Anti-tissue factor pathway inhibitor (TFPI) therapy: a novel approach to the treatment of haemophilia

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Abstract

Novel approaches to the treatment of haemophilia are needed due to the limitations of the current standard of care, factor replacement therapy. Aspirations include lessening the treatment burden and effectively preventing joint damage. Treating haemophilia by restoring thrombin generation may be an effective approach. A promising target for restoring thrombin generation is tissue factor pathway inhibitor (TFPI), a multivalent Kunitz-type serine protease inhibitor that regulates tissue factor-induced coagulation via factor Xa-dependent feedback inhibition of the tissue factor–factor VIIa complex. Inhibition of TFPI reverts the coagulation process to a more primitive state evolutionarily, whilst regulation by other natural inhibitors is preserved. An aptamer and three monoclonal antibodies directed against TFPI have been investigated in clinical trials. As well as improving thrombin generation in the range associated with mild haemophilia, anti-TFPI therapies have the advantage of subcutaneous administration. However, the therapeutic window needs to be defined along with the potential for complications due to the novel mechanism of action. This review provides an overview of TFPI, its role in normal coagulation, the rationale for TFPI inhibition, and a summary of anti-TFPI therapies, previously or currently in development.

Keywords Tissue factor pathway inhibitor · Haemophilia · Monoclonal antibodies · Coagulation

Introduction

Haemophilia and the current approach to its treatment

Haemophilia A and B are inherited bleeding disorders. Haemophilia A is characterised by the deficiency or absence of factor VIII (FVIII) and is the most common form, with an incidence of 1 in 5000 live male births. Haemophilia B, which is a result of missing or deficient factor IX (FIX) has an incidence of 1 in 30,000 births [1]. Haemophilia patients are classified into mild, moderate, and severe groups based on plasma levels of the deficient factor as recommended by The FVIII and FIX Subcommittee of the International Society of Thrombosis and Haemostasis [2]. In the majority of patients, the severity predicts the bleeding phenotype.

Recurrent, spontaneous, and trauma-related bleeding are characteristics of severe haemophilia, where factor levels are < 1% [2]. The typical sites of bleeding are joints and muscles and, in the minimally treated state, severe patients present with up to 30–40 bleeds a year resulting in irreversible joint damage and secondary disability [3]. If left untreated, internal bleeding into organs or from mucosal surfaces are the most frequent causes of mortality [4]. Patients with moderately severe disease have factor levels of 1–5% and have far fewer spontaneous bleeds than severe patients. Patients with mild haemophilia, classified by factor levels of > 5–40%, mostly present with bleeding in the context of trauma and surgery [5].

For the last 50 years, factor replacement therapy has been the foundation of haemophilia treatment, which consists of both bleed management and prophylaxis for bleed prevention [1, 6]. The aim of prophylaxis is to convert haemophilia patients from a severe to a moderate bleeding phenotype by administering frequent infusions of FVIII or FIX, often between two and four times a week. This raises the baseline factor level from < 1 to $\geq 1\%$ and reduces the number of spontaneous bleeds [7]. The benefits of prophylaxis have

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been confirmed in a randomised controlled trial [8] and it is the current standard of care [5].

Current haemophilia care: challenges and limitations

Prophylaxis with factor replacement therapy results in fluctuating factor levels and coagulation potential. In most cases, however, achieving the goal of a trough level of $\geq 1\%$ seems to be effective in decreasing the number of spontaneous bleeds to single figures, although that may be considered a modest goal [9]. In addition to the magnitude of trough levels, the number of hours per week spent at $< 1\%$ has an impact on the annualised bleed rate [10] and is influenced by the frequency of infusion, dose, and the number of missed or delayed doses [11]. Dose and infusion frequency are, in turn, influenced by the half-life of the factors, cost effectiveness of the regimen, and patient acceptability [12, 13]. Furthermore, a trough level of 1% is effective in preventing most spontaneous bleeds but does not prevent traumatic bleeds, and thus, individual patients require treatment administration to be modified to their personal circumstances [12, 14]. Personalising treatment to age, circumstances, disease phenotype, and complications, whilst having the potential to derive considerable benefit, can be resource intensive for both patients and physicians [12, 13].

