The potential of serpins for future treatment for haemophilia

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Serpins (serine protease inhibitors) are the largest family of protease inhibitors and include several key players in the regulation of coagulation. Antithrombin, protein C inhibitor (PCI), α1-antitrypsin (α1AT), protein Z-dependent protease inhibitor (ZPI) and heparin cofactor II are examples of members of this superfamily of protease inhibitors. The molecular mechanisms behind the serpin functions are somewhat different from the majority of other inhibitors. Serpins are so called “suicide” or “single-use” inhibitors. The determinant of the specificity of a serpin is the reactive centre loop (RCL), a stretch of amino acids that the target protease recognises as a substrate. Cleavage initiates a major conformational change in the serpin that ultimately traps and irreversibly inactivates the target protease.

Due to the anticoagulant properties of several serpins, combined with their relatively long half-lives, they are increasingly investigated as potential therapeutic tools in the treatment of haemophilia. Haemophilia A and B are the most well-known bleeding disorders described to date, caused by factor (F)VIII or FIX deficiency, respectively. Currently available treatment involves replacement of the missing coagulation factor with recombinant or plasma derived concentrates. However, there are many limitations of factor replacement therapies, including the development of inhibitory antibodies, affecting ~30-50% and 3-5% of previously untreated individuals with severe haemophilia A and B, respectively [3, 4], the need for frequent intravenous infusions due to the relatively short half-life of FVIII and FIX concentrates, as well as the high cost of treatment. Over the past decade several new approaches have emerged to develop alternative ways to treat FVIII and FIX deficiencies in haemophilia. This has been done either by boosting procoagulant forces or by inhibiting anticoagulant pathways. Emicizumab (hemlibra, Hoffmann-La Roche) represents a new promising haemophilia A therapy [5]. The mode of action of Emicizumab differs from all other therapies on the market by substituting part of the cofactor function of FVIIIa through bridging of FIXa and FX, the other two components of the intrinsic tenase complex. There are currently four therapies blocking the natural anticoagulants undergoing clinical trials. Of these, three monoclonal antibodies block the tissue factor pathway inhibitor (TFPI) pathway: concizumab (Novo Nordisk), BAY1093884 (Bayer) and PF-06741086 (Pfizer).[6-8] A fourth therapy, fitusiran (Alnylam/Sanofi), uses a synthetic siRNA to reduce circulating antithrombin levels by preventing synthesis.[9] Both strategies have given positive results in initial human investigations.[8, 10]

Another attractive candidate for modulation of haemostasis in haemophilia is the anticoagulant activated protein C (APC) pathway (Figure 1). APC resistance through FV Leiden, the most common risk factor for deep venous thrombosis, is associated with reduced clinical severity in haemophilia.[11] This would suggest that the haemophilia bleeding phenotype can be improved by inhibiting the APC pathway, thereby prolonging the life-span of the prothrombinase complex. Several groups have
attempted to develop small molecule inhibitors against APC. However, these have a relatively high inhibition constant and, as is common with peptides, poor pharmacokinetics, suggesting that while the target may be suitable, the approach may not be the most optimal.[12, 13] An alternative, and more efficient, way of modulating the APC pathway has recently been developed.[14] The approach was to engineer a serpin that specifically inhibits APC activities, based upon the endogenous APC inhibitors, PCI and α1AT. While neither PCI nor α1AT are specific to APC in their natural forms, this was accomplished by substituting the amino acid residues in and around the P1-P1’ bond.[14] An engineered candidate (serpinPC) was shown to promote thrombin generation and restore fibrin deposition in a murine haemophilia B model. However, APC also has anti-inflammatory and cytoprotective roles which is a safety concern that must be considered. How efficiently serpinPC inhibits the anticoagulant versus anti-inflammatory activities of APC still remains to be determined.

