Factor VIIa gets even bigger

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Since its introduction onto the market in 1996, recombinant factor VIIa (rFVIIa: NovoSeven®; Niastase®; produced by Novo Nordisk, Baegsvaerd, Denmark) has become an increasingly important therapeutic option for physicians caring for haemophilia patients with inhibitors. Both because of its short terminal half-life of approximately three hours (1, 2), and the ways in which it bypasses factor VIII or IX deficits to restore haemostasis (3, 4), rFVIIa must be used in large, supraphysiological doses of at least 90 μg/kg bodyweight, often repeatedly. Notwithstanding this limitation, the safety and efficacy of rFVIIa (5) in meeting often urgent clinical needs have propelled the product to annual sales in excess of $1 billion. The expansion of rFVIIa use into more general surgical and trauma care has, not without controversy, also begun to be explored (6).

In this issue of Thrombosis and Haemostasis, Weimer et al. contribute to this theme of FVIIa enlargement — but at the molecular level (7). In this first report of a biologically-active rFVIIa fusion protein (rFVIIa-FP), cDNAs encoding human serum albumin (HSA) and FVII, separated by an intervening 93 base oligonucleotide spacer, were combined. At the protein level, rFVIIa-FP therefore contained all 585 amino acids of HSA, as an extension of the carboxy terminus of the FVIIa heavy chain, separated by a 31 amino acid glycine/serine linker. This strategy distanced the albumin moiety from the amino-terminal Gla and first epidermal growth factor-like (EGF-1) domains of rFVIIa in rFVIIa-FP, domains which are essential for high-affinity binding to the FVIIa cell surface receptor and enzymatic co-factor, tissue factor (TF). Consequently, purified rFVIIa-FP bound TF with an affinity comparable to NovoSeven. The specific molar enzymatic activity of rFVIIa-FP in vitro, determined chromogenically, was 60–70% of unfused wild-type rFVIIa controls, irrespective of whether rFVIIa-FP was expressed in human HEK 293 or rodent CHO cells. In vivo the plasma half-life of rFVIIa-FP in rats was extended by six to seven fold versus unfused rFVIIa, and recovery of the initial injected dose was also increased. Most impressively, the procoagulant effects of rFVIIa-FP were still readily apparent 16 hours after administration to FVII-depleted rats, a time at which the effects of equimolar NovoSeven had completely dissipated.

While these promising findings need extension into bleeding models, using both normal and haemophilic animals, Weimer et al. convincingly demonstrate that FVIIa is another example of a therapeutic protein whose biological function can be conserved, and its clearance from the circulation slowed, by genetic fusion to albumin. This strategy, first reported by Patrice Yeh et al. in 1992 (8), has been shown to confer at least some of the stability and plasma longevity of albumin onto smaller fusion partners, such as the leech thrombin inhibitor hirudin (9), interleukin 2 (10), brain natriuretic peptide (11), and interferon-α-2b, the latter being in phase 3 clinical trials (12). Albumin fusion can allow fusion partners to evade glomerular filtration by virtue of their increased molecular volume and charge density, and likely also benefits from albumin recycling mediated by the nonclassical major histocompatibility complex-I molecule FcRn (13). The outcome of albumin fusion experiments, however, can by no means be taken for granted, as shown by the finding that a coagulation factor IX-albumin fusion protein failed to acquire an albumin-like clearance profile, but instead exhibited a plasma half-life close to that of factor IX (14). The more desirable results reported by Weimer et al. for rFVIIa-FP may derive from a lesser contribution of specific receptors, such as endothelial collagen IV, to the clearance of FVII(a) than to that of factor IX (15).

Recently, it has been reported that FVIIa binds with high affinity to the endothelial cell protein C receptor (EPCR), although it is not yet established whether or not this binding leads to degradation of rFVIIa, or confirmed that it operates in vivo (16). This finding suggests the intriguing possibility of clearance of rFVIIa-FP being a competition between EPCR and FcRn, with FcRn dominating either kinetically or due to interference by the HSA moiety of rFVIIa-FP with FVIIa-EPCR binding. These issues, and similar fundamental questions, such as whether or not rFVIIa-FP can be regulated by tissue factor pathway inhibitor and/or antithrombin in its complex with TF and factor Xa, remain to be addressed. Some of these questions, in combination
with the critical question of efficacy in bleeding models, will also have to be answered for a NovoSeven analogue that has been “glycopegylated”, modified by the addition of polyethylene glycol chains to its glycans (17). Whether neither, one, or both of these novel forms of rFVIIa will enlarge its already considerable therapeutic role, and not just its molecular volume and terminal half-life, remains to be seen.

References