

Innovations in Factor Therapies for People with Bleeding Disorders

Chair: David Page, Chair, WFH Blood Product Safety and Supply Committee, Toronto, ON, Canada

Solvent-Detergent Treatment of Mini-pool Cryoprecipitate at Local Blood Centres

Magdy El-Ekiaby, Shabrawishi Hospital, Cairo, Egypt

Solvent detergent (SD)-treated mini-pools of cryoprecipitate are more effective and safer than what is currently available, and can be used at local blood centres, Dr. Magdy El-Ekiaby said. While there is a surplus of treatment in developed countries, many countries have no treatment at all and these virally inactive mini-pools may provide a solution, he added.

The economics of plasma is very complicated and the raw material and direct manufacturing costs are quite high, Dr. El-Ekiaby explained. Furthermore, there is limited availability of plasma and the cost of a fractionation facility is prohibitive in many cases. Contract fractionation is another option, but is not cheap either, he said.

Dr. El-Ekiaby's group is working on the production of enriched, virally-safe mini-pool plasma components. By turning fresh frozen plasma (FFP) into cryoprecipitate and cryopoor plasma, they have managed to increase the concentration of therapeutic proteins 3-5 fold compared to FFP. The resulting product also maintains 90% of the potency of starting plasma.

SD treatment has a good history – there has been no HIV, HCV, or HBV transmission from SD-treated products in 25 years, Dr. El-Ekiaby reported. The World Health Organization, U.S. Food and Drug Administration, European Medicines Agency, and the National Institutes of Health (U.S.) have all endorsed SD treatment. The viral inactivation process Dr. El-Ekiaby's team is using targets lipid enveloped viruses using a 3-micron filter for cell debris and bacteria. Small bags are produced labelled with FVIII and fibrinogen levels.

Dr. El-Ekiaby described the virus inactivation bag. It is actually 2 oval bags to avoid dead ends and poor mixing. A third, funnel shaped bag is used for SD removal. Filtration completely removes SD and bacteria. It takes 7 hours to produce 2,700 I.U. of FVIII. The SD used is TNBP triton x-45. The group has observed no change in FVIII and VWF activity after SD treatment. Dr. El-Ekiaby said the proteins are in very good shape. Other colleagues have done viral validation in worst case conditions. A company is now manufacturing the bags and field testing will begin soon in four countries. The cost per I.U. will be 6-8 cents if plasma is available.

The Prospects for Longer-Lasting and Less Immunogenic Factor VIII and IX Concentrates

Paul Giangrande, United Kingdom

Longer-lasting concentrates could be available within five years, Dr. Paul Giangrande said. He noted that the life expectancy of people with hemophilia is approaching that of all males thanks to state of the art treatment. Today, in many countries, children receive prophylaxis and it would be nice to have factor that lasts for a week or so instead of two or three days. The goals for longer-acting factor are prolonged half-life and reduced immunogenicity. Perhaps one day oral administration will also be possible.

One product under development is a factor VIII molecule that's attached to a molecule of fat using polyethylene glycol, Dr. Giangrande said. A trial in Russia showed longer protection against bleeds. Another group is making a FVIII molecule that is resistant to inactivation. This means active molecules with a five times greater half-life compared to the current product.

Another strategy under investigation is pegylation—the addition of polyethylene glycol polymer chains to factor concentrates to extend half-life. Studies of one such product currently under development show there is some loss of activity, but it still works. In a mouse model, the half-life is doubled. Pegylation of factor IX and von Willebrand factor is also under development.

There is also work underway with recombinant factor VII (rFVIIa). One group is using site specific glycopegylation to extend its duration of action. This process increases the half-life to about 16 hours (versus 2-3 hours with conventional rFVIIa). Another technique being studied is albumin fusion. Using genetic engineering, rVIIa is fused to albumin. Initial studies reveal an up to 9-fold increase in half-life.

Efforts to extend the half-life of factor IX are also underway. Two groups are working to increase the half-life of FIX through endothelial recycling, by attaching the FIX molecule to the immunoglobulin molecule. The FDA has approved clinical studies.

Elsewhere, a recombinant porcine B-domain deleted factor VIII is being developed for patients with inhibitors.

Dr. Giangrande touched on other innovations that are on the horizon – oral treatment using FVIII parceled in fat particles; attempts to develop a FIX molecule for nasal or oral administration; and work with tissue factor pathway inhibitor. Dr. Giangrande concluded that genetic engineering to enhance therapy is a very realistic medium term goal.

The Prospects for Gene Therapy in the Treatment of Bleeding Disorders
David Lillicrap, Canada

Dr. David Lillicrap spoke about the prospects for gene therapy, or gene transfer, in the treatment of bleeding disorders. He noted during the first 10 years or so of a person with hemophilia's life, we can keep the joints normal with prophylaxis, but eventually chronic arthropathy develops. We have already seen the benefits of cloning genes – first they allowed a greater understanding of the pathogenesis of hemophilia, then the diagnosis, and finally therapy.

Dr. Lillicrap discussed the therapeutic rationale for gene transfer. Patients with factor levels of 1-5% rarely bleed and do not develop chronic musculoskeletal disability. We know low dose prophylaxis prevents and reduces bleeds and arthropathy. The potential benefits of long term maintenance of FVIII or FIX levels of 1-5% will be the prevention of long-term harm. However, treatment products might still be required for surgery or trauma.

Dr. Lillicrap said that he is a believer in gene transfer for hemophilia, although there has been a lot of hyperbole in the past. There are four basic approaches to gene transfer: viruses, stem cells, non-viral methods to transfer genes, or repair mutations.

Dr. Lillicrap discussed what has happened in gene transfer for hemophilia up to today. There have been six clinical trials of hemophilia gene transfer, three each for hemophilia A and B, all in the United States. A total of 43 patients have been treated. There have been transient minimal factor increases and no significant adverse effects. A study conducted by Kathy High and Mark Kay achieved 12% FIX by week 2, but fell back to 0% by week 12. There was also some evidence of liver damage due to immunologic response. Two upcoming trials will attempt to address this immune response; one will use high transient immunosuppression, while a U.K. trial will use an alternative AAV serotype.

Another possible risk with gene transfer is gene insertion events. Two children with X-linked severe combined immunodeficiency developed leukemia after gene transfer. Dr. Lillicrap said that insertional mutagenesis is possible in hemophilia gene transfer but FVIII and FIX transgenes will not give transduced cells a proliferative advantage, which means the development of cancer is unlikely.

The increase in factor levels that we have seen with gene transfer trials so far has not lasted very long, but Dr. Lillicrap called this a limitation rather than a complication. He noted that in a recent Canadian hemophilia gene transfer survey on patient acceptability of gene transfer, 65% of respondents said they would accept a therapy that had to be done every year. Subcutaneous administration is most acceptable to 75%. At this time, we don't know if gene transfer would be a lifelong cure or if it would have to be administered every year or so.

Finally, there is evidence that gene transfer can incite inhibitors, but it can also induce tolerance (this may depend on the vector that is used). There is evidence in dogs that gene transfer can produce inhibitors but then tolerance

In conclusion, Dr. Lillicrap said that hemophilia gene transfer has produced a cure in many mice and about 50 dogs, the risk of cancer is small, and while the risk of inhibitors is greater, gene transfer can also induce tolerance.