"Development of a PSAQ-SRM assay to detect and quantify growth hormone and insulin-like growth factor 1"

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Project Review

Growth hormone (GH) is included in the WADA list of prohibited doping substances. The detection of GH misuse is currently achieved using immunological assays to determine the abundance ratio between the 22 kDa and the 20 kDa GH isoforms. Another strategy, which profiles indirect markers of GH administration (mainly blood IGF-1 increase), has also attracted some interest. The aim of the present project is to develop an innovative liquid chromatography - mass spectrometry-based assay to detect and quantify simultaneously the 22 kDa GH isoform and IGF-1 in serum. We anticipate that this assay will also provide information on the GH isoform ratio. Serum sample treatment will be designed: (i) to circumvent the use of antibodies to overcome the current dependence on antibody manufacturers and (ii) to be compatible with highthroughput sample analysis. Mass spectrometry analysis will be performed using the Selected Reaction Monitoring (SRM) mode renowned for its high specificity and sensitivity. Quantification will be achieved using the PSAQ method (Protein Standard Absolute Quantification) which uses stable isotope-labeled versions of the targeted proteins as quantification standards. We expect that this PSAQ-SRM assay, which targets both direct and indirect biomarkers of GH misuse, will improve the reliability of the biological information delivered. This should be an important contribution to efficient sports drug testing.

Results and Conclusions

Growth hormone (GH) is included in the WADA list of prohibited doping substances. The recombinant form of GH and its endogenous counterpart (22 kDa) are structurally analogous, making detection of GH misuse challenging. A strategy to overcome this issue is based on the determination of the abundance ratio between the 22 kDa and the 20 kDa GH isoforms in athlete's serum samples. This is effective because exogenously administered recombinant GH exerts negative feedback on endogenous expression of the 20 kDa isoform, resulting in a modified 20 to 22 kDa abundance ratio (Wu et al., 1999).

In this context, this project aimed at developing an innovative liquid chromatography - mass spectrometry -based assay to detect and quantify both the 22 kDa and 20 kDa GH isoforms in serum. Mass spectrometry analysis was performed using Selected Reaction Monitoring (SRM) acquisition (Lange et al., 2008). Quantification was performed using the PSAQ method (Protein Standard Absolute Quantification) (Brun et al., 2007).

The first task of the project consisted in the synthesis of PSAQ standards, i.e. isotope labeled versions of the 22 kDa and 20 kDa GH isoforms. Both standards were successfully produced with a final purity over 95% and an isotope incorporation greater than 99%. These standards are now available as validated products for the anti-doping community. Then, using these standards as model proteins, we implemented a LC-SRM method on a 6500QTRAP mass spectrometer to simultaneously detect and quantify GH isoforms. This method was specifically optimized to allow the detection of peptides discriminating the 22 kDa from the 20 kDa isoform. In a third step, we assessed different biochemical methods to extract GH isoforms from serum matrix. Although immunocapture was effective to capture GH isoforms, it did not allow the discrimination of the 2 GH isoforms. Detection sensitivity was determined at 50 ng/ml of serum.

Biochemical optimizations are still ongoing to improve the detection limit of the method and investigate the abundance ratio between the 2 GH isoforms.