PROJECT REVIEW

"Chip technology for the detection of growth hormone abuse- Extension study"

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Human growth hormone (GH) is necessary for metabolism and growth following birth. As such, under non-pathological conditions every human produces GH that comprises a family of related, but structurally different molecules. To combat GH deficiency a recombinant analogue (a single isoform) has been produced that has to be administered subcutaneously. In healthy individuals the administration of this recombinant isoform leads to the down-regulations of the bodies-own production and, as a consequence, the isoform distribution is altered. Thus, monitoring the isoform distribution of GH in body fluids is a valid approach for the detection of GH abuse in athletes, Within the first three years of the project the most important results has been the development of a test, capable of monitoring the ratio between 20 and 22 kDa GH in both urine and plasma. This method is the only one at the moment hat addresses the two most important GH isoforms through an immunological approach. Completion of this development (beta-testing phase) is the main objective of the current application. Also the transformation of the ELISA based approach to a LUMINEX platform will be worked-out. Furthermore, several other aspects of the original proposal could be concluded during the extension. First, the generation of the 17 KDa GH isoform in sufficient amounts for functional testing; a non-recombinant approach to this isoform has been developed already. Secondly, the characterization of a monoclonal anti-5kDa antibody that has recently been produced. Thirdly, the completion of an excretion study in Barcelona with analysis employing both the developed ELISA as well as multiplexed approach (Luminex), recently implemented by one of the partners. Finally, the technique of surface Plasmon resonance could be used to characterise the antibodies used in alternative differential ELISAs.

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Results and Conclusions

Human growth hormone (hGH) abuse amongst athletes has been suspected for a number of years and the only solid evidence for this assumption has been obtained from seized material transported by or belonging to elite athletes. Several approaches aim at development of robust analytical methods to determine hGH doping. Within the framework of this project a clinical trial with recombinant human GH (rhGH) was conducted on nine healty individuals (2 controls without treatment + 7 rhGH recipients) during 7 days with a 2-week wash-out period to evaluate the potential of a direct approach focussed on the ratio between 22 and 20 kDa hGH.

The immuno assays were conducted both in Japan and Spain using an ELISA and Luminex® platform. Overall, the data obtained from the different laboratories and techniques correlate well (over 90% for all correlations except for the 20 kDa ELISAs were 85% was reached) albeit that as a result of an unusually high background for the 20 kDa immunoassay quantitation of this isoform resulted more cumbersome than in earlier stages of the assay development. Two relevant aspects are that during the 7-days of administration no significant decrease in the 20 kDa hGH values was observed, contradicting the generally established negative feedback mechanism for this hormone. In general, 12 hours after administration nearly all samples displayed an elevated ratio between 22 and 20 kDa (> 20) whereas in the timeframe between 12 and 24 hours post administration approximately 50% of the samples gave a ratio superior to the basal level. Collected material was partially assayed with other formats such as the “rec” and “pit” differential immunoassays (both kit A and kit B) yielding a valuable comparison. The “rec” assays correlate well with the 22 kDa immuno assays but the “pit” assays do less well with the 20 kDa immuno assay indicating that in essence different analytes are being targeted. Finally, also indirect marker proteins insulin-like growth factor I (IGF-I) and mannose binding lectin (MBL) were analysed to enable a best possible overview for antidoping purposes. Whereas IGF-I displayed the expected trend (with a unexpected dip in the concentrations four hours post administration that lateron is compensated) MBL also displayed significant concentration increases in most cases. Yet, the large dynamic range encountered renders the latter a difficult candidate for anti-doping purposes.