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Go long! A touchdown for factor VIII?

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Half-life extension of biologics through bioengineering has impacted the treatment of a number of diseases. In this issue of Blood, Mei and colleagues present their novel bioengineering strategy to extend the half-life of factor VIII (FVIII) through targeted conjugation of polyethylene glycol (PEG) polymers. A longer-acting FVIII could significantly enhance prophylactic therapy for hemophilia A.

The recurrent hemarthroses and resultant crippling hemophilic arthropathy of hemophilia have almost been completely eradicated through aggressive prophylactic therapy with clotting concentrates in the developed world. Quality of life measures now track similar to their unaffected peers and patients engage regularly in sporting activities unimagined in previous generations. Prophylaxis for severe hemophilia is now recognized as the standard of care with optimal initiation very early in life before the onset of repeated hemarthroses, typically between ages 1 to 3 years. Nevertheless, barriers remain to realizing the benefits of prophylaxis universally.

The primary determinant of FVIII residence time in plasma is interaction with von Willebrand factor (VWF), which protects it from proteolysis and cellular uptake. With a hemostatic challenge, thrombin activation releases FVIII from VWF so that it can exert its procoagulant function. The majority of infused FVIII is cleared in the liver through interaction with the low-density lipoprotein receptor–related protein (LRP) family of cell-surface receptors. A PEGylated form of FVIII would have reduced cellular uptake and a resultant prolongation of plasma half-life. The elimination of PEG-FVIII that is internalized in the hepatocyte has not been fully characterized but may follow urinary and fecal excretion routes limiting intracellular accumulation.

The costs for prophylactic replacement therapy are much greater than $100 000 USD per patient per year. Repeated venous access (typically 3 times per week to every other day) is still a barrier for many with frequent need for central venous access devices in the youngest boys. Suboptimal adherence to a prophylactic regimen also compromises outcomes.

Recombinant FVIII (rFVIII) has proven to be a remarkable facsimile of its plasma-derived counterpart. The broad adoption of rFVIII throughout the developed world has increased the worldwide supply of FVIII concentrates and helped advance the implementation of prophylaxis. However, at the heart of recombinant DNA technology is not just the ability to mimic native proteins, but to exploit insights gleaned from their structure and function to engineer targeted modifications to enhance their functional properties. A number of bioengineering strategies have led to rFVIII variants with improved efficiency of expression, increased potency and resistance to inactivation, and resistance to inhibitors. A major emphasis of current bioengineering efforts has been on half-life extension. A longer-acting FVIII holds promise to overcome some of the barriers to adoption of and adherence to prophylaxis.

The primary determinant of FVIII residence time in plasma is interaction with von Willebrand factor (VWF), which protects it from proteolysis and cellular uptake. Clearance of FVIII has only recently begun to be elucidated. FVIII is too large in molecular weight to be cleared by the kidney. Cellular clearance, primarily in the liver, occurs through interaction with a family of low-density lipoprotein receptor–related proteins (LRP) and heparan sulfate proteoglycan receptors among others (see figure). Some of the half-life extension strategies under investigation include sustained delivery, first attempted through association of rFVIII with PEGylated liposomes, chemical modification (eg, direct PEGylation) and bioengineering of FVIII itself through mutagenesis or the generation of fusion proteins.
(eg, with an Fc antibody fragment). It may be surprising that PEGylated rFVIII has not reached the clinic sooner. This has been employed for a number of biologics and successfully transitioned to the clinic (eg, PEG-asparaginase for acute lymphoblastic leukemia). However, the primary advantage of PEGylation for a biologic is the incorporation of many water molecules within the hydrophilic PEG structures, functionally increasing the effective size of the conjugated protein above the filtration size of the kidney. This is of no particular advantage for FVIII since it is already too large for kidney filtration. Any advantage to PEGylation of FVIII is likely through disruption of interaction with cellular clearance receptors. However, such chemical modification could be a disadvantage to FVIII if it interfered with key protein–protein interactions (eg, VWF, thrombin, factor IXa). These potential hazards of chemical modification of FVIII are particularly problematic without the ability to target the sites where PEG polymers are conjugated to the protein.

