

Review Article

Tissue factor pathway inhibitor: structure, biology and involvement in disease

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Abstract

Tissue factor (TF)-initiated coagulation plays a significant role in the pathophysiology of many diseases, including cancer and inflammation. Tissue factor pathway inhibitor (TFPI) is a plasma Kunitz-type serine protease inhibitor, which modulates initiations of coagulation induced by TF. In a factor (F) Xa-dependent feedback system, TFPI binds directly and inhibits the TF–FVII/FVIIa complex. Normally, TFPI exists in plasma both as a full-length molecule and as variably carboxy-terminal truncated forms. TFPI also circulates in complex with plasma lipoproteins. The levels and the dual inhibitor effect of TFPI on FXa and TF–FVII/FVIIa complex offers insight into the mechanisms of various pathological conditions triggered by TF. The use of selective pharmacological inhibitors has become an indispensable tool in experimental haemostasis and thrombosis research. *In vivo* administration of recombinant TFPI (rTFPI) in an experimental animal model prevents thrombosis (and re-thrombosis after thrombolysis), reduces mortality from *E. coli*-induced-septic shock, prevents fibrin deposition on subendothelial human matrix and protects against disseminated intravascular coagulation (DIC). Thus, TFPI may play an important role in modulating TF-induced thrombogenesis and it may also provide a unique therapeutic approach for prophylaxis and/or treatment of various diseases. In this review, we consider structural and biochemical aspects of the TFPI molecule and detail its inhibitory mechanisms and therapeutic implications in various disease conditions.

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Introduction

Normal coagulation activation proceeds through the tissue factor (TF)-dependent pathway, whereby TF forms a 1 : 1 stoichiometric complex with native factor (F) VII (FVII–TF). FVII binds to the lipid portion of TF in the presence of calcium, which acts as a bridge between TF and FVII. As a result, FVII is auto-cleaved within the complex to FVIIa. The TF–FVII/FVIIa complex activates FX and FIX. The action of this complex on its natural substrates (FX and FIX) is inactivated in a FXa-dependent fashion by a circulating endogenous coagulation inhibitor, known as tissue factor pathway inhibitor (TFPI); previously called anti-convertin, lipoprotein-associated coagulation inhibitor (LACI), extrinsic pathway inhibitor (EPA) or tissue factor inhibitor (TFI). In this way, and in a FXa-dependent manner, TFPI prevents further production of FXa and FIXa by the TF–FVII/FVIIa complex. Thus, additional FIXa and FXa can only be generated through the lower part of the classical intrinsic pathway, which involves the activation of FX by the FVIIIa–FIXa complex, where FIXa results from the initial action of TF–FVII/FVIIa and its generation by FXIa (Figure 1).

The FXa-dependent inhibition of the TF–FVII complex by TFPI is not absolute, as TFPI also inhibits the activation of FIX by TF–FVII in the absence of FXa [1]. However, this inhibition is believed to be achieved in two steps. First, TFPI binds to and inactivates FXa in a 1 : 1 stoichiometric complex [2,3]. Second, in a calcium-independent mode, the TFPI–FXa complex binds to and inhibits the TF–FVII complex [2,3]. Thus, TFPI switches much of the coagulation process from the TF–FVII-dependent system to the lower part of the previously known intrinsic pathway of blood coagulation (Figure 1). TFPI recognizes FXa most effectively when the enzyme is in the prothrombinase complex, which contains FXa, FV, calcium ions and phospholipids [4]. The optimal inhibition of TF–FVIIa activity is obtained by the full-length TFPI molecule rather than its C-terminus truncated form, hence the higher rate formation between the full-length TFPI molecule and FXa (TFPI–FXa complex) [5]. The C-terminus is also required for binding other cell-surface receptors, including the low-density lipoprotein receptor-related proteins [6]. Animal experiments have shown that TFPI-related proteins all have the ability to inhibit TF-mediated coagulation activation

