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The Potential Close Future of Hemophilia Treatment – Gene Therapy, TFPI Inhibition, Antithrombin Silencing, and Mimicking Factor VIII with an Engineered Antibody

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Summary
Hemophilia is one of the best researched monogenic diseases. Hemophilia A will affect approximately 1:5,000 male live births. In recent decades, great progress has been made with the introduction of recombinant proteins in the 1990s for therapy and prophylaxis, securing adequate availability and, with the introduction of the prophylaxis concept, reducing the negative impact of hemophilia on morbidity (especially arthropathy). Despite this progress, there are still challenges to overcome to secure adequate prophylaxis and treatment: for the time being, causal pharmacological hemophilia prophylaxis and therapy requires repeated i.v. application on a regular basis. Although this approach leads to a reduced comorbidity, it does not yet represent an optimized approach with continuous reversal of the hemophilic defect, which would be the ideal solution. This review summarizes the very new treatment strategies for the treatment of hemophilia A and B.

Introduction

Hemophilia is one of the best researched monogenic diseases. Hemophilia A will affect approximately 1:5,000 male live births. In the 1990s, great progress has been made with the introduction of recombinant proteins for therapy and prophylaxis, securing adequate availability and, with the introduction of the prophylaxis concept, reducing the negative impact of hemophilia on morbidity (especially arthropathy). Despite this progress, there are still challenges to overcome to secure adequate prophylaxis and treatment: for the time being, causal pharmacological hemophilia prophylaxis and therapy requires repeated i.v. application on a regular basis. Although this approach leads to a reduced comorbidity, it does not yet represent an optimized approach with continuous reversal of the hemophilic defect, which would be the ideal solution.

Therefore, various approaches are actively explored. One path is to improve pharmacokinetics of factor concentrates by prolonging their respective biological half-lives; this approach is reviewed elsewhere in this volume. Another path is to look into completely new approaches with very different modes of action than used for the treatment of hemophilia so far. Four of them have recently received renewed attention as clinical studies have been performed to prove the respective concept: reversal of the hemophilic defect through gene therapy, inhibition of tissue factor pathway inhibitor (TFPI), downregulation of antithrombin by RNA silencing, and circumventing the absence of factor VIII (FVIII) using a bispecific antibody recognizing factor X (FX) / activated factor Xa (FXa) as well as factor IX (FIX) / activated factor IXa (FIXa).

This paper will give an overview on the pathophysiology behind these 'old' and new approaches and will provide additional data on their development, as appropriate.

Inhibition of TFPI

TFPI has been known to play an important role in controlling the tissue factor (TF) associated procoagulant response for quite some time [1]. TFPI is a multivalent, Kunitz-type proteinase inhibitor occurring in three isoforms: TFPI-α, TFPI-δ (a truncated form with two Kunitz domains), and glycosyl phosphatidyl inositol

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(GPI) anchored TFPI-β. Endothelial TFPI-α represents the greatest in vivo reservoir of TFPI containing three Kunitz domains, while TFPI-β, through alternative splicing, contains only two Kunitz domains and has an alternative carboxyterminus that directs the attachment of a GPI anchor [2, 3].TFPI inhibits the TF / activated factor VIIa (FVIIa) complex via the Kunitz 1 domain as well as FXa in the prothrombinase complex via the Kunitz 2 domain [4, 5], whereas the Kunitz 3 domain seems to be responsible for the cell surface localization of TFPIs [5]. Two decades ago, inhibition of TFPI was already shown to shorten ex vivo clotting times early on in a dose-dependent manner [6]. Thus, inhibiting TFPIs results in shortening of the bleeding time in acquired hemophilia [7] and reduction of surrogate markers of hemophilia in hemophilic animals as well as hemophilic patients [8–10]. In knock-out studies, platelet TFPI was found to be a primary physiological regulator of bleeding in hemophilia [11]. Inhibition of TFPI and the respective influence on hemostasis can be achieved via various approaches such as the use of aptamers [12, 13] or specific antibodies [10, 11, 14].

