhibits TF in a two-step mechanism. It binds via its second Kunitz domain to the active site of factor Xa, thus inhibiting the proteolytic capacity of factor Xa. The following step is the inhibition of the catalytic activity of TF-factor VIIa complexes by formation of the quaternary complex TF-factor VIIa / TFPI-factor Xa [15]. Due to its factor Xa dependency, inhibition by TFPI is slow but it completely inhibits TF/factor VIIa activity [16]. The function of the third Kunitz domain, that contains a heparin-binding site, is not clear. TFPI is mainly synthesized by vascular endothelial cells [17] and the majority (50–90%) of the intravascular pools of this inhibitor is associated with these cells [18,19]. Less than 2.5% of total TFPI is located in platelets and the remaining in plasma in a free or lipoprotein-associated form [20,21].

3. Cellular expression of tissue factor

In normal vessels, TF is synthesized at the subendothelial level by smooth muscle cells in the tunica media and predominantly by fibroblasts in the adventitia surrounding the vessels [22,23]. Hence, disruption of the endothelial layer exposes TF to circulating blood. Peripheral blood cells and endothelium do not express TF under normal conditions, consistent with the hypothesis that expression of TF is anatomically separated from blood. However, in response to a variety of stimuli, TF expression is induced in different cell-types and this has been the subject of many studies. Some important findings are listed below.

3.1. Monocytes and macrophages

In addition to increasing procoagulant activity with age [24] different mechanisms may trigger increased procoagulant activity by mononuclear cells.

Lymphocytes may interact with monocytes and macrophages by two main parallel and independent models of cellular interaction [25]. One pathway is mediated by specific cytokines like interleukin (IL)-2, tumor necrosis factor-β and interferon-γ. These cytokines, produced by Th1 cells, induce TF expression in mononuclear cells [26]. Conversely, inhibition of TF expression is mediated by Th2 cell derived cytokines IL-4, IL-13 and IL-10 and may be important in preventing thrombosis [26,27]. The second pathway is thought to be due to T cell binding to the CD40 receptor present on the membranes of monocytes and macrophages [28], which induces active TF expression on human monocytes and macrophages [29,30].

In addition to cell-induction, C-reactive protein, of which plasma levels are increased in patients with unstable angina [31], stimulates monocyte TF expression [32]. Furthermore, an isofrom of platelet-derived growth factor (PDGF), PDGF-BB, is released by activated platelets and induces TF expression on the surface of both vascular smooth muscle cells and human monocytes [33,34]. Another feature of PDGF-BB is the stimulation of vascular smooth muscle cells to produce monocyte chemoattractant protein-1 [35], which is produced within the plaque and most likely involved in the recruitment of monocytes into the plaque [36]. It also leads to a dose- and time-dependent induction of TF expression in arterial smooth muscle cells and monocytes [34,37]. Recently, it has been demonstrated that human platelets may also express and release TF during activation [38]. These findings indicate a positive
feedback mechanism of additional TF mediated thrombin generation.

Monocyte-derived macrophages that are enriched with lipids or intracellular free cholesterol (foam cells) produce greater amounts of TF compared to non-lipid-enriched macrophages [39,40]. Conflicting results however, have been reported on the effect of lipoproteins on the procoagulant activity of monocytes and macrophages. Studies have shown no [41,42] or even an inhibitory [43] effect, whereas others demonstrated an induction by native or oxidized low-density lipoprotein of TF expression and activity on mononuclear cells [44,45]. Experimental exposure of monocytes to very low-density lipoprotein and high densities lipoprotein has resulted either in no effect [41] or in increased TF activity [43,44]. More uniform are the reports concerning acetylated low-density lipoprotein, which is a potent inducer of TF expression in monocytes and macrophages [40,42,46].