Coagulation Initiation

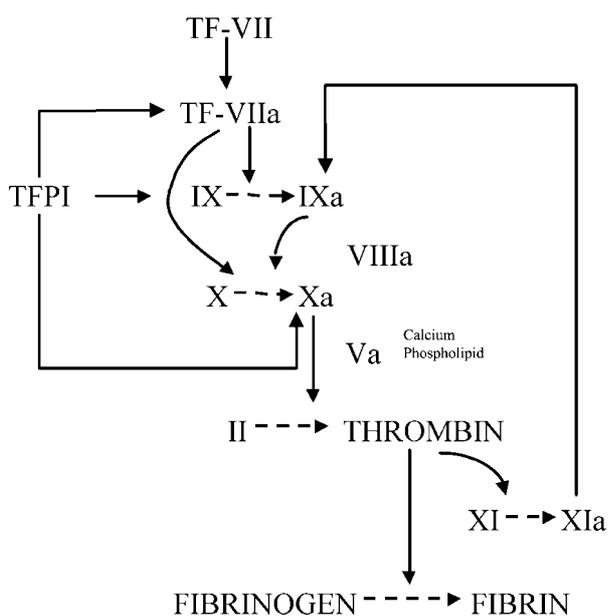


Figure 1. A scheme of the current concept of the coagulation process. *In vivo*, coagulation is initiated when tissue factor (TF) binds to factor (F) VII or co-factor FVIIa at a site of blood vessel injury or on activated monocytes/endothelial cells. The TF–FVIIa complex activates FX and FIX. Thrombin is formed from prothrombin (FII) through the action of FXa, FVa, calcium and phospholipid (prothrombinase complex). Tissue factor pathway inhibitor (TFPI) inactivates TF–FVIIa complex in concert with FXa. Thrombin activates FXI which, in turn, converts FIX to FIXa. The generation of FXa is then amplified through the action of the FVIIIa–FIXa complex

[7] and that TFPI–Xa complex is a much more potent inhibitor of the TF–FVIIa complex than TFPI alone [8].

We have previously reviewed the nature and the clinical importance of TF, urinary TF (uTF) and monocyte TF (mTF) levels in disease [9–11]. In this review, we aim to focus on the natural inhibitor of the TF-mediated pathway of the coagulation process (tissue factor pathway inhibitor, TFPI). We will concentrate on the structure and biology of TFPI, its role in thrombosis and thrombogenesis and its therapeutic potential.

TFPI — a historical perspective

TFPI is an endogenous anticoagulant protein, a serine protease inhibitor and is the only known regulator of the TF-dependent pathway of blood coagulation. It is known to play an important role in the control of thrombogenesis at both cellular and plasmatic sites. Experiments performed early in the second half of the last century demonstrated the presence of an endogenous inhibitor of TF-induced coagulation activation [12–14]. Subsequently, Hjort (1957) showed that the inhibitory activity was directed against ‘convertin’, which is a TF–FVII–Ca⁺⁺ complex [15]. In 1984, it was shown that FXa exerted a negative feedback

on its own formation and on the activation of FIX by TF–FVII complex in human plasma [16]. Further evidence for the requirement for FXa was provided [17] and this was subsequently confirmed [18–19]. The name used for the inhibitor varied until a consensus meeting of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis in 1991 agreed on the name ‘tissue factor pathway inhibitor’ (TFPI).

TFPI levels in the normal population

The half-life of TFPI is rather short (60–120 min) [20,21]. The total normal human plasma TFPI concentration is about 1.0–2.5 nM [1,22]. The average activity in normal human plasma is by definition 1 U/ml. The mean (\pm SD) functional concentration is 55 ± 8.2 ng/ml and by antigenic assay is 60 ± 13 ng/ml [23]. This may represent only a small fraction of the total endogenous TFPI.

Structure and some physicochemical properties of TFPI

The *TFPI* gene is localized on chromosome 2 [24]. TFPI consists of three multivalent Kunitz-type domains with an acidic amino-terminal region and a basic carboxy-terminal end (Figure 2). The first domain (on the left) binds to FVIIa and the second (in the middle) binds to and inhibits FXa [3,19,25]. The function of the third domain (on the right) has yet to be determined, but it may be involved in the association of TFPI with lipoproteins [26]. The basic C-terminus of TFPI is required for the binding of TFPI to cell surfaces, and cell-bound TFPI mediates the internalization and degradation of FX and the down-regulation of surface TF–FVIIa activity [6,7]. TFPI is also needed for the inhibition of smooth muscle cell proliferation *in vitro* [7].

Normal human plasma TFPI exists in both full-length and variably carboxy-terminal truncated forms [27]. *In vivo*, approximately 80% of plasma TFPI circulates as a complex with plasma lipoproteins, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) [1,3]. The most predominant forms of plasma TFPI have a molecular weight of 34–41 kDa; less abundant forms of high molecular mass are also present [1]. These predominant forms appear to afford protection against thrombosis [28,29]. About 5–20% of plasma TFPI circulates free, uncomplexed [22,30], containing both full-length and C-terminal truncated forms [31]. Lipoprotein-associated TFPI has less anticoagulant effect than the free TFPI [30]. Free TFPI is released from the vessel wall by heparin [32]. This may additionally contribute to the TFPI antithrombotic activity [33].

