

## Editorial Focus

# The next generation of anti-haemophilia factor, factor VIII

## Long-lasting protection from spontaneous bleeding, are we there yet?

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**H**aemophilia A is an X-linked bleeding disorder caused by a deficiency of factor VIII (FVIII), a necessary cofactor for the generation of thrombin. The life expectancy of a child born with severe haemophilia A has improved dramatically over the past 30 years, from less than 20 years to nearly normal, primarily due to the development of FVIII concentrates, but also due to the efforts of comprehensive haemophilia centers that provide speciality medical care. However, as so clearly documented in the 2006 world survey of haemophilia care by the World Federation of Haemophilia, major challenges remain for the haemophilia community. Haemophilia A is the most common inheritable coagulation protein deficiency, and occurs with equal frequency in every population studied (1). However, in this age of globalization, discrepancies in the provision of haemophilia care among geographic regions have become glaringly apparent (2).

Despite optimal medical care and the availability of recombinant FVIII preparations, individuals with haemophilia A still face major medical complications. Spontaneous bleeding into joints can lead to orthopedic problems even after only a few such bleeds (3). Spontaneous intracranial bleeding occurs all too often in individuals with severe haemophilia A, and can lead to death or neurological deficits, even when treated promptly after clinical symptoms appear. In addition, some individuals suffer from the development of neutralizing antibodies to infused FVIII, rendering the therapeutic infusions of FVIII no longer useful for coagulation (1). The half-life of FVIII protein is only 8–12 hours, and therefore, therapeutic intravenous infusion of FVIII protein concentrates must be administered frequently for haemostasis after serious bleeding episodes, or to sustain prophylactic FVIII concentrations, infusions are often administered every other day. Such treatment regimens are very expensive. Clearly, the development of a preparation of FVIII that would prevent spontaneous bleeding and could be infused once every two weeks would represent a major breakthrough for treating haemophilia A.

In this issue of *Thrombosis and Haemostasis*, Spira et al. (4) add to the clinical data that support the view that such a breakthrough may be developing. The initial goal of their research was to extend the time in circulation of infused FVIII. A commonly used method for prolongation of the circulating half-life of rec-

ombinant proteins is the covalent incorporation of polyethylene glycol (PEG). Another commonly used method to extend the half-life of pharmacological compounds is to incorporate the compound into liposomes. Use of these proven technologies should safely provide benefit for FVIII also. Previously, Spira et al. (5) reported initial results with the use of PEGylated liposomes with recombinant FVIII (Kogenate FS). Such a formulation for FVIII has at least two potentially significant advantages. First, FVIII is a complex protein that has to interact precisely in order to fulfill its cofactor function in coagulation reactions. Second, a major clinical risk with currently available therapeutic FVIII is the development of an immune response and neutralizing antibodies. The addition of the PEG moiety to the FVIII protein directly would incur the risk of altering unfavorably the precise cofactor interactions, and simultaneously incur the risk of boosting the immune response. Baru et al. (5) avoided these potential risks by incorporating the PEG with the liposome rather than the FVIII protein directly.

Spira et al. (6) previously reported results using PEGylated liposome formulated FVIII in subjects with severe haemophilia A that showed prolonged intervals to the next spontaneous bleeding episode after infusion of PEG-liposomal FVIII compared to infusion of recombinant FVIII alone. The mean number of days without spontaneous bleeding was  $13.3 \pm 4.8$  days after infusion of PEG-liposomal FVIII at FVIII dose of 35 IU/kg compared with 7.2 days  $\pm 1.7$  days after infusion of a standard FVIII dose of 35 IU/kg ( $p < 0.05$ ). These exciting results generated much discussion in the haemophilia community, and raised a number of interesting questions for further study, as reported in the current publication.

