Project Review

“Heparin: (more than) a masking agent in Epo-doping control”

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The detection of doping with recombinant peptide and protein hormones (e.g. erythropoietin – Epo, human growth hormone - hGH) is one of the most challenging analytical problems in doping control. The WADA-accredited method for the detection of doping with recombinant human erythropoietins (rhEpo) is based on isoelectric focusing (IEF) in carrier ampholytes.

Due to the risk of suffering a stroke or heart attack some athletes already admitted (e.g. the triathlet Lisa Huetthaler; published interview) having used anticoagulants (e.g. aspirin) in combination with Epo-doping. Heparin is one of the oldest and cheapest anticoagulants.

Our preliminary results showed that heparin has an at least threefold destructive effect on the Epo IEF-method, i.e. either (1) the Epo-profile gets completely destroyed or smeared, (2) the NESP-profile gets shifted to the endogenous area, or (3) a negative profile gets shifted to the basic area and thus leads to a false positive result.

The project investigates the effect of different heparin pharmaceuticals (unfractionated heparins, fractionated “low molecular mass” heparins) on both the Epo IEF-PAGE and SDS/Sarcosyl-PAGE methods and after administration of heparin to humans. Both biological matrices will be studied, urine and blood serum/plasma. Additionally, the capability of Epo immunoaffinity purification on the removal of heparin will be studied. What we already know is that ultrafiltration is not able to remove higher molecular mass heparins from urinary Epo.

Hence, the effect of a targeted enzymatic degradation of heparins on various Epo IEF and SDS-PAGE profiles (endogenous, recombinant) will be studied. This latter strategy might be a solution to the destructive effect of heparins on Epo analysis.

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Result and Conclusion

The detection of doping with recombinant peptide and protein hormones (e.g. erythropoietin - Epo, human growth hormone - hGH) is one of the most challenging analytical problems in doping control. The WADA-accredited method for the detection of doping with recombinant human erythropoietins (rhEpo) is based on isoelectric focusing (IEF) in carrier ampholytes.

Due to the risk of suffering a stroke or heart attack some athletes already admitted (e.g. the triathlete Lisa Hütthaler; published interview) having used anticoagulants (e.g. aspirin) in combination with Epo-doping. Heparin is one of the oldest and cheapest anticoagulants. Our results showed that heparin has an at least threefold destructive effect on the Epo IEF-method, i.e. either (1) the Epo profile gets completely destroyed or smeared, (2) the NESP profile gets shifted towards the endogenous area, or (3) a negative profile gets shifted to the basic area and thus leads to a false positive result. The project investigated the effect of different heparin pharmaceuticals (unfractionated heparins, fractionated "low molecular mass" heparins) on the analysis of endogenous and recombinant epoetins by IEF-PAGE and SDS/SAR-PAGE with spiked samples and after administration of heparin to humans. Both, urine and blood serum were used as sample matrices. It was demonstrated that Epo immunoaffinity purification is able to remove heparin and prevents its harmful effect on the Epo IEF profile. Alternatively, targeted enzymatic degradation of heparins prior to IEF- or SDS/SAR-PAGE can be used to reverse the destructive effect of heparin. No destructive effects of heparin were observed for SDS- and SAR-PAGE of EPO standards and urine/serum samples (independently of that result, urine and serum samples have to be immunoaffinity purified in order to avoid gel overloading with high abundant proteins).