PROJECT REVIEW

"The application of cellular chemistry and proteomic approaches to the detection of gene doping."

J. Roberts, P. Teale, R. Kay (Immunochemistry & Biotechnology Section, HFL Ltd., New Market, UK), **C. Creaser, C. Rees, G. Ball, S. Mian** (Nottingham Trent University, Nottingham, UK), **G. Goldspink, S. Harridge** (UCL Medical School, University of London, UK)

The development of molecular biological technology over the last decade has resulted in dramatic advances in our ability to monitor, control and manipulate cellular systems. As a result the genetic modification of athletes (gene doping) considered a distant threat no more than five years ago is now considered a real threat to the integrity of sport.

Recently the Netherlands Centre for Doping Affairs published a monograph on the issue of Gene doping. Genes suitable for use as doping agents were identified, as were suitable vectors for their introduction. The threat to sport of gene doping is made more pressing by a number of factors:

• By its nature Gene doping cannot be controlled by the application of technologies currently in use in sports testing laboratories.

• Vectors and genes that have been identified as potential doping agents reflect current knowledge and will change with our understanding of molecular biology.

• The possible side effects and potential for serious impact upon the subjects undergoing gene doping has not yet been quantified but are likely to be profound.

The introduction of a gene for production of IGF-1 is considered a high probability target for potential gene doping. The potential to increase muscle mass has already been demonstrated in model systems and its use in gene therapy is under investigation. For these reasons IGF-1 has been chosen as the model for gene doping for this research project. Modern biochemical techniques such as those employed for research into gene expression are immensely powerful, and provide tools for investigating subtle changes in the genetic makeup of organisms or the presence of foreign DNA.

A radically different approach to detection, possibly more applicable as a screening methodology, could be developed through the application of proteomic or transcriptomic techniques. Following gene doping (or the application of an anabolic agent) the expression of one or more proteins will be altered, this expression will also be reflected in the translated RNA. Proteomic and transcriptomic approaches are targeted at identifying such changes in expression/translation. The application of multiple techniques to identify gene expression using cellular chemistry

(London group), advanced mass spectrometry with ESI (Newmarket group) and MALDI (Nottingham group) for circulating proteins significantly improves the possibility of success.

The Application of Cellular Chemistry and Proteomic Approaches to the Detection of Gene Doping

Results and Conclusions

The administration of GH and IGF-I gene therapy to a murine population, and the generation of mass spectral profiles produced suitable ANNs models for discriminating between doped and control populations. The GH gene therapy batches showed inconsistent results between batches, however the initial batch showed very promising results. The IGF-I gene therapy administration generated models that were not as accurate as the first GH batch, but were significantly better than the large GH gene therapy batch.

The application of ANNs to the human GH administrations also generated a number of highly accurate models, and in the case of the LC-MS analysis, an important peptide biomarker ion was characterised as being derived from the protein leucine-rich alpha-2-glycoprotein. The subsequent development of a 5 minute UPLC-MS/MS assay enabled a partial validation of the protein and comparison with IGF-I, an existing biomarker to GH administration. It has been demonstrated that the combination of quantitative data for the A2GL and IGF-I proteins increases the separation of the placebo and treated states compared to IGF-I alone. However, the data from both GH administrations show that the combined values still cannot be used in isolation to discriminate doped from normal populations.

This is the first demonstration that quantitative data from an established biomarker related to GH may be combined with data from an unrelated protein to enhance predictive discrimination between treated and untreated samples. The A2GL protein could therefore be used in combination with existing biomarkers of GH such as PIIINP, IGF-BP3 and IGF-I , and with further method development, the application of UPLC-MS/MS could give quantitative information on all four proteins in serum or plasma in a single analysis.

We have demonstrated that the use of ANNs is a valid and potentially useful approach to the detection of gene and protein doping, and that it could possibly be used to study the effects of other protein targets, for example erythropoietin. The project has also resulted in the generation of a high throughput and sensitive assay for the quantitation of IGF-I in human serum and, following further method development, in human plasma. Recent publications on the analysis of IGF-I by LC-MS/MS require the use of antibody coated magnetic beads and subsequent analysis using 35 minute runtime. The IGF-I assay we have developed requires only acetonitrile for the extraction process in a 96 well plate format, and a 5 minute UPLC-MS/MS analysis. This approach gave similar sensitivity levels to the recent paper, with a significantly higher throughput LC-MS/MS analysis.

Publications

A. Posters

1. International Mass Spectrometry Society Conference (IMSC) 2006.
The development of a robust and reproducible extraction method for the analysis of low molecular weight biomarkers in serum and plasma.

2. IMSC 2006
Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry Combined with Artificial Neural Network Models for the Identification of Proteomic Biomarkers Indicative of Growth Hormone Administration in Mice.

3. American Society of Mass Spectrometry (ASMS) 2007
The use of MALDI/MS, LC/MS and artificial neural networks for detecting serum biomarkers of growth hormone administration in human subjects.

4. British Mass Spectrometry Society annual conference 2007
MALDI-MS and LC-MS combined with artificial neural networks for the detection of GH gene doping.

5. Abstract submitted to HUPO 7th world conference 2008.
High throughput quantitative analysis of a new biomarker to GH abuse using UPLC-MS/MS.

B. Articles

1. Joshua Boateng, Lee Lancashire, Pamela Brown, Murrium Ahmad, Balwir-Matharoo Ball, Robert Davy, Shi Yu Yang, Jane Roberts, Phil Teale, Cristiana Velloso, Robert Rees, Graham Ball, Geoffrey Goldspink, Colin Creaser: The Use Of Proteomic And Bioinformatics Techniques For The Detection Of Protein Biomarkers Following Growth Hormone Administration. *The Internet Journal of Genomics and Proteomics.* 2007. Volume 2 Number 2