Comparative studies of pituitary (NIDDK, USA) and recombinant (Thyrogen and IPEN) human thyroid stimulating hormone (hTSH) for what concerns carbohydrate structures and charge heterogeneity

Oliveira, J.E.; Loureiro. R.F.; Carvalho, C.M.; Damiani, R.; Bartolini, P.; Ribela, M.T.C.P.

Biotechnology Department, IPEN-CNEN São Paulo, Brazil

Three thyrotropin preparations, two of them CHO-derived (Thyrogen and r-hTSH IPEN) and the third pituitary-derived (p-hTSH from NIDDK. USA) were analyzed for monosaccharide composition by combined gas chromatography/mass spectrometry (GC/MS) of the per-O-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced by acidic methanolysis. It was found that the glycosylation of r-hTSH differ from that of p-hTSH in both the extention and type of glycosylation. The overall level of glycosylation of the recombinant hTSH was found higher than that of the pituitarypreparation. A total carbohydrate content of 14.5%, 20% e 6% respectively for Thyrogen, r-hTSH IPEN and p-hTSH was found. Unlike the native protein (p-hTSH), no sulfated N-acetyl galactosamine (GalNAc) was detected on the recombinant protein. Higher contents of N-acetyl glucosamine (GlcNAc) were found in the recombinant compared with the pituitary preparation: 1.8 and 2.6 times for Thyrogen and r-hTSH IPEN respectively. Levels ~8 times higher of galactose (Gal) were found for the two recombinant preparations. Higher mannose (Man) levels were also observed in recombinant preparations. N-acetyl neuraminic acid (Neu Ac) or sialic acid, the most critical sugar for what concerns circulatory half life, was also observed in both, CHO-derived and in pituitary-derived hTSH. The highest NeuAc content was found in r-hTSH IPEN: 3.4% against 1.4% and 0.4% for Thyrogen and p-hTSH respectively.

Structural investigations on the N-glycans of the r-hTSH preparations revealed N-complex type glycans with bi (predominantely), tri and tetraantenarity (in a minor level) forms, with a variable level of sialylation. Of these structures, 86.9% were found to be sialylated while 13.7% were neutral. Both fucosylated and nonfucosylated species have been identified. The predominant (36.02%) oligosaccharide species was monosialylated biantennary complex type oligosaccharide.

Since differences in glycosylation can generate charge heterogeneity, we examined the isoelectric focusing profiles (IEF) of these three hTSH preparations. Surprisingly the major bands appearing on IEF were the same for the three preparations, focusing on pI 5.75, 6.03 and 6.46. For Thyrogen, six bands were visible in the pI range 5.20–7.35. For r-hTSH IPEN, the distribution of charge isomers was similar, but three more bands were observed at extremes pI: 4.71; 5.04; 7.88. In the case of p-hTSH nine bands were observed in the pI range 4.55 - 7.35. The higher sialylation observed in the two recombinant preparations is probably responsible for the higher activity that we previously observed in the in vivo bioassay in mice. This fact may have important clinical effect.

Supported by FAPESP and CNPq

11433