Tissue factor pathway inhibitor: from unknown coagulation inhibitor to major antithrombotic principle

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1. Introduction

Acute thrombosis is the major immediate cause of cardiovascular death. The thrombotic process is also a stimulus for developing and continuing the atherosclerotic process. To control thrombus formation has been the aim of scientists during several decades. Exposure of tissue factor (TF) to blood, for instance on rupture of atherosclerotic plaques or on endothelial cell damage, may cause acute obstruction of a vessel due to thrombus formation. Thus, the physiological TF inhibitor, tissue factor pathway inhibitor (TFPI), is of interest both pathophysiologically and for its pharmacological potential in inhibiting TF-induced thrombus formation. Exposure of TF to circulating blood starts the coagulation cascade by binding to Factor VII(a) (FVIIa), and the complex of FVIIa and TF activates Factor X (FX) and Factor IX (FIX). TFPI is dependent on some degree of coagulation activation and formation of activated FX (FXa) in order to inhibit the FVIIa/TF complex. Physiologically, TFPI’s main role is inhibition of small amounts of tissue factor, and this inhibition is probably essential for maintaining a normal hemostatic balance. It is not known whether total lack of this inhibitor is compatible with life, since no such deficiency has been found. Serial measurements of increasing plasma TFPI during the course of a disease may signal poor prognosis. Heparin accelerates TFPI’s inhibitory effect and free TFPI has great heparin affinity. TFPI probably contributes significantly to the anticoagulant properties of the endothelium, and may be particularly important for the outcome of vascular injury. The discovery of the inhibitory principle of TFPI has opened a new arena for pharmacological intervention in the thrombotic process. This may well be a major breakthrough for the control of acute thrombosis in cardiovascular disease. Recombinant TFPI has proved effective in the treatment of experimental disseminated intravascular coagulation, sepsis and thrombosis. Whether TFPI or TFPI derivatives will be as effective in the treatment of patients remains to be determined.

2. The molecular basis of inhibition

TFPI has a double inhibitory effect; it inhibits FXa 1:1 by binding at or near its active site, and it inhibits TF by forming a quaternary inhibitory complex consisting of TFPI, TF, FVIIa and FXa (Fig. 1). The FXa binding is reversible and calcium-independent, while the binding of TF/F VIIa to TFPI/FXa is irreversible and calcium-dependent [1–3]. The clue to this mechanism of inhibition is that TFPI does not exert its TF-inhibiting effect unless the coagulation cascade has been activated. Once FXa has been formed, TFPI inhibits FXa, and through this binding becomes able to feedback-inactivate the TF-triggering of the coagulation through complexing with Factor VIIa/TF.

TFPI is a Kunitz-type serine protease inhibitor of 276 amino acids and approx. 42 kDa molecular weight [4]. The complete gene structure of TFPI has been established and localized to gene 2q [5]. TFPI has been purified from human endothelial cells and megakaryocytes, from culture supernatants of HepG2 cells and several other cancer cell lines. It has been recombinantly produced in mouse fibroblasts and baby hamster kidney cells transfected with mammalian expression plasmids as well as from yeast and E. coli. TFPI consists of three Kunitz-type structures, of which the first inhibits FXa, while the second inhibits the TF/FVIIa complex [6]. The C-terminal region of the TFPI molecule is involved in FXa inhibition, and TFPI molecules without the C-terminal have a reduced anti-Xa effect and reduced anticoagulant effect in vitro [7]. The role of Kunitz domain 3 was until recently less understood, but there are
now indications that regions within the third domain contribute to the lipoprotein binding of TFPI [8]. Domain 3, and even more so the carboxyl terminus of the full-length TFPI molecule, is necessary for optimal FXa inhibition, and also for TFPI’s binding to heparins [7]. The heparin-induced enhancement of TFPI’s FXa-inhibition has been shown to become gradually greater as more of the C-terminal portion is intact.