Monocytes from patients with unstable angina showed a higher TF like procoagulant activity in vitro than control subjects or patients with stable angina [47]. This activity correlated with activation of the coagulation system as assessed by fibrinopeptide A plasma levels. Conceivably, the exposure of lymphocytes to an (unknown) inducer triggers a series of reactions leading to monocyte activation, increased thrombin generation and instability of coronary artery disease [47]. A higher monocyte TF-like activity was shown in patients who had recently suffered an acute myocardial infarction compared to healthy controls [48]. This was paralleled by an increase in plasma TFPI activity, which may be of importance for maintenance of the hemostatic balance. However, in these patients, heparin-induced increases in plasma TFPI levels may be partly responsible for this effect. Unstable angina patients exhibit higher levels of TF expression both in unstimulated and endotoxin-stimulated monocytes, compared to patients with stable angina and acute myocardial infarction [49]. Only in the unstable angina patients monocyte activation was correlated with increased fibrinogen levels [49]. Balloon angioplasty in stable and unstable angina patients seems to have no significant effect on the level of TF expression in stimulated and unstimulated monocytes within the first 24 h after the procedure [50]. Increased monocyte TF expression is seen in patients with an acute coronary syndrome and less pronounced, in patients with chronic stable angina [51].

An interesting experiment demonstrated abundant TF staining in thrombi formed in human blood that was exposed to surfaces which either was devoid of TF or did not contain stainable TF (see Fig. 2) [52]. ‘Blood-borne’ TF from neutrophils and monocytes may be thrombogenic and involved in thrombus propagation at the site of vascular injury. Moreover, it may serve as a mechanism of initiating thrombus formation independent of procoagulants in the atherosclerotic plaque. This hypothesis is supported by the demonstration of transfer of TF from monocytes and possibly polymorphonuclear leukocytes to platelets, making the platelets capable of triggering and propagating thrombosis [53].

Thus, monocytes and macrophages not only play an important role in the pathogenesis of atherosclerosis [54], they also are potent activators of blood coagulation through the ability to express TF upon activation. This might aggravate or even trigger thrombosis in patients with acute coronary syndromes.

3.2. Vascular smooth muscle cells

Platelet-derived growth factor-BB, as mentioned previously, and thrombin are capable of inducing TF expression on cultured vascular smooth muscle cells [33,55]. However, the induction of surface-bound active TF represents only a small percentage (±20%) of total cellular TF [55]. This could represent an adaptive mechanism for limiting the procoagulant potential of the vessel wall. The remainder of TF was present in an intracellular pool and as encrypted surface TF [55], which may provide a rich source of TF after smooth muscle cell damage, such as plaque rupture or balloon injury. Native low-density lipoprotein also increased surface TF on human arterial smooth muscle cells, but surface TF activity remained limited [56]. Ligation of the CD40 receptor, present on the membrane of smooth muscle cells, augments the expression of both TF protein and TF activity on the surface of human vascular smooth muscle cells [57]. Because TF and CD40 colocalize on lesional smooth muscle cells in human atheroma, this pathway may contribute to the TF expression during inflammatory responses of atherogenesis and arterial injury.

3.3. Endothelial cells

Many studies have investigated the induction of TF-mediated procoagulant activity in cultured human endothelial cells, and several stimulating factors have been identified including interleukins [58–60], tumor necrosis factor-α [58,60–62], endotoxin [63,64], and shear stress [65]. In some studies no or only minimal TF activity at the (apical) endothelial cell surface was detected after stimulation with tumor necrosis factor [62,66,67]. TF expression was more prominently localized at the subendothelial matrix [67], at the basolateral surface [62], or it remained intracellular in the endothelial cell [67]. This suggests a tightly regulated TF expression in endothelial cells which may only gain access to the blood after disruption of the endothelial monolayer. Some pathways of TF expression described for monocytes–macrophages and/or vascular smooth muscle cells, have also been identified in endothelial cells. Oxidized low-density lipoprotein is a potent stimulus for TF expression in cultured human endothelial cells [68]. Ligation of the CD40 receptor (expression of which might be upregulated after exposure with interferon-
γ) by T cells or activated platelets increases TF expression on endothelial cells [69,70]. Platelets have also been shown to stimulate TF production in human endothelial cells [71].

