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

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Background and Aims: This study was designed to synthesize, purify and study invivo 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 invivo 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). Invivo 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 >1molar 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 invivo biological activity of the product was comparable to standard drug.

Keywords: GCSF; PEGylation; Ion exchange HPLC; ANC