Other important challenges exist in paediatric care. Initiating primary prophylaxis at 1–2 years of age, before or after their first joint bleed but prior to a second joint bleed [15], rather than at birth exposes haemophilia patients to risk of potentially fatal intracranial bleeding. Adequacy of venous access for regular infusions is also a challenge in paediatric patients [16].

To address the challenge of treatment burden, which has a huge impact on patient acceptability and adherence [17], extended half-life factors have been introduced into clinical practice [18]. Whilst these have been successful in decreasing the treatment burden significantly in patients with haemophilia B, the impact in haemophilia A has been more modest [19]. The latter is related to the limited prolongation of FVIII half-life with individual patients demonstrating a 1.2–1.8-fold extension in half-life. Furthermore, FVIII half-life is heavily influenced by the half-life of von Willebrand factor, the carrier protein for FVIII [20].

Development of inhibitory antibodies that render treatment ineffective [21] is another major challenge in the current treatment. Unlike other severe haemophilia patients, patients with inhibitors have been the recipients of suboptimal prophylaxis until 2017, when emicizumab was approved for prophylaxis in this group of patients [22]. Traditionally bypassing agents, i.e., recombinant activated factor VIIa (FVIIa) or activated prothrombin complex concentrate (aPCC), were used for the management of bleeds [21, 23].

These bypassing agents restore thrombin generation to achieve haemostatic efficacy via pathways that, in normal haemostasis, may contribute only a limited amount of thrombin [24–26]. In addition to on-demand therapy, they have also been used for prophylaxis, but provide less effective bleed control when compared to prophylaxis in non-inhibitor patients [27–30].

In this context, extensive research has been conducted investigating other therapeutic interventions for the management of haemophilia with and without inhibitors.

New approaches to haemophilia treatment

To overcome some of the limitations of factor replacement therapy, a new therapeutic approach being explored is the restoration of thrombin generation. Furthermore, there is an aspiration to achieve therapeutic goals that are more effective in the prevention of joint damage and less burdensome on patients. Currently, two broad approaches are being investigated in clinical trials. First, for use in haemophilia A, a bi-specific antibody (emicizumab) that is able to bridge activated FIX (FIXa) and factor X (FX) resulting in the generation of activated FX (FXa), mimicking FVIII function, has been developed and is approved for use in haemophilia A patients with inhibitors [31]. Second, downregulation of natural inhibitors resulting in improved thrombin generation is being investigated. This has been achieved through attenuation of antithrombin (AT) activity, the serine protease inhibitor responsible for inhibition of common, initiation, and amplification pathways [32], and through inhibition of the activity of tissue factor pathway inhibitor (TFPI) that inhibits the initiation pathway [33–36].

Tissue factor pathway inhibitor (TFPI)

TFPI is a glycoprotein and a multivalent Kunitz-type serine protease inhibitor. It is the principle inhibitor of the initiation pathway and inhibits tissue factor (TF)-induced coagulation [37] through FXa-dependent inhibition of the TF:FVIIa complex that initiates coagulation [37–39]. In addition, it also inhibits the common pathway in the early stages of thrombin generation when prothrombinase is constituted by FXa-activated factor V (FV) [40].

TFPI was cloned and characterised in 1988 and has multiple isoforms due to alternative messenger ribonucleic acid (mRNA) splicing events. The amino acid sequence results in a negatively charged amino acid terminus, three tandem Kunitz-type inhibitory domains (K1, K2 and K3), and a positively charged carboxy terminus [41]. The K1 and K2 domains bind and inhibit FVIIa and FXa respectively. The K3 domain has no known inhibitory function [42, 43].

TFPI α and TFPI β are the two major isoforms of TFPI [43]. TFPI α is a 276-residue glycoprotein with an acidic amino terminus followed by the three Kunitz domains and a basic carboxy terminus. TFPI β contains a glycosylphosphatidylinositol (GPI) anchor replacing the K3 domain in the carboxy terminus [42].