Tissue factor pathway inhibitor

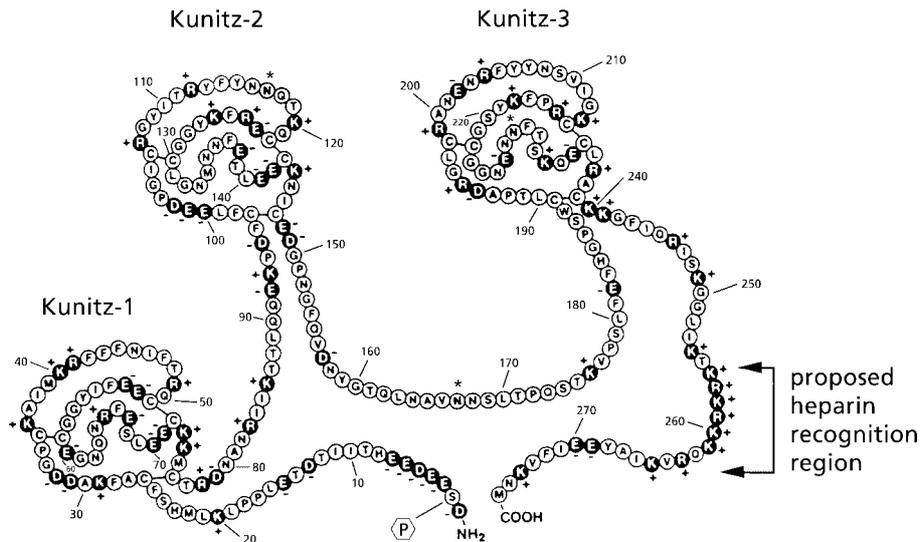


Figure 2. Model of tissue factor pathway inhibitor (TFPI), showing the Kunitz-type domains and the positively- and negatively-charged amino acid groups. The arrows point to the site of heparin binding at the C-terminal end of the TFPI [21]. Reproduced with permission from Brose GJ, Tissue factor pathway inhibitor and the current concept of blood coagulation. *Blood Coagul Fibrinolys* 1995;6:7–13

In addition to heparin, TFPI can also be displaced from the endothelium by non-heparin glycosaminoglycans [21,30,32]. Other anticoagulant/antithrombotic drugs that are known to release TFPI include pentosan polysulphate, hypersulphated heparin, low-molecular weight heparins, and tissue plasminogen activator. In most cases the kinetics of this effect are reportedly similar to that of heparin [32,34,35]. Generally, the TFPI anticoagulant effect and its ability to bind heparin depend on the presence of a positively charged carboxy tail [36]. After heparin injection, both free and endothelium-associated TFPI forms are in equilibrium *in vivo* [37]. Protamine, polybrene or platelet factor 4 administrations *in vivo* do not always neutralize the anticoagulant effect of heparin, which suggests that the remaining activity may be due to the release of TFPI by heparin. It therefore appears that TFPI is important for and contributes to the anticoagulant properties of heparin [2,32,38].

Sites for TFPI production

TFPI is produced constitutively by microvascular endothelial cells [39] and pooled in the endothelium (50–80%), plasma (10–50%) and platelets (less than 2.5%) [25,40]. It is also produced by the liver and monocytes/macrophages. Production of TFPI has also been demonstrated in cultured human umbilical vein endothelial cell (HUVECs), the endothelium of capillary cells, megakaryocytes, venules and lymphatic channels, the U937 monocyte cell line, glomerular cells and in many, but not all, neoplastic cell lines [18,30,41]. Pharmacokinetic studies showed that TFPI is cleared by the liver and the kidneys [27,42]. Recently, TFPI was quantified in human

semen [43]. Correlation with conventional fertility parameters suggest a role for TFPI in the seminal liquefaction process and hence an effect on global fertility [43]. The source of this form of TFPI is not yet clear.

Regulation of TFPI production

The last decade has brought remarkable progress in our understanding of the molecular basis and the role of the TFPI molecule in the haemostatic process. TFPI is mainly produced by and bound to the vascular endothelium, possibly by glycosaminoglycans [21,30,32]. A major portion of plasma TFPI is bound to LDL [44] and therefore may regulate its activity [45]. Separation of LDL-bound and -free TFPI from human plasma showed that the inhibitory activity for TF-induced coagulation was limited to the LDL-free fraction [46]. The close relationship between serum-free TFPI levels and serum thyroid hormone levels suggest that thyroid hormones might influence the synthesis or metabolism of TFPI on the surface of the endothelial cells [47]. Since plasma TFPI is largely truncated at the C-terminus and a poor inhibitor of blood coagulation [31,48], down-regulation of extravascular TF initiated coagulation by TFPI most likely requires the release of TFPI from activated platelets or the transfer of endothelial-associated TFPI into the extravascular space [49].

