“Development of a multiplexed detection assay for PEGylated proteins in doping control samples”

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Project overview:

Erythropoiesis-stimulating agents (ESAs) have been frequently detected in doping control samples in the past and are among the illicitly used drugs that are most often mentioned by confessing athletes. New ESA therapeutics represent new options for cheating athletes and new challenges for sports drug testing authorities as comprehensive detection assays are required. With the introduction of the recently approved EPO-mimetic drug Omontys (formerly referred to as Hematide and Peginesatide), a first peptidic ESA is principally available that necessitates complementary analytical methods as Omontys will not be detectable with conventional EPO analytical methodologies. First successful procedures for the analysis of Omontys from plasma, serum, and dried blood spots were recently reported; however, the most frequently available doping control samples are urine specimens. Since pharmacokinetic studies have demonstrated that a significant amount of Omontys is renally excreted, the transfer of the methodology to urine and the analysis of proof-of concept samples from human in-vivo studies are desirable.

In the present study, the completion of a detection assay for Omontys from urine samples by means of liquid chromatography-mass spectrometry is aimed. Pilot studies have demonstrated the capability of extracting, enzymatically hydrolysing and subsequently measuring a proteotypical peptide of Omontys from spiked urine specimens; in order to apply the methodology to authentic doping control samples, the method will be fully characterized and an administration study with the EPO-mimetic agent will be conducted with six human volunteers. The in-vivo derived samples will be measured with the developed assay to demonstrate its utility for the determination of the prohibited drug in authentic specimens.

Results and Conclusions:

PEGylation of protein therapeutics by covalently attaching polyethylene glycol (PEG) polymers results in an increased molecular mass and, therefore, slower renal clearance, reduced proteolytic susceptibility, and decreased immunogenicity. Endogenously, PEGylated substances do not exist and, hence, the concept of targeting PEGylated compounds as an initial testing approach towards prohibited substances was considered. In this research project, different strategies including immunoaffinity purification & LC-MS/MS, commercial PEG-specific ELISAs, and SDS-PAGE in combination with iodine staining were tested to assess the capability of these approaches to allow for multiplexed detection of PEGylated proteins in doping control serum

samples. In the course of the studies, ELISA kits and iodine staining were found to be of limited specificity. In addition, the diversity of PEGylations regarding size and composition resulted in limited recognition of PEGylated model compounds by anti-PEG antibodies used for immunoaffinity purification, suggesting that (to date) testing merely for the presence of PEG polymers in human serum does not facilitate sports drug testing approaches per se. However, Pegvisomant (i.e. PEGylated and sequence-modified human growth hormone) was successfully detected in serum samples with the employed methodologies, which demonstrates that in general mass spectrometric assays can be established if antibodies binding with appropriate efficiency to a broader spectrum of PEG conjugates are available.