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School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

N-terminal PEGylation of recombinant human erythropoetin and evaluation of its biological activity and physicochemical stability

A. Roholamini Najaf Abadi[1], H. Faghihi[1,*], M. Hedayati[2]

[1]Department of Pharmaceutics, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Cellular Biology, Pasteur Institute of Tehran, Tehran, Iran

Background and Aims:rHu EPO is widely used in the treatment of anemia but its application is limited due to short half life. PEGylation ,the covalent attachment of PEG to proteins, can increase drug volume so decrease its renal clearance. Although PEGylation decreases *in vitro* biological activity due to less binding capacity to receptors, *in vivo* biological activity is prominently increases.
Methods: In acidic pH conditions PEG- Propionaldehyde of 20 KDa was reacted with N – terminal amino group of rHu EPO, because the PKa of this amine is less than other existing amine groups. After 15 h the reaction was stopped following Anion-exchange chromatography and size –exclusion chromatography to prepare a purified mono N –Terminally PEGylated rHuEPO. UT-7 cell lines assay was used to assess biological activity of PEGylated –EPO.
Results: Results showed declined *in vitro* biological activity in comparison to unmodified EPO .while*in vivo* activity would be increased significantly as a result of long duration of action. Stability test was performed in 4°C , the results showed more stability in PEGylated –EPO evaluated by *in vitro* biological activity.
Conclusions:N-Terminal PEGylation of Recombinant Human Erythropoetin was performed and the product showed enhanced stability and half life as a sheilding effect of PEG moiety. Although *in vitro* biological activity decreased as a result of PEG conjugation to protein molecule, long maintenance of protein would compensate this limitation to a larger extent.

Keywords: PEGylation; Erythropoetin; Biological activity; Stability