TFPI — possible role in cell signalling

Cell signalling and coagulation activation are interconnected through multiple pathways. Binding of TF to FVIIa induces intracellular signals in several cell types, including monocytes, endothelial cells,

HUVECs, human fibroblasts, the HaCaT keratinocyte cell line, the J82 bladder carcinoma cell line and the breast cancer cell line MDA-MB-231. The TF-cytoplasmic domain mediates signalling through the G-protein-coupled protease activated receptors (PARs) [50,51]. PAR-2 is involved in FVIIa and FXa signal transduction. PAR-2 but not PAR-1, has also been shown to lead to the phosphorylation of the TF-cytoplasmic domain in endothelial cells [52]. Epidermal growth factor receptor and proline-rich tyrosine kinase 2 participate in TF–FVIIa signalling, as formation of the TF–FVIIa complex increases the phosphorylation of these proteins. FVIIa protease activity and available TF are necessary for the generation of signal [53]. TF-induces PAR-1 and PAR-2 signalling is inhibited by recombinant TFPI-1 (rTFPI-1), suggesting that TFPI-1 regulates TF-mediated signalling through PARs [52]. In addition, TFPI-1, known to be anchored by glycosyl-phosphatidylinositol [54,55], also suppresses signalling in HUVECs, in which TF was up-regulated by inflammatory cytokines [52].

Comparison of TFPI across species

Plasma TFPI levels vary across species [56]. The homology of rabbit and rat TFPI to human TFPI is about 60% [57,58], whereas the homology of monkey TFPI to human TFPI is about 94% [59]. The homology of the Kunitz-type domains of TFPI among the three species is 57%, 86% and 69% in the first, second and third domains [57]. The differences between rabbit and rat TFPI and those of human or monkey may be due to differences in the amino acid sequence, post-translational modification of TFPI, or species differences in the properties of the lipoproteins [60]. The cDNA and predicted protein sequence for the full-length mouse TFPI show significant homology to that of rat [61]. Mouse TFPI expressed in and purified from human kidney cell line 293 inhibited human FVIIa, FIXa, FXa and FXIa [61]. Cloning and expression of the mouse *TFPI* gene may offer useful information and material for coagulation studies performed in a mouse model system. However, in studying the regulatory roles of TFPI in thrombosis and atherosclerosis, species differences should be taken into consideration, especially if rabbits and rats are to be used as the animal model [60]. Recently, a two-Kunitz (Ixolaris) and a five-Kunitz (Penthalaris) molecule with TFPI-like properties have been described in the tick saliva from *Ixodes scapularis* [62,63]. In contrast to TFPI, the five-Kunitz (Penthalaris) TFPI-like molecule binds FX and FXa tightly as scaffolds for inhibition of the TF–FVIIa complex [63].

Methods for TFPI determination

Several methods are available to assess TFPI levels at plasma, cellular and molecular levels, and in other

body fluids, with substantial clinical potential. These include:

1. Functional assays [15,64,65].
2. Clotting assays [66,67].
3. Anticoagulant activity assay; a novel approach for TFPI measurement [68].
4. Chromogenic substrate assays [69].
5. Immunochemical assays [70].
6. Immunostaining and immunomorphometric analysis [71–74].
7. *In situ* hybridization for TFPI mRNA and immunogold electron microscopy [74,75].
8. Gene expression analysis [73,76,77].

Variables that could potentially influence TFPI activity

A number of factors could modulate TFPI activity *in vivo*. These are considered briefly below.

Platelets

TFPI anticoagulated whole blood exhibits a remarkable decrease in platelet count [78] and TFPI promotes platelet aggregation [79]. TFPI may facilitate interaction between platelets and release of intracellular binding molecules from platelets through the GPIIb receptor [79]. Thrombospondin-1 released from platelet α -granules may also interact with TFPI at the surface within the extravascular matrix, where it could enhance TFPI inhibitory activity [49], which in turn down-regulates the procoagulant activity of TF. This interaction could occur *in vivo* and it is not known how this would affect TFPI measurements.

Leukocytes

Human leukocyte elastase (HLE) proteolytically cleaves TFPI [80]. It not only affects the ability of TFPI to inhibit TF–FVIIa but also significantly decreases the inhibitor function with respect to TFPI's inhibition of FXa [80]. Interestingly, both purified HLE and stimulated neutrophils regenerate TF activity from a preformed FXa–TFPI–FVIIa–TF inhibitory complex [80], suggesting putative effects of the neutrophil leukocyte stimulation on the regulation of the TF-mediated pathways of blood coagulation. Cleavage of TFPI by cathepsin G was observed, indicating that the formation of FXa–TFPI complex may reduce or modulate the proteolytic potential of stimulated leukocytes by temporary inhibition of cathepsin G [81].

Plasma lipoproteins

Among middle-aged healthy men, plasma free-TFPI levels correlated significantly with total cholesterol, LDL, triglycerides and apolipoprotein B [44]. In familial hypercholesterolaemia, TFPI activity was higher than in normal subjects and correlated with LDL

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cholesterol [82]. Patients with abetalipoproteinaemia and hypolipoproteinaemia, with no risk of thrombosis, showed very low levels of TFPI [70]. Elevated plasma free-TFPI levels in hypercholesterolaemia may represent a compensatory mechanism to prevent coagulation activation via the TF–FVII/FVIIa complex [83]. Cholesterol lowering therapy induces a marked drop in the total TFPI activity due to specific drop in LDL TFPI complexes [84]. In hypercholesterolaemic patients, total but not free-TFPI levels were significantly decreased after 6 months of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitory (fluvastatin) therapy [83]. Such data indicate that the anticoagulant activity of lipoprotein-associated TFPI is markedly lower than that of free TFPI [85] and that both activation and anti-activation of the coagulation system may occur in association with hypercholesterolaemia [83].

