PROJECT REVIEW

"Development of a Test Method to Detect Growth Hormone Abuse in Sports"

Recently, we have demonstrated that it is possible to detect the administration of recombinant human growth hormone (hGH) based on a "differential immunoassay approach" (Wu et al., The Lancet, *353 (9156),* 1999). The rationale for this approach is that the protein hormone hGH as secreted by the pituitary gland consists of several different isoforms (major hGH isoforms: 22,000 Dalton isoforms, minor isoforms: 20,000 Dalton, 12,000 Dalton and various other chemically modified isoforms and fragments), whereas recombinant growth hormone is a purified form of hUH with a distinct molecular weight of 22,000 Dalton only

Screening a large panel of monoclonal antibodies raised against pituitary and recombinant hGH, respectively, we were able to identify specific antibodies and to set up two different immunoassays: One assay, which recognizes all different isoforms of hUH, and another assay, which preferentially recognizes 22,000 Dalton-hUH monomers.

The administration of recombinant hGH leads to a dramatic proportional increase in the 22,000 Dalton-hGH isoforms in circulation, whereas the other isoforms~ relative abundance is diminished. Measuring a blood sample by both assays, i.e. the permissive assay and the 22,000 Dalton monomer hGH specific assay, allows to calculate the relative abundance of the 22,000 Dalton-hUH isoform.

Measuring a series of blinded samples, we were able to identify all those sera which had been drawn after administration of recombinant hUH, because the "22,000 Dalton-hUH specific assay result" in comparison to the "all hUH isoforms assay result" indicated a much higher proportion of 22,000 Dalton-hUH in these samples compared to the placebo group samples.

Funded by the German "Bundesinstitut für Sportwissenschaften", this test method for blood samples is currently being evaluated by a blinded analysis of a large series of samples obtained from the GH2000 double blind study.

Proposal for an independent test method, possibly to be used as a confirmatory test

The test method described above can be interpreted as a "proof of principle", that the detection of the administration of recombinant hGH is possible by the analysis of the hUH isoforms composition in a sample. It is most likely that a more detailed analysis of these isoforms will lead to an improved detection method. To make such a method court proof, it is necessary to demonstrate the change in isoforms composition by a test independent from the above described method. This test should include the analysis of other isoforms than the 22,000 Dalton-hUH. Recent experiments in our laboratory indicate that especially the analysis of complexed

hUH (dimers and oligomers) and of the 12,000 Dalton isoforms are promising approaches.

The most appropriate technology for the identification of protein isoforms in biological fluids is the two-dimensional gel electrophoresis. This technique involves the separation of proteins by two independent physicochemical properties: Jsoelectric point and molecular weight.

As we have developed a large panel of monoclonal antibodies against hUH, we are in the unique position to have the opportunity of combining the highly specific recognition of a protein by a specific antibody and the high resolution of proteins provided by gel electrophoresis. Screening many of our antibodies generated against growth hormone, we have been able to detect as little as 1 pie ogramm of recombinant hUH in one dimensional gel electrophoresis. Furthermore, we were able to demonstrate the presence of various hUH isoforms by this technique. This has demonstrated the potential of the combination of gel electrophoresis with specific antibodies against hUH. However, at present the development of a test method based on this technique is hindered by some specific difficulties:

- The concentration of hUH in serum (and even more in urine) samples is rather low. Thus, an affinity concentration step is required before the less abundant isoforms of hUH can be analysed by this method. Alternatively, the affinity of the antibodies has to be improved to visualize small amounts of hUH isoforms.

- For gel electrophoresis, proteins are diluted in a specific buffer and loaded on a SDS containing gel. This treatment destroys the original three-dimensional structure of the protein and thereby can abolish the recognition by specific antibodies. Therefore, antibodies suitable for analysis of unpretreated serum samples are not neccessarily suitable for gel electrophoresis. Specific antibodies have to be selected which are capable to recognize the "dc-naturalized" protein-hormone molecule on a gel.

- Reproducible identification of protein isoforms by gel electrophoresis requires a high degree of standardization of the experimental conditions: Small changes in the quality of the gel, in the pH or in the voltage can lead to major variations in the protein spot pattern detected. This strict criteria of standardization and quality control can not be met by the more simple and variable in-house gels and gel running devices frequently used (and sufficiently precise) for research purposes.

- Long term stability and exact documentation of the protein spot pattern

derived from gel electrophoresis requires high end detection and documentation systems, especially when a very small amount of protein — as it is the case for hUH isoforms — has to be detected.

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Results and Conclusions

In this investigation, we have shown that all the 4 immunoassays for GH doping detection have very lower cross-reaction with the most hGH-related homologous proteins or peptides, implicating that the assays are very specific and their performance will not be disturbed by such proteins or peptides in physiological concentration. According to our window-of-opportunity hGH-application study, growth hormone abuse can be detected up to 36 hours with this approach after a single injection of a dose normal for hGH replacement therapy. Doses applied in abuse for performance enhancement are expected to be higher than the doses applied in this study. As an indicator of hGH doping, the ratio calculated from result from the two paired differential immunoassays changes so dramatically after hGH injection that a cut-off can be selected easily to ensure that no false positive result will occur. For the formal establishment of nominative data with regard to the ratios, a study of sera from a large number of hGH-untreated subjects will be performed and analyzed.