TFPI also inhibits a variety of serine proteases, such as trypsin, α -chymotrypsin, plasmin, and cathepsin G, demonstrating that this inhibitor has a relatively broad spectrum of inhibition [44]. TFPI is also known to be degraded by several proteinases, including thrombin, plasmin, FXa, matrix metalloproteinases, and neutrophil elastase [45].

The major sites of TFPI synthesis in humans are endothelial cells and megakaryocytes [46, 47]. Human endothelial cells secrete TFPI α , which is present in plasma and is the only form of TFPI in platelets [48, 49]. In contrast to TFPI α , TFPI β is predominantly expressed on the endothelial surface where it is maintained through a GPI anchor [50, 51]. Heparin infusion releases cell-surface-associated TFPI, with TFPI α being the main releasable isoform, and results in a two-to-fourfold increase in TFPI levels [42]. Although the precise mechanism is poorly understood, this heparin-releasable pool is potentially bound to cell-surface glycosaminoglycans via its basic carboxy-terminal region [47]. TFPI α is stored within quiescent platelets, but is not localised within the α -granule and is available for release after platelet activation where it can demonstrate its inhibitory activity [52]. Platelets accumulating at the site of vascular injury cause a steep increase in local TFPI concentrations by releasing TFPI, which is facilitated by dual activation of platelets with collagen and thrombin, potentially limiting the growth of the thrombus [51].

In plasma, 80% of TFPI is carboxy-terminal truncated and circulates bound primarily to low-density lipoproteins (LDL), with the levels of the latter having an impact on plasma TFPI levels [42]. The other 20% is unbound free-form TFPI characterised by the K3 domain. It comprises either full-length (FL)-TFPI α , half of the free-form TFPI that contributes the most to the anticoagulant activity, or various forms of carboxy-terminal truncated TFPI [42]. Platelet TFPI is exclusively FL-TFPI α , the amount is equal to circulating active FL-TFPI [51]. The circulating plasma TFPI pool is approximately 3% of the total vascular TFPI pool with the endothelial TFPI β constituting around 94% and heparin-releasable TFPI α constituting another 3–4% of the vascular pool [53].

In non-haemophiliacs, the mean plasma-circulating TFPI concentration has been estimated to be approximately 1.6–2.5 nM or ~70 ng/mL [42, 54]. A recent study evaluated the influence of age, gender, race, and oestrogen use on levels of total TFPI, TFPI α , FV, and protein S in both plasma and platelets in 485 healthy volunteers [55]. The study confirmed that the mean plasma concentrations of total

TFPI and TFPI α were approximately 60 ng/mL (62.2 ng/mL in males, 54.3 ng/mL in females) and 12.5 ng/mL (14.7 ng/mL in males, 11.3 ng/mL in females), respectively. TFPI α accounted for approximately 20% of total TFPI in plasma. Females had significantly lower levels of plasma total TFPI and TFPI α than males. An influence of age was not evident on plasma TFPI concentration in males, but females showed age-dependent increases achieving similar levels as males when > 70 years of age. Oestrogen use was associated with lower plasma total TFPI and TFPI α . There was a marked inter-individual variation in the TFPI concentration in plasma and platelets. This five-to-tenfold variation was also influenced by the ethnic origin of the participants with Caucasians showing higher levels of TFPI when compared to Asians and African Americans, and this was particularly evident in the platelet TFPI concentration. Platelet TFPI was not influenced by gender, age, or oestrogen use [55].

Normal coagulation, TFPI, and the rationale for TFPI inhibition

Blood coagulation is one of the host defence systems, and a major function includes maintenance of the integrity of the endothelium and vasculature [56, 57]. A breach in the integrity results in a haemostatic response that transiently covers the gap until tissue repair bridges the wound. This haemostatic response includes platelet activation and aggregation, thrombin generation, and clot formation, followed by dissolution of the clot with regeneration of the normal tissue [58, 59]. This response is tightly regulated by the natural anticoagulants, present in the circulation and extensively localised to the endothelium [60]. Essentially, a breach results in loss of the anticoagulant surface and presentation of TF and exposure of subendothelial collagen. The resulting haemostatic response is temporally and spatially localised. This is vital for restricting blood loss without interrupting blood flow through excessive clotting [61, 62]. Essentially impaired platelet activation, platelet aggregation, thrombin generation, and poor clot stability all result in a bleeding diathesis [63]. Restoration of the deficient or defective factor results in the amelioration of bleeding tendency [5, 21].