Drugs

Treatment with HMG-CoA reductase inhibitor was shown to induce a significant reduction of total TFPI antigen levels [83,86]. This reduction in TFPI levels may result from normalization of an up-regulated clotting system rather than an unfavourable reduction of a natural anticoagulant [86]. Eprosartan and losartan have been shown to reduce plasma TFPI levels [87], while infusion of tissue-type plasminogen activator for myocardial infarction increased plasma TFPI by about 45% [88,89]. However, neither warfarin or dermatan sulphate showed any significant effect [88,89]. Intravenous or subcutaneous injection of heparin increased plasma TFPI levels [85,89].

Diet

There was no significant difference between various diets with respect to fasting or non-fasting plasma TFPI concentration [90]. Although postprandial activation of FVII was accompanied by a moderate increase in plasma TFPI levels in normal subjects, no significant difference between three diets (olive oil, sunflower oil and rapeseed oil) with respect to postprandial plasma TFPI levels was observed [90]. In healthy subjects, neither high fat load nor hypertriglycerolaemic patients showed an effect on TFPI activity [91,92]. However, in a normal population, a significant difference was found between TFPI and total cholesterol levels [82]. A therapeutic lipid-lowering diet showed significant reduction in the total TFPI antigen concentration [86,93].

Age and gender

In a normal population, plasma TFPI levels increase with age and healthy newborns have about 40–50% of normal adult levels. A slight increase in plasma TFPI levels has been reported in older men [94], with mean levels lower in women [95]. Pregnant women in the

third trimester showed slightly increased plasma TFPI levels compared to non-pregnant women [89,91]. In the early stages of labour, a 29% increase in antigenic TFPI levels was observed compared to sex and age-matched controls [70]. Pre-eclamptic women did not show a significant difference in TFPI levels when compared to healthy pregnant women [89]. However, women on hormone replacement therapy showed a significant decrease in TFPI activity [96].

Individual variation and physical activity

There seems to be little variation in the plasma levels of healthy individuals, either during the day, after meals or from month to month [91]. This group has also reported a high variation (74–159% of pooled reference plasma) in a single sample taken from 21 healthy individual under 60 years [91]. However, the TFPI levels of a given individual did not vary on repeated sampling over weeks to months [91]. In healthy men TFPI levels increased slightly after exercise [97].

Cigarette smoking

Thrombosis is a primary cause of morbidity in cigarette smokers and increased TF expression in human atherosclerotic plaques (carotid and mice aortic root plaques) is associated with smoking [98]. In cell culture supernatants, a significant decrease in endothelial cell-derived TFPI was observed [99]. This suggests either a decrease in TFPI production or an increased translocation into the caveolae [71,100]. In addition, smokers showed a relatively increased TF : TFPII ratio and a significant negative correlation with serum cotinine levels, indicating a possible dose-related effect of smoking on TFPI [99].

The clinical value of TFPI

The dual inhibitor effect of TFPI on FXa and TF–FVIIa complex offers insights into the mechanisms of various pathological conditions triggered by TF. rTFPI has been used in clinical trials and several animal studies have found a beneficial effect of rTFPI as an antithrombotic agent.

Thrombosis and atherosclerosis

TFPI levels correlated with age-related cholesterol levels in the normal population. Positive associations were observed between TFPI and apo B levels [45,101]. This was highest in subjects with genotypes containing the E4 allele, in whom there was a high lipid risk profile associated with increased LDL cholesterol levels [101]. Subjects with combined hyperlipaemia may have increased thrombotic risk related to the activation of FVII during postprandial hyperlipaemia [101]. Thus, increased TFPI

levels in this condition may be related to activation of the TF-dependent pathway. Monocytes/macrophages in lipid-rich plaques may also play a determinant role after plaque disruption by promoting thrombin generation and thrombosis via TF [102]. As LDLs accumulate in atheroma, where thrombosis is likely to occur, the increase in LDL-associated TFPI may exert an antithrombotic effect in preventing local thrombosis. Lipid-rich plaques are more thrombogenic than less advanced atherosclerotic plaques [103].

