

1044 adults were evaluated. Platelet dose was not predictive for bleeding in any age group; however, children in all age groups had a significantly higher risk of grade 2 or higher bleeding (grade 3 but not 4) versus adults, earlier bleeding after HSCT, and more days of grade 2 bleeding than adults. Moreover, the effect of age on grade 2 bleeding differed by disease treatment, with autologous transplants being the most pronounced. Josephson et al concluded that children were at higher risk of bleeding over a wide range of platelet counts, indicating that this increased bleeding risk was likely due to factors other than the platelet counts.

On the surface these data appear surprising because most hospitals that transfuse pediatric subjects employ specific dosing with a mL/kg or random donor equivalent/5–10 kg whereas adults often receive a single apheresis unit defined as $> 3 \times 10^{11}$ platelets/unit (*AABB Standards for Blood Banks and Transfusion Services*²). Thus, adults in a population with increasing body mass with successive generations could lead to “underdosing” in larger patients and increased bleeding due to relatively fewer platelets given per transfusion. In direct contrast, the PLADO analysis did not demonstrate that bleeding correlated with (1) the morning platelet count, (2) prophylactic platelet dose, or (3) that pediatric patients would benefit from a higher prophylactic transfusion trigger, suggesting that factors other than platelets appear to impart the bleeding risk in children. These factors likely include the treatment intensity as illustrated by the most pronounced bleeding differences in children receiving autologous HSCTs (vs adults) because the former received “conditioning” regimens for solid tumors, specifically neuroblastoma, brain tumors, and Wilms tumor, while the adults received pretransplant treatment for multiple myeloma or lymphoma. When analyzed against the other children in this study in the different disease categories, the children who underwent autologous/syngeneic HSCT had similar to increased bleeding risk whereas the adults who received autologous/syngeneic HSCT had markedly lower risk of bleeding than the other adult patients in the different treatment groups. Chemotherapy-induced mucositis may also have increased bleeding risk in children because they experienced more oral, nasal, and gastrointestinal bleeding versus adults who

had more skin, soft tissue, and musculoskeletal bleeding.

Children are different from adults because they tolerate more intensive chemotherapy, may have discordant structure and function of their vascular endothelium, and may have different rates of vascular regeneration after treatment-based injury resulting in accelerated platelet consumption and increased bleeding events.^{3–5} Josephson et al appropriately allude to these differences; however, other factors may impart bleeding risks including fevers, infection, and graft-versus-host disease (GVHD). The PLADO study design precluded GVHD because it was initiated immediately after HSCT, and patients were followed to day 36, which would be prior to when most patients develop GVHD, although 3 patients did manifest GVHD, which was likely acute GVHD. In addition, allergic rhinitis as a cause of epistaxis independent of platelet count was not studied and although it may represent the cause of epistaxis for many children, it would likely not impact the PLADO data because significant myelotoxicity is known to decrease allergic and atopic

reactions. Future randomized controlled trials should account for the possible increased bleeding risks in febrile or infected patients as well as the effects of GVHD and aggressive treatment regimens for solid tumors, especially in pediatric patients, and must increase the sample size to ensure robust statistical analyses of the data.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Josephson CD, Granger S, Assmann SF, et al. Bleeding risks are higher in children versus adults given prophylactic platelet transfusions for treatment-induced hypoproliferative thrombocytopenia. *Blood*. 2012;120(4):748–760.
2. American Association of Blood Banks. *AABB Standards for Blood Banks and Transfusion Services* (27th Ed). Bethesda, MD: AABB Press; 2011.
3. Kitchens CS. Amelioration of endothelial abnormalities by prednisone in experimental thrombocytopenia in the rabbit. *J Clin Invest*. 1977;60(5):1129–1134.
4. McPherson ME, Owusu BY, August KJ. Platelet transfusion requirements are associated with endothelial cell injury and angiogenesis during allogeneic hematopoietic stem cell transplantation [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2010;116:1433.
5. Milligan RG Jr, Wangenstein OD. Pulmonary microvascular filtration and reflection coefficients in two- and four-week-old rabbits. *Microvasc Res*. 1978;15(3):331–338.

