

# THE HEMOPHILIA BULLETIN

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It's always a pleasure to go a meeting at the superb convention center in San Diego! Most days, we had the sunny weather for which the town is known.

The most intriguing paper of the meeting was that presented by Tom Howard of Emory University, Atlanta, on **polymorphisms in the factor VIII molecule**. In the early 1970's, in the committee designing the Bethesda test for inhibitors, Roger Edson of Minnesota warned that we ought to use POOLED normal plasma, not any single normal plasma, as the source of FVIII. He said, all FVIII molecules might not be alike: they might vary from person to person. When we later realized that our hemophilia patients of black African descent were twice as likely to develop inhibitors as our white patients, I wondered whether there were race-specific variations in FVIII and homologous proteins such as factor V and ceruloplasmin, and whether black patients were being infused with predominantly-white FVIII molecules that might be more "foreign" to them than to white patients.

Dr. Howard compared the FVIII genes of normal subjects of different races in the southeast USA and found five common single nucleotide polymorphisms. White subjects were found to have only two of the allele patterns, and 93% of white subjects had one specific allele. These two alleles plus three others were found in blacks. Two of the three black-specific alleles have polymorphisms in the A2 or C2 domains, which contain major inhibitor epitopes. A large minority of black subjects had only black-specific FVIII alleles. Thus, FVIII is more polymorphic in Americans of black African descent than in whites. That's not surprising; geneticists are aware of the much greater genetic diversity in Africa than on other continents. Current recombinant FVIII concentrates consist of one or the other of the two alleles found in whites.

Dr. Howard wonders whether black patients with missense mutations may be particularly vulnerable when exposed to "white" FVIII, if they don't have a "white" allele and might recognize the infused "white" allele as foreign. He is undertaking a study of the FVIII alleles of persons with hemophilia of black African descent, with and without inhibitors.

I wonder whether homologous molecules might have corresponding polymorphisms. If so, a patient with severe hemophilia due to a null mutation for FVIII might still recognize "self" from the homologous molecule, and, if that were structurally different in these polymorphic sites from the infused FVIII, would he be more likely to

develop an inhibitor than if his homologous molecules had structures similar to the infused FVIII?

Might this mean that FVIII needs to be matched to the patient, to avoid provoking inhibitors? Just as red cells sometimes are typed in detail? Is it dangerous to infuse patients with FVIII with alleles that are not natural to them? African-only alleles might be dangerous to white patients, and white ones to some African patients. It's way in the future, but, specific alleles of human FVIII, needed by a minority of the population, might be developed. Licensure of any product intended for a specialty audience is always a challenge. But I'm jumping the gun. First, Dr. Howard's findings need to be extended, and their relationship to inhibitors examined.

## **What circumstances favor inhibitor development?**

Elena Santagostino and colleagues in Italy studied 102 children with severe hemophilia A (FVIII < 2%) treated exclusively with recombinant FVIII concentrates and tested for inhibitor every three months. Of these, 37 developed inhibitors of more than 5 BU and ten developed lower-level inhibitors. They could **not** confirm the suggestion, made in recent years, that early age at first exposure to exogenous FVIII increased risk. A significantly decreased risk of inhibitor ( $p = < .0001$ ) was seen among children who started prophylaxis early, before 20 exposure-days had elapsed (at a median of 11 months of age), versus those who started prophylaxis later (at a median of 35 months of age.) Prophylactic doses varied; frequency typically was twice or three times weekly. This level of prophylaxis is similar to the lowest doses used in some programs for induction of immune tolerance, notably in The Netherlands.

We're learning more and more about the **immunologic response of hemophilic mice to FVIII**. Christine Hausl of Austria and colleagues found that FVIII-specific memory B cells are inhibited by supra-physiologic doses of FVIII but not by physiologic doses. We must beware of extending mouse results to expectations in humans, but their studies tend to support the need for high-dose FVIII in induction of immune tolerance for established inhibitors.

Fiona Rawle of Canada and her colleagues showed that oral administration of the C2 domain of human FVIII induced tolerance in some but not all strains of mice, a study that built on their previous demonstration that oral feeding of the entire molecule induced tolerance. I am very interested in the potential of inducing tolerance with oral feeding. Such tolerance also has been observed with feeding of human factor IX from the milk of transgenic pigs, a potentially abundant source.

## Snippets from the VWD session:

Margaret Rand of Canada notes that genes in platelets affect the expression of **platelet GP1b-alpha binding sites for VWF**. The size of the site is affected if the gene stutters, that is, if there are variable numbers of tandem repeats (VNTR, more jargon) in the gene. In other words, part of the gene may repeat itself. The more it repeats itself, the larger the binding site. We presume that a larger site binds better. A VNTR coding for three repeats (a large repeat) is half as common in type 1 VWD as in normal controls. This variation in a platelet gene may affect the expression of a bleeding phenotype. If there are few repeats, a smaller binding site, a person may be more susceptible to bleeding and may come to medical attention and, if his levels of VWF are borderline, be diagnosed as having VWD.