Aptamers to Inhibit TFPI

The best documented aptamer – initially developed as ARC19499, later BAX499 – was generated through systematic evolution of ligands by exponential enrichment using recombinant human TFPI. Iterative rounds of selection with identification of individual clones resulted in the generation of a 32-nucleotide core aptamer, appended with a 3′-idT and a 5′-end 40-kDa PEG moiety [12]. BAX499 seems to be able to bind to TFPI simultaneously to FXa. BAX499 inhibits TFPI (different than domain-specific antibodies) via the Kunitz 1-, Kunitz 3- and C-terminal domains; and its inhibitory activity is reduced in the presence of protein S [15]. After binding of BAX499 to TFPI, the TFPI/BAX499 complex retains FXa inhibitory activity, BAX499 delayed TFPI-mediated inhibition of extrinsic tenase activity, and BAX499 reversed TFPI inhibition of the prothrombinase complex [16]. In an experimental setting, the TFPI inhibition effect is (expectedly) dependent on TF density [13]. Accordingly, BAX499 was found to improve surrogate markers of hemostasis in whole blood (thrombelastography) and plasma (thrombin generation) from hemophilic patients ex vivo [9] and in a dose-dependent manner [13, 17]. However, unexpected bleeding occurred in clinical studies – possibly due to an increase in TFPI half-life (and thus accumulation) through binding of BAX499 without complete inhibition of TFPI activity [18].

Antibodies to Inhibit TFPI

Antibodies to TFPI have long been known to shorten clotting times [6], and they were shown to enhance generation of both FXa and thrombin [14, 19]. TFPI levels in patients with hemophilia do not seem different from those without [20]. Thus, various antibodies have been or are currently evaluated in order to be used in the treatment of hemophilia patients [14, 21].

The anti-TFPI antibody concizumab (mAb 2021) binds TFPI via the Kunitz 2 domain, thus preventing interaction of TFPI with the FXa-active site [10]. It can be applied i.v. or s.c. and displays a high bioavailability [22]. A phase 1 study of concizumab suggested a favorable safety profile and a concentration-dependent procoagulant effect in healthy volunteers and hemophilia patients [23]. In this double-blind, placebo-controlled trial of escalating concizumab doses given i.v. or s.c. to healthy volunteers (n = 28) or hemophilia patients (n = 24), no serious adverse events and no anti-concizumab antibodies were seen. A dose-dependent procoagulant effect was evidenced by D-dimers and Prothrombin Fragments F1 + F2 [23]. Concizumab was shown to augment results of a thrombin generation assay in vitro in plasma of hemophilia patients as well as ex vivo after s.c. injections in healthy volunteers [19]. A phase 2 study is underway to assess the efficacy and safety of concizumab administered s.c. once daily in preventing bleeding episodes in hemophilia A and B patients with inhibitors.

BAY 1093884 is a neutralizing anti-TFPI antibody that can be applied i.v. and s.c.; it induces a dose-dependent decrease of free TFPI [21]. In vitro and in silico immunoprofiling supporting the design of BAY 1093884 suggests a low toxicity potential and immunogenicity in humans [24]. A phase 1 study to investigate the safety, tolerability, and pharmacokinetics of BAY 1093884 after i.v. and s.c. administration of increasing single doses in patients with severe hemophilia A or B with or without inhibitors is currently underway.

PF-06741086 is another inhibitory antibody of TFPI that inhibits thrombin generation in a dose-dependent manner [25] and seems to restore hemostasis in an on-demand hemophilia mouse injury model when administered after the onset of a bleeding injury [26]. PF-06741086 is being developed for the treatment of hemophilia A and hemophilia B with and without inhibitors.

Another observation of clinical importance with regard to TFPI in hemophilia patients is that, during the treatment of patients with an inhibitor, TFPI levels are found to be higher after treatment with activated prothrombin complex concentrates (aPCCs) as compared to rFVIIa [27], suggesting that the use of aPCCs might, beside the anticipated procoagulant effect, also induce an anticoagulant effect [28] (whereas the net sum is procoagulant).

Downregulation of Antithrombin

Reduction of coagulation gene transcription through the use of small interfering RNA has been described for various coagulation proteins [29]. Silencing of antithrombin has been shown to induce a clinically relevant hypercoagulable state outside the hemophilia setting [30]. As clinical experience suggests that prothrombotic mutations might attenuate the clinical course of hemophilia, an antithrombin RNA interference (RNAi) approach was developed that improves thrombin generation in a mouse model of hemophilia [31]. In another animal model, a 50% to near complete reduction in antithrombin levels was achieved in a dose-dependent manner through weekly dosing [31]. This approach was then translated into an early clinical study, proving that roughly 50% reduction of antithrombin levels can be achieved in hemophilia patients through RNAi therapy (fitusiran) with lower doses and weekly dosing; and up to 80% reduction can be achieved with higher doses and monthly dosing [32]. In this trial of fitusiran with different doses evaluated, the mean peak plasma levels of the drug were ob-

served after 2–6 h. The drug levels showed a rapid decrease in plasma, with a mean elimination half-life of roughly 3–5 h. Plasma levels of the drug increased in proportion to the dose applied. Anti-fitusiran antibodies were not observed. Plasma exposures were similar after first and last doses, suggesting that there was no accumulation of fitusiran after repeated administration. There was a clear association between the lowering of the antithrombin level and an increase in thrombin generation in patients with hemophilia, but not in healthy volunteers. This relationship was similar in participants with hemophilia A and hemophilia B. A reduction in the antithrombin level from baseline by 75% or more resulted in median peak thrombin values that correspond to the lower end of the range observed in healthy volunteers. The exploratory character of the study allowed to identify a monthly, fixed dose of 80 mg that lead to a consistent reduction of antithrombin by 87%, suggesting that a stable hemostatic protection can be achieved. In this first, small study (all in all 28 patients), adverse events were mainly mild to moderate and consisted of injection site reactions and transient liver enzyme elevations. Three various serious singular adverse events were observed, but no thromboembolic complications were seen in these early studies. In a post-hoc analysis, an apparent lower bleeding rate was seen under fitusiran as compared to before study enrolment. All bleeding events were controlled with the use of regular hemophilia therapy (factor concentrates). However, definitive conclusions on the frequency of toxicities and the efficacy are not possible due to the small sample size. An extension of this first study in order to evaluate the long-term safety and tolerability of fitusiran in male patients with moderate or severe hemophilia A (phase 1) or B (phase 2) is ongoing. In this extended study, however, a fatal thrombotic complication occurred in relationship with additional use of FVIII concentrate [33]. All fitusiran trials were halted transiently; the U.S. Food and Drug Administration (FDA) lifted the hold on clinical studies after trial's protocol was amended to better mitigate risks [34].

FVIII Mimetic, Bispecific Antibody

Recently, the use of a bispecific antibody to act as aFVIII-mimetic agent, thus allowing to generate the tenase complex even in the absence of FVIII, has been established [35]. The antibody initially identified from a screen, hBS23, has been further engineered to create ACE910 or emicizumab [36]. Emicizumab is a humanized bispecific antibody that binds to and therefore bridges FIXa and FX. Because of its structure and the associated mode of action, emicizumab is not expected to induce or be affected by FVIII inhibitors. Also, antibodies to ACE910 do not seem to inhibit FVIII [36]. Further research has shown that the non-antigen-contacting regions in emicizumab (an IgG antibody) are potential and important targets for engineering to improve the biological activity of IgG antibodies. For example, the tertiary structure determined by the inter-chain disulfide bonds in emicizumab was found to strongly affect the FVIII-mimetic activity [37].

Emicizumab has good subcutaneous bioavailability and a long half-life (4–5 weeks) in healthy volunteers [38]. Emicizumab has been shown to have in vivo hemostatic activity in non-human primates with acquired hemophilia [39] as well as in patients with congenital hemophilia [40].

Plasma emicizumab concentrations increase in a dose-dependent manner, and reach steady state trough levels by week 12, with loading doses of 1–3 mg/kg and weekly doses of 0.3–1 mg/kg. With a loading and weekly dose of 3.0 mg/kg, a steady state is not observed by week 12; rather, concentrations continue to increase [40].

Long-term application of emicizumab for up to 33 months, although reported in a small cohort of 18 patients, seems to be well tolerated [41]. Early evidence suggests that emicizumab can be applied s.c. every 4 weeks [42]. In November 2017, the FDA approved emicizumab for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and pediatric patients with hemophilia A (congenital FVIII deficiency) with FVIII inhibitors [43, 44].

Gene Therapy

Although the prevention of bleeding in hemophilic patients after a single therapeutic intervention and without the anticipated need for further intervention is an important goal, earlier approaches to gene therapy in hemophilia have not reached the anticipated efficacy or were associated with actual or potential toxicity [45, 46]. But progress was seen later on [47], and recently two landmark trials on successful gene therapy for hemophilia have been published, one in hemophilia A [48] and one in hemophilia B[LG1] [49]. These two trials – for now – seem to show that gene therapy in hemophilia is coming of age.

In the hemophilia A gene therapy trial, a single i.v. dose of an adeno-associated virus serotype 5 (AAV5) vector encoding a B-domain-deleted human FVIII (AAV5-hFVIII-SQ) was used to treat 9 men with severe hemophilia A. Low- (1 patient), intermediate- (1 patient) and high-dose (7 patients) vector doses were used, and patients and were followed up for 1 year. FVIII levels remained low with the low or intermediate dose. In the high-dose cohort, the FVIII activity increased to more than 5 IU/dl 2–9 weeks after gene transfer in all 7 patients and normalized (and stayed normal, >50 IU/dl) in 6 of them. Median annualized bleeding rate in the high-dose cohort decreased from 16 events before to 1 event after gene transfer; and FVIII use for bleeding stopped in this cohort by week 22. The primary adverse event was an elevation in the aspartate transaminase up to 1.5 times the upper limit of the normal range. No neutralizing antibodies to FVIII were detected [48].

In the hemophilia B gene therapy trial, a single i.v. dose of a single-stranded adeno-associated viral (AAV) vector was used to introduce a FIX Padua (factor IX-R338L) transgene in 10 men with hemophilia B who had FIX coagulant activity of 2% or less. No serious adverse events occurred. Therapy induced vector-derived FIX coagulant activity that was sustained in all patients with a mean FIX activity of 34% (range 14–81%). On cumulative follow-up of 492 weeks (individual follow-up 28–78 weeks), the annualized mean bleeding rate decreased from 11.1 events/year (range 0–48 events/year) to 0.4 events/year (range 0–4 events/year); factor use decreased accordingly (2,908 IU/kg (range 0–8,090 IU/kg) before and 49.3 IU/kg (range 0–376 IU/kg) after therapy). Eight of 10

patients did not use factor after therapy; and 9/10 patients did not bleed after therapy. Asymptomatic increases in liver enzymes occurred in 2 patients and resolved with short-term prednisone treatment [49].

Conclusions

New approaches to achieve a procoagulant response in hemophilia patients (other than coagulation factor concentrates) comprise inhibition of TFPI, downregulating antithrombin, and the use of a bispecific antibody to mimic the biological activity of FVIII. All of these approaches have been evaluated in clinical proof of principle studies in hemophilia patients. These studies have shown that all of these approaches are able to induce a procoagulant response or to shift the system more towards an attenuated bleeding phenotype. Therefore, all these approaches seem to be promising in order to further improve hemophilia care and allow continuous, long-term reversal of the hemophilic defect and/or phenotype. For the bispecific antibody, this has already led to product approval for a well-defined indication in the US. Gene therapy seems to be coming of age and is very promising; more data will be needed in order to adequately judge potential long-term side effects. Also, more studies are needed in order to obtain reliable information which intervention is most suited for which occasion in order to reliably increase the quality of hemophilia therapy.

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