PROJECT REVIEW

"Detection of the non-human *N*-glycolyl-neuraminic acid (Neu5Gc) using immunopurification and chipLC/MS/MS. Acronym: GLYCOCHIP"

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Recombinant glycoproteins expressed in non-human cells and in particular EPO and NESP has shown to contain small amounts of *N*-glycolyl-neuraminic acid (Neu5Gc), a sialic acid for which humans are devoid of the suitable hydroxylase. The detection of such non-human component of EPO (or other proteins) will constitute an unequivocal evidence of their exogenous origin.

As part of previous research projects funded by WADA, we showed the presence of such monosaccharide in rEPO and NESP and later developed a capillary hplc method with fluorescence detection for the determination of small amounts of Neu5Gc (LOD ca. 5 fmol). By using such method in combination with an immunoaffinity purification developed in parallel using microwell plates coated with an anti-EPO antibody we were also able to detect the presence of Neu5Gc in plasma samples of EPO users. This method is already at the cutting-edge of the current technology using fluorescence detection. Furthermore, according to anti-doping regulations, the use of mass spectrometry is preferred, or required whenever possible.

With this precedent, the aim of the present project is to move on following the same strategy, taking profit of our long experience in the field, and develop a method with a better sensitivity and able to detect Neu5Gc using mass spectrometry as unequivocal identification. The method will use nanoLC (hplc-chip from Agilent technologies) as the optimum sensitivity set-up and triple quadrupole MS. The instrument to be used has as specifications the detection of a chromatographic peak of 0.8 fmol reserpine (S/N =20) under conventional LC conditions. Using the hplc-chip (nanoLC) conditions this sensitivity should be increased ca. 50-100 times, fully supporting the feasibility of the attempt. The low amounts of Neu5Gc expected in urine of EPO users (ca.≥ 35 fM) will be detectable in 20 mL of urine or less. Although some publications suggest that Neu5Gc can be incorporated into different glycoproteins through its ingestion as part of the diet, our preliminary results suggest that once a sample is immunopurified, there are no traces of Neu5Gc in negative samples, thus making the method specific for the purified glycoprotein. Although we will apply the method for EPO, using already developed immunopurification strategies, the same approach can be applied to the detection of the same analyte in different recombinant glycoproteins and matrices provided the corresponding specific immunopurification is applied.