● ● ● THROMBOSIS & HEMOSTASIS

Comment on Shibeko et al, page 891

Factor VIIa: on its own and loving it

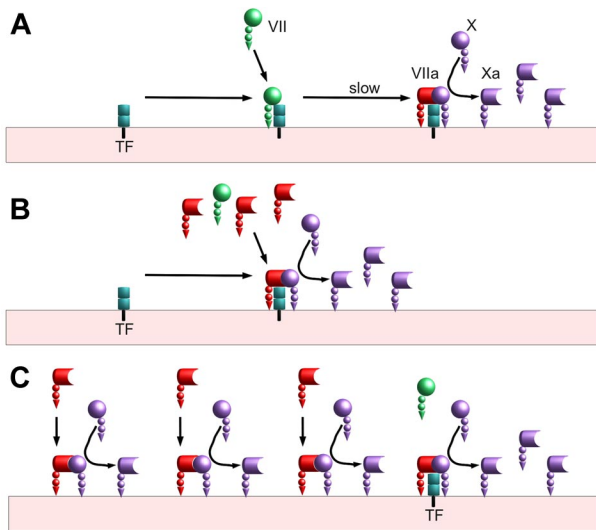
Dougald M. Monroe UNIVERSITY OF NORTH CAROLINA SCHOOL OF MEDICINE

In this issue of *Blood*, Shibeko et al have used a variety of experimental studies and mathematical models to investigate the mechanism of action of high-dose factor VIIa as a bypassing agent in hemophilia.¹

These studies address a vexing intellectual problem regarding the use of factor VIIa as a bypassing agent in hemophilia. The activity of factor VIIa, like that of coagulation factors IXa and Xa, is significantly enhanced when bound to its coagulation cofactor. Thus, factor IXa and factor VIIIa form a functional complex in vivo and the absence of either protein results in hemophilia. Similarly, lipid surface factor Xa activity is enhanced several thousand-fold when bound to factor Va and the complex of factor Xa with factor Va is required for physiologic thrombin generation. Because factor VIIa activity is significantly enhanced in the presence of its cofactor, TF, one might suspect that relatively low doses of factor VIIa would saturate available TF and provide the needed hemostatic activity.

In treating hemophilia patients with inhibitors, some of the initial dosing strategies included administering as little as 35 $\mu\text{g}/\text{kg}$ factor VIIa. This dose was predicted to lead to levels on the order of 0.5 mg/mL in plasma; a concentration roughly equal to the plasma concentration of factor VII (10nM). A dose of 35 $\mu\text{g}/\text{kg}$ was effective at stopping bleeding in some patients but was suboptimal in many others.² Clinical studies comparing doses of 35 $\mu\text{g}/\text{kg}$ against 70 $\mu\text{g}/\text{kg}$, 90 $\mu\text{g}/\text{kg}$, and 120 $\mu\text{g}/\text{kg}$ suggested that increasing dose were, in general, associated with increasing efficacy.^{2,3} Given that TF is almost certainly limiting in vivo, it was unclear why higher doses gave greater efficacy.

Biochemical studies approached this question and 2 theories emerged (see simplified



Factor VIIa activation of factor X. (A) Baseline mechanism: On a lipid surface, factor VII binds to tissue factor (TF) and is slowly activated, either by the 1% of plasma factor VII that is activated or by factor Xa generated by other VIIa/TF complexes. This VIIa/TF complex activates factor X to factor Xa before ultimately being shut off by tissue factor pathway inhibitor (TFPI; not shown). **(B) Tissue factor–dependent (competition) mechanism:** On a lipid surface, factor VIIa can compete with factor VII for binding to TF. The VIIa/TF complexes can immediately begin activating factor X without requiring a slow activation step. The VIIa/TF complexes can also activate any TF-bound factor VII, further speeding factor X activation. **(C) Tissue factor–independent mechanism:** In addition to binding to tissue factor, factor VIIa can bind lipid surfaces and convert factor X to factor Xa. This reaction is much slower than the reaction of TF-bound factor VIIa; however, the amount of surface-bound factor VIIa may exceed the amount of TF-bound by orders of magnitude. This reaction is also limited by the amount of available surface. Unlike factor VIIa in a TF complex, factor VIIa without TF is not inhibited by TFPI.

illustrations in figure). These theories were generally described as TF-dependent⁴ and TF-independent.^{5,6} In a sense this is an unfortunate nomenclature; even if factor VIIa at pharmacologic doses has measurable activity independent of TF, the overall coagulation process still requires initiation by TF just as it still requires a surface, calcium, factor X, factor V, and prothrombin. On average, investigators agree that it is biochemically possible for the reactions in both panels B and C of the figure to occur; the 2 theories differ in the degree to which the reactions shown in panel C are relevant to the physiology of high-dose factor VIIa as a therapy.