TFPI levels were significantly raised in patients with ischaemic heart disease (IHD) compared to normal subjects and a positive correlation was observed between TF and TFPI levels in these patients [104]. As with other cell-mediated clotting activation processes [cancer or disseminated intravascular coagulation (DIC)], in IHD both TF and TFPI are elevated; monocytes and endothelial cells are thought to be the likely source [104]. Higher concentrations of plasma TFPI were observed in postmyocardial infarction patients compared to age-matched controls. This was associated with an elevated procoagulant state that might in fact exacerbate the disease process and increase the risk of subsequent acute ischaemic events [105]. Hyperactivation of the coagulation system in IHD patients may, in part, be compensated for by TFPI, which may not be sufficient to attenuate the elevation of circulating TF [104]. Variations in both TF and TFPI levels were observed in patients with unstable angina, effort angina and myocardial infarction and both factors were raised in patients with coronary lesions [104]. Specific inhibition of the TF activity by either rTFPI or a polyclonal antibody against human TF reduced plaque thrombogenicity and inhibited both platelet and fibrin deposition [103].

Venous thrombosis

Deficiency in TFPI is difficult to define, as circulating plasma-TFPI represents only a small fraction of the total TFPI pool *in vivo*. The major fraction is that which is released after heparin injection. In addition, homozygous TFPI deficiency could conceivably be a lethal condition. However, a threshold effect between low TFPI levels and increased risk of thrombosis has been suggested [106,107]. In particular, post-heparin TFPI levels were significantly decreased in patients with venous thrombosis and hereditary defects, compared to controls [108,109]. In almost all patients the defect was not temporary [108]. This was confirmed independently but the inheritance fault was not addressed [110]. In the study of Ariëns *et al* [108], six patients had at least one first-degree relative with low TFPI levels, suggesting that this defect could have been inherited. The TFPI levels measured in these patients were approximately half those found in controls, indicating that familial TFPI deficiency in the heterozygous state occurs in thrombotic patients [108].

Thrombosis may also result from resistance to coagulation inhibitors, and the genetic defects of the function of clotting factors are often associated with low levels of the circulating proteins. The most common inherited abnormality known to lead to venous thrombosis is activated protein C resistance [111,112]. In this context, it has been shown that 69% of patients with TFPI resistance presented with a family history of venous thrombosis, and it was postulated that TFPI resistance may correspond to a novel haemostatic genetic risk [113]. Indeed, few polymorphism sites in the *TFPI* gene have been described in the normal population or in patients with venous thrombosis. Interestingly, while the *TFPI-C536T* mutation is statistically associated with a high risk of venous thrombosis [114], both the *TFPI-V264M* and *TFPI-C399T* mutations were not [115,116]. Low levels of TFPI could also be a risk factor for thrombosis when combined with FV Leiden mutation (*FVQ506*) [117–119]. Resistance to TFPI has also been shown to occur in patients with venous thromboembolism who otherwise have no evidence of hereditary thrombophilia [120].

The use of exogenous female hormones is a well-recognized risk factor for venous thrombosis. This was also associated with a significant reduction in TFPI levels [107,121]. A study reported up to 12% (TFPI activity) and 30% (TFPI antigen) decrease which, interestingly, was independent of blood lipids [122]. However, it remains to be established whether such hormonal treatment and increased thrombotic risk is related to the observed low levels of TFPI.

In contrast to venous thrombosis, TFPI levels on the whole have been found to increase in atherothrombotic disease or DIC. The reason for this is not clear, but low plasma TFPI levels in some patients could be attributed to consumption, while higher levels may be caused by mobilization of TFPI from the vessel wall [123]. The severity of endothelial damage [124] and TFPI synthesis or clearance could also play a part. In addition, the reported increase in atherothrombotic conditions is primarily due to increase in the TFPI antigen levels. A compensatory increase in TFPI may possibly result from atheroma-induced TF production. TFPI levels decreased in the circulation during thrombus formation. Genetic abnormalities of TFPI, which may predispose to the development of venous thrombosis, could also affect various functions of the TFPI protein, such as altered inhibitory activity, secretion by endothelial cells, binding to the endothelial membrane, proteolysis in the vascular space or association with lipoproteins.

Stroke

TFPI levels were significantly decreased in stroke patients compared to controls matched for age and cholesterol levels [125]. This may not be related to the

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clinical subtypes of stroke, as TFPI activity was low in atherothrombotic infarction and lacunar infarction but not in cardioembolic infarction [125]. On the other hand, TFPI levels in subdural fluid and venous blood samples obtained from patients with chronic subdural haematoma and those with subdural effusion were similar to those obtained from normal subjects [126]. The amount of TFPI is elevated in frontal cortex samples obtained from Alzheimer's diseased brains, thus TFPI may play a cell specific role in proteinase cerebral regulation [127].

