“Novel strategies to Improve Recombinant Factor VIII Production and its in vivo Recovery”

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Dear Editor,

Hemophilia is a prototype disease for successful gene therapy, as a small percentage increase in factor VIII (F VIII) is all that is required to reverse the clinical manifestations. But the experimental studies conducted during the last decade or two have not yet translated into successful therapeutic options for the clinicians dealing with severe hemophilia A patients. Though further studies on gene therapy will go on, we cannot expect gene therapy in routine clinical practice in the near future. Hence replacement therapy with recombinant factor VIII concentrates will remain to be the main treatment option for some time to come. There is huge shortage of recombinant F VIII concentrates, more so in developing countries. It is inevitable that we have to look at some novel therapeutic strategies aimed at increasing the yield of recombinant F VIII protein and at increasing the survival of the transfused F VIII concentrates.

Following are some of the strategies on which some preliminary work has been done and are worth studying in more detail;

1. Strategies improving the potency of transfused recombinant F VIII,
2. Strategies aimed at decreasing the clearance of transfused F VIII,
3. Strategies to increase the secretion of recombinant F VIII during its production,
4. Strategies to increase the transcription of F VIII.

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Strategies Aimed at Improving the Potency of Transfused Recombinant F VIII

The activated F VIII (F VIIIa) is a heterotrimer consisting of a relatively stable non covalently linked A1/A3-C1-C2 dimer which is relatively weakly associated with A2 domain through electrostatic interactions. Spontaneous dissociation of this A2 domain from the F VIIIa heterotrimer results in loss of procoagulant function. This is the major mechanism of F VIII inactivation in vivo.

The duration of transfused recombinant F VIII can be increased by delaying or abolishing the dissociation of A2 domain. This approach is already under laboratory testing [1, 2]. A novel disulfide link between cysteine residues of the A2 and A3 domains can be engineered during preparation of recombinant F VIII.

Strategies Aimed at Decreasing the Clearance of Transfused F VIII

LDL receptor related protein (LRP) is a broad specificity hepatic clearance protein of wide spectrum of proteins including F VIIIa. A few other proteins in whose clearance LRP plays a role are, F Xa, F IXa, tissue pathway factor inhibitor (TFPI), thrombin-antithrombin complexes and plasminogen activators.

Two separate F VIII sites are involved in binding with LRP, (1) within the C2 domain and (2) within the A2 domain. LRP mediated endocytosis is facilitated by cell surface heparin sulfate proteoglycan. In animal studies, mutation of both LRP and heparin sulfate proteoglycan binding sites of recombinant F VIII protein showed a synergistic effect on F VIII clearance. F VIII half-life was prolonged by 3.3 fold, when LRP binding alone was

blocked, as compared to 5.5 fold prolongation of F VIII half-life when both LRP and heparan sulfate binding were blocked [3].

Strategies to Increase the Secretion of Recombinant F VIII During its Production

Production of recombinant F VIII in heterologous cell types has given us much insight into the biosynthesis of F VIII [4]. F VIII protein folds into its tertiary structure after it is translocated into the endoplasmic reticulum (ER). In the ER, three chaperones are important in the correct folding of the protein. They are calnexin, calreticulin, and immunoglobulin binding protein, BiP. ATPase domain of BiP interacts with recombinant F VIII through an eleven residue hydrophobic β -sheet that occurs in the A1 domain of F VIII. Mutation of Phe309 with in the β -sheet to serine, increases the secretion of recombinant F VIII several fold [5]. This needs to be studied in further detail, and can be used to increase the production of recombinant F VIII.

Strategies to Increase the Transcription of F VIII

The 1.2 kb of 5' flanking region of F VIII gene contains numerous transcription binding sites. These include transcription factors hepatocyte nuclear factor-1 (HNF-1), nucleation factor-kB (NF-kB) and CCAT/Enhancer Binding Protein (C/EBP). It is known that F VIII is an acute phase protein. This is due to the binding of NF-kB and C/EBP to their binding site located in the 5' untranslated region [6]. This mechanism can be exploited to increase the production of recombinant F VIII from heterologous cell types.

As plasma derived F VIII products carry the risk of viral transmission, recombinant F VIII products are the preferred F VIII concentrates. However, there is huge scarcity of these products, especially in developing countries where almost all the F VIII concentrates are imported from the West. Though clinical trials of gene therapy for severe hemophilia A are underway, it is unrealistic to expect gene therapy to come into clinical practice in the near future. So the need today is to find out strategies to increase the yield of F VIII. The above mentioned strategies should be seriously pursued in more detail so that they could fruitify into better yield and recovery of transfused recombinant F VIII concentrates.

References

1. Gale AJ, Pellequer JL (2003) An engineered interdomain disulfide bond stabilizes human blood coagulation factor VIIIa. *J Thromb Haemost* 1:1966
2. Gale AJ, Radtke KP, Cunningham MA et al (2006) Intrinsic stability, functional properties of disulfide bond-stabilized coagulation factor VIIIa variants. *J Thromb Haemost* 4:1315
3. Sarafanov AG, Ananyeva NM, Shima M et al (2001) Cell surface heparan sulfate proteoglycans participate in factor VIII catabolism mediated by low density lipoprotein receptor-related protein. *J Biol Chem* 276:11970
4. Miao HZ, Sirachainan N, Palmer L et al (2004) Bioengineering of coagulation factor VIII for improved secretion. *Blood* 103:3412
5. Swaroop M, Moussalli M, Pipe SW et al (1997) Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation factor VIII. *J Biol Chem* 272:24121
6. Begbie M, Notley C, Tinlin S et al (2000) The Factor VIII acute phase response requires the participation of NF-kappaB, C/EBP. *Thromb Haemost* 84:216