Title: **Characterization of recombinant human erythropoietin glycosylation by capillary electrophoresis and mass spectrometry.**

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The purpose of this work has been to investigate the differences in the glycosylation pattern of both recombinant human erythropoietin (rhEPO) and neuroEPO, an epoetin with low sialic acid content. It has been demonstrated in various animal models of stroke that erythropoietin has neuroprotective effects when delivered intranasal. Since neuroEPO does not have erythropoiesis-stimulating activity, it does not show the problems rhEPO has when administered systematically, thus it has a big potential in this field. Intranasal delivery is a non-systemic route that allows the drug to cross the blood-brain barrier (BBB) to be delivered to the central nervous system in a few minutes.

In order to accomplish a differentiation of both epoetins (rhEPO and neuroEPO) two analyses were carried out. First, they were analyzed as intact glycoproteins by capillary electrophoresis (CE) coupled to UV detection using the methodology proposed by the European Pharmacopoeia (EurPh) for the separation of rhEPO glycoforms. This methodology was modified for the separation of neuroEPO glycoforms optimizing the pH of the separation buffer in order to achieve a better baseline resolution and separation. In second place, a ZIC-HILIC-MS methodology for the analysis of N-glycans was applied to both glycoproteins. Glycans were released with N-glycosidase F and labelled with [$^{12}C_6$]-aniline. The pre-established methodology allowed determining properly even minor glycans of rhEPO but it didn't work as well for neuroEPO glycosylation characterization. Intensities for the second glycoprotein were lower and the less abundant glycans couldn't be as well identified as for the first one.

In the future, the study of the characterization of neuroEPO will have to be carried on to be able to keep on developing its neuroprotective function.

Keywords: Erythropoetin, neuroEPO, capillary electrophoresis, mass spectroscopy, stroke, nasal delivery, glycoforms, glycans.