Diabetes

It is widely accepted that a procoagulant state exists in diabetic patients [128,129], and TFPI activity is influenced by such a state [130]. Patients with diabetic angiopathy showed an imbalance between the haemostatic and thrombosis-protecting systems [131], where TFPI may play a significant role as an inhibitor of the TF-dependent pathway. TFPI levels were higher in Type 1 diabetic patients compared to age and gender-matched controls and its levels correlated with fasting glycaemia and glycated haemoglobin (HbA_{1c}) [130,132]. TFPI levels were also high in Type 2 diabetic patients and those with impaired glucose tolerance (IGT) compared to normal or those subsequently found to be normal [130]. Increased TFPI levels in patients with Type 1 diabetes could also result from endothelial dysfunction commonly associated with diabetes, which seems directly dependent on hyperglycaemia-related endothelial aggression [133,134]. Induced hyperglycaemia-hyperinsulinaemia in normal subjects (characteristic of Type 2 diabetes) caused increased plasma TFPI levels [135], which were independent of hyperinsulinaemia, hypertriglyceridaemia and hyperosmolality [135] or hyperlipidaemia [132]. This increase in TFPI levels may constitute a potential for reducing thrombin generation and subsequent thrombus formation when TF mediates thrombogenicity of a disrupted atherosclerotic plaque [103].

Haemophilia

Evidence obtained from TFPI-immunodepleted rabbits suggests that TFPI protects against a prothrombotic phenotype and its role in haemophiliacs as a potential therapeutic modality is being investigated. However, no thrombotic disease due to a hereditary deficiency of TFPI has been reported [136]. Bleeding disorders in haemophilia patients can be treated with rFVIIa [137]. This suggests that enhancing the TF-dependent pathway may compensate for the lack of FVIII and FIX. Indeed, both clotting time and bleeding time in haemophilia plasma was normalized by blocking TFPI [67,138]. This suggests that TFPI is an important factor for the prolonged bleeding time in haemophiliacs and that blocking TFPI may be potentially haemostatic in haemophilia patients. TFPI is not

only found in plasma but also in platelets and endothelium. Thus, complete neutralization of *in vivo* TFPI levels would only be achieved by inhibiting both circulating (plasma) as well as non-circulating TFPI.

Surgery

Low levels of TFPI have been observed in patients undergoing hip surgery [89]. Plasma TFPI levels also fell slightly in other surgical procedures that caused fibrinogen levels to rise, suggesting that TFPI is not an acute phase reactant [91]. On the other hand, in patients undergoing vascular surgery, plasma TFPI levels increased after intravenous and subcutaneous injection of heparin [89]. However, in the latter group, patients had high TFPI levels even before heparin injection [85]. Repeated injection of heparin does not exhaust the release of TFPI [30]. TFPI levels were also elevated following the administration of enoxaparine in patients undergoing general or orthopaedic surgery [139].

Cancer and inflammation

Activation of blood coagulation and fibrinolytic pathways in patients with cancer is a well-known phenomenon. TFPI levels increased significantly before or after heparin administrations in patients with cancer [30,85] and levels correlated with disease progression [30,140]. Patients with adenocarcinoma of the pancreas showed significant increased TFPI levels at diagnosis; these were also related to disease progression [30]. TFPI decreased to normal levels after surgical removal of the tumour [30] and correlated strongly with bilirubin levels. The TFPI levels were significantly decreased after the relief of cholestasis [30], suggesting that pre-operative cholestasis may have contributed to this increase. TFPI levels were also increased in other types of advanced cancer [88,141]. The mechanisms by which TFPI is increased in patients with cancer is not fully understood; however, increased synthesis by tumour cells or host cells is likely. Tumour-associated macrophages [39] and various cancer cells have been shown to express TFPI [18,142,143], whereas small lung cell carcinoma, renal cell carcinoma and malignant melanoma did not [39]. The absence of appropriately configured TF-FVII: Xa complex, required for TFPI-binding, may be the cause.

Activation of coagulation induces a pro-inflammatory response *in vitro*; similarly, coagulation factors can be initiated by inflammatory mediators. TFPI levels were high in patients with inflammatory bowel disease; this was coupled with endothelial cell lesions and sustained coagulation activation [144]. TFPI levels were also significantly high in patients with chronic inflammatory liver disease and in those with unexplained juvenile thrombosis [145,146]. However, TFPI concentrations decreased in patients with liver cirrhosis, suggesting that this may be a contributory factor for portal vein thrombosis in at least some cases [147].

In infectious states with a strong acute phase reaction, such as pneumonia, the TFPI levels were stable [148]. This further supports the notion that TFPI is not an acute phase reactant protein.

Glomerulonephritis

TFPI is found in the kidneys of normal rabbits and in a crescentic model of glomerulonephritis (GN) [41,149], where fibrin deposition might be a key mediator of injury [41], probably through TF-mediated coagulation activation [150]. TFPI protects renal function in experimental crescentic GN [30,149]. In fibrin-dependent crescentic GN, glomerular TFPI synthesis and expression was initially decreased and subsequently returned to normal values. TFPI was detected in cellular crescents and was more prominent in fibrous or fibrocellular crescents [149]. Thus, TFPI may reflect the chronicity of the crescentic lesion [149]. However, plasma TFPI levels were increased throughout the evolution of the disease. TFPI was strongly expressed in the latter stages of crescent formation and correlated inversely with the presence of fibrin-related antigen in human crescentic GN [149]. This late induction of TFPI may inhibit TF activity and favour reduced fibrin deposition in the chronic stages of crescent formation [149].

