Increasing the *in vivo* half-life of factor VIIa by attachment of the natural polysaccharide heparosan

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Objective

- Investigate the size-dependent effect of heparosan-conjugation on the pharmacokinetics (PK) and *in vitro* activity of factor VIIa (FVIIa)

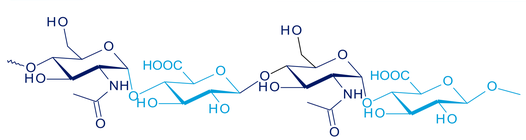
Conclusions

- The naturally occurring polysaccharide, heparosan, was found to be as effective as PEG in prolonging the half-life of FVIIa in rats
- At an optimal polymer size of 40-kDa, the heparosan-FVIIa conjugate retained a higher proteolytic activity compared to PEG
- The use of heparosan as a potentially superior half-life extension principle for FVIIa is currently being further explored

Introduction

- While FVIIa is safe and efficacious for on demand treatment of bleedings in haemophilia patients with inhibitors, a longer acting FVIIa molecule would be desirable for routine prophylaxis
- Polyethylene glycol (PEG) conjugation is effective in prolonging the *in vivo* half-life of FVIIa, however, at the expense of a reduction in activity[1,2]
- Heparosan (HEPtune™, Caisson Biotech) is a new promising polymer for half-life extension that is naturally occurring and composed of [-β1,4-*N*-acetylglucosaminyl-α1,4-glucuronyl-] disaccharide repeats (Figure 1). Heparosan can be produced chemo-enzymatically, which allows for tailoring of size and incorporation of handles for conjugation to payloads[3]

Figure 1 Structure of heparosan disaccharide repeat



Methods

Polymer conjugation

- Heparosan polymers were produced in sizes from 13 to 157 kDa and functionalized with a maleimide moiety at the reducing end. Each polymer was then conjugated site-specifically to a FVIIa variant containing a free C-terminal cysteine (FVIIa 407Cys). For reference, similar size PEG-FVIIa 407Cys conjugates were prepared.

Pharmacokinetics

- The FVIIa conjugates were administrated as a single intravenous (iv) bolus dose of 20 nmol/kg in the tail vein of three Sprague Dawley rats. Blood samples were collected as appropriate, diluted in assay buffer and analysed using a sandwich ELISA specific for human FVII. Results were subjected to non-compartmental analysis using Phoenix WinNonlin (Pharsight).

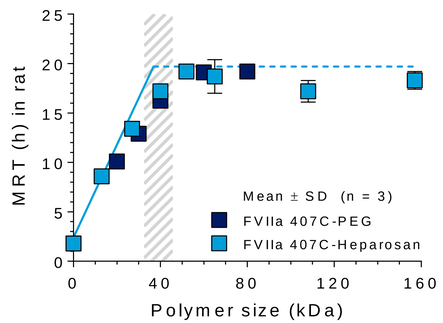
Proteolytic activity

- The proteolytic activity of FVIIa and polymer conjugates (10 nM) was measured under k_{cat}/K_m conditions at a single FX concentration (40 nM) in 50 mM HEPES, 100 mM NaCl, 10 mM $CaCl_2$, pH 7.4 buffer containing 0.1% PEG8000, 1 mg/ml BSA and 25 µM PS:PC vesicles.

Results

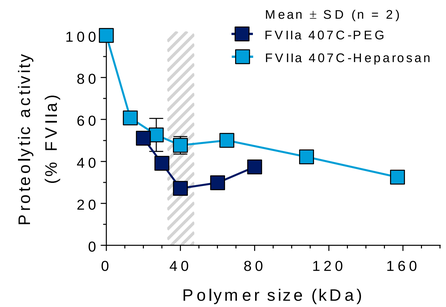
- For both the heparosan and PEG-conjugates, the effective half-life (mean residence time, MRT) of FVIIa was observed to increase with increasing polymer size up to approximately 40 kDa. Above this size no further extension of half-life was observed (Figure 2)

Figure 2 Relationship between polymer size and effective half-life (MRT) of the FVIIa conjugates in rat



- The effective half-life was 17.1 ± 0.7 h for the 40 kDa heparosan conjugate and 13.9 ± 0.1h for the 40kDa PEGylated conjugate. In comparison the effective half-life of FVIIa was 1.7 ± 0.1 h (n = 3 rats)
- The heparosan conjugates retained higher proteolytic activity as compared to the corresponding PEG conjugate. At the optimal polymer size of 40 kDa the specific proteolytic activity was (48±4)% as compared to (27±1)% for the corresponding PEG conjugate (Figure 3)

Figure 3 Relationship between polymer size and *in vitro* proteolytic activity of the FVIIa conjugates



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3. DeAngelis, *et al.* PL Espert Opin Drug Deliv 2015; 12(3):349-352.

Conflict of interest disclosure

The authors are employees at Novo Nordisk A/S and Caisson Biotech LLC respectively.

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