

Project Review

"The Development of a Methodology for Detecting Abuse with IGF-I in Sport"

R. Holt, **P. Sönksen**, (University of Southampton, UK), **E. Bassett** (University of Kent, UK), **D. Cowan** (King's College London, UK)

Growth hormone (GH) is a naturally occurring endogenous peptide hormone produced by the pituitary gland. GH has strong anabolic properties regulating body composition and is widely accepted as being a major drug of abuse in sport. The majority of its anabolic actions are mediated through the generation of Insulin-like growth factor-I. There are reports that IGF-I is being abused by athletes either alone or in combination with GH. Its use is banned under the International Olympic Committee (IOC) and World Anti Doping Agency (WADA) list of prohibited substances.

The detection of exogenously administered GH and IGF-I poses a formidable challenge, as they are identical to that which is produced naturally in the body. While androgenic anabolic steroids and related substances can be measured by mass spectroscopy, no such methods have been developed for testing for abuse with GH and IGF-I.

The GH-2000 and GH-2004 teams have developed a test for the detection of abuse with GH based on GH-dependent markers. The GH-2000 group showed that the administration of rhGH leads to significant rises in GH-sensitive markers which can then be used to construct formulae that give good discrimination between those taking GH and those taking placebo. IGF-I and P-III-P were selected as the best of the markers of GH action

At present there is no specific test to detect IGF-I abuse. The aim of the current project is to assess whether a marker approach can be used to detect the administration of exogenous IGF-I.

IGF-I alone is associated with significant side effects of hypoglycaemia and jaw pain. Recently a combination of recombinant human (rh)IGF-I and IGFBP-3 has been developed by Insmed that is much better tolerated. We will be using this combined rhIGF-I/ rhIGFBP-3 complex for our studies.

Detection of growth hormone doping by gene expression profiling of peripheral blood in humans

Results and Conclusions

Initial microarray analysis was undertaken using the Affymetrix platform. We have performed 98 Affymetrix Human U133 Plus 2.0 microarrays spanning four treatment groups and forty individual subjects. To determine the effects of treatment, we have compared an individual's baseline (week 0) and final treatment sample (week 8). Due to the small changes in gene expression, a second analysis was conducted using the Agilent Technologies platform, a more recently developed transcript profiling platform with enhanced sensitivity. The two colour methodology of the Agilent 44K G4112F array allows direct comparison of an individual's week 0 and week 8 sample on the same microarray. We performed 40 Agilent microarrays for 20 GH treated men and women. Validation of the Agilent microarray expression data was performed for seven genes using quantitative real time PCR (qPCR). For both gene expression profiling platforms, the GH-induced alterations were small, and similar in magnitude to variations between individuals. Results from both platforms demonstrate that maximal regulation was of the order of two fold, and that few genes were regulated greater than 1.5 fold. Further, whilst both platforms identify differentially expressed genes, these signatures were different between men and women treated with GH. Using data from the Affymetrix platform at the probe set level, testosterone alone had little effect, whilst the administration of testosterone and GH together resulted in regulation of probe sets that showed little overlap with those regulated by GH alone.

We conclude that it is unlikely that gene expression analysis of peripheral blood leukocytes would be a viable approach for the detection of GH doping.