4. Tissue factor in atherosclerotic plaques

TF was identified in atherosclerotic plaques from various arterial sites. TF-antigen, TF-activity as well as TF-mRNA have been detected in different cell types within the plaque including endothelial cells, vascular smooth muscle cells, monocytes and especially in macrophages or foam cells (see Fig. 3) [22,72–77], which represent up to 60% of the cells in the plaque [78]. TF colocalizes with TFPI in these cells [79]. The presence of TFPI in human atherosclerotic plaques is associated with reduced TF activity [80].

TF expression was shown in all stages of atherosclerotic lesions [72,74,75]. Abundant TF protein (see Fig. 3) and TFPI was found in the extracellular matrix of the necrotic core of the plaques [22,73–75,77,79,81,82]. TF found in the lipid-core is thought to be largely derived from the macrophages present in the plaque [73,81,82]. However, a contribution of vascular smooth muscle cells and endothelial cells cannot be ruled out. Extracellular TF in apoptotic areas is derived from shed membrane apoptotic microparticles originating from macrophages and lymphocytes [82]. This accounts for almost all the TF activity of the plaque extract.

TF in atherosclerotic plaques may play a major role in the initiation of thrombus formation. Fibrin deposition in atherosclerotic intima is occasionally located around foamy or non-foamy macrophages and smooth muscle cells, which overexpress TF [74]. Compared to fibrous, collagen-rich lesions and other arterial components, more in vitro
Fig. 3. Immunohistochemical stains of adjacent sections of human coronary atherectomy specimen. (A) A macrophage-rich area, (B) stained for TF antigen; and (C) a smooth muscle cell-rich area, (D) stained for TF antigen. The acellular area seen in center of C (*) corresponds to an area of extracellular staining for TF in D (*). Note that most of the TF appears intracellular, and compared to macrophages, the intracellular staining of smooth muscle cells for TF is less intense. (E) A cholesterol crystal-rich area (*), typical of the core of an atherosclerotic plaque with diffuse staining for TF antigen in the extracellular debris surrounding the crystals. (F) An organizing thrombus containing both cellular and extracellular TF antigen. Reproduced from Marmur et al. [77], with permission.
platelet deposition and more intense TF staining are found on lipid-rich atheromatous core material [81].

In human coronary atherectomy specimens, concentrations of TF antigen and activity were found to be higher in plaques taken from patients with de novo angina, unstable angina or myocardial infarction as compared to stable patients or patients with re-stenosis [83–86]. In addition, thrombus was detected only in TF-positive plaques (see Fig. 3) [77] and fibrin deposition was mainly observed around the massive infiltration of TF-positive macrophages in patients with unstable angina [86]. These findings suggest a role of TF in the progression to unstable coronary syndromes.

5. Plasma levels of soluble tissue factor in acute coronary syndromes

Significantly higher levels of circulating soluble TF are found in patients with acute myocardial infarction and unstable angina compared to patients with stable angina and control subjects [87–90]. It is not certain, however, if soluble TF in blood reflects surface-associated TF or the potential for TF mediated activation of coagulation. A significant decrease of TF levels was seen after successful treatment of the acute coronary syndrome, except in patients with acute myocardial infarction with prodromal unstable angina whose TF levels remained high after 2 weeks. Moreover, patients with prodromal unstable angina before the acute myocardial infarction showed higher TF levels than in patients with sudden onset of the infarction [87,88]. This may reflect repeated episodes of coronary thrombosis. Plasma levels of free TFPI [89,91,92], as well as TFPI-activity [93] are increased in patients with an acute coronary syndrome, compared to control subjects or patients with stable angina.

These results express the hypercoagulable state of these patients, which is inhibited by TFPI. The source of circulating TF is unclear, but enhanced TF expression in coronary atherosclerotic plaques and circulating monocytes are likely candidates.