Potential therapeutic applications of TFPI

The use of selective pharmacological inhibitors has become an indispensable tool in experimental haemostasis and thrombosis research, in which rTFPI may be a beneficial therapeutic agent as a natural anticoagulant to attenuate pathological clotting activation. Two forms of rTFPI are available for therapeutic purposes — the full-length and the two-domain rTFPI. Each has a different pharmacokinetics and activity profile.

Humans

Local administration of TFPI prevents venous thrombosis [151] and may offer an excellent alternative to systemic heparin plus dextran and avoids the risks of systemic anticoagulation [152]. Traumatized arteries treated via luminal irrigation with TFPI at a dose of $\mu\text{l/ml}$ yielded 91% patency rates at 1 day and 73% at 7 days postoperatively, compared to 8% for normal saline vehicle (control) and 40% for heparin, at both evaluation times [153]. Thus, the use of TFPI as a 'topical' antithrombotic agent is effective for the prevention of thrombosis in microvascular anastomoses [153]. Brief inhibition of coagulation by rTFPI administration sustains the patency of arteries recanalized by pharmacological fibrinolysis, without markedly perturbing haemostatic mechanisms [154]. Intact functional TFPI can be tethered to the cell surface [155]. This suggests that genetic manipulation of, for example, endothelial cells leading to the stable expression

of TFPI may inhibit the development of coronary artery disease following cardiac allo-transplantation, and may inhibit thrombosis in the context of xenotransplantation [155]. rTFPI has been used in clinical trials in patients with sepsis and in those following microvascular surgery [156]. Infusion of TFPI caused a dose-dependent reduction of the procoagulant response to endotoxin in healthy humans *in vivo* [157]. TFPI may prevent haematogenous metastasis of colorectal carcinoma, especially at the pre-operative stage [158] and in acute conditions, such as the abrupt disseminated phase subsequent to surgical manipulation of tumours [159]. Expression of TFPI strongly inhibited the invasive ability of ovarian tumour cells *in vitro* but did not affect their migratory ability, suggesting an experimental basis for treating human ovarian tumours with gene therapy [76].

Experimental animal models

The importance of TFPI as a therapeutic agent has also been demonstrated in an animal model. Immunodepletion of TFPI lowers the threshold by which TF induces DIC. Infusion of rTFPI protects against thrombosis and DIC in numerous experimental models [40]. Endotoxin-induced activation of blood coagulation was completely prevented by TFPI [157]. In animal sepsis models, TFPI blocked the coagulant response and prevented death, with concurrent reduction of cytokine release [160–162]; thus, TFPI may attenuate the cytokine response by endothelial cells [157]. Administration of TFPI 4 h after *E. coli* challenge reduced mortality in a baboon model [136]. Infusion of two-domain TFPI analogue significantly counteracts endotoxin-induced coagulopathy in rabbits [163]. In a murine experimental model, intravenous injection of murine rTFPI immediately before the introduction of tumour cells reduced metastasis by 83%. B16 murine melanoma cells stably transfected with a *TFPI* expression vector exhibited an 81% reduction in lung seeding following intravenous injection. Mice receiving intravenous somatic gene transfer of a sense *TFPI* expression vector developed 78% fewer lung nodules than controls [164], suggesting that TFPI may have a significant antimetastatic effect [165]. Circulating and tumour cell-associated TFPI appear to play a role in haematogenous metastasis [159]. Within circulating blood, TFPI may have an anticoagulant and antimetastatic effect, while during extracellular matrix expression, a pro-invasion and/or pro-metastatic effect should also be considered [159].

In vivo inhibition of TFPI by anti-TFPI during the development of GN in rabbits significantly increased glomerular fibrin deposition (GFD) and exacerbated renal impairment [41]. Infusion of human rTFPI significantly reduced GFD development, proteinuria and renal impairment. Thus, TFPI is down-regulated in the early response to glomerular injury. Endogenous glomerular TFPI and treatment with rTFPI reduces GFD and injury in fibrin-dependent GN. Glomerular

expression of TFPI may be critical in determining the outcome of GFD in renal injury [41].

In conclusion, abnormal coagulation activation may contribute to the pathogenesis of many disease processes. TFPI is essential for maintaining normal haemostatic balance by inhibiting the TF-dependent pathway of blood coagulation. Full-length rTFPI has been found to be an effective antithrombotic agent in many clinical and animal studies. It has undergone extensive preclinical, phase II and III testing and may yet have a role in routine clinical practice.

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