Three macromolecular complexes generated in the initiation and amplification pathways drive thrombin generation: extrinsic tenase, intrinsic tenase, and prothrombinase complexes. The initiation (extrinsic) pathway results in the formation of the extrinsic tenase or initiation complex following tissue injury. The extrinsic tenase complex includes TF released following tissue injury and circulating FVIIa, and generates small quantities of FXa and FIXa from their proenzymes. A small part of the FXa produced cleaves prothrombin, to generate thrombin which activates platelets and cofactors V and VIII. The

amplification (intrinsic) pathway becomes the source of additional FXa via the formation of an intrinsic tenase complex consisting of membrane bound FIXa and activated FVIII (FVIIIa). This is important, particularly with the rapid inhibition of extrinsic tenase complex by TFPI [37, 39]. The intrinsic tenase has evolutionarily arrived quite late [64, 65]. Prothrombinase complex with FXa bound to activated cellular surfaces in the presence of its co-factor FVa, converts prothrombin to thrombin (common pathway). Thrombin mediates platelet activation and fibrin deposition enabling blood clot formation [61, 62]. The prothrombinase complex is responsible for the explosive alpha-thrombin generation which is 10^5 -fold over the FXa rate alone [66].

The binding of the K2 domain of TFPI to FXa results in the formation of a binary complex and initiates the inhibition of the latter. Further generation of FXa is inhibited by the formation of a quaternary complex, when the K1 domain binds to FVIIa in the TF:FVIIa binary complex. Here, the inhibition of FXa is the rate-limiting step not the inhibition of TF:FVIIa [38, 39]. This inhibition of FXa by the K2 domain is enhanced by protein S. The K3 domain of TFPI α is localised to membrane surfaces when it is bound to protein S, which is most important at physiological levels of TFPI [67–69]. In addition, early in the initiation pathway TFPI α inhibits FXa-activated FVa and platelet FVa but not thrombin-activated FVa with decreased thrombin generation secondary to inhibition of the prothrombinase complex. A high-affinity exosite interaction between the FV acidic region and basic region of TFPI α mediates this inhibitory interaction [40].

Essentially, TFPI attenuates the response to TF, such that magnitude of the early thrombin generation determines the response through the amplification pathway. This attenuation is secondary to the inhibition of two major protease–co-factor complexes: extrinsic tenase complex generating FXa, and early prothrombinase complex that includes FXa-activated FVa generating thrombin [43].

Attenuation of TFPI inhibition in the absence of the amplification pathway can result in restoration of thrombin generation. TFPI is a particularly attractive target as its inhibition results in reversal to a more primitive coagulation pathway that has been in existence for millions of years prior to development of the amplification pathway [64, 65]. TFPI inhibition is being investigated extensively and four molecules are in various stages of clinical development. The first molecule to enter clinical trials was aptamer, and more recently, three monoclonal antibodies (mAbs) directed against the various Kunitz domains have been studied in clinical trials.

Anti-TFPI therapeutics in development

Anti-TFPI aptamer (BAX 499)

Aptamers are oligonucleotides, such as RNA and single-stranded deoxyribonucleic acid (ssDNA) or peptide molecules that bind targets of interest with high affinity and specificity due to their specific three-dimensional structures [70]. Aptamers have found increasing applications in the diagnostic and therapeutic arena due to their versatility.

