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Targeting TFPI for hemophilia treatment

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Abstract

Hemophilia is a severe bleeding disorder treated by infusion of the missing blood coagulation protein, factor VIII or factor IX. The discovery and characterization of the anticoagulant protein tissue factor pathway inhibitor (TFPI) led to the realization that inhibition of TFPI activity could restore functional hemostasis through the extrinsic blood coagulation pathway in a manner that does not require the activity of factors VIII or IX. There are currently several therapeutic agents that inhibit TFPI in development for treatment of hemophilia. A comprehensive understanding of TFPI structure, biochemistry, and cellular expression is necessary to understand how it modulates bleeding in hemophilia and the physiological impact of therapeutic agents targeting TFPI.

Keywords

hemophilia; TFPI; blood coagulation; factor VIII; factor IX; prothrombinase

Hemophilia A and B are severe bleeding disorders caused by deficiency of the blood clotting proteins, factor VIII (FVIII) or factor IX (FIX), respectively, and occur in 1:5000 male births [1]. Total absence of either protein produces nearly identical bleeding symptoms characterized by spontaneous bleeding into joints and soft tissues. Hemophilia was first documented in the second century. It has had a significant impact on European history as Queen Victoria of England passed the disease to the royal families of Russia, Spain, and Germany. Alexi, the son of the Russian Tsar Nicholas II and Tsarina Alexandra, had hemophilia. The treatment of his disease is thought to have altered the course of Russian history surrounding the 1917 revolution. The FVIII and FIX genes are located on the X chromosome. The FVIII gene has 26 exons that are susceptible to spontaneous mutations. The FVIII gene also has six large introns. Of these, introns 1 and 22 frequently undergo intronic inversions that produce severe hemophilia [2]. The FIX gene is smaller and less susceptible to mutation than the FVIII gene. However, elegant forensic studies found that hemophilia in the royal families was caused by a splice acceptor mutation just upstream from Exon 4 in FIX [3].

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Tissue factor (TF) pathway inhibitor (TFPI) is a Kunitz-type serine protease inhibitor that exerts anticoagulant activity by blocking early procoagulant stimuli [4;5]. In doing so, TFPI modulates fibrin formation in human FVIII or FIX deficient plasma [6] and bleeding severity in animal models of hemophilia [7-11]. These studies have led to development of therapeutic agents that block TFPI for treatment of hemophilia. Knowledge of TFPI structure, biochemistry and cellular expression is necessary to understand how it modulates bleeding in patients with hemophilia and the physiological impact of therapeutic agents targeting TFPI.

TFPI Structure

TFPI is produced as two major isoforms in humans, TFPI α and TFPI β that result from alternative splicing within the C-terminal portion of the gene [12]. The two isoforms have identical N-terminal regions consisting of two Kunitz-type serine protease inhibitor domains (K1 and K2) separated by a short stretch of amino acids. These domains inhibit factor VIIa (FVIIa) and factor Xa (FXa), respectively. TFPI α is a soluble protein with a third Kunitz domain (K3), which binds to Protein S [13;14], and a basic C-terminal tail. Protein S localizes TFPI α to membrane surfaces, thereby enhancing inhibition of FXa by K2 [15]. Notably, TFPI β does not have the K3 domain, yet is an effective inhibitor of FXa in the absence of Protein S. This results from a C-terminal glycosylphosphatidyl inositol (GPI) anchor attachment sequence that directly binds TFPI β to the cell surface [16].

TFPI Biochemistry

Blood coagulation advances through a pathway of augmenting proteolytic reactions climaxing with the generation of thrombin, which activates platelets and facilitates production of fibrin networks. Blood clot formation initiates at the site of vascular injury, where exposed subendothelial TF interacts with factor VIIa (FVIIa). The TF-FVIIa complex activates FX and FIX. In the presence of calcium ions and a membrane surface, factor Va (FVa) combines with FXa to form prothrombinase, a protein complex that catalyzes conversion of prothrombin to thrombin. TFPI suppresses early stages of coagulation by inhibiting TF-VIIa [4;17] and early forms of prothrombinase [18].

Efficient inhibition of TF-VIIa requires inhibition of FXa [4]. Kinetic studies of these reactions have shown that the rate limiting step for inhibition of TF-FVIIa by K1 is the inhibition of FXa by K2 suggesting that the two Kunitz domains simultaneously inhibit TF-FVIIa and FXa immediately after FX is activated by TF-FVIIa [19]. It has more recently been recognized that early forms of prothrombinase are inhibited by TFPI α through interaction of its basic C-terminal tail with an acidic region within the B domain of forms of FVa generated early in the procoagulant response [18]. FV contains a heavy chain and light chain separated by a B domain. Interactions between acidic and basic regions within the B domain maintain FV in an inactive conformation [20]. Remarkably, a well-conserved amino acid sequence within the basic region of the FV B domain is nearly identical to a region within the TFPI α basic C terminal tail [5;18]. Before thrombin is generated in the clotting cascade, FXa removes a portion of the B domain containing the basic region producing a form of FVa retaining the acidic portion of the B domain [21]. Similar forms of FVa are

released from α -granules upon platelet activation [22]. TFPI α inhibits prothrombinase harboring these forms of FVa through high affinity binding of the TFPI α basic C terminal tail to the acidic region of FVa that allows for efficient interaction of K2 with the active site of FXa [18].