One of the important issues raised in the previous studies involved how to account for the mechanism of action that led to the prolongation of the bleeding free interval. Initially the assumption was that the PEG and liposome prolonged the circulating half-life of the infused FVIII. However, in clinical trials the half life of PEG-liposomal FVIII was not different from standard FVIII (5, 7). Several interesting hypotheses to explain the prolonged efficacy in the absence of prolongation of the circulating half-life of FVIII will need to be addressed in animal studies and

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Received July 22, 2008  
Accepted July 22, 2008

Prepublished online August 14, 2008  
doi:10.1160/TH08-07-0462

in future clinical trials. These results so far emphasize how much remains to be defined in how coagulation works *in vivo* to prevent spontaneous bleeding. Experiments of nature may help shed some light on the complexities involved. A patient with severe haemophilia A by genetic testing did not have severe haemophilia by clinical experience, and the circulating half-life of infused FVIII was not different than expected. Further analysis revealed that he had another mutation of another coagulation protein, factor V Leiden, and therefore presumably did not suffer clinical spontaneous bleeding due to the prolonged activity of the active form of FVIIIa (8). An intriguing possibility is that the PEG-liposomal formulation of FVIII may not prolong the circulating half-life of FVIII, but may prolong the residency time in tissue of either FVIII itself or the active FVIIIa, thus protecting against spontaneous bleeding episodes.

In this manuscript Spira et al. (4) detail clinical studies, using randomized, subject-blinded, four-way cross-over design, comparing various doses of pegylated liposome in the formulation with a fixed dose of FVIII. One of the questions raised by their previous work, that the order of infusion of the standard FVIII and the test FVIII might influence the subsequent bleed free interval, has clearly been answered. No, the order of infusion does not influence the subsequent time to the first bleeding episode. The results of this study are helpful for this important area of research to understand how the formulation components contribute to the overall clinically significant effect of prolonging the interval to the next spontaneous bleeding episode. In this study the results support the conclusion that increasing the PEGylated liposome dose will enhance the clinical effect, with increasing benefit and no apparent decrease in the safety profile. Although there appears to be the suggestion of a plateau for the liposome dose, future studies will be needed to determine clearly what dose of FVIII with what dose of pPEGylated liposome is optimal for both the clinical outcome as well as to minimize side effects. To date, after over 90 subjects infused at least once with the PEG-liposomal FVIII, the preparation appears to be very well tolerated. Of course, critical questions remain to define the tolerability of repeated infusions in adults and especially in young growing children. While there is no cholesterol in the liposomal FVIII, and therefore the transient elevations in lipid laboratory values are due to mobilization of lipid from stores, these parameters will be watched closely in future clinical trials using repeated infusions.

Now that we have two clinical trials using PEGylated-liposomal FVIII, the results are both encouraging and raise interesting questions. It is encouraging to see that in two different clinical cohorts of severe haemophilia A, the time to the next spontaneous bleeding episode was prolonged compared to standard FVIII. Since both of these cohorts of subjects involved individuals with severe orthopedic problems due to treatment with on demand protocols using low doses of FVIII (by Western standards), it will be interesting to determine whether similar results of prolonged bleed-free intervals after infusion occur in subjects accustomed to higher doses of FVIII infused more frequently and with much better orthopedic scores for their joints. Indeed, can one contemplate prophylaxis soon with infusion once every two weeks? Only carefully designed clinical trials will answer these questions.

In addition to the immediate benefits of prolonged bleed-free intervals, there are two potentially interesting opportunities. One of the currently most worrisome consequences of therapeutic infusion of FVIII is the development of neutralizing inhibitors. It is possible that the use of PEG-liposomal FVIII formulation might shield certain immunogenic epitopes from the immune system, leading to decreased incidence of significant neutralizing antibody formation. It is reassuring that so far in over 90 individuals who have received PEG-liposomal FVIII no inhibitors have been detected. Again, this possibility can only be fully tested in future long-term clinical trials of repeated infusions.

Finally, as both animal and clinical studies proceed with this formulation, it will be informative and interesting to determine how the use of PEG-liposomal FVIII, with its as yet undetermined mechanism of action underlying its clinical benefit, interacts with other approaches to support coagulation in patients with severe haemophilia. Ultimately, it may prove useful to combine different approaches interacting with different aspects of the complex pathways of coagulation to support full clinical benefit. As with many early exciting new studies, these thoughts raise the comment: please, more studies needed as soon as possible. These are exciting times for haemophilia treatment, and our goal should be to treat haemophilia as a disorder of bleeding, and not simply as one of orthopedic problems: Haemophilia A patients with no bleeds at all, no neurological sequelae, no central catheters with risks of infection, no neutralizing inhibitors and no orthopedic disabilities. The manuscript by Spira et al. (4) marks one step in the journey toward that goal.

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