In pre-clinical and clinical studies, an oligonucleotide aptamer against TFPI, BAX 499 (formerly ARC19499, Baxter Healthcare, Cambridge, MA, USA) showed potent and specific inhibition of TFPI. In vitro studies demonstrated procoagulant activity with effective inhibition of TFPI, restoration of thrombin generation and clot formation in haemophilic plasma. In a nonhuman primate haemophilia model, saphenous vein bleeding time decreased following administration of the aptamer in vivo [71]. However, the subsequent clinical study was discordant with the pre-clinical observations.

In a phase 1 dose-escalation study in patients with haemophilia with and without inhibitors, BAX 499 unexpectedly showed an increased number of bleeding events in the cohort receiving the highest dose (72 mg subcutaneous (SC)), resulting in premature termination of the study [72]. Plasma levels of FL-TFPI increased over 25-fold in patients receiving the highest dose, which was associated with substantially reduced thrombin generation. Multiple biological mechanisms appeared to underlie the increase in FL-TFPI plasma levels [72].

BAX 499 increased the release of intracellularly-stored TFPI, similar to heparin with no increase in transcription of mRNA or release of endothelial-associated TFPI β . TFPI half-life was found to be increased secondary to inhibition of proteolysis and decreased receptor clearance. Inhibition of proteolysis was secondary to a fourfold decrease in elastase-catalysed cleavage of TFPI. This cleavage is mediated at the Lys86–Gln90 region, a hot spot for proteolytic cleavage by a wide variety of proteases including thrombin, plasmin, FXa, elastase, and chymase [45], resulting in removal of the K1 domain with loss of activity. Furthermore, BAX 499 strongly reduced TFPI binding to LDL receptor-related protein 1 (LRP1), which is involved in receptor-mediated endocytosis [73]. Pharmacokinetic (PK) studies in mice also showed that, while human FL-TFPI was rapidly cleared, anti-TFPI aptamer with and without pegylation prolonged the clearance [72, 73].

The inhibitory mechanisms were also investigated with specific reference to domain interactions between aptamer and TFPI [74]. Binding experiments demonstrated that FL-TFPI was required for tight aptamer binding, and binding

affinity was decreased if the carboxy-terminal region of TFPI was missing, very weak when TFPI contained only K3 and carboxy-terminal domains, and non-existent when only K1 and K2 domains were present. Importantly it had no effect on TFPI β , in spite of being an excellent inhibitor of FL-TFPI. Additional studies also demonstrated that TFPI was able to bind aptamer and FXa simultaneously [74]. In in vitro assays measuring the inhibition of FXa activity by TFPI, antibodies against the K2 domain fully inhibited TFPI and restored FXa activity. In contrast, BAX 499 and antibodies against the K1, K3, or carboxy-terminal domains of the protein did not fully inhibit TFPI, resulting in only partial restoration of FXa activity [74, 75]. These experiments demonstrate that the interaction between aptamer and TFPI is complex, and the inhibitory mechanisms are distinct from that of other TFPI inhibitors. Suggested mechanisms include interference with FXa interactions outside the K2 domain or conformational change in TFPI, weakening the ability of the K2 domain to interact with FXa.

To further explain the bleeding tendency, the inhibitory activity of BAX 499 against TFPI was tested with a wide concentration range of FL-TFPI in two model systems and in two global haemostatic coagulation assays including thrombin generation and rotational thromboelastometry (ROTEM). BAX 499 demonstrated a paradoxical effect, efficiently neutralizing TFPI at low concentrations and less efficiently inhibiting TFPI at high TFPI levels, where TFPI maintained more than half of its inhibitory capacity [76]. This, in conjunction with an increase in FL-TFPI levels, appeared to be responsible for the increased bleeding tendency.

Anti-TFPI antibodies

The procoagulant effect of an anti-TFPI antibody was first demonstrated both in an in vitro laboratory study of haemophilic plasma where it shortened the activated partial thromboplastin time (aPTT) [77] and an animal model where the antibody decreased the bleeding time without complete normalization of aPTT [78]. Additional studies have demonstrated that blood loss continued to decrease beyond complete inhibition of plasma and endothelial TFPI pools, suggesting a putative role for the inhibition of platelet TFPI released at the site of injury [79].

