"Novel methods for identification of recombinant glycoprotein hormone extension"

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Project overview:

Prohibited glycoprotein hormones such as ESA, hCG, FSH etc. are currently tested by immunological methods, and the decision is made by immuno-blotting electrophoresis or by comparing concentration with the applicable threshold. However, heterogeneity of the markers or multiple sources of the target compound, e.g. pregnancy, hormone producing tumor, doping etc. can sometime cause in difficulty of decision making. We confirmed that all recombinant glycoproteins from CHO cell line studied were lacking certain human type glycanes, which are not always identifiable by mass spectrometry. Our first year results represented that recombinant and human glycoprotein hormone can be isolated by stereo specific lectin-glycane interactions, and the recombinant fraction does not show any human type glycane specific lectin interactions. Thus, findings of our 1st year project supported the possibility of origin specific detection of glycoproteins by lectin specific extraction coupled to a normal immunoassay instruments that commonly used by all WADA laboratories.

Results and Conclusions:

Genetically manufactured glycoprotein has the unique isoform profile and the heterogeneity can sometimes cause in interpretation difficulties. Prohibited glycoprotein hormones are currently tested by immunological methods with the result evaluation using the applicable threshold or by comparing the isoform profile with that of the reference standard as no procedure yet available to identify their origins. Glycoproteins from human show a strong lectin-glycan interaction with certain lectins whereas those expressed in CHO-cell lines do not show any interaction with the selected lectins. Immobilized SSA lectin was suspended with a sample aliquot and non-reactive glycoprotein was collected by an exclusion chromatography. Rest of captured hormone was then eluted with Lactose solution and each fraction was measured by immunoassays. SSA captures LH and FSH from human, and Lutropin (rLH) and Follitropin (rFSH) are collected in the Filtrate. IEF gel electrophoresis western blotting analysis and well-evaluated immunoassays confirmed that FSH in the Filtrate and the Eluate were identical to those of rFSH and uFSH respectively. Pre-analysis lectin fractionation coupled with immunoassay has enabled an origin identification of LH and FSH. Some other possible markers to indicate origin of glycoprotein were evaluated.