PER977 binds to four sites on dabigatran, disallowing it from inhibiting Factor IIa. PER977 31.25 mg/kg significantly reduced blood loss mass in rats treated with dabigatran etexilate 15.5 mg/kg orally ($p<0.001$).

PER977 binds to a site on argatroban that does not interfere with argatroban's binding to Factor IIa. As such, rats administered argatroban 5 mg/kg subcutaneously followed by PER977 100 mg/kg intravenously, remain anticoagulated.

PER977 showed no binding to common cardiac drugs such as lisinopril, propafenone, and digoxin, among others, or to antiepileptic drugs such as gabapentin, lamotrigine, phenytoin, and valproate.

In conclusion, said Dr. Laulicht, PER977 reverses new generation oral anticoagulants *ex vivo* in human blood and decreases bleeding *in vivo* in a standard rat tail bleeding model.

Protein Bioengineering Strategies Improve Upon Hemophilia Treatments

Written by Wayne Kuznar

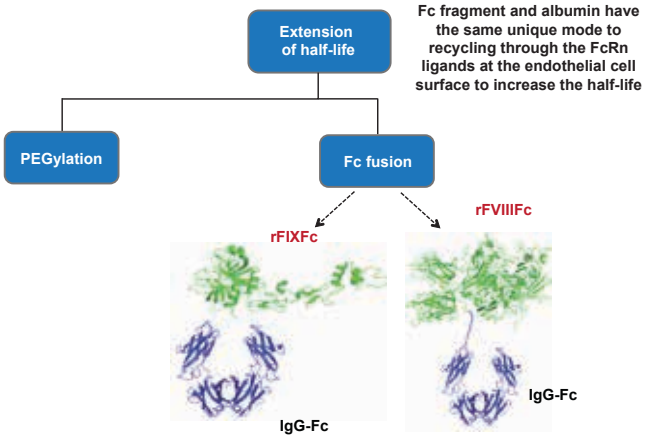
A new era in the treatment of hemophilia has emerged with a new generation of protein therapeutics. Flora Peyvandi, MD, PhD, University of Milan, Milan, Italy, provided an overview of the future of coagulation factor replacement therapy.

Protein engineering has increased the clinical potential and reduced rapid clearance of antihemophilic drugs from the body. Current therapy for hemophilia is safe and effective but immunogenic. Next generation antihemophilic drugs should have enhanced efficacy, greater safety, reduced immunogenicity, and improved delivery. Bioengineering technologies that have been applied successfully to other therapeutic proteins are now being applied to Factor VIII, Factor IX, and Factor VIIa. These technologies include the addition of polyethylene glycol (PEG) polymers and polysialic acids, alternative formulations with PEG-modified liposomes (PEG-Lip), and fusion proteins technologies. Pegylation of a protein extends its half-life and increases drug efficacy.

Glycopegylation allows for targeted pegylation so that the PEG can be attached to specific parts of the coagulation factors, such as the B domain for Factor VIII and the activation peptide for Factor IX. Upon activation, the pegylated portion of the protein is cleaved off, leaving the native activated coagulation factor.

An alternate strategy to extend the half-life of proteins is to fuse them to another protein with a much longer half-life, such as the fragment crystallizable (Fc) region of an immunoglobulin (Figure 1). Fc-containing proteins that are internalized by endothelial cells bind to the neonatal Fc receptor (FcRn) present in the acidified endosome, and are recycled back to the cell surface and subsequently released back into plasma at physiologic pH. These approaches markedly increase molecular weight, which reduces renal clearance.

Figure 1. Half-Life Extension



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Albumin fusion technology yields an altered version of a protein by fusing the gene for human albumin to the gene that encodes the active protein drug. This technology increases the protein's molecular weight, prolonging the half-life *in vivo*. The albumin molecule also masks the protein, rendering it resistant to proteases and less immunogenic.

Modified long-acting recombinant Factor VIII products in late-phase clinical studies have half-lives of 1.5- to 1.6-fold longer than their unmodified versions (Figure 2). Similar strategies have been used to extend the half-life of Factor IX by 3- to 5-fold.

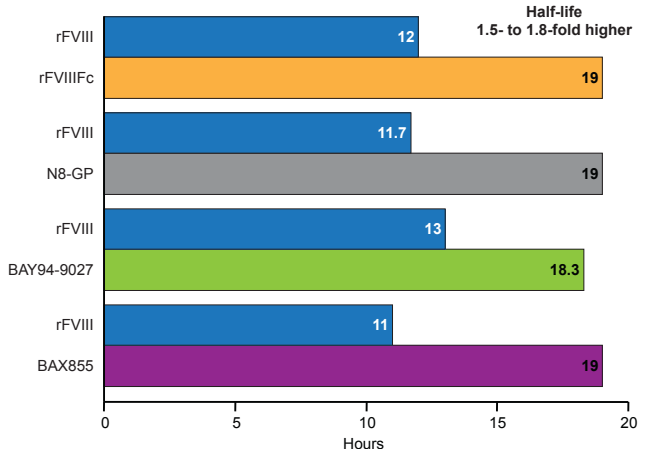
Current prophylactic treatment requires infusion 2 to 3 times weekly using Factor VIII and 2 times weekly using FIX products. In the future, the frequency of administration will be significantly reduced to once or twice weekly and every 1 to 2 weeks with long-acting recombinant FVIII and FIX products respectively, said Prof. Peyvandi.