Three mAbs against TFPI: concizumab, PF-06741086, and BAY-1093884, are currently in various phases of clinical development as summarised in Table 1. The anti-TFPI antibodies differ in their specificity against the domain structure and potentially clearance from circulation.

Concizumab

Concizumab (Novo Nordisk A/S, Bagsvaerd, Denmark) is a monoclonal, humanized IgG4 antibody specific for the K2 domain that has demonstrated the restoration of thrombin generation in pre-clinical and clinical samples [34, 80]. In animal models, it has arrested bleeding under haemophilia conditions [34]. In phase 1 single-dose and multiple-dose-escalation studies, concizumab demonstrated a dose-dependent decrease in TFPI levels, with concurrent increase in d-dimers and prothrombin fragment 1 + 2 [33, 81]. A tendency to reduced bleeding was observed with increasing dose of concizumab [81]. Efficacy and the dose-response relationship of concizumab will be determined in phase 2 proof-of-concept studies. At the highest dose levels in the single-dose-escalation study, decreased fibrinogen was noted

Table 1 Status of clinical development of anti-TFPI antibodies for the treatment of haemophilia

Antibody (company)	Description	Completed clinical studies	Ongoing studies
Concizumab (Novo Nordisk)	Monoclonal, humanized IgG4 antibody specific for the K2 domain	Explorer TM 1: Phase 1 single-escalating dose study in haemophilia A and B patients [31] Explorer TM 3: Phase 1 multiple-dose study in haemophilia A patients (NCT02490787) [74]	Explorer TM 4: Phase 2 proof-of-concept multiple-dose study in inhibitor patients with haemophilia A or B (NCT03196284) Explorer TM 5: Phase 2 proof-of-concept study in haemophilia A patients without inhibitors (NCT03196297)
PF-06741086 (Pfizer)	Fully human mAb (IgG1) against the K2 domain	Phase 1 study in healthy volunteers [33]	Phase 2 multiple-dose study in haemophilia A and B patients without inhibitors (NCT02974855)
BAY-1093884 (Bayer)	mAb against K1 and K2 domains	N/A	Phase 1 single-escalating dose and multiple-dose study in haemophilia A and B patients (NCT02571569) [34, 75]

Ig immunoglobulin, K2 Kunitz 2, mAb monoclonal antibody, N/A not applicable

with no change in platelet count, AT or protein C or S suggesting consumption [33], but the mechanisms are not completely elucidated [33]. The elevated D-dimers and prothrombin fragment 1 + 2 are believed to represent a procoagulant tendency. No significant adverse events were observed in the cohort, except for an episode of a short segment of thrombophlebitis in a healthy volunteer, which was diagnosed by ultrasound 5 days after SC injection. In the haemophilia patients randomised to the highest SC dose cohorts (1000 or 3000 µg/kg), transient elevation of troponin T was noted in three patients with no significant electrocardiogram abnormalities. Similarly, in the intravenous (IV) cohort, fibrinogen concentration decreased without a concurrent decrease in protein C, protein S, AT, or platelet count at the highest doses (1000 or 3000 µg/kg) [33].

PK profiles were consistent with target-mediated drug disposition (TMDD) in which binding of concizumab (drug) to TFPI (target) influenced drug distribution, elimination, and plasma concentration [33]. This essentially results in a disproportional increase in drug concentration with increasing drug dose at higher levels, secondary to saturation of the target. To understand this disproportionate dose–response relationship, it becomes important to identify the drug- and target-specific parameters that influence exposure–response relationships, and patient-specific characteristics that account for inter-subject pharmacodynamic variability [82].

