NRC·CNRC

Extending *in vivo* half-life of therapeutic proteins (L-11944)

Highlights

Post-translational modifications of therapeutic proteins are commonly made to improve their circulating half-life, thereby enhancing their efficiency. Numerous strategies have been employed towards this end, including covalent modification, such as through PEGylation – the chemical addition of chains of polyethylene glycol (PEG) to therapeutic proteins – which improves protein stability and solubility, prevents proteolytic degradation, and reduces the clearance rate from the blood-stream. However, PEGylation relies on chemical conjugation of PEG chains to free amino groups or engineered cysteine residues on the protein, which can lead to heteroge-neously-modified proteins whose activity can be adversely affected.

To address this, the NRC has developed a site-specific, two-step *in vitro* modification process whereby polysialic acid (PSA) is enzymatically added to existing glycans, resulting in proteins with greater stability, solubility and circulating half-life.

Technology transfer

› A commercial exploitation licence for the technology

Market applications

› Improve the half-life of therapeutic proteins used to treat a wide range of diseases

A1AT construct	AUC (hr*ng/ml)	t1/2a (hr)	t1/2b (hr)	CL (ml/hr)
A1AT	149.9 ± 47.12	0.44 ± 0.10	5.01 ± 1.47	14.33 ± 4.77
diSA-A1AT	984.3 ± 234.3	0.42 ± 0.031	13.45 ± 1.34	2.11 ± 0.51
PSA-A1AT	2691 ± 303.7	1.12 ± 0.14	27.32 ± 1.29	0.75 ± 0.085

How it works

Many therapeutic proteins are glycosylated during production, both when recovered from human serum and when expressed in cell lines. The terminus of these glycans acts as a handle for further modification. The NRC two-step modification process consists of deploying proprietary enzymes at the N-linked glycan handle to achieve site-specific polysialylation. The technology can be used to modify a variety of glycan structures including bi-, tri-, and tetra-antennary glycans with either α-2,3- or α-2,6-linked terminal sialic acids.

The NRC has demonstrated the ability of this system to modify three glycoproteins with varying N-linked glycan compositions including the human therapeutic proteins alpha-1-antitrypsin (A1AT) and factor IX, as well as bovine fetuin. Polysialy-lation of A1AT was achieved without adversely affecting its function as an elastase inhibitor. In mouse models, the modified A1AT has shown a marked increase in biological half-life with no loss of bioactivity, a shift in elimination route towards the liver, and no observation of abnormal uptake of PSA-modified A1AT in other organs. The process has been shown to achieve an 18-fold greater bioavailability with PSA-A1AT compared to unmodified A1AT.

Benefits

› Improved stability, solubility and circulating half-life of therapeutic proteins

› Lower doses of therapeutic proteins and lower frequency of dosing required

Patents

NRC file 11944: Patents granted in the United States and China, pending in Canada, Europe and Japan

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