"Characterization of erythropoietin (EPO) produced in liver, a potential source to atypical EPO profiles in doping samples"

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Project Overview

Erythropoietin (EPO) is a hormone that stimulates the production of red blood cells in the spine. EPO is predominantly produced in the kidney, and recombinant EPO

(rHuEPO) is used therapeutically in the treatment of anaemia seen with chronic kidney disease and certain cancers. Athletes have in addition misused this hormone as a doping agent in order to improve endurance capacity. Endogenous EPO and rHuEPO have different degree of glycosylation in addition to different combinations of charged groups on the glycan, and detection of misuse today is based on the different isoelectric profiles these charge-differences give rise to. Recently new recombinant EPO analogous (epoetins) have entered the market, some of which display a different and more basic isoform distribution than the traditional rHuEPO. In addition, DynepoTM, the new recombinant EPO that is expressed in a human cell line, has a slightly less basic isoform profile than rHuEPO. The current method for EPO-analysis is based on the profiles of the traditional epoetins, and hence there is a need to further develop this method to meet the new challenges. This project aims to characterize endogenous EPO found in atypical urines and to determine differences in the protein and glycan-groups from the new epoetins, by the use of affinity chromatography, enzymatic deglycosylation and gel electrophoresis.

Results and Conclusions

Erythropoietin (EPO) is a hormone that stimulates the production of red blood cells in the spine. EPO is predominantly produced in the kidney, and recombinant EPO and analogues (epoetins) are used therapeutically in the treatment of anaemia seen with chronic kidney disease and certain cancers. Athletes misuse epoetins to improve endurance capacity. Endogenous EPO and the epoetins have different degree of glycosylation and different combinations of charged groups on the glycan, and detection of misuse is based on the different isoelectric profiles these charge-differences give rise to. New epoetins which display a more basic isoform distribution than the traditional epoetins, and the deviating (more basic) isoelectric EPO-profiles seen in active urines and in effort-urines, pose a challenge to the current EPO-

method. The aim of this project was to characterize endogenous EPO found in atypical urines and to determine differences in the protein and glycan-groups from the new epoetins, by the use of affinity chromatography, enzymatic deglycosylation and gel electrophoresis.

We found that EPO isolated from umbilical cord blood (predominantly produced in the liver) has more basic isoelectric profile than EPO from adult blood, and similar to that seen in some effort urines. Unlike the recombinant epoetins with hyper basic profiles, umbilical cord EPO, as well as EPO from effort urines, display the same electrophoretic mobility on SDS/SAR-PAGE as normal urinary and blood EPO.

EPO produced from a human liver cell line, HepG2, has a hyper basic isoelectric profile, and similar WGA-affinity as EPO from umbilical cord blood and effort urines. However, HepG2 EPO migrates differently from endogenously produced EPO when analysed with SDS-PAGE. HepG2 EPO and also EPO from adult blood, was differently (less) affected by enzymatic deglycosylation than urinary and recombinant EPO, indicating structural differences in the glycan groups. EPO was isolated from human liver tissue, to our knowledge for the first time. The results show that EPO produced in the liver has a different (more basic) isoelectric profile than EPO produced in the kidney, and the same electrophoretic mobility when analysed with SDS/SAR-PAGE.

Publications and Presentations

Poster, Manfred Donike Workshop for Dope Analysis, 2008: Y. Dehnes, M. Borgen, M. Lönnberg and P. Hemmersbach. ”Shifted isoelectric profiles of endogenous erythropoietin possibly due to different physiological origin”

Mini-publication: Y. Dehnes, M. Borgen, M. Lönnberg and P. Hemmersbach ”Shifted isoelectric profiles of endogenous erythropoietin possibly due to different physiological origin” in W. Schänzer, H. Geyer, A. Gotzman, U. Marek (Eds.), Recent Advances in Doping Analysis, vol. 16, Sportverlag Strauß, Cologne, 2008, p359-362