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Glycosylation and pharmacokinetics of human thyrotropin

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Human thyrotropin (hTSH) is an heterodimeric glycoprotein hormone consisting of two peptide chains or subunits (α and β), the α-subunit with two N-glycosylation sites (at Asn 52 and Asn 78) and the β-subunit with only one (at Asn 23). Considering that glycans (and their structures) play a critical role in the synthesis and biological activity of hormones, the aim of this work was to analyze the impact in pharmacokinetic properties of three thyrotropin preparations with differences in their glycidic portion. Two of them are CHO-derived and have different terminal glycan configurations: sialic acid linked to galactose in α2,3 (rhTSH) and in α2,3 + α2,6 (human like sialylated recombinant hTSH, hlsrhTSH). The third analyzed preparation was pituitary-derived (phTSH) which has α2,3 + α2,6 linkages. Monosaccharide composition analysis showed that while N-glycan structures of the two recombinant preparations were all of the complex type, in the native hTSH preparation high mannose, hybrid and complex structures were identified. About 84-86 % of the oligosaccharides of these three preparations were negatively charged: the recombinant preparations having terminal sialic acid and the native preparation with terminal sulphate and/or sialic acid. The latter showed that most of the charged carbohydrate structures had terminal sulphate while only ~ 1/3 had terminal sialic acid. About one half of the sialylated structures were also sulphated. Variable levels of sialylation (mono to tetra) were found in the recombinant preparations, while in the native preparation only monosyalylated structures were identified. In the terminal sulphate, a variable level (mono to tri) was observed. The overall level of glycosylation of the recombinant preparations was found higher than that of the pituitary preparation, being the total amount of carbohydrate 17.1%, 14.8% and 9.5%, respectively for rhTSH, hlsrTSH and phTSH. Glycosylation site-occupancy (glycans/hTSH molecule) for these preparations were ~ 2 for rhTSH and p-hTSH while a lower occupancy was found for hlsr-hTSH (1.2 glycans/molecule). The most critical sugar, for what concerns hormone circulatory half life, is N-acetyl neuraminic acid (Neu Ac) or sialic acid (SA), a higher content of SA (2.8-3.4 fold) being found in recombinant compared to pituitary preparation. The influence of these glycosylation differences on hTSH in vivo stability was evaluated by a comparative analysis of serum half-life and of the area under the elimination curve ($AUC_{0\,5h}$ μg/min/ml) of the three hTSH preparations in mice. The two recombinant hTSH with different sialic acid linkages did not differ significantly, not showing in mice an increased therapeutic efficacy due to CHO sialylation against human-like sialylation. On the other hand, these preparations revealed a ~ 2-fold increased half-life compared with pituitary hTSH, the AUC increasing also ~ 1.7-fold. These findings indicate that the increased sialic acid content of the recombinant hTSH preparations improved hTSH in vivo effects due to an increased circulation time.