Structural comparison analysis of Erythropoietin and its biosimilars by a proteomic approach

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The upcoming patent expiration for many biotechnology drugs increases the interest in developing biosimilar products. The structural complexity of biological products requires structural and functional comparability studies between the 'originator' and biosimilars before their authorization.
This represents a driving force for the development of analytical workflow able to characterize the primary structure of the proteins and at the same time to detect products differences and impurities.
This work shows the proteomic approach (as reported in figure 1) developed to characterize the biotechnological drugs erythropoietin (rhEPO) and its biosimilars. The major aim of this comparison is to verify the identity of primary sequence and to individuate the post-translation modification for the protein products. [1-3].
In this study qualitative and quantitative analyses were performed by Tandem time of flight Matrix-Assisted Laser desorption/ionization mass spectrometry (MALDI-TOF/TOF) and liquid chromatography (HPLC) respectively.
Briefly, we performed a drug purification both by 2DE gel-electrophoresis and by HPLC.
In order to establish the exact molecular weight of the biopharmaceuticals under studied, the purified intact proteins, derived from separation by HPLC, were analyzed by MALDI-TOF/TOF mass spectrometer in linear ion mode.
Then, to verify the identity of primary amino acid sequence, spots derived by 2DE gel electrophoresis were subjected to in-gel trypsin digestion and the peptides were analyzed by MALDI-TOF/TOF mass spectrometer in reflector ion mode. The Peptide mass fingerprinting obtained, that is the set of peptide masses derived from the digestion, were matched with the theoretical peptide masses generated by specific protein database (e.i. MASCOT database), in order to identify the proteins.
2DE gel electrophoresis allowed to identify the different isoforms, derived from glycosylation of proteins.
The limit of MALDI-TOF/TOF mass spectrometry is the lack of ability to perform quantitative analysis. In order to verify the exact concentration of proteins in the three pharmaceutical formulations, we performed quantitative analysis by HPLC-UV. In addition, this technique allowed to individuate the possible presence of impurities or of degradation products.
In conclusion, the analytical strategies allowed to compare the rhEPO with their biosimilar, highlighting some differences due to the glycosylation patterns of proteins.
These results demonstrate that this approach could be a valid method to perform comparability studies for biosimilar products.

References:
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