3. Measurements of TFPI in health and disease

Estimations based on the amount of heparin-releasable TFPI (see below) indicate that as much as 75–90% of total body TFPI is located in relation to the endothelial cells. The ratio between endothelium intracellular and extracellular TFPI is not known. The remaining 10–25% is found in circulating blood in two main fractions: most of it is lipoprotein-bound TFPI (95%); the smaller fraction (5%) is free TFPI [9]. Both of these fractions are measurable in TFPI assays. Functional assays, like the amidolytic assay [10], measures the total TFPI, while the ELISA assays can differentiate between full-length, free TFPI and lipoprotein-bound TFPI [11]. Coagulation assays (clotting assays) may best reflect the anticoagulant function of the different TFPIs, as this mimics the in vivo coagulation and takes the kinetics of the inhibition into account [12].

The mean plasma concentration of TFPI in a healthy adult population was 2.25 nM or 89 ng/ml (95% range: 53–142 ng/ml) measured by an immunological method [13]. The TFPI levels are lowest at birth, they increase slightly with age and there is no gender difference [10,13]. Most studies of clinical materials have been performed with functional end-point assays [14,15]. However, the number of reports of TFPI results using a commercially available ELISA assay is increasing [11]. A third, clotting-based, dilute prothrombin time activity assay has also been used [12,16,17]. Results from these three different kinds of TFPI assays are not directly comparable.

Increased levels of TFPI have been observed in a number of illnesses, including malignancy and septicemia, and more recently also in uremic patients, in poorly regulated diabetics and in patients with hyperlipidemia [14–19]. Increased plasma TFPI levels have been reported in malignant disease and inflammatory processes also in children [20]. The reason for the increased TFPI levels in these clinical situations is mostly unknown. In hyperlipidemia, it mainly reflects increased levels of its carrier, the lipoproteins. It has been shown that when LDL is lowered pharmacologically, the TFPI levels normalize [21]. In other clinical situations, the inflammatory process itself may be the signal for increased TFPI production. In vitro studies have earlier indicated that there might be a slight increase in TFPI produced by endothelial cells and monocytes by stimulation with endotoxin [22]. However, van der Logt and co-workers did not find increased mRNA or TFPI protein in isolated peripheral blood monocytes after stimulation [23]. The increased levels reported are only slightly increased compared to age-adjusted normal controls. One single measurement in a patient is difficult to interpret. However, serial TFPI plasma measurements in patients with sepsis and malignant disease have shown steadily increasing TFPI levels in patients with fatal outcome compared to survivors [14,15] and serial measurements may be clinically useful in predicting outcome for patients with fatal illnesses.

4. Anticoagulant activity versus antigenic measurements of TFPI

The full-length TFPI has more anticoagulant activity in the dilute prothrombin time assay than lipoprotein-bound or otherwise truncated TFPI [12]. Accordingly, a TFPI derivative expressed in yeast, containing only the first 161 amino acids, and thus lacking the third Kunitz domain, showed similar activities to full-length TFPI toward FXa/TF/FVIIa in chromogenic substrate assays with purified factors [16]. However, the anticoagulant activity in the dilute prothrombin time assay was about 50-fold lower, and comparable to the effect of antibodies to TF in the same assay [16]. It is known that TFPI from different sources—mammalian cell lines, normal plasma, post-heparin plasma (plasma drawn after an i.v. bolus of heparin) and plasma fractions—with similar inhibitory activity in an amidolytic assay has shown significantly different anticoagulant effects in clotting assays [17]. Hubbard and Weller found that the antigen methods measure up to 10 times increased levels in post-heparin plasma compared with only 2–3 times increase with a functional method [11]. The difference in TFPI levels measured by the antigen assay and the functional assay may be understood if
TFPI antigen assay is mainly directed against domain 3, which is exposed only where the TFPI molecule is not bound to lipoprotein. Another explanation that has been advocated is that the lipoprotein somehow ‘masks’ the TFPI from being detected by antigenic methods. In post-heparin plasma the fraction of TFPI not bound by lipoprotein increases the most [17].