Shibeko et al have attempted to bring some clarity to this question and to improve on earlier mathematical models of this process. It has previously been shown that zymogen factor VII decreases the rate of VIIa/TF activation of factor X by competing with factor VIIa for TF; increasing the concentration of factor VIIa can overcome this inhibition effect of plasma factor VII.⁴ Shibeko et al also observe this competition on both lipid vesicles and on cells. Further, they add to the possible significance of the TF-dependent mechanism by studying how binding of factor VIIa to TF

might increase the rate of factor VII autoactivation. In part these studies involve some clever modifications to the existing mathematical models; these modifications account for TF density and for the fraction of factor VIIa bound to a surface (in a model that for all other reactions assumes infinite surface). Shibeko et al find that both models contribute to some extent to the enhanced thrombin generation in their biochemical and mathematical models but find that the TF-dependent mechanisms dominate. Their findings lower slightly the concentration of factor VIIa required to achieve a plateau because they find enhanced factor VII activation to be a major player.

The data of Shibeko et al add to our understanding of the biochemical mechanisms involved in factor X activation by factor VII(a) and TF. But in some sense these data still leave us with an intellectual problem regarding therapy. The authors note that it is not clear why increasing doses of factor VIIa would benefit a patient if the TF-dependent mechanism is predominant. And yet clinical studies looking at factor VIIa doses greater than 200 µg/kg suggest significantly increased

efficacy in the high-dose group compared with the lower dose groups.⁷

It may be that future studies can further add to our understanding. Starting from the conclusion that the TF-dependent mechanism is dominant in vivo, it is possible to make 3 specific, testable hypotheses. One hypothesis is that factor VIIa molecules engineered to have increased TF-independent activity with the same level of TF-dependent activity will have similar dosing requirements to existing factor VIIa molecule in vivo. The second hypothesis, an extension of the first, is that a factor VIIa molecule engineered to have no TF binding would be ineffective in vivo regardless of the level of TF-independent activity. Finally, a factor VIIa molecule engineered to have no (or very reduced) activity (zymogen-like factor VIIa) unless bound to TF would be predicted to be effective in vivo with dosing requirements similar to existing factor VIIa molecules.

Understanding the in vivo mechanism has potential consequences for new therapies. If the dominant part of the mechanism of high-dose factor VIIa is mediated through TF, it suggests that therapies that enhance TF function, such as tissue factor pathway inhibitors, may be sufficient to correct hemophilia. By contrast, if the dominant part of the mechanism of high-dose factor VIIa is independent of TF, then factor VIIa molecules with enhanced TF-independent activity,⁸ that are specifically targeted to the platelet surface, or other molecules that have directed enhancements of platelet surface thrombin generation⁹ may be better future directions for new therapeutics.

Conflict-of-interest disclosure: The author has received institutional research grants from Novo Nordisk and Inspiration Biopharmaceuticals as well as honoraria from Novo Nordisk for speaking. ■

REFERENCES

- Shibeko AM, Woodle SA, Lee TK, Ovanesov MV. Unifying the mechanism of recombinant FVIIa action: dose dependence is regulated differently by tissue factor and phospholipids. *Blood*. 2012;120(4):891–899.
- Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomised trial of two doses of rFVIIa (novoseven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost*. 1998;80(5):773–778.
- Lusher JM, Roberts HR, Davignon G, et al. A randomized, double-blind comparison of two dosage levels of recombinant factor VIIa in the treatment of joint, muscle and mucocutaneous haemorrhages in persons with haemophilia A and B, with and without inhibitors. rFVIIa study group. *Haemophilia*. 1998;4(6):790–798.

4. van 't Veer C, Golden NJ, Mann KG. Inhibition of thrombin generation by the zymogen factor VII: implications for the treatment of hemophilia A by factor VIIa. *Blood*. 2000;95(4):1330-1335.
5. Rao LV, Rapaport SI. Factor VIIa-catalyzed activation of factor X independent of tissue factor: its possible significance for control of hemophilic bleeding by infused factor VIIa. *Blood*. 1990;75(5):1069-1073.
6. Monroe DM, Hoffman M, Oliver JA, Roberts HR. Platelet activity of high-dose factor VIIa is independent of tissue factor. *Br J Haematol*. 1997;99(3):542-547.
7. Parameswaran R, Shapiro AD, Gill JC, Kessler CM. Dose effect and efficacy of rFVIIa in the treatment of haemophilia patients with inhibitors: analysis from the hemophilia and thrombosis research society registry. *Haemophilia*. 2005;11(2):100-106.
8. Holmberg HL, Lauritzen B, Tranholm M, Ezban M. Faster onset of effect and greater efficacy of NN1731 compared with rFVIIa, APCC and FVIII in tail bleeding in hemophilic mice. *J Thromb Haemost*. 2009;7(9):1517-1522.
9. Ivanciu L, Toso R, Margaritis P, et al. A zymogen-like factor Xa variant corrects the coagulation defect in hemophilia. *Nat Biotechnol*. 2011;29(11):1028-1033.