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POSTER ABSTRACT FORM

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THE ROLE OF SULFATION AND SIALYLATION ON THE BIOACTIVITY AND METABOLIC CLEARANCE RATE OF RECOMBINANT HUMAN TSH (rhTSH) AND rhTSHβ-hCGβ CARBOXY TERMINUS EXTENSION PEPTIDE CHIMERA.

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Begin Text ➤

Previous studies from our laboratory have shown that unlike N-linked oligosaccharides of pituitary TSH which primarily terminate in N-acetylgalactosamine-sulfate, those on recombinant human TSH expressed in Chinese hamster ovary (CHO) cells terminate in galactose-sialic acid. Recently, N-acetylgalactosamine-transferase and sulfotransferase have been demonstrated in human embryonic kidney (293) cells. We thus reasoned that rhTSH and its variants when expressed in 293 cells and possibly monkey kidney (COS-7) cells would produce TSH with N-linked oligosaccharide structures terminating in sulfate. Because previous studies with chimeric FSH produced in CHO cells showed increased half-life, we constructed a similar chimera of hTSHβ-subunit with the carboxy terminus extension peptide (CTEP) of hCGβ subunit. COS-7 and 293 cells were cotransfected with plasmids containing either WT or chimeric TSH with hCGαcDNA. Bioactivity was determined in two FRTL5 assays (cAMP production; ^{3}H Thymidine uptake). Both chimeric and WT TSH expressed in 293 and COS-7 cells displayed similar bioactivity suggesting that terminal sulfation & sialylation of oligosaccharides does not alter *in vitro* bio-activity. The metabolic clearance rate (MCR) of chimeric TSH and WT TSH secreted by COS-7 and 293 cells was compared to that of WT TSH produced by CHO cells. The presence of sulfate had a dramatic effect on the MCR of WT TSH (293) which was cleared 3 times faster than WT TSH expressed in CHO cells. Interestingly, COS-7 cells had a clearance rate closer to that of CHO cells than 293 cells suggesting the presence of sialic acid. The maximum increase in circulatory half-life was demonstrated by chimeric TSH (COS-7) which showed significantly reduced MCR (3-4 fold). Despite the presence of CTEP in chimeric TSH produced by 293 cells, its MCR was identical to that of WT TSH from COS-7 cells indicating the absence of sialic acid. These results suggest that chimeric TSH produced in various cell lines can be used as a tool to delineate the role of sulfate and sialic acid in MCR and thereby the *in vivo* bioactivity.

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