Carolyn Miller of London investigated the correlation of ABO blood groups, von Willebrand factor antigen clearance and a polymorphism (Tyr1584Cys) in the A2 domain of the VWF gene. The median **half-life of VWF released after DDAVP** stimulation was 4.1 hours in patients with type 1 VWD and 9.5 hours in patients with mild hemophilia A. The half-lives in the VWD group did not vary according to ABO group or the version of that polymorphism. Why should the half-life of VWF:Ag be so much shorter in VWD than in mild hemophilia?

Patricia Lamont and Margaret Ragni of Pittsburgh infused DDAVP into two men whose severe hemophilia A had been ameliorated by **liver transplants** (performed because of liver failure related to chronic hepatitis C). After transplantation, their FVIII levels stabilized at more than 30%. After DDAVP, FVIII levels did **not** increase but levels of VWF antigen and ristocetin cofactor did rise substantially. They concluded that **extra-hepatic FVIII synthesis must be necessary for DDAVP-stimulated release of FVIII**. Why? Tune in next year.

Laurence Goodnough, a noted transfusion specialist who was, until very recently, at Barnes Hospital in St. Louis, described the difficulties in providing **oversight for off-label uses of rVIIa** (NovoSeven). He said that on-label usage, for FVIII inhibitors, was consuming a fairly stable amount of rVIIa but off-label use is increasing. Many hospitals appoint someone or some department to give permission for its use, largely to avoid runaway costs. At various institutions, the "gate-keepers" may be the blood bank, the pharmacy, the operating-room committee, the critical care committee and so on. At Barnes, the transfusion medicine service was the gate-keeper. For reversal of the warfarin effect, he recommended 10 mg of vitamin K, plus, for serious bleeding, a 1.2 mg vial of NovoSeven for an adult (rather than an exact per-kg dose, a policy limiting wastage), and fresh-frozen plasma, 15-20 ml/kg. One entire 4.8 mg vial of NovoSeven was recommended for an adult with bleeding due to a platelet disorder unresponsive to platelet transfusion, or for uncontrolled bleeding after

trauma or surgery. Use of whole vials rather than units-per-kg dosage decreased the total dosage and the cost.

Dr. Goodnough advised a dose of 80 micrograms/kg for sustained correction of a prolonged prothrombin time in liver disease (lower doses often were associated with the need for a repeat dose). For patients who have a fundamentally normal coagulation system, a lower dose may suffice, such as the 20 micrograms/kg used for retropubic prostatectomy.

One of the off-label uses of **rVIIa** that has been well-evaluated is **intracerebral hemorrhage (ICH)**. In a corporate symposium, a neurologist, Stephan Mayer, of Columbia University, reviewed the data. ICH causes 15% of all strokes in western countries and 30% in Asia. Hypertension is the underlying cause (60-70% of cases). ICH results in higher mortality (30-40%) and worse functional outcomes in survivors than other types of strokes. Major predictors of a poor outcome are greater volumes of blood in the hematoma and older age. The volume of blood can be determined fairly precisely by CT scan. In a prospective study, 103 patients with ICH were CT-scanned within three hours of onset of symptoms and again after another hour or so, and again after 20 hours or so. Significant growth of the hematoma, more than a third larger, was seen in 38% of patients on repeat CT scan. Most hematoma growth occurred within the first six hours, especially within the first hour. Would improvement of early hemostasis improve outcomes?

To test that hypothesis, 400 patients who had ICH and a baseline CT scan within three hours of onset were randomized to receive placebo or a dose of rVIIa. At 24 hours, the mean increase in hematoma volume was 29% after placebo, 16% after rVIIa at 40 micrograms/kg, 14% after rVIIa at 80 ug/kg, and 11% after rVIIa at 160 ug/kg. Overall, patients given rVIIa had 5 ml less blood in the hematoma, and 12-14 ml less total lesion volume (blood plus surrounding edema) at 24 hours. Mortality was 29% on placebo and 18-19% on rVIIa. At 90 days, patients who had been treated with rVIIa had better function than those given placebo; higher doses of rVIIa were related to better outcomes. An increased risk of thromboembolic adverse events was seen: 2% on placebo, dose-dependent on rVIIa, up to 10% on the highest dose. So there's a trade-off, more adverse events but better outcome with higher doses. This report is one of the best-documented beneficial off-label uses of rVIIa. One needs a quick CT scan of the brain to document that a fresh stroke is hemorrhagic, not thrombotic. I hope that our emergency rooms are ready for the challenge.

Good news! A new plant for the manufacture of **recombinant porcine factor VIII** is to be dedicated in Massachusetts in March. Hearty congratulations to Ipsen! I'll be glad to see a form of porcine FVIII back on the market. It's a great use in the right circumstances in patients with inhibitors.