Bioengineering strategies have also been employed to extend the half-life of recombinant Factor VIIa through site-specific pegylation, albumin fusion, or modification of amino acid sequence.

RNA interference is a cellular pathway of gene silencing in a sequence-specific manner at the mRNA level. A short interfering RNA, ALN-AT3, which employs a hepatocyte-targeting ligand, has been developed against antithrombin. In nonhuman primates, ALN-AT3 yielded potent and

durable knockdown of antithrombin with an up to 4-fold increase in peak thrombin generation. Clinical trial using this novel drug will start at the end of this years.

Figure 2. Half-Life Extension of Long Acting rFVIII



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Another approach is a humanized bispecific antibody to Factors IXa and X, termed hBS23 (ACE910), which is able to restore Factor VIII hemostatic activity. It is delivered via intravenous injection and has a 2-week half-life. Subcutaneous bioavailability of ACE910 is 84%. A clinical trial using this product has already started in Japan [JapicCTI-121934; http//www.clinicaltrials.jp].

Complement Inactive in *E. coli* Shiga Toxin-Producing Diseases

Written by Rita Buckley

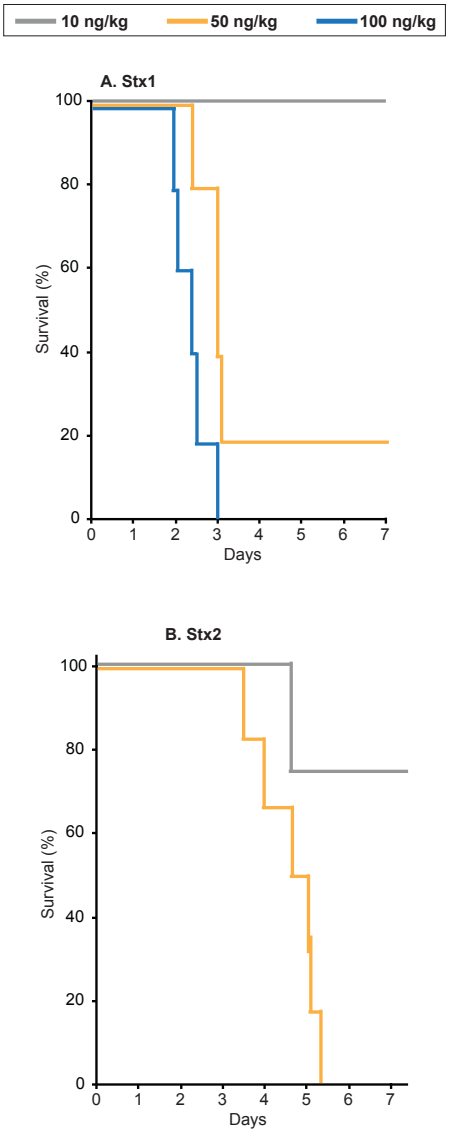
In a nonhuman primate model of hemolytic uremic syndrome (HUS), complement was not activated despite clear microvascular thrombosis and cellular injury. Complement is an important immune defense mechanism. Shinichiro Kurosawa, MD, PhD, Boston University School of Medicine, Boston, Massachusetts, USA, presented outcomes from a study on the role of complement in HUS and thrombotic microangiography induced by *Escherichia coli* (*E. coli*) Shiga toxins.

Enterohemorrhagic Shiga toxin-producing *E. coli* (EHEC), the leading cause of acute renal failure in otherwise healthy children, is associated with the potentially lethal complication of HUS. EHEC are food- and water-borne bacteria, contributing to the estimated 76 million illnesses, 325,000 hospitalizations, and 5200 deaths each year in the United States attributable to foodborne outbreaks, with a total annual cost of $10 to $83 billion [Bavaro MF. *Curr Gastroenterol Rep* 2012]. Toxins from these bacteria cause kidney, intestinal, and neurologic damage.

A 2011 outbreak of an *E. coli* strain secreting Shiga toxin type-2 in Germany infected 3842 individuals, many after consuming contaminated fenugreek sprouts. More than 50% of them required hospitalization; 855 developed HUS; and 54 died [Werber D et al. *BMC Med* 2012].

In order to develop a clinically-relevant animal model of HUS, Stearns-Kurosawa and colleagues [*Infect Immun* 2010] studied the effects of Shiga toxin types 1 and 2 (Stx1, Stx2) in nonhuman primates, comparing the *in vivo* consequences of the toxins in a parallel and reproducible manner. They found that the time course, pathology, and cytokine profiles differed between Stx1 and Stx 2, but that both induced HUS (Figure 1).

Figure 1. Time Course, Pathology, and Cytokine Profiles Differ Between Stx1 and Stx2



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