The improved factor concentrate

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Summary
The current treatment of haemophilia with coagulation protein replacement therapy is both effective and safe. Nevertheless, this therapy requires frequent, repeated intravenous infusions and approximately 25% of treated haemophilia A patients develop antibodies to the replacement protein. Furthermore, the cost and limited availability of current concentrates has restricted access to therapy to less than 30% of the global haemophilia population. With this background, efforts are now underway to develop coagulation concentrates with enhanced biological properties that further improve the quality of care for haemophiliacs. The specific areas of enhancement that are being explored include improved biosynthetic processes, prolonging the circulating half-life and reducing concentrate immunogenicity. Coincident with these approaches, it is hoped that there will be more widespread availability of these concentrates and that their cost will be contained.

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Over the past four decades, we have witnessed a progressive advancement in the products used to treat and prevent bleeding in haemophilia (7). In developed countries, current treatment protocols involve the administration of either high-purity plasma-derived or recombinant concentrates either on a prophylactic basis or on demand. This treatment is effective and safe from the previous concerns relating to infectious agent transmission (4). Nevertheless, the requirement for repeated intravenous administration of these concentrates and the development of neutralizing anti-FVIII antibodies in approximately 25% of treated haemophilia A patients indicates that improvements can still be made (Tab. 1). The two strategies being pursued to achieve this goal are

- the development of novel delivery systems and protein constructs or
- clotting factor gene transfer (3, 8, 9).

This brief review will focus on enhancements in factor VIII (FVIII) protein development.

Given the rising utilization of concentrate prophylaxis, the search for means to extend the circulating half-life of the infused proteins has become the leading area of interest in the development of new products (Tab. 2). To minimize bleeding events, a variety of prophylactic infusion protocols have been developed with the infusion of concentrate as frequently as daily to once per week, depending upon a number of factors including individual pharmacokinetic responses and patient preference. Nevertheless, most clinicians would support the goal of developing a concentrate that would provide adequate prophylactic haemostatic coverage in all patients through once weekly infusions. Currently, there are two broad approaches being pursued to achieve an extension of FVIII protein circulating half-life:

- the development of FVIII conjugates that interfere with its clearance and
- the generation of variant forms of FVIII in which the FVIII protein sequence is changed or fused to another molecule.

One of the major problems in developing strategies to extend FVIII half-life is that our basic knowledge of FVIII clearance is limited (5). We know that while the average FVIII half-life is approximately 12 h, there is significant interindividual variability, the cause of which is incompletely understood. Plasma levels of VWF certainly play a role in influencing FVIII clearance and thus indirectly other genetic factors such as ABO blood group may also have some effect. It appears that both FVIII and VWF are cleared predominantly in the liver and spleen by cells of the reticuloendothelial system (6) and that, at least for FVIII, the LDL receptor-related protein (LRP) and heparan sulphate proteoglycans play a role in this mechanism (14). However, much more needs to learnt about this phenomenon if we are to develop biologically rationalized means of interfering with FVIII clearance.

FVIII conjugate development

FVIII conjugate development is being prioritized by a number of industrial groups. The aim of all these projects is to provide FVIII with a molecular coating that prevents its interaction with clearance receptors. The forms of conjugates under development include various forms of polyethylene glycol and polysialic acid that are attached to FVIII through a variety of either site-specific or random linkages. The first of these concentrates to reach the clinic, developed by Bayer Inc. utilizes a pegylated FVIII molecule that is in turn attached to and delivered via synthetic liposomes. Preliminary in vivo analysis of this concentrate suggests that the conventional pharmacokinetic characteristics of FVIII are unchanged by this conjugation process (11) but that the concentrate results in a prolonged bleed-free interval following administration (15). How this effect is achieved is as yet unclear. There are plans to evaluate this product in a large prospective clinical trial in the near future.

Given the key role of VWF in influencing FVIII clearance, it is rational that some investigators are focusing attention on molecular conjugates of VWF as a means to indirectly extend FVIII half-life. Preinfusion VWF plasma levels are an important regulator of FVIII clearance and extending VWF half-life would be expected to have a therapeutically beneficial effect.

There are a number of drawbacks, both potential and already realized to these various conjugation strategies. In addition to interfering with clearance receptors for FVIII, all of the direct FVIII conjugation strategies are very likely to adversely affect FVIII pro-
coagulant participation in the intrinsic tenase complex. Thus, at least initially after infusion, and before in vivo processing of the novel conjugated molecules, one would expect that FVIII’s specific activity would be reduced to a variable extent. The predictability of this phenomenon may be further complicated by the fact that the concentrates will comprise a heterogeneous mix of conjugated molecules. Where VWF is conjugated, an adverse effect on primary hemostasis needs to be excluded.

The other unresolved question about these concentrates is the long-term fate of the conjugate material. While pegylated drugs have been used for the treatment of several diseases for up to a year, there is no experience with chronic long-term infusions of pegylated proteins. The site of conjugate clearance and potential adverse effects on physiological functions needs further investigation.

