PROJECT REVIEW

"Analysis of growth hormone isoform profiles in human plasma using proteomics strategies"

M. Thevis (German Sport University, Cologne, Germany), **A. Tonevitsky** (Russian Research Institute, Moscow, Russia), **W. Schänzer** (German Sport University, Cologne, Germany)

Growth hormone (GH) therapy has become a frequently employed strategy to fight dwarfism, and with the advent of biotechnologically produced recombinant GH, purity and sufficient amounts have been guaranteed. However, the need to prepare recombinant peptide hormones and repetitively inject drugs has led to gene therapy approaches that aim to generate GH-producing cells. Early attempts were based on myoblasts and fibroblasts modified to produce GH as growth hormone gene therapy does not necessarily need to be pituitary-specific because targets of GH are peripheral organs.

The abuse of GH in sports has been a serious issue since years, and first assays enabling the discrimination between natural isoforms and recombinant GH have been established using a so-called differential immunoassay approach. More detailed information on GH isoforms used for the determination of GH administration is obtained using proteomics approaches employing 2D gel electrophoresis followed by mass spectrometric identification and characterization of GH and its fragments. In order to provide fundamental information for new methods complementing the commonly accepted assay and to provide basics for another option to cope with the problem of GH misuse, which includes administration of recombinant GH as well as gene therapy, GH isoforms shall be isolated of from human plasma followed by 2D gel electrophoresis, visualization, and subsequent mass spectrometric identification. Using this strategy, GH isoforms can be considered individually in terms of a profile, and more information on the variability and stability of single items is obtained. Owing to the well known fact that endogenously produced GH is down-regulated upon GH administration, the considerable change in GH isoform profiles should be detected using proteomics technology. Moreover, the fact that future GH gene therapy may include cells different from the pituitary, significantly different isoform profiles are possible and should principally be detectable employing the technology established in this project.

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Results and Conclusions

Growth hormone is widely abused by athletes for its anabolic and lipolytic as well as growth promoting effects. The presented method is capable of discriminating endogenous and recombinant growth hormone in plasma or serum samples if the concentration is high enough to detect endogenous isoforms which is the case for samples with a normalized spot volume of the main 22 kDa spot of > 0.52. The capability to detect and visualize discrete isoforms of hGH represents an important advantage for confirmatory GH analyses in sports drug testing and could complement currently employed assays to reveal GH misuse. While endogenous samples show two or four isoforms on the blots, samples containing recombinant growth hormone lead to detection of one spot only. In addition to the volume limit, the order of appearance is considered and the detection of the main isoform must be accompanied by the 20 kDa splice variant prior to the detection of a more acidic 22 kDa spot, which is phosphorylated in endogenous samples but may be an artefact from protein production in samples containing recombinant growth hormone. The suggested discrimination limit of 0.52 may be corrected to even lower values if larger samples populations are analyzed.

Additionally all growth hormone variants that are detected in the doping control method were identified by mass spectrometry approaches. This makes the doping control method even more powerful and allows a better evaluation of results.

Publications

Kohler M, Thomas A, Püschel K, Schänzer W, Thevis M. Identification of human pituitary growth hormone variants by mass spectrometry. J Proteome Res. (2009); 8(2):1071-6.

Kohler M, Püschel K, Sakharov D, Tonevitskiy A, Schänzer W, Thevis M. Detection of recombinant growth hormone in human plasma by a 2-D PAGE method. Electrophoresis (2008);29(22):4495-4502.