PROJECT REVIEW

"Evaluation of Membrane Assisted Isoform Immunoassay (MAIIA) for direct detection of rhEPO in doping, a new rapid method with potential for on-the-site- as well as for out-of- competition testing."

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Membrane Assisted Isoform Immunoassay has been identified as a possible technique candidate for a new EPO doping test (Evaluation report of the urine EPO test, March 11, 2003 by Dr. G. Peltre and Prof. Dr. W. Thormann).

MAIIA, which combines a chromatographic separation, based on charge or bio-affinity, with a sensitive and specific immunoassay detection, all integrated in a small microporous sheet has recently been shown to be a sensitive, rapid, simple and inexpensive method for determination of protein isoforms in biological fluids (Lönnberg, M. (2002) Membrane-assisted immunoassay, separation and determination of protein isoforms, Acta Universitatis Upsaliensis, Comprehensive summaries of Uppsala dissertations from Faculty of Science and Technology 691).

Recent findings have indicated that protein isoforms play an important role in healthy and diseased organisms, and their determination should therefore be beneficial in clinical diagnosis (Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct, Glycobiology 3, 97). In a joint research project the Dept. of Surface Biotechnology at Uppsala university and R&D at Pharmacia Diagnostics AB in Uppsala, Sweden, set out to develop a technique for the quantitative, measurement of protein isoforms, at low concentration, in complex media.

The MAIIA technique, the result of this research effort, can be used to measure glyco-protein isoforms with different glycosylation-patterns based on their different charges (mainly the sialic acid content) or their varying oligosaccharide structures leading to different behaviour in binding to carbohydrate specific lectin ligands. The concept has been thoroughly demonstrated by measuring carbohydrate-deficient isoforms of transferrin, analytes that e.g. can be used to detect alcohol abuse, in serum (Lönnberg, M., Carlsson, J. (2000) Membrane assisted isoform immunoassay- a rapid method for the separation and determination of protein isoforms in an integrated immunoassay, J. Immunol. Meth. 246, 25 ; Stibler, H., Borg, S., Joustra, M., (1986) Micro anion-exchange chromatography of carbohydrate-deficient transferrin in serum in relation to alcohol consumption, Scand. J. Lab. Inves. 58, 55).

Preliminary experiments have shown that the MAIIA technique also might be used in a sensitive, rapid and inexpensive test procedure for the discrimination of endogenous EPO from different recombinant EPO-forms in urine. Such a method should be an interesting alternative, as a doping test, to the presently used rather lengthy and expensive IEF-method (Lasne, F. et al (2002) Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones, Anal. Biochem. 311, 119).

Before starting a project aiming towards a routine EPO doping test, we feel that further research activities on the different test steps (sample treatment, chromatography and immunoassay detection) as well as improvement of reagents and test components are needed. We also need input about the legal and technical requirements related to the present routine doping testing and access to clinical knowledge and samples for testing. We have therefore established contact with Dr Garle at the doping control laboratory, Huddinge university hospital, Stockholm, Sweden, who has expressed an interest in participating as a coinvestigator to evaluate the MAIIA technique as an EPO doping test.

EVALUATION OF MEMBRANE-ASSISTED ISOFORM IMMUNOASSAY (MAIIA) FOR DIRECT DETECTION OF rhEPO IN DOPING

Results and Conclusions

The purpose of this eight-months pre-project was to evaluate a new technology as a basis for a rapid and easy-to-use erythropoietin (EPO) doping test. The proposed test-procedure will distinguish recombinant EPO and EPO analogues from the endogenous forms by utilizing the differences in their glycosylation structures.

The urine specimen is applied on a small anti-EPO column, which rapidly and efficiently captures EPO in the urine. After a washing step, the bound EPO is released by lowering the pH in the eluent. The novel, chip-based technology, Membrane Assisted Isoform ImmunoAssay (MAIIA), is thereafter used for chromatographic ion-exchange separation of the EPO varieties in the eluate and their on-line detection by a sensitive and quantitative immunoassay in the same small and disposable chip. This procedure will take about one-two hours when processing about 15-20 samples.

The obtained results look very promising. Urine specimens from patients (suffering from kidney-related deficiency of EPO) receiving recombinant EPO as well as urine specimens from healthy individuals were tested. The difference in results, between the tested patient-urines and the upper normal value (mean +2 stand. dev.), were roughly 3 times larger with the MAIIA technology (average for different test-settings) than for the results obtained with the present IEF EPO doping test, as tested by the Oslo doping laboratory.

The implementation of the MAIIA EPO doping test would most significantly reduce the cost of required investments, hands-on time and total test time. The easy-to-use set-up will make it possible to perform the testing in laboratories of different degrees of sophistication as well as in the field.

The technology should also have the potential to reveal new varieties of EPO and its analogues, as several interesting selective carbohydrate binders (lectins) can be introduced on the chip as a complement the charge-based separation.