5. TFPI and plasma lipoproteins

The formation of a TFPI–lipoprotein complex is dependent on the presence of TFPI’s third Kunitz domain [8]. Lesnick and co-workers found that the major plasma carriers of TFPI are small, dense subtypes of LDL, HDL and lipoprotein (a) (Lp(a)), whereas very-low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL) do not seem to transport TFPI [24]. TFPI in plasma is 50% associated with HDL, 40–45% associated with LDL, and about 5% is free TFPI [9]. In patients with hypercholesterolemia treated with the cholesterol-lowering drug, simvastatin, the reduction in TFPI after treatment correlated closely with the LDL cholesterol concentration [21]. In a recent study of young male survivors after myocardial infarction, the TFPI activity levels amidolytic assay were found to be increased in patients with hyperlipidemia (1.25 ± 0.23 vs. 1.17 ± 0.20 U/ml in controls), particularly in those with type IIb hyperlipoproteinemia [25]. The increase in TFPI was significantly correlated with the dense LDL apoB-levels (r = 0.46) and the small dense HDL subspecies HDL3b (r = 0.34). The role of increased TFPI plasma level in hyperlipemic patients is unclear. It may represent an increased resistance to TF-induced coagulation, but, more likely, it may just reflect the binding of TFPI to the increased amount of lipoprotein. Lipoprotein-bound TFPI is less anticoagulant than free TFPI [8,17], and the binding of TFPI to lipoprotein may impair the anticoagulant activity of TFPI in hyperlipidemic subjects. It remains to be investigated whether this plays a role in the pathogenesis of premature coronary disease.

6. The release of TFPI into the blood by heparin injection

When heparin is injected into the blood, a rise in plasma TFPI is observed after 5–10 min [26]. This release of TFPI seen on injection of unfractionated heparin and also on injection of low-molecular-weight heparin, contributes significantly to the anticoagulant effect of heparin in vitro and probably also in vivo [12,17]. Other glycosaminoglycans (e.g., dermatan sulphate and pentosan polysulphate) are also able to induce TFPI release, but to a lesser extent than heparin [14], probably due to lower binding affinity. The binding of TFPI to heparin is dependent on the molecular mass of heparin, with a maximum over 10 kDa [27]. On long-term continuous heparin infusion, the TFPI levels stay elevated [28]. The increase in TFPI seems to be dose-dependent and correlates quite well with the activated partial prothrombin time (APTT) (r = 0.50–0.76) and anti-Xa activity (r = 0.45) [27]. TFPI is most probably released from endothelial cells upon heparin infusion, although the binding sites for TFPI on the endothelial cells have not been identified. It has been suggested that TFPI is bound to endothelial cell glycosaminoglycans, especially heparan sulphate, owing to the known heparin binding of TFPI. However, a recent in vitro study found conflicting results with this hypothesis: the binding of TFPI to the endothelial cell surface was not influenced by pretreatment of the endothelial cells with glycosaminoglycan-degrading compounds [29]. On repeated heparin injections, repeated peaks in plasma TFPI are seen, indicating that TFPI is released into the blood by the presence of the heparin, and that most of the TFPI ‘slips back onto’ the endothelium as heparin is cleared from the circulation [14].

Cancer patients have an increased TFPI-releasing response to heparin injection compared with normals [30]. Kalbas and co-workers have shown that malignant HepG2 cells released more than 5 times the baseline value of TFPI, while human umbilical vein endothelial cells released only twice the baseline value. The release was independent of protein synthesis [31]. Some patients with thrombophilia without known other cause are ‘poor responders’ and release less TFPI on heparin infusion than others [28]. Also, patients with poorly regulated diabetes release more TFPI on heparin injection than well-regulated diabetics [19]. These studies indicate that in selected patient groups, a ‘heparin challenge’ test with the measurement of releasable TFPI upon heparin injection could give valuable additional information.

7. TFPI as protection against DIC in everyday life?

If the level of exposure of endotoxin or tissue factor exceeds a certain level, DIC and septic shock results. Smaller amounts of endotoxin or tissue factor will not result in DIC. However, if the normal level of TFPI is reduced, as was done by antibodies in rabbits in experimental DIC studies, the same low level of endotoxin or tissue factor resulted in fulminant DIC [32]. This indicates a physiological role for TFPI in handling the levels of endotoxin or tissue factor that we may be exposed to repeatedly during minor illnesses or everyday life.

8. Blocking TFPI as hemophilia treatment

Patients with hemophilia have longer dilute prothrombin times than normals, and the prolongation is normalized after treating the plasma sample with antibodies to TFPI.
9. Pharmacological potential of TFPI

Various forms of TFPI may show different pharmacological as well as anticoagulant properties. Bregengaard and co-workers found that in rabbits the distribution volume of a full-length TFPI was about 10 times larger than for a TFPI containing only the first two Kunitz domains [35]. The full-length TFPI disappeared from the circulation quickly as a result of binding to components in the vessel wall. Heparin interfered with that binding, and injection of heparin recovered the full-length TFPI, giving a release similar to vessel-wall-bound endogenous TFPI. Also, the site of interaction is probably located within the third Kunitz domain or in the C-terminus, since the two-domain TFPI was not recovered by the presence of heparin, but required lower doses to maintain the same plasma concentration [35].

