

PROJECT REVIEW

“Bitter-Sweet Differences Between Native and Recombinant Glycoprotein Hormones-Application to Antidoping Strategies”

R. Gutierrez-Gallego, J. Segura, R. Ventura, R. Ramirez Llanelis
(Municipal Institute of Medical Research Foundation, Barcelona, Spain)

Human chorionic gonadotropin (hCG) and human lutropin (hLH) are hormones synthesised or purified by certain pharmaceutical companies for treatment of infertility in women. However, both hormone act through the same receptor in the body and may trigger the production of testosterone. As such these hormones may be attractive molecules for athletes looking for illicit performance enhancement.

Both hormones belong to the family of glycoproteins, which means that protein carry a particular decoration known as glycosylation. The glycosylation of both hormones has been found to be of utmost importance for the correct functioning of the molecules. Furthermore, it is known that glycosylation is a phenomenon that is not primarily regulated by particular genes. In stead, glycosylation is the result of the concerted action of many primary gene products, donor and acceptor substrate availability, and additional external factors (temperature, presence of growth factors, etc.).

As such, the glycosylation is cell type specific. Recombinant glycoprotein hormones will therefore carry glycans synthesised by the machinery of the host cell and these glycans may be different from those synthesised by the natural producers. Detailed structural analysis of the glycosylation of the endogenous and exogenous hormones will reveal the cell-type specificities.

Once established, the cell-type specific structural elements may be used as targets for the development of new, faster, and more reliable screening and confirmation procedures.

Results and Conclusions

“Bitter-sweet differences between native and recombinant glycoprotein hormones- application to antidoping strategies –”

Human chorionic gonadotropin and human lutropin belong to the family of glycoprotein hormones that are used in the treatment of female fertility problems. Under normal circumstances both hormones result in the production of testosterone. This is the main reason for its potential abuse by athletes: increasing the testosterone concentration without affecting the ratio between testosterone and epitestosterone. While both male and female have lutropin (produced in the pituitary), chorionic gonadotropin is produced by the placenta under term conditions, and as such restricted to the female gender. Even though under ordinary circumstances hCG is not produced by men, its production has been described under certain pathological situations. As such, its sole detection in a biological specimen is not sufficient.

These hormones, being glycoproteins, carry the particular signature of their origin through the carbohydrate decoration. This post-translational modification depends very much on the environmental settings and could be different in recombinant and endogenous species. As such, the basic principle of this application was the analysis of the glycosylation of exogenous and endogenous CG and LH with the aim of establishing the existence of structural differences that would enable unambiguous discrimination.

Both hormones share the α -subunit, glycosylated at Asn52, Asn78, and Thr39 (the latter only described in CG), and have different β -subunits glycosylated at Asn30 in both and at Asn13 only in CG. Furthermore, O-glycosylation has been described for CG at Ser121, Ser127, Ser132, and Ser138. Pure glycoproteins (both prepared from pooled urine of pregnant women, isolated from pituitary material, or recombinant from different sources) were prepared or purchased. Glycoproteins were analysed at the intact level by mass spectrometry and sugar analysis. While the former revealed subtle molecular weight differences mainly in the α -subunit the latter proved too insensitive. A directed analysis of the sialic acid residues that end-cap most of the glycans revealed the presence of the nonhuman residue N-glycolyl neuraminic acid both in proteins expressed in CHO, as well in murine cells. The identification of this residue in hCG and LH, and already previously identified in recombinant erythropoietin, represents a single target to address recombinant glycoprotein pharmaceuticals. Analysis of the N-linked glycans revealed that murine glycosylation is different with the existence of terminal Hex(α 1-x)Hex-R epitopes. CHO derived material also displays a slightly different glycosylation for hCG. The endogenous glycosylation machinery results in a significant part of hybrid type structures (predominantly at the α -subunit) that is much less pronounced in the exogenous material. Similar results were obtained for the lutropin preparations. Analysis of the de-N-glycosylated hCG and proteolytic peptides showed that no significant differences exist in terms of other peptide modifications or potential O-glycosylation occupancy although a thorough comparison of the glycans is still ongoing.