6. Tissue factor and (coronary) artery interventions

Balloon angioplasty results in plaque fracturing and dissection, and the extent of vascular injury appears to be related to the extent of thrombin generation [94–96]. Exposure of circulating clotting factors to procoagulants within the vessel wall, especially after formation of a neointima, accelerates thrombus formation [97–99]. This is of particular clinical importance as patients develop a neointima after angioplasty, and a significant proportion needs re-intervention. In one clinical study, percutaneous intervention in patients with stable or unstable coronary disease, resulted in elevated soluble TF levels measured in the coronary sinus after 4 h. This was accompanied by increased levels of thrombin–antithrombin complexes after 24 h [100]. However, this increase was not observed by the study of Marco et al., who did not find significant changes in plasma levels of TF and thrombin–antithrombin complexes after angioplasty in patients with stable and unstable angina [90].

TF induction in vascular smooth muscle cells may importantly contribute to thrombosis associated with arterial intervention. Intimal smooth muscle cell expression of TF plays an important role in the fibrin-rich thrombus formation and subsequent neointimal development following balloon injury to rabbit aortas [101]. TF overexpressing smooth muscle cells in a rat arterial neointima accelerated reendothelialization and increased smooth muscle cell migration via an autocrine manner in vivo and in vitro, of which the in vitro response occurred by coagulation-dependent and -independent pathways. This contributed to enhanced intimal thickening [102]. Arterial balloon injury in normal rabbit and rat aortas resulted in a rapid increase (after 1 or 2 h) in TF-mRNA both in the media and adventitia, associated with induced TF activity in the media [103–105] that persisted for 24 h after injury [104,106]. Thrombin generation was attenuated in injured vessels preincubated with an antibody to rabbit TF [106]. Moreover, TF expression also persisted during the subsequent weeks in smooth muscle cells, which proliferated to form the neointima [105]. Balloon angioplasty in porcine coronary arteries resulted in a bi-phasic increase of TF antigen and activity in the vascular smooth muscle cells of the media, first peaking between 1 and 24 h with a second increase at 4 weeks after angioplasty [107]. In a study by Gertz et al., neointimal TF expression, induced by balloon angioplasty in rabbit femoral and porcine coronary arteries, persisted 1 month after intervention [108]. However, Giesen et al. recently found prominent medial TF staining only 24 h after first balloon injury in normal rat aortas [99]. In addition, despite abundant TF in the neointima before and after a second balloon inflation 2 weeks following the first injury, this was not accompanied with an increased luminal TF activity and only small platelet-fibrin microthrombi were present after the double injury. Possible explanations for this phenomenon are that active TF is rapidly complexed with inhibitors such as TFPI, that active TF does not appear to be anchored to the arterial wall and can be readily washed away, or due to induction of fibrinolytic activity [99].

Comparable to PDGF, TF in complex with factor VIIa, induces chemotactic migration of cultured rabbit smooth muscle cells [109,110].

In summary, these studies suggest that TF not only influences the acute thrombotic response, but also may contribute to atherogenesis, coronary restenosis or prolonged thrombin generation after interventions.
7. Therapeutic approaches

Two groups of agents inhibiting TF or TF/factor VIIa complex have been developed and tested. The first group contains specific inhibitors of TF whether or not in complex with factor VIIa. The second group includes a variety of drugs with non-specific TF inhibiting properties.