Studies in rabbits and baboons have shown that increasing the plasma levels of TFPI by infusing recombinant TFPI in large doses protects against disseminated intravascular coagulation (DIC) and septic shock induced by tissue factor or E. coli [36,37]. In the baboon study, pretreatment with TFPI prevented the E. coli septic shock and improved survival significantly. Also, TFPI prevented the induced septic shock and death in the baboons even when administered several hours after the E. coli infusion. Thus, TFPI effectively prevented septic shock, and was also effective in the treatment of already ongoing sepsis [37].

10. TFPI as antithrombotic drug

Recombinant TFPI proved effective as an antithrombotic drug in an ex vivo model of thrombogenesis on TF-containing subendothelium in the presence of both FXa and FVIIa [38]. Infusion of various types of recombinant TFPI has been shown to prevent venous and arterial thrombosis in animal models [39–43]. In a rabbit ear model of microvascular anastomosis, local application of TFPI increased patency rates significantly above controls (patency at 7 days: controls 0%, TFPI treated 73%), and patency was higher with TFPI than with heparin (at 7 days: 73% vs. 40%) [40]. Also, TFPI has been shown to prevent arterial reocclusion after thrombolytic therapy in a dog model [39]. Promising results have recently been found in the baboon for the antithrombotic effect of a TFPI containing only the two first Kunitz domains [41]. TFPI prevented acute arterial thrombus formation after surgical endarterectomy performed on baboon aorta which was exposed to flowing arterial blood for 60 min in a shunt model. The thrombus was 90% reduced with TFPI compared to controls, as measured with indium-labelled platelet deposition and with iodine-labelled fibrinogen. Another TF-inhibiting compound, active site inhibited FVIIa, has shown similar antithrombotic effects also on thrombus formation on in vivo surgical carotid endarterectomies [43]. These studies underline the important triggering effect that TF in the subendothelium has on thrombus formation, and, extrapolating, may indicate great effects for prevention of thrombi formed on TF-rich atherosclerotic plaques upon rupture or in conjunction with interventional therapy. The long-term beneficial effect of TFPI treatment has been verified in a rabbit model of balloon angioplasty, where inhibition of tissue factor by either TFPI or an inactivated Factor FVIIa showed significant reduction of neointimal thickness after 21 days. The reduction in neointimal thickness was significant compared with controls (minimal luminal diameter for controls, 0.88 ± 0.21 mm; for TFPI-treated, 1.32 ± 0.21 mm), but also when compared with treatment with hirudin (0.97 ± 0.22 mm), which inhibits thrombin directly, or TAP, which inhibits Factor Xa (0.98 ± 0.14 mm) [44].

11. Future perspectives

Inhibition of tissue factor is one of several paths for pharmaceutical intervention of the thrombotic process. It seems particularly promising for stopping processes induced by tissue damage and exposure of TF, and there is evidence that inhibition of TF is more potent than inhibition further down the coagulation cascade both for acute thrombus and for neointimal hyperplasia. However, the size of the TFPI molecule indicates that exploitation of the TF-inhibiting principle is the main gain from these discoveries, and there is a continuous search for smaller and maybe even more effective compounds. Measuring plasma TFPI is not yet routine in most hemostasis laboratories. The best method for measuring the in vivo effect of TFPI is still being debated. An elevated plasma TFPI may indicate a poor prognosis in certain patient groups, especially if found to be increasing in serial measurements. Also, the heparin challenge, the TFPI release upon heparin injection, may be useful in the investigation of thrombophilia. The results of preclinical and clinical trials of TFPI in humans are expected to bring an important new principle of inhibition of thrombus formation into everyday medical practice. If TFPI or other TF-inhibiting compounds in addition are able to inhibit the formation of neointimal hyperplasia after interventional procedures like angioplasty, stenting or surgery, a major breakthrough in reducing cardiovascular mortality and morbidity will have been made.
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