**FVIII variants and fusion proteins**

The alternate strategy to impeding FVIII clearance through a conjugate-based approach is to alter the sequence of the protein directly. This has involved two types of protein engineering, either the introduction of sequence changes to the primary structure of FVIII or, more recently, the generation of FVIII fusion molecules with partner expected to extend half-life.

The initial attempts to change the FVIII sequence to benefit its clearance properties involved the introduction of mutations to the LRP and HSPG interactive sites. Results in mice have shown that LRP deficiency results in higher plasma FVIII levels and there is preliminary data which suggests that certain polymorphic variants of LRP in humans influence FVIII clearance. However, despite these observations, the mutagenesis of the LRP and HSPG interactive sites has not yielded results that are likely to be translated into the clinic in the near term. There are two major reasons for this shortcoming:

- These same sites on FVIII are also critical for its interaction with other participants in the tenase complex and thus significant loss of procoagulant potency is lost with most of the substitutions.
- The influence of LRP and HSPG-mediated clearance appears to be only one of what may be a more complex interplay of clearance mechanisms.

The second group of changes made to FVIII to influence its half-life has been aimed at minimizing the two processes involved in FVIIIa inactivation: spontaneous dissociation of the A2 domain and proteolytic cleavage by activated protein C (APC). Studies by Pipe and Kaufman have evaluated an APC resistant FVIII variant referred to as IR8. In this molecule, two amino acid substitutions have been introduced at residues 336 and 562, the sites of cleavage by APC (10). This molecule is indeed resistant to APC-mediated proteolysis and possesses prolonged procoagulant activity, however, the substitutions introduced into IR8 interfere with the binding of FVIII to VWF and thus the protective effect of VWF in plasma is lost.

**Tab. 1** Limitations of current factor VIII concentrates and potential solutions

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<th>Limitation</th>
<th>Potential solution</th>
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<td>Short circulating half-life</td>
<td>Direct or indirect extension of FVIII half-life</td>
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<td>Inhibitor development</td>
<td>Reduced immunogenicity FVIII molecules</td>
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To prevent the spontaneous dissociation of the A2 domain after FVIII activation, Gale and colleagues have engineered a new disulfide linkage between the A2 and A3 domains (2, 12). Two different A2 stabilized molecules have been generated and these proteins have been successfully studied in mice.

The final group of variant FVIII molecules involves the development of FVIII fusion proteins in which the FVIII sequence is linked by a short peptide linker (approximately 30 residues) to a second protein. These fusions involve either the Fc domain of the immunoglobulin molecule or albumin. The rationale for this choice of fusion partners relates to two factors. Both immunoglobulin and albumin circulate as highly abundant plasma proteins with prolonged half-lives (> 20 days) and both proteins utilize the FcRn recycling receptor found on the surface of endothelial cells (13). It is assumed that the FVIII fusion proteins will use this same recycling mechanism and, that as a result, they will attain half-lives that will tend towards that of the two FcRn-associated partners. These novel proteins are currently under development although encouraging preclinical data has recently been documented with a FVIIIa-albumin fusion and a FIX-Fc fusion molecule has very recently entered into a phase I/II clinical study.

While the direct modification of FVIII to attain a prolonged half-life is perhaps a more elegant therapeutic approach, there are certain limitations to these strategies that are inherently more problematic. All of the substitutions and fusions detailed above may adversely interfere with FVIII’s interaction with FIXa and FX, thus reducing its specific procoagulant activity. However, of even greater concern is the potential for neoimmunogenicity due to these alterations. This complication will be very difficult to evaluate in preclinical models and this assessment may have to be left until early phase human studies are undertaken.

**FVIII proteins with reduced immunogenic potential**

With approximately 25% of haemophilia A patients at risk of developing antibodies that...
neutralize the function of their FVIII infusions the development of concentrates with reduced immunogenicity would be a highly beneficial therapeutic advance. Multiple studies over the past two decades have now established that the major B and T-cell epitopes on FVIII are in the A2, A3 and especially C2 domains. Patients usually develop an oligoclonal response to FVIII and thus simultaneously possess antibodies to several epitopes.

Lollar and colleagues are developing a strategy to substitute less immunogenic porcine FVIII sequences into the regions where the major B and T cell epitopes are located (1). This is a complex process, as many of these sites overlap with regions of FVIII that interact with its procoagulant partners. Furthermore, while the substituted porcine sequences may prove less immunogenic in some patients, it is unclear whether the chimeric proteins will ultimately avoid the development of an immune response.

Conclusions

Despite the excellent efficacy and safety record of currently available clotting factor concentrates there is still room to enhance the quality of haemophilia care with products that require less frequent administration and are less immunogenic. The first of these new products is already undergoing clinical trials and it is likely that additional novel concentrates will reach the clinic in the next five years. Nevertheless, the development of these new molecules will not be easy, and a variety of potential limitations will need to be thoroughly evaluated before widespread clinical exposure.

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References


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