7.1. Specific inhibitors of tissue factor and tissue factor/factor VIIa complex

7.1.1. Active site inactivated recombinant factor VIIa

Administration of active site inactivated recombinant factor VIIa (factor VIIai) results in a highly stable TF/factor VIIai complex that is unable to initiate coagulation. However, even at high concentrations, this protein does not replace TF bound to factor VIIa [16]. It competes with factor VIIa for TF binding and thus a certain amount of active TF/factor VIIa complexes may be formed during administration of factor VIIai [16]. In animal models, intravenous, topical and intracarotid administration of factor VIIai exerted a pronounced antithrombotic effect, which was not accompanied by bleeding or a prolonged bleeding time [111–113]. In addition, a 3-day infusion of DEGR-VIIa (a drug with comparable mechanism and function as factor VIIai) following balloon angioplasty significantly reduced neointimal formation in rabbit atherosclerotic femoral arteries [114]. Administration of factor VIIai during sequential balloon injury to the rabbit abdominal aorta, reduced both fibrin deposition and loss of lumen. The extrinsic coagulation cascade may therefore contribute to the blood vessel remodeling response, following balloon injury, that is independent of neointimal growth but leads to extensive loss of lumen [115]. However, in combination with a thrombolytic agent, factor VIIai did not improve the results in a dog model of thrombolysis [116].

7.1.2. Monoclonal antibodies

Monoclonal antibodies to TF, markedly reduce plaque thrombogenicity in human arterial segments [117]. In rabbits, these antibodies prevented thrombus formation in carotid arteries [118], reduced thrombotic occlusions in everted femoral arteries [119], and significantly shortened lysis time and decreased reoclusion rates following tissue-plasminogen activator administration in a model of carotid thrombosis [120]. Recently, it was suggested that these antibodies reduce myocardial ischemia/reperfusion injury by reducing TF-initiated intravascular thrombosis [121] and by inhibiting thrombin-mediated inflammation resulting in decreased chemokine expression and leukocytes recruitment [122].

7.1.3. Soluble recombinant human tissue factor

Kelley et al. used a soluble form of human TF, the 219-residue extracellular domain, and converted it into an antagonist of membrane bound TF by alanine substitution of lysine residues 165 and 166 [123]. This molecule, termed hTFAA, binds factor VIIa and greatly reduces the catalytic capacity of activated factor X compared to the complex TF/factor VIIa. When administered to rabbits undergoing balloon carotid arterial injury, hTFAA showed potent antithrombotic effects while causing smaller increases in bleeding tendency relative to heparin doses of equal antithrombotic potential [123].

7.1.4. Recombinant tissue factor pathway inhibitor

Of all agents inhibiting the extrinsic coagulation pathway, recombinant TFPI (rTFPI) is the most extensively investigated drug. On human atherosclerotic arterial segments, it reduced significantly both platelet and fibrinogen deposition [117]. When administered with recombinant-plasminogen activator in a dogs model of electrically induced arterial thrombosis, rTFPI attenuated or even prevented reocclusion and markedly inhibited cyclic flow variations [124,125]. However, in a different study by Lefkovits et al., rTFPI or DEGR-VIIa compared to tick anticoagulant peptide, administered after thrombolytic treatment with recombinant-plasminogen activator, did not prevent acute reocclusion in a similar model [116]. Differences in methodology and thrombus preparation may be responsible for these discrepancies. Therefore, the value of inhibiting the extrinsic TF-dependent pathway as adjunctive treatment to a thrombolytic agent, remains to be established. Most experimental studies using rTFPI, investigated the inhibitory properties of this agent on thrombosis and restenosis in arteries undergoing balloon angioplasty or intimeectomy. rTFPI markedly inhibited fibrin formation and accumulation of thrombus at the site of injury [89,126]. The same findings were reported in a recent study on local gene transfer by a vector encoding human TFPI at the site of balloon injury in porcine carotid arteries [127]. This even prevented platelet-dependent thrombosis under increased shear stress without detectable hemorrhagic risk. However, it takes at least a few hours before a transferred gene becomes expressed as a protein and even longer before the protein reaches a concentration that is sufficient for a biological effect. rTFPI gene transfer in combination with rTFPI irrigation in the initial 24 h after angioplasty may overcome this shortcoming, and has recently been investigated [128]. Whereas each method separately led to a significant reduction, the combination of these two methods achieved an additional reduction of neointima formation after balloon angioplasty in rabbit aortas. Other studies administered rTFPI via (local) bolus injection or continued infusion varying from 1 to 7 days after angioplasty or intimeectomy in different animal models, and histological examination was performed 3–4 weeks after arterial intervention [89,114,129–131]. All experiments showed a significant reduction in neointimal formation or intimal hyperplasia in rTFPI-treated animals compared to controls. As rTFPI reduces fibrin and throm-
bus formation, different mechanisms may contribute to the inhibition of intimal hyperplasia and restenosis. Fibrin and fibrin fragments are chemotactic for smooth muscle cells in vitro [132]. Thrombin has been shown to activate platelets producing PDGF, and both thrombin and PDGF stimulate smooth muscle cell proliferation [96,133]. In addition, the migration of cultured rabbit aortic smooth muscle cells, induced by the complex TF/factor VIIa, was markedly inhibited by rTFPI [110]. It has also been reported that rTFPI alone prevented the proliferation of cultured human neonatal aortic smooth muscle cells [134]. Therefore, rTFPI seems to reduce neointimal development through both a direct and indirect action on smooth muscle cells.

