"In vivo administration of EPO Biosimilars with low molecular weight: how to improve detection by anti-doping laboratories"

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Project overview

The increasing number of biosimilars of the first-generation EPOs (epoetin alfa and beta) produced all over the world raise the question of their detection. Due to small structural changes, for some EPO Biosimilars the identification criteria edicted by WADA for rEPO may not always be reached, which would render their use by athletes undetectable. Preliminary results have identified Hemax and Epotin authorized in Algeria and Jimaixin authorized in China as rEPOs with apparent molecular weights lower than the original epoetin alfa (Eprex) and closer to endogenous EPO. Thus identification using SDS/SAR-PAGE method could be problematic while their IEF profiles always remain very basic and distinct from endogenous EPO. To go further, evaluating detection from samples resulting of a real administration in healthy subjects is needed.

The objectives of this project are:
- to analyze blood and urine samples using IEF and SAR-PAGE methods according to the TD2014EPO and to compare identification capacities and establish the window of detection for each rEPO tested.
- to test complementary strategies to improve detection of EPO biosimilars: neuraminidase treatment that has been shown to improve the migration distance between rEPO and endogenous EPO as well as a 2-D separation gel approach (mixing IEF and SDS separation) will be tested by AFLD on samples that show a problematic rEPO identification.

In addition, specific glycosylations of each Biosimilar will be characterized by LC-MS coupled to fluorescence.

Results and Conclusions:

Recombinant erythropoietin (rEPO) biosimilars are generic epoetin drugs developed following an expired patent. All licensed EPO biosimilars shall demonstrate the same safety and efficacy for therapeutic use as the original drug but small structural differences compared to the reference product due to some variations in the production process can be accepted. After analyzing various EPO-biosimilars, three different kinds were selected for further characterization due to their slightly lower apparent molecular weight (MW) compared to the original epoetin alpha drug Eprex®. Jimaixin™ authorized in China, and Hemax® and Epotin™ authorized in Algeria.

The aims of this research were:

i) to study the electrophoretic profiles obtained by IEF and SDS-PAGE of the three Biosimilars spiked in urine and plasma and to evaluate their identification following WADA's criteria (TD2014EPO),

ii) to test complementary strategies to improve detection of EPO biosimilars using a two-dimensional electrophoresis approach (SDS separation following IEF) and a neuraminidase treatment of the sample shown to increase the separation between recombinant and endogenous EPO by SDS-PAGE,

iii) to evaluate by mass spectrometry the specific N-glycosylation pattern of each biosimilar and compare with the original rEPO Eprex®.

Experiments with spiked urine and plasma samples showed that samples spiked with the Biosimilars were more challenging to identify for rEPO compared to Eprex in particular at low amount. Differences were seen according to the method of identification used and the biosimilar spiked. Epotin and Jimaixin were more difficult to identify by IEF compared to SDS-PAGE while it was the opposite for Hemax. The SDS-PAGE method applied to urine samples had the higher identification rate considering the three biosimilars. Two-dimensional electrophoresis experiments did not improve the detection. This analysis proved complex to perform and no clear criteria could be used to identify rEPO.

Samples pre-treated with Neuraminidase gave promising results. Using the SDS-PAGE, the EPO bands were slightly broader compared to untreated samples. Neuraminidase-treated Dynepo could be used as a separation marker between endogenous and exogenous signals and this could improve the identification of low doses of biosimilars. By IEF, an interesting pattern for the biosimilars treated with neuraminidase was found. Using a 2-10 pH gradient gel was necessary to detect three thin additional bands inserted between the main EPO isoforms. This characteristic was observed for the three biosimilars while these bands were totally absent in non-spiked samples.

N-glycosylations of Eprex® and the biosimilars were identified by MALDI-TOF and abundance of the various glycan forms were compared.

All three biosimilars were enriched in bi and tri antennae forms while a decrease was observed in particular for the main glycan forms of Eprex® (tetraantennae tri- and tetra-sialylated forms). Hemax had the glycan profile the closest to Eprex® while Epotin™ and Jimaixin™ presented more loss of

sialic acids. These results on N-glycan were in good agreement with the presence of additional basic isoforms in their IEF-PAGE profile.

In conclusion, even if these biosimilars may present some challenges at low concentration, current methods used in the anti-doping laboratories and if necessary, an easy-to-implement neuraminidase pretreatment of the samples can assure detection of these compounds.