PROJECT REVIEW

"Mass Spectrometry of Peptide Hormones"

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To establish a facility within ASDTL which is capable of meeting the increasing demands for confirmation of peptide hormones and other large biologically active molecules using liquid chromatography mass spectrometry (LC/MS).

There is an urgent need to develop the skill and resource base needed to carry out the mass spectral analysis of bio-molecules used for doping. Whilst at present the use of immunoassays and other immuno-reactive techniques is accepted as proof of doping this is unlikely to continue once it has been demonstrated that mass spectral confirmation is possible. Recently published work has shown that it is now possible to detect and identify proteins in biological matrices at the extremely low concentrations found naturally.

At present the confirmation of the presence of peptide hormones and other large bioactive molecules is done using techniques that rely on specific antibody reactions to large molecules. Unfortunately, such reactions are not completely specific and the current IOCIWADA anti-doping code includes the need for two separate antibodies to confirm doping with HCG. All drugs that are detected, other than peptide hormones, must be confirmed by the use of mass spectrometry using gas chromatography mass spectrometry. The reason the peptide hormones were excluded from this requirement was that it was not practicable to attempt mass spectrometric analysis of large bio-molecules both because of their high molecular weight and because of the very low concentrations found in blood and urine. However with the ever increasing demands of proteomics research the use and capabilities of mass spectrometry using LC/MS for the analysis of bio-molecules has increased dramatically in the last few years and will continue to do so.

The aim is to research processes to allow:

- A validated method to confirm cases of HCG doping using LC/MS.
- A [C/MS method to identify and confirm the presence of haemoglobin based blood substitutes.
- A mass spectral method to distinguish between recombinant EPO and urinary EPO
- Mass spectral methods to detect and identify other significant biologically active molecules such as NESP, EPO mimetics, growth hormone isomers, IGF1 etc at the low levels found in blood and urine samples.

Mass Spectrometry of Peptide Hormones

Results and Conclusions

In the first year of this three year project we completed a validated method for confirming the presence of haemoglobin based oxygen carriers in serum and began to investigate methods that could be used for confirmation of the abuse of peptide hormones. The first has been achieved and a paper describing the work has been published (Goebel, 2005). The latter activity was focused on two peptide hormones, human chorionic gonadotropin (hCG) and erythropoietin (EPO). Mass spectrometric analysis of peptides and proteins provides structural information which can uniquely identify the compound being examined. WADA rules mass spectrometry as the definitive method for confirmation except in the case of peptide hormones. It has been seen with the development of new techniques such as carbon isotope ratio mass spectrometry that, once a mass spectral technique can be shown to replace or supplement a less rigorous procedure, its use becomes essential both to confirm guilt and to demonstrate innocence. The same will apply to the peptide hormones in the near future and positive cases will require mass spectral confirmation once it is possible to do so.

It was intended that in the second stage of this project that we would establish capillary chromatography coupled with high resolution mass spectrometry (HRMS) as a routine procedure for the analysis of peptide digests and proteins. We have completed the development and validation of a precise MS method for the reliable identification and quantitation of hCG in urine at physiological levels. In addition we have extended this procedure to assist in developing a method with the potential to distinguish between recombinant and urinary hCG.

We have developed of methods for the concentration and purification of hormones such as hCG, EPO, insulins and IGF-I from urine and serum to assist in their subsequent analysis by LC/MS/MS. A method has been developed and validated for the detection of synthetic insulins in both serum and urine (Goebel et al 2008). The method will soon be an ISO17025 method for routine use in our laboratory. Work has also proceeded on methodology for the detection and quantitation of IGF-I and related analogues such as long R3 IGF-I in serum.

Our ability to detect and identify peptide hormones at physiological levels has recently been enhanced by our purchase of a Thermo LTQ-Orbitrap XL with an Eksigent 2D Nano LC and a Michrom Advance Nanospray source. Using this instrument we are capable of detecting natural gonadotrophin releasing hormone (GnRH) in urine at levels significantly below those previously reported (Thomas et al 2008)

Publications:

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