7.1.5. Nematode anticoagulant protein c2

A peptide with a mode of action comparable to TFPI is nematode anticoagulant protein c2 (NAPc2), isolated from the saliva of the hookworm Ancylostoma caninum [135]. In contrast to TFPI, NAPc2 binds to factor X at a site distinct from its catalytic center, therefore obviating the need for formation factor Xa prior to forming the resultant binary complex which inhibits the TF/factor VIIa complex. A recombinant form of NAPc2 has been tested in a pig and rat model of acute coronary and carotid thrombosis, and was significantly more potent than low molecular weight heparin [136]. To prevent deep venous thrombosis, recombinant NAPc2 showed promising results in patients undergoing knee arthroplasty [137]. At a dose of 3.0 μg/kg bodyweight, administered within 1 h after surgery, the rate of deep venous thrombosis in this group of patients was 12.2%, which is half the rate achieved with the current standard treatment with low molecular weight heparin, with no excess in major bleedings. This drug is currently investigated in different clinical trials on preventing arterial thrombosis, including in patients undergoing elective coronary angioplasty.

7.2. Non-specific inhibition of tissue factor and tissue factor/factor VIIa complex

7.2.1. Unfractionated and low molecular weight heparin

Both unfractionated and low molecular weight heparin increase plasma TFPI antigen and activity, and thereby provide an additional anticoagulant mechanism [18,138,139]. It appears that heparin releases TFPI from vascular endothelium [18,138,140] by displacing the inhibitor from endothelial cell surface glycosaminoglycans with subsequent release into the circulation and formation of heparin–TFPI complexes [141,142]. In addition, heparin inhibits monocyte TF expression after binding to activated monocytes [143]. However, heparin releasable TFPI antigen and activity are depleted during repeated or continued intravenous heparin administration [144] and during percutaneous transluminal coronary angioplasty [145]. A rapid decrease in TFPI concentration impairing physiological vascular thromboresistance may contribute to a sudden burst of thrombin generation as a rebound phenomenon after abrupt cessation of unfractionated heparin administration [146].

7.2.2. Lipid lowering treatment

Dietary lipid lowering reduces TF expression by cells (predominantly macrophages) present in rabbit atheroma [147]. Data regarding the effects of n−3 fatty acids on TF activity are conflicting. Some studies found that dietary enrichment with fish oil or administration of n−3 fatty acids inhibit stimulated and non-stimulated monocytes to express TF activity in healthy subjects [148,149], patients with hypertriglyceridemia [149] and nonhuman primates [150]. Others reported no effect of n−3 fatty acids on resting or endotoxin-stimulated mononuclear cells in healthy volunteers [151], and that an increased dietary intake even resulted in a tendency towards a stimulation of monocyte procoagulant activity [152].

