Synthesis and in vivo evaluation of PEGylated granulocyte colony stimulating factor (PEG-GCSF)

G. Yousefi, A. Tamaddon, F. Hassanshahi

Department of Pharmaceutics, Pharmacy Faculty, Shiraz University of Medical Sciences, Iran

Background and Aims: This study was designed to synthesize, purify and study in vivo effect of PEG-GCSF. Granulocyte colony-stimulating factor, GCSF is a growth factor which regulates the proliferation of neutrophilic granulocytes in order to treat cancer therapy-induced neutropenia. G-CSF shows short serum half-life, which necessitates multiple injections. In order to overcome this shortcoming, site-specific PEGylation of G-CSF was performed and in vivo biological activity of protein was studied.

Methods: Different molar ratio of GCSF and polyethylene glycol(PEG)-propionaldehyde 20kd in different pH and temperatures in the presence of sodium cyanoborohydride were reacted. Product of the reaction (monoPEG-GCSF) was characterized by Sodium dodecyl sulfate polyacrylamide gelelectrophoresis (SDS-PAGE) and size exclusion chromatography and then purified by ion exchange high performance liquid chromatography (HPLC). In vivo biological activity of product was evaluated by injecting the final product, standard PEG-GCSF and GCSF to male rats and measuring their white blood cells (WBC) and absolute neutrophil count (ANCs).

Results: The results show that in >1 molar ratio of PEG and GCSF, acidic pH and low temperatures the yield of reaction was >70%. Synthesis of PEG-GCSF could be confirmed by size exclusion HPLC and SDS-PAGE (staining with comasie blue and Iodine method). Ion exchange HPLC could separate monoPEG-GCSF from unreacted GCSF and PEG and purify the product. The profiles of increasing of WBCs and ANCs were similar for our product and standard PEG-GCSF, and both were higher than GCSF.

Conclusions: The technique was able to produce monoPEG-GCSF with high yields and in vivo biological activity of the product was comparable to standard drug.

Keywords: GCSF; PEGylation; Ion exchange HPLC; ANC