Tissue Factor in Brain Is Not Saturated With Factor VIIa
Implications for Factor VIIa Dosing in Intracerebral Hemorrhage

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The recent commentary by Dr Torbey on “Intracerebral Hemorrhage: What’s Next?” has prompted us to share some data and thoughts on the use of activated factor VII (FVIIa, NovoSeven, Novo Nordisk A/S) in the management of intracerebral hemorrhage. FVIIa is approved to promote hemostasis in hemophiliacs with inhibitors. Studies have also suggested that it promotes hemostasis in nonhemophilic patients with bleeding attributable to trauma or with intracranial hemorrhage.

The commercial FVIIa product is the activated form of the naturally occurring human protein. The precursor, FVII, is readily activated by a number of coagulation and noncoagulation proteases once it has bound to its cofactor, tissue factor (TF). FVIIa has little proteolytic activity in solution. Binding to a suitable phospholipid surface enhances its activity somewhat, but binding to TF greatly enhances proteolytic activity toward its substrates, FIX and FX. Plasma levels of FVIIa are normally around 100 pmol/L. Because of the very low activity exhibited by FVIIa in the absence of TF and the very high affinity of TF for FVII, it is thought that FVIIa only exhibits significant activity in vitro when complexed to TF. Exogenous FVIIa was originally thought to enhance hemostasis by increasing activation of FX by the TF pathway. However, very high levels of FVIIa are required to promote hemostasis in hemophilia: up to 1000× the normal plasma level and much more than should be needed to saturate the available TF. Evidence has now accumulated from in vitro, ex vivo, and animal studies to suggest that at these high concentrations FVIIa binds to activated platelets where it directly activates FX. The resulting FXa binds to its cofactor FVa to produce hemostatically meaningful levels of thrombin on the platelet surface. Thus, FVIIa probably acts primarily by a TF-independent mechanism in hemophilia.

It the presence of a full complement of the coagulation factors, FVIIa can still act in a TF-independent manner on platelet surfaces to promote thrombin generation. However, somewhat lower doses of FVIIa are required to significantly enhance of platelet-surface thrombin generation in nonhemophilic states, such as thrombocytopenia or trauma.

We have recently shown that most or all of the TF around vessels in the skin has bound FVII(a), even in the absence of an injury. This occurs when coagulation factors, including FVII, leave the vasculature in amounts roughly proportional to their molecular weight, and percolate through the extravascular space. Thus, no TF-dependent enhancement of hemostasis by exogenous FVIIa would be expected, because TF is already saturated with endogenous FVII. We now provide evidence that this scenario may be different in the central nervous system.

We hypothesized that the level of “free” TF (TF that is not bound to FVII) might be high in brain for three reasons: (1) the total amount of TF in the brain is extremely high; (2) brain tissue is separated from the plasma coagulation factors by the blood-brain-barrier; and (3) clinical trials suggest that lower levels of recombinant FVIIa might be sufficient to reduce hematoma growth in brain compared to other types of hemorrhage.

Biotinylated active site–inhibited FVIIa (FVIIai-biotin) has been used as a probe to detect active TF in frozen sections of human tissue. We have used the mouse version of this reagent to detect “free” TF in formalin-fixed sections of mouse skin. In our modification of the technique, fixation with formalin crosslinks TF to adjacent molecules. The FVIIai-biotin reagent cannot bind to crosslinked FVIIa/TF complexes, because its binding site is blocked by the endogenous FVIIa.

In the current study we used FVIIai-biotin to examine brain tissue from the same mice (C57B) used for the skin studies, under a protocol approved by the University of North Carolina IACUC. Tissue fixation, paraffin embedding, sectioning and staining were carried out as previously described.

We found that FVIIai-biotin bound extensively to the brain parenchyma (Figure, A), as well as around vessels (Figure, C and D). In addition, the ependyma bound the probe strongly (Figure, E). Background nonspecific staining from endogenous biotin was minimal (Figure, B and F).

Our findings potentially have implications for dosing of recombinant FVIIa in patients with intracerebral bleeding. In this setting, it seems likely that the exogenously administered...
FVIIa in plasma will gain access to “free” TF at sites that are damaged by trauma or dissecting blood.

If FVIIa acts in a TF-dependent manner at sites of bleeding in the central nervous system, the concentration of FVIIa required for optimal hemostasis will likely be much lower than when FVIIa acts in a TF-independent manner on the surface of activated platelets. The affinity of TF for FVIIa is quite high, with a dissociation constant around 50 picomoles/L. By contrast, the affinity of activated platelet membranes for FVIIa is orders of magnitude worse, with a dissociation constant in the micromolar range. We do not expect these affinities to translate directly into the dose required for efficacy, because there may be so much “free” TF in brain tissue that it depletes the local concentration of FVIIa. However, we do suggest that the optimal dose of FVIIa for intracerebral hemorrhage will be considerably lower than the optimal dose for hemophilic bleeding (120 to 270 µg/kg) or even surgical or trauma-associated bleeding at non-CNS sites. This hypothesis is consistent with trials examining the effects of FVIIa in acute intracerebral hemorrhage. In one of the trials doses as low as 40 µg/kg reduced hematoma expansion, and there was a trend toward reduction in hematoma expansion at 20 µg/kg in the second trial. However, the risk of arterial thrombosis appeared to increase with increasing dose. We speculate that the dose-response curve for reducing hematoma expansion may not run in parallel with the risk of thrombosis. If this is so, it may be possible to select a dose and dosing schedule of FVIIa that provides maximal hemostatic benefit at a minimal thrombotic “cost.”

We agree with Dr Torbey that future studies should include combinations of treatments, rather than a single drug or procedure. It seems as though FVIIa may have a role to play in the management of intracerebral hemorrhage, and a low-dose regimen may be of particular benefit when combined with other interventions.

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### References


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