PROJECT REVIEW

"Determination of the epitope specificity of anti-20 kDa antibodies by SPR – search for complementary immunoglobulins"

R. Gutierrez Gallego, J. Segura, J. Bosch, G. Such (IMIM, Barcelona, Spain), **M. Wu, S. Wang, H. Du, G. Cheng** (China Doping Control Center, Beijing, China)

The detection of human growth hormone in body fluids (plasma or urine) is a cumbersome task for several reasons being the low concentrations and the molecular heterogeneity the most important ones. Thus, at present for reasons of sensitivity and specificity immunoglobulins are indispensable in any analytical approach and also for the detection of growth hormone abuse antibodies play an important role. It is known that the administration of growth hormone results in a down regulation of the bodies-own production and that during a certain time frame only the exogenous molecule will be found in circulation. As the pharmaceutical product consists in a single isoform (the endogenous material is composed of several isoforms) this difference is used for anti-doping purposes.

Currently, two strategies are being followed: -1- an approach in which the amount one isoform (22 kDa) is compared all other isoforms and -2- an approach in which the amount of one isoform (22 kDa) is compared to the amount of a second isoform (20 kDa). For the second approach only a few specific antibodies are known and available. In order to have a reliable test for anti-doping purposes at least two specific antibodies, with distinct epitopes, should be available. In the course of the development of antibodies to the 20 kDa isoform fourteen different clones were generated by the research team. One, #7-clone 1B3, has now been fully explored. This antibody has shown superior surface properties with respect to the other known anti-20 kDa mAbs. Now, within the framework of this project the other clones will be produced at larger scale and characterised for their binding properties. The most promising clones will be further developed with the aim of having at least two distinct anti-20 kDa antibodies. The characterisation and comparison of the generated antibodies will be done by means of surface plasmon resonance and employing the entire arsenal anti-GH antibodies, GH isoforms, proteolytic GH fragments, synthetic GH isoforms, growth hormone binding proteins, sandwich assays, etc. This approach has already been applied successfully in the characterisation of the antibodies employed in the differential immunoassay that addresses the ratio between "pit" and "rec" isoforms.

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Results and conclusion

Human growth hormone (GH) in humans is a family of different molecules that come from a single gene. The composition of the family members fluctuates very little both at the intra- and interindividual level. However, when the GH pharmaceutical is administered the composition, and thus the ratio between the family members, is altered, and this sets the basis of the so-called direct approaches. One is the established rec/pit differential immuno assay approach that is implemented in most WADA accredited laboratories. An alternative approach, based on the specific ratio between 22 and 20 kDa GH is in the final stage of development with a multi-lab validation study. The major difficulty of the latter approach is the fact that finding a pair of antibodies (for screening and confirmation) with a different binding epitope for 20 kDa is very difficult as this variant differs only from the 22 kDa in that the amino acid Phe31 is linked to Asn47 instead of Glu32 (splicing-out of AA32-46). In this study we have evaluated and characterised 14 new anti-20 kDa antibody clones. We have identified 2 (antibody #4 and antibody #7) with excellent surface properties that are highly specific for 20 kDa GH. However, all antibodies that displayed binding to 20 kDa after immobilisation appear to have the same epitope as sandwich type studies reveal no complementary binding. When we compared the two best performers with one other established ultra specific and good surface antibody from a different source we could establish that this antibody, and the two identified here (#4 and #7) do bind different epitopes. As such, the 22 vs 20 kDa approach to address GH doping may be set up in compliance with the requirements for screening and confirmation procedures.