Treatment with HMG-CoA reductase inhibitors (statins) reduced monocyte TF expression in hypercholesterolaemic patients [153], and attenuated TF antigen, TF activity and TF-mRNA levels in cultured stimulated and unstimulated human macrophages, differentiated from monocytes [154]. The latter results indicate that statins interfere with the TF biosynthesis. Nordøy et al. showed in patients with combined hyperlipidemia a significant reduction of plasma low density lipoprotein levels induced by simvastatin. This was paralleled by a reduction of TFPI activity, which was possibly due to a decrease in low density lipoprotein–TFPI complexes [155]. The free form of plasma TFPI was unchanged by statins alone but decreased modestly by adding n−3 fatty acids to statin treatment. Berrettini et al. also showed a significant correlation between TFPI activity and low density lipoproteins [156]. However, dietary supplementation of n−3 polyunsaturated fatty acids in patients with chronic atherosclerotic disease increased both TFPI plasma levels and low density lipoprotein-cholesterol, reduced plasma levels of prothrombin fragment 1+2, while the plasma factor VII clotting activity did not change. Clearly, statins and n−3 fatty acids have complex effects on the clotting cascade and more research is needed.

7.2.3. Other non-specific tissue factor inhibiting agents

L-Arginine, the endogenous precursor of nitric oxide, significantly blunted TF expression in stimulated monocytes after angioplasty of the iliac arteries in a rabbit model of atherosclerosis [157].

Cyclosporin A inhibited procoagulant activity in stimulated and unstimulated cultured human monocytes and macrophages [158], and in a dose-dependent manner reduced monocyte TF activity and TF-mRNA expression in cardiac transplant recipients [159].

Recently, it has been demonstrated that smoking increases TF immunoreactivity in atherosclerotic plaques and
treatment with aspirin attenuates this effect [160]. Dual antiplatelet therapy of ticlopidine and aspirin versus aspirin alone for at least 24 h before angioplasty, seems to inhibit release of TF into the coronary circulation in patients with stable and unstable coronary disease [90]. Specific thrombin inhibition by hirudin attenuates intimal TF expression induced by balloon angioplasty in animal models [108].

Finally, ACE inhibitors and angiotensin II receptor antagonists have shown to downregulate TF expression (including TF-mRNA) by endotoxin-stimulated human monocytes [161]. ACE inhibitors also decreased plasma levels of TF antigen and monocyte chemoattractant protein-1 in patients with acute myocardial infarction [162]. These latter results suggest that the antithrombotic mechanism of ACE inhibitors and angiotensin II receptor antagonists could be, at least in part, related to their ability to reduce TF expression and to inhibit the accumulation of monocytes and macrophages.

8. Conclusion

Thrombosis plays a key role in atherosclerotic diseases and causes mortality and morbidity in patients with acute coronary syndromes. It becomes clear that the enhanced expression of TF in patients with (coronary) atherosclerosis and during arterial interventions may play a significant pathogenic role. Factors that induce TF may initiate atherothrombotic complications. This hypothesis is confirmed by experimental studies on inhibiting (directly or indirectly) TF and thus preventing acute thrombosis and/or restenosis after intervention. Studies on new TF inhibiting agents have been performed largely in animals. Future research will investigate the impact of these drugs in patients with coronary or other atherosclerotic diseases, and in patients undergoing catheter-based interventions.

References


[23] Osterud B, Bajaj MS, Bajaj SP. Sites of tissue factor pathway inhibitor (TFPI) and tissue factor expression under physiologic and pathologic conditions. On behalf of the Subcommittee on Tissue Factor Pathway Inhibitor (TFPI) of the Scientific and Standardization Committee of the ISTH. Thromb Haemost 1995;73:873–875.

[27] Olesen LT, Wiestvik AB, Joo GB, Okkenhaug C, Kierulf P. Inhibition of IL-1 induced tissue factor (TF) synthesis and procoagulant activity (PCA) in purified human monocytes by IL-4, IL-10 and IL-13. Cytokine 1996;8:822–827.


[53] Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993;82:513–520.


[59] Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993;82:513–520.


Atsushi N, Nishida T, Marutsuka K et al. Combination of a brief irrigation with tissue factor pathway inhibitor (TFPI) and adenovirus-mediated local TFPI gene transfer additively reduces neointima formation in balloon-injured rabbit carotid arteries. Circulation 2001;103:570–575.