In this issue of Blood, Mei et al have used a unique bioengineering approach to identify promising PEGylated rFVIII molecules with extended half-lives. They screened targeted PEGylated rFVIII mutants achieved through linkage to free surface exposed cysteine residues introduced through mutagenesis. They identified variants that retained full procoagulant function and VWF binding in vitro, exhibited improved pharmacokinetics in hemophilic mice and rabbits, and prolonged efficacy in bleeding models in mice consistent with their enhanced half-life in vivo. Their results confirm that the site of PEGylation on FVIII is critical to PEGylation efficiency, preservation of procoagulant activity and pharmacokinetic impact.

This would appear to be a touchdown on a very competitive playing field of hemophilia pipeline products if the preclinical studies can be replicated in clinical trials. A doubling of the FVIII half-life, suggested as achievable in this study, could potentially realize once per week prophylactic infusions, maintaining trough FVIII levels in plasma above critical thresholds for prevention of spontaneous bleeding (typically 1%). This would be welcome innovation for parents struggling to find venous access every other day in an infant or toddler, or for the busy adolescent striving to adhere to a regular prophylactic regimen. In addition, adults with hemophilia who may still utilize an on-demand regimen could gain some of the benefits of prophylaxis due to the extended plasma half-life.

There are a couple of issues that may temper enthusiasm for such an approach. Firstly, PEG is not biodegradable on any meaningful timescale and the PEG polymers themselves must be small enough for kidney excretion or rely on fecal elimination. Otherwise, higher molecular weight PEG could accumulate in the liver. Although PEGylation of biologics has a proven track record in the clinic, its application to rFVIII should bring some pause. This would be a unique setting in which a PEGylated biologic, whose target for clearance is the liver, would be administered prophylactically beginning in infancy and continuing for perhaps the life of the individual. The potential for unintended toxicity from PEG accumulation in the liver must be addressed fully in preclinical in vivo studies in the available animal models. Second, the benefits of a full prophylactic rFVIII regimen may not be solely due to maintaining FVIII plasma levels above key threshold levels. Implicit in an every other day infusion regimen is the regular achievement of fully corrective (≥50%) plasma levels immediately following each infusion. It is difficult to dismiss the impact of this aspect of prophylaxis as it pertains to the impressive joint preservation and active lifestyle enjoyed by those on such a regimen. Would once per week dosing provide sufficient plasma levels of FVIII throughout the week such that patients could continue to engage in work and play as they do currently? Perhaps extended half-life FVIII will serve as a backbone for prophylactic therapy with additional episodic prophylaxis still required to achieve plasma levels intermittently in the fully corrected range. Even so, Mei et al have presented a significant achievement through unique and innovative bioengineering for which we will look forward to its hopeful translation to the clinic.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

REFERENCES


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**REDCELLS & IRON**

**Comment on Salomao et al, page 267**

**Throwing out the baby**

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In this issue of *Blood*, Salomao and colleagues reveal the novel mechanism of aberrant protein sorting during enucleation, resulting in additional membrane protein deficiencies in hereditary elliptocytosis and spherocytosis. Whereas red cells of all birds, fish, reptiles, and amphibians retain their nucleus, the unique signature of definitive mammalian red cells is that they lose their nucleus before entering the blood stream. Investigators in the 1960s argued over the mechanism by which this occurred, some favoring karyolysis. Microcinematography of bone marrow cell suspensions by Bessis and Bricka in 1952 provided evidence in favor of expulsion, later confirmed by electron microscopy studies. But questions remained: Was this just a curious elimination of the unwieldy nuclear structure to prepare the flexible but strong red cell for its travels, or did this process have any clinical relevance? Careful study of this process has revealed that enucleation involves regulated segregation of cytoskeletal and cell-surface proteins between the plasma membrane of the extruded nucleus and the reticulocyte. Major cytoskeletal proteins, including band 3, spectrin, ankyrin, and...
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