"Detection of circulating anti-Neu5Gc antibodies as a proof of use of recombinant glycoproteins: rEPO and analogues"

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Project Summary

All humans express antibodies against N-glycolyl-neuraminic acid (Neu5Gc), a sialic acidhumans cannot produce that is present in glycoproteins from animal sources. Small amounts ofNeu5Gc can metabolically be incorporated to human proteins resulting in the development of an immune response.

Neu5Gc is present in recombinant glycoproteins (e.g. rEPO and analogues) since they are expressed in non-human cell lines. It has been proven that mice receiving injections of a recombinant glycoprotein, containing Neu5Gc, immediately produce antibodies against it.

The hypothesis is that the concentration of antibodies against Neu5Gc must be much much higher in subjects treated with recombinant proteins (e.g. rEPO) than in the general population whre the exposure to Neu5Gc happens through a complex mechanism from the diet.

The main objectives of the project are:
1- Developing a test to quantify antibodies against Neu5Gc circulating in human blood (serum or plasma) based on what has already been described in the literature.
2- Test human blood samples from healthy individuals and from patients being treated with rEPO.
3- Refine the analytical test in order to increase specificity and minimise background by testing different Neu5Gc containing antigens and approaches (inhibition).
4- Validate the test for anti-doping purposes establishing thresholds to ascertain the illegal exposure to recombinant glycoproteins as well as studying the pharmacokinetics of antibody production.

Results and Conclusions

Humans do not possess the enzymatic capability of producing the sialic acid N-glycolyl-neuraminic acid (Neu5Gc). It has been described that, as Neu5Gc is present in animal products, small amounts of Neu5Gc can metabolically be incorporated into human proteins resulting in the development of an immune response. Anti-Neu5Gc antibodies (with different linkages to other monosaccharides in glycans) have been described as measurable in almost all human beings. We hypothesised that, as Neu5Gc is present in recombinant glycoproteins (e.g. rEPO and analogues), expressed in non-human cell lines, treatment with these substances should give rise to the development of a higher title of specific antibodies.

Following the findings published in the literature, the overall aim of the project was the detection of circulating antibodies raised specifically against Neu5Gc, with the α2,3-linkage to Galactose found in recombinant EPO and analogues.

Following the project plan, a specific antigen Neu5Gc-α2,3-Gal-β1,4-GlcNAc was synthesised linked to biotin (through an hexaethylenglycol bridge) or to KLH.

Serum samples were obtained from patients under chronic NESP treatment as well as healthy untreated volunteers. Additionally samples were also obtained from volunteers participating in a clinical trial receiving rEPO treatment for 3 weeks.

Detecting particular human antibodies in serum samples has proven to be very challenging as the concentration of total IgG is huge and non-specific binding is a major drawback.

Different antigens were tested including Neu5Gc or Neu5Ac linked to a polyacrylamide (as described in the literature), the whole rEPO and our specific rEPO trisaccharide.

After optimising all ELISA parameters, and being able to minimise background, small differences in anti-trisaccharide titre could be observed between patients treated with NESP and healthy volunteers. However those mean differences would not allow setting a cut-off to differentiate between them. However, when those tests were applied to samples from healthy volunteers submitted to rEPO treatment (e.g. resembling doping cases) there was no measurable increase in antibody titres.

In conclusion, despite the many reports in the literature suggesting that circulating anti-Neu5Gc antibodies are ubiquitous, those measurements could not be consistently reproduced nor a clear titre of Anti-Neu5Gc antibodies could be detected either in patients using a rEPO analogue chronically nor in volunteers submitted to rEPO treatment.