PF-06741086

PF-06741086 (Pfizer, New York City, New York, USA) is a fully human mAb (IgG1) that targets the K2 domain of TFPI with high affinity. Pre-clinical studies demonstrated a procoagulant effect as evidenced by thrombin generation assays (TGAs) and dilute prothrombin time (dPT) with increased thrombin generation and shortened dPT [83]. Animal studies that demonstrated the haemostatic efficacy of PF-06741086 included tail-transection models in both haemophilia A and haemophilia B mice [35, 84]. In a phase 1 study that included 41 healthy male volunteers and SC and IV administration of the drug, the pharmacological effects of TFPI inhibition were seen. Parameters suggesting coagulation activation in vivo included elevated D-dimers and prothrombin fragment 1 + 2, and ex vivo parameters included dPT and TGA. Responses were dose-dependent, and interestingly, the change in TGA parameters was modest, indicating downstream regulation. Anti-drug antibodies were detected in 15 volunteers and three tested positive for low levels of neutralizing antibody measured with a novel assay [35]. Similar to concizumab, TMDD was apparent. Increasing doses were associated with an increase in total TFPI levels, which measures both free and antibody complexed TFPI, most likely reflecting the slow turnover of the complex, and an ongoing

pharmacodynamic effect was observed [35], unlike with BAX 499 aptamer.

BAY-1093884

BAY-1093884 (Bayer AG, Leverkusen, Germany) is a mAb against both K1 and K2. Administering either SC or IV doses of BAY-1093884 in nonhuman primates showed that the drug shortened dPT clotting time, which correlated with free TFPI levels and the plasma concentration of BAY-1093884 [85]. BAY-1093884 is currently being investigated in a phase 1 single and multiple-dose-escalation study [36, 85].

Opportunities and challenges for anti-TFPI therapies

The treatment burden in haemophilia is related to the need for IV replacement therapy and the frequency of infusions needed to achieve a reasonable trough level that is protective. Standard recombinant FVIII and FIX require between two and four infusions a week, and extended half-life recombinant FVIII and FIX require infusions between one and two times a week. Interventions that result in simpler drug administration such as SC treatment are well received by the community. In addition, interventions that result in less variability in coagulation potential, without reaching the patient's baseline, give patients freedom to undertake activities without having to plan them around their treatment. They also present clinicians with opportunities for better bleed control.

The TF:FVIIa complex formed at the site of injury, as described, is rapidly inactivated by TFPI that is released from both thrombin-activated platelets and present on the surface of endothelial cells. The duration of activity of the TF:FVIIa complex is dependent on the relative concentrations of TF and TFPI, and these may vary at a particular site of vascular injury [86]. Indeed, higher levels of TFPI have been noted in synovial fluid from haemophilic joints [87]. Furthermore, the increased D-dimers and prothrombin fragment 1 + 2 may represent an exaggeration of normal baseline coagulation due to the presence of some background TF activity. It is not uncommon to see patients with D-dimers beyond the normal range, and concerns about slightly higher D-dimer levels may be misplaced. Indeed, patients present with elevated D-dimers and low fibrinogen secondary to cavernous malformations for decades with no obvious deleterious effects [88]. Other challenges include the influence of TMDD on the infusion frequency of the anti-TFPI antibodies with patients having to receive injections more frequently. The intravascular pool of TFPI is large and it appears that blocking the plasma pool may be adequate to prevent bleeds,

but the therapeutic window is to yet to be defined. It is also worth noting that TFPI essentially rolls back the coagulation cascade in haemophilia patients to very primitive coagulation that appeared too effective and that potentially may not be precise.

Conclusion

Restoration of thrombin generation is an exciting therapeutic approach in the management of haemophilia. Whilst anti-TFPI therapies may not normalise thrombin generation, restoration appears to get patients into a range consistent with mild haemophilia providing sufficient protection for most activities. The long-term consequences of these approaches are not known and close vigilance is required, especially with regards to thrombotic complications and any signals where there is cross talk between coagulation and inflammation.

Compliance with ethical standards

Conflict of interest PC has worked as an investigator on the anti-TFPI mAb for Novo Nordisk and Bayer; has received honoraria from Baxalta/Shire, Biogen Idec, CSL Behring, Novo Nordisk, Pfizer, Roche, and Sobi; has served on advisory boards for Bayer, Baxalta/Shire, Biogen Idec, CSL Behring, Chugai, Freeline, Novo Nordisk, Pfizer, Roche, and Sobi; and has received research funding from Bayer, CSL Behring, Novo Nordisk, Pfizer, and SOBI.

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