TFPI cellular expression

TFPI α and TFPI β exhibit differential expression in platelets and endothelial cells [23-25]. TFPI α is secreted from cultured human endothelial cells [26] and activated platelets [24;27]. It is found in human plasma and is a heparin-releasable protein with plasma concentrations increasing 2- to 4-fold immediately following heparin infusion [28;29], suggesting that TFPI α binds to cell surface glycosaminoglycans through its basic C-terminal tail. Murine TFPI α is not produced by endothelial cells and is not present in plasma, but it is released from activated platelets [25]. TFPI β is produced by cultured human endothelial cells [30] and murine endothelium [23]. TFPI β is not present in human or murine platelets [24;25].

Inhibitory activity of TFPI α and TFPI β

While TFPI α is uniquely capable of prothrombinase inhibition, studies comparing the TF-FVIIa inhibitory activities of TFPI α and TFPI β have shown that cell surface associated TFPI β is a slightly more potent inhibitor of TF-mediated generation of FXa processes than exogenous, soluble TFPI α [31]. The over-expression of either TFPI α or TFPI β impacts several TF-mediated cellular functions *in vivo*. Over-expression of TFPI α diminished tissue invasion of a mesothelioma cell line following intrapleural injection into nude mice with associated decreases in inflammation, and fibrin and collagen deposition associated with tumor growth in this model [32]. Over-expression of TFPI β diminished TF-dependent CHO cell infiltration into lungs following tail vein injection into SCID mice and blocked consumptive coagulopathy induced in this model [31]. Over-expression of TFPI α through a smooth muscle cell promoter blocks vascular remodeling and angiogenesis in mice [33]. These *in vivo* anticoagulant effects of TFPI are potentially modified by a wide range of enzymes including metallo [34;35], neutrophil [36], fibrinolytic [37], and blood clotting proteases [38;39] that cleave at sites within the connecting regions between the Kunitz domains or at their active site. For example, matrix metalloproteinase-8 (MMP-8), a zinc-dependent protease released by neutrophils and connective-tissue cells at sites of acute inflammation [40;41] and by endothelial cells within atherosclerotic lesions [42], cleaves TFPI within the linker region between the second and third Kunitz domains and within the N-terminal region. MMP-8 cleavage of TFPI decreases its ability to inhibit factor Xa in amidolytic assays and results in decreased anticoagulant activity of TFPI in factor Xa initiated clotting assays [35]. Factor XIa cleaves TFPI between K1 and K2 and at the active sites of K2 and K3. Similar to what is observed with MMP-8, incubation of plasma with FXIa decreased anticoagulant activity of TFPI in clotting assays [39].

TFPI and hemophilia

The identification of TFPI as an inhibitor of the extrinsic coagulation cascade led to early studies of how it modulates blood coagulation in hemophilia. These studies found that TFPI

modulates TF-FVIIa mediated generation of FXa in a manner that was dependent on the presence of FVIII and FIX [43], and suggested that inhibition of TFPI would enhance hemostatic pathways initiated by TF-FVIIa. It was found that injection of a polyclonal anti-TFPI antibody shortened the bleeding time in a rabbit nail clip model [7]. These initial studies have been followed by studies in mouse [11], dog [8], rabbit [10] and monkey [9] hemophilia models all demonstrating that inhibition of TFPI mitigates hemophilia bleeding and have led to the development of pharmaceutical agents for treatment of human hemophilia.

Several different types of compounds directed against TFPI have been developed as potential therapeutic agents, including aptamers [9], monoclonal antibodies [10], fucoidans [8] and peptides [44] with an aptamer and a monoclonal antibody advancing to clinical trials. The aptamer, which bound to the first and third Kunitz regions of TFPI [45] was withdrawn from further development after producing bleeding during clinical trials. The bleeding was associated with large increases in the plasma TFPI concentration that were thought to occur from release of TFPI from endothelial cells and decreased clearance of TFPI induced by the aptamer [46]. An initial safety clinical trial for a monoclonal antibody directed against the second Kunitz domain of TFPI had no serious adverse events. Antibody infusion produced a dose-dependent increase in plasma D-dimers and prothrombin fragment 1+2 [47]. These findings are similar to those observed in mice lacking TFPI rescued from embryonic lethality by deficiency of protease activated receptor 4 (PAR4), which have elevated plasma thrombin-antithrombin complex [48]. Since PAR4 is the major platelet thrombin receptor on murine platelets, these data suggest that endothelial associated TFPI β , rather than platelet TFPI α , acts to dampen the intravascular procoagulant stimuli responsible for the elevation in the plasma coagulation markers observed when TFPI is inhibited or absent. Therefore, selective inhibitors of platelet TFPI α , which has been shown to be a primary physiological regulator of bleeding in hemophilia mice [11], may be an optimal therapeutic agent for treatment of hemophilia.

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