PROJECT REVIEW

"Development of Antibodies to Human Asialo Erythopoietin. Possible Application to a Confirmation Procedure in Anti-Doping Control of Recombinant Erythropoietin"

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At the present time, anti-doping control for recombinant human erythropoietin (rHuEPO) relies on isoelectric focusing of ultra-filtered urine and double blotting of this hormone. This method results in an image of the isoelectric pattern of erythropoietin (EPO) present in urine and allows to differentiate between natural and recombinant hormone. Indeed, some of the isoforms composing the isoelectric profiles of natural urinary EPO are collocated with the isoforms of rHuEPO but others, more intense, present more acidic pI. This difference enables the detection of rHuEPO in urine but till now has no structural explanation. It is important to explore this structural aspect both from a fundamental scientific and anti-doping analytical points of views.

We have undertaken investigations on this topic by studying the role of sialic acid residues in the isoelectric profiles of recombinant and natural urinary EPO. Our preliminary results show that in addition to sialic acid. Some other unidentified residues are responsible for the more acidic properties of natural hormone since after desialylation, the two hormones present different isolelectric patterns.

This observation is very important since apparently, some isoforms corresponding to molecules devoid (or with very few) of these additional structures are observed in the asialopattern of rHuEPO but not in that of natural urinary hormone.

The presence of these isoforms in a asialo pattern would thus constitute an absolute demonstration of the presence of recombinant hormone.

It is thus envisaged to develop a confirmation test based on analysis of the asialopattern EPO. However, an essential condition to these investigations is obtaining specific antibodies well-recognizing the asialo-erythropoietin molecules and we have planed to produce such antibodies.

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Results and Conclusions

The objectives of this first part of the project was to obtain antibodies to asialo EPO in order to investigate the asialo EPO patterns obtained in various situations (negative, positive, unstable, atypical EPO profiles from urine samples) and from various recombinant drugs (Epoetin alfa, beta, omega, delta, Darbepoetin alfa). These antibodies would be used as primary antibodies in immunoblot following isoelectric focusing (IEF) of desialylated EPO.

Immunization of two rabbits produced antiserum with very low titres that did not give any results on immunoblots.

Immunization of 3 mice gave rise to better results and the most reactive mouse, after an additional injection of immunogen, was chosen for the fusion step.

Sera and hybridoma (after purification of immunoglobulins by protein G affinity chromatography) were tested on immunoblot experiments. Whereas good results were obtained by dot blot, no results were obtained after IEF of desialylated Eprex.

It is probable that the tested antibodies have conformational epitopes that are lost during IEF with urea. Since urea is necessary to perform IEF of EPO, the project was suspended.