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SDS-PAGE of recombinant and endogenous erythropoietins: benefits and limitations of the method for application in doping control

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Abstract

Biosimilar erythropoietins, chemically modified erythropoietins (MIRCERA), or latest generation of recombinant erythropoietins (Dynepo) all show isoform profils which slightly or markedly differ from the standard profiles of epoetin alfa (Erypo, Eprex), beta (NeoRecormon), and NESP. Thus, the criteria of the WADA Technical Document on Epo-analysis (TD2007EPO) need to be adapted to the current situation. As has been shown in previous as well as more recent publications [1, 2] SDS-PAGE holds the potential of being a complementary confirmation tool for the worldwide practiced IEF-method [3] due to differences in molecular mass of the various recombinant and endogenous erythropoietins. By combining molecular mass information (SDS-PAGE) with IEF-profile (pI) information the interpretation of analysis results (e.g. effort urines) can be simplified. On several examples out of the daily practice the usefulness of this approach can be demonstrated. Due to the lower molecular mass of serum/plasma Epo compared to most recombinant erythropoietins the method can be also used to directly detect Epo-doping in blood [4].

However, SDS-PAGE in its currently performed version has also serveral limitations: While it is ideally suited for resolving NESP and MIRCERA the resolution of the method is somewhat low for older generation erythropoietins, biosimilars, and endogenous erythropoietins (urinary Epo, serum/plasma Epo). In addition, it is prone to be unable to detect recombinant erythropoietins with similar apparent molecular masses as endogenous erythropoietins.

Results and Discussion

SDS-PAGE and IEF-PAGE are two orthogonal separation methods which complement each other in the detection of doping with recombinant erythropoietins. While IEF separates proteins according to charge, SDS-PAGE separates them according to their apparent molecular mass. Due to the lower masses of uhEPO and shEPO compared to most rhEPOs

(e.g. epoetins alfa, beta, and delta) and genetically (darbepoetin alfa) or chemically (MIRCERA) modified EPOs a differentiation between endogenous and exogenous erythropoietins is possible by SDS-PAGE. Despite the fact that Dynepo is produced in a human cell line (human fibrosarcoma cell line HT-1080) instead of a Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cell line, the IEF-profile is not identical to human endogenous EPO. On SDS-PAGE epoetin delta (Dynepo) produced a very sharp band, which was unusual when compared to epoetin alfa, beta, omega, darbepoetion alfa, PEGylated epoetin beta (MIRCERA), biosimilars, and human urinary and serum EPO. Due to this very characteristic band shape of Dynepo on SDS-PAGE on the one hand and a relatively intense alpha band on IEF-PAGE on the other hand the detection of doping with Dynepo appeared to be more simple by SDS-PAGE. The latter method also revealed additional information about the nature of active urines and atypical or effort-type IEF-profiles. Preferably, the amount of EPO applied on an SDS-PAGE gel should be equal for each lane. This can be accomplished by estimating the EPO concentration of the urinary retentates with a commercial ELISA kit before the immunoaffinity extraction. However, due to the limited mass resolution of SDS-PAGE bands of uhEPO and epoetin alfa or beta were slightly overlapping, but nevertheless with a clear difference in the average apparent molecular mass. Perfect separation was obtained for NESP and MIRCERA. Blood (serum, plasma) in combination with immunoaffinity purification and SDS-PAGE appears to be a very promising strategy for detecting EPO doping with most types of erythropoietins (including biosimilars) and latest generation of PEGylated erythropoiesis stimulating agents (e.g. MIRCERA, Hematide). [5]

References

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