Recently, another serpin has been investigated in the context of haemophilia treatment. ZPI together with its cofactor, protein Z (PZ), forms a fourth anticoagulant pathway (Figure 1). In contrast to antithrombin, which is a universal serine protease inhibitor, ZPI is a specific inhibitor of FXa and FXIa. ZPI reacts with the active sites of FXa and FXIa via its P1-Tyr in the RCL, thereby trapping them in inactive modified serpin-protease complexes, similarly to the mechanisms of other serpins. While ZPI inhibits FXIa in the absence of PZ, the inhibition of FXa is strictly dependent upon the cofactor. PZ enhances the reactivity of ZPI with FXa ~1000-fold by bringing ZPI to negatively charged phospholipid surfaces, where it encounters membrane-bound FXa.[15] This is further accelerated by a direct interaction between the PZ and FXa Gla domains.[15, 16]

The potential of PZ-ZPI as a therapeutic target in haemophilia was initially demonstrated by Girard and colleagues.[17] They showed that gene-deletion of either ZPI or PZ improved coagulation in a haemophilia A mouse model. Furthermore, they developed a monoclonal antibody against PZ and showed that blocking PZ activity resulted in increased thrombin generation in human haemophilia plasma.

In this issue of the Journal of Thrombosis and Haemostasis, Huang has developed a novel variant of ZPI, which functions as an antagonist of endogenous ZPI[18]. Rather than blocking endogenous ZPI, the strategy was to reduce its anticoagulant properties by outcompeting it with an inactive variant. Huang substituted two key amino acid residues, Tyr387, which is the RCL P1 residue and crucial for the recognition by FXa and FXIa, as well as Lys239, which strongly enhances the affinity of ZPI for PZ. [19, 20] The resulting variant, ZPI-2A (K239A/Y387A), has no inhibitory activity towards FXIa or FXa, while the >20-fold increase in affinity towards PZ meant that it could outcompete endogenous ZPI in binding to PZ, leaving the endogenous ZPI without a cofactor due to the formation of an inactive ZPI-
2A-PZ complex. As a result, ZPI-2A successfully reduced the inhibition of free and prothrombinase-bound FXa by wild-type PZ in pure-component and plasma based assays. Huang then repeated the experiments in haemophilia plasma, which led to a dose-dependent increase in thrombin generation as well as a reduction in lag time. Due to the high affinity between ZPI-2A and PZ, a 3-fold molar excess of ZPI-2A over the 60nM wild-type/endogenous ZPI was enough to completely reverse the anticoagulant properties of ZPI in all assays. However, whether this concentration is high enough to reverse ZPI activities in vivo, as well as the feasibility to administer ZPI-2A within this concentration range still remains to be determined.

In contrast to the other anticoagulant pathways, ZPI or PZ deficiency alone has not been shown to be associated with increased risk of thrombosis.[15] Reduced PZ levels are associated with thrombosis only in FV Leiden carriers.[15] These observations suggest that the role of the PZ-ZPI pathway in vivo may be less important than those of the other endogenous anticoagulant pathways. Consequently, it is therefore questionable whether blocking PZ-ZPI causes a strong enough procoagulant response in individuals with haemophilia to restore haemostasis. However, the results by Girard et al. and Huang both clearly show that reversing the activity of PZ-ZPI lead to an increased thrombin generation in haemophilia plasma [17, 18]. We also know that restoring the thrombin generation to “normal levels” is not necessary to substantially reduce the bleeding phenotype in individuals with haemophilia. It is therefore possible that even a relatively modest reduction in FXa inhibition, as is achieved by blocking the PZ-ZPI pathway, may be sufficient to significantly improve haemostasis in individuals with haemophilia. Blocking the PZ-ZPI also carries fewer potential risks than blocking any of the other anticoagulant pathways, which all have a critical importance for maintaining haemostasis as well as, for APC, anti-inflammatory/cytoprotective roles.

There are several additional possible advantages of developing a serpin or a serpin antagonist, such as serpinPC or ZPI-2A, respectively, as haemophilia therapies. Compared to standard FVIII and FIX concentrates, their half-lives are relatively long, days vs hours. SeprinPC or ZPI-2A could also potentially be administered subcutaneously, and due to their similarity to endogenous proteins, they are less likely to cause an immunological response and are also not affected by any existing FVIII or FIX inhibitory antibodies. Targeting the PZ-ZPI pathway specifically is a novel approach and also has the advantage that it is not limited to one particular bleeding disorder. ZPI-2A could be used for both haemophilia A and B. It may also be interesting to extend the research to include other coagulation factor deficiencies, such as FV deficiency, where no factor replacement concentrates are currently available.
ADDENDUM

J.A. wrote the manuscript.

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DISCLOSURE OF CONFLICTS OF INTEREST

The author has no conflicts of interest to disclose.
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