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Generation of long acting therapies using glycosylated linkers

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Abstract

Rationale: The current therapeutic drugs such as, growth hormone (GH), granulocyte colony stimulating factor (GCSF) and leptin require once daily injections, which are inconvenient and expensive. Therefore, a number of approaches to reducing therapeutic regimens clearance have been tried mainly through conjugation with another moiety. One such technology already being employed is PEGylation; however this has been shown to be non-biodegradable and toxic. A previous study by Asterion has shown that the use of glycosylated linkers between two GH ligands to create protein tandems resulted in their glycosylation and an increased molecular weight (MW) whilst maintaining biological activity. The use of this technology using GCSF as an example will be presented, but can be easily applied to other molecules such as leptin. Hypothesis: The incorporation of variable glycosylated linkers between two GCSF ligands will create a construct with high molecular weight and protected from proteolysis resulting in reduced clearance without blocking bioactivity. Methodology: GCSF tandems with linkers containing between NAT glycosylation motifs and their respective controls (Q replaces N in the sequence motif NAT so there is no glycosylation) were cloned, and sequenced. both native GCSF and the non-glycosylated tandem protein
Following expression in Chinese hamster ovary (CHO) cells, expressed protein was analysed by SDS PAGE to confirm molecular weights. In vitro bioactivity was tested using an AML proliferation assay.
Immobilized metal affinity chromatography (IMAC) was used to purify the protein. Pharmacokinetic and pharmacodynamics properties of the purified GCSF tandem proteins were measured in normal Sprague Dawley rats with full ethical approval. Results: Purified glycosylated tandems show increased molecular weight above that of controls when analysed by SDS PAGE. All GCSF tandems show increased bioactivity in comparison to native GCSF. Following intravenous administration to rats, GCSF2NAT, GCSF4NAT, GCSF8NAT containing 2, 4 & 8 glycosylation sites respectively and GCSF8QAT (non-glycosylated GCSF tandem control) showed approximately fold longer circulating half-life compared to that reported for the native GCSF (1.79 hours). Both GCSF2NAT and GCSF4NAT show a significant increase in the percentage of neutrophils over controls at 12 hours post injection. This effect however is short lived as the counts at 24+ hours are not significantly different to controls. GCSF8NAT shows an increase in the percentage of neutrophils that is only significant at 48 hours. Conclusion: Results show that the use of glycosylated linkers to generate GCSF tandems results in molecules with increased molecular weight, improved in vitro bioactivity, longer circulating half-lives and enhanced neutrophilic population when compared to

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