PEGYLATION OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (R-H-G-CSF) PRODUCED IN ESCHERICHIA COLI: A NEW DRUG TO OVERCOME NEUTROPENIA

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Human Granulocyte Colony-Stimulating Factor (h-G-CSF) is a haematopoietic glycoprotein which acts on neutrophilic precursor cells stimulating proliferation, differentiation and functional activation of neutrophil granulocyte progenitor cells and mature neutrophils. The therapeutic potential of G-CSF for treating neutropenic patients has led to the development of different forms of recombinant human G-CSF (r-h-G-CSF) either produced as a glycoprotein, or as a non-glycosylated protein in bacterial cells, differing from the native protein by the presence of an additional methionine residue at the amino-terminal site of the r-h-G-CSF polypeptide chain. As other therapeutic proteins, G-CSF has a short *in vivo* half-life, but the covalent attachment to polyethylene glycol (PEG) is currently being employed to prolong its circulation time in the bloodstream. PEGylated proteins possess enhanced pharmacokinetic and pharmaco-dynamic profiles, along with sustained activity, thereby circumventing the necessity to administer frequent injections to patients. We propose a new PEGylated form of this cytokine, obtained by an enzymic reaction via bacterial transglutaminase between met-G-CSF and PEG 20K. Physico-chemical characterization of the new product (BK0026) identified the 135th glutamine as the only amino acidic residue involved in the covalent attachment to PEG. Our objectives are to demonstrate how this new PEGylation does not interfere with the protein bioactivity and that this innovative met-G-CSF is comparable to the commercial product Neulasta® (Amgen), PEG20K-N terminal-met-G-CSF. The pharmacological investigation showed that BK0026 is biologically active and its efficacy is equivalent to the reference product, when tested *in vitro* both in proliferation test, employing murine myeloblastic cell line NFS-60, and in receptor binding assay. When administered to normal and neutropenic CD rats, studied as an animal model for chemotherapy-induced neutropenia, BK0026 boosted significantly the dose-dependent proliferation of neutrophil cells. In neutropenic rats, BK0026 was able to control myelotoxicity: the recovery of neutrophils and white blood cells was dose-dependent. Pharmacokinetic study results have demonstrated that both drugs share the same pharmacokinetic profile. All these studies clearly demonstrate the comparability of biological activity and bioavailability of BK0026 to that of the reference product Neulasta. In conclusion, this new PEG